

First indication of Japanese mitten crabs in Europe and cryptic genetic diversity of invasive Chinese mitten crabs

Sarah Hayer¹, Dirk Brandis¹, Günther B. Hartl², Christine Ewers-Saucedo¹

1 Zoological Museum of the Christian-Albrechts University, Hegewischstrasse 3, 24105 Kiel, Germany **2** Institute of Zoology, Christian-Albrechts University, Am Botanischen Garten 1-9, 24118 Kiel, Germany

Corresponding author: Christine Ewers-Saucedo (ewers-saucedo@zoolmuseum.uni-kiel.de)

Academic editor: Adam Petrusek | Received 1 April 2019 | Accepted 15 July 2019 | Published 28 August 2019

Citation: Hayer S, Brandis D, Hartl GB, Ewers-Saucedo C (2019) First indication of Japanese mitten crabs in Europe and cryptic genetic diversity of invasive Chinese mitten crabs. NeoBiota 50: 1–29. <https://doi.org/10.3897/neobiota.50.34881>

Abstract

The Chinese mitten crab (*Eriocheir sinensis*) is a prominent aquatic invader with substantial negative economic and environmental impacts. The aim of the present study was to re-evaluate the genetic diversity of mitten crabs throughout their native and invaded ranges based on publicly available sequence data, and assess if multiple introductions or rapid adaptation could be responsible for biologically divergent mitten crabs in Northern Europe. We assembled available genetic data of a fragment of the mitochondrial cytochrome c oxidase subunit one gene (COI) for all species of the genus *Eriocheir*. We applied phylogenetic and population genetic analyses to compare native and invasive populations, and to identify possible source populations. The phylogenetic reconstruction revealed that five COI sequences from Europe, morphologically identified as Chinese mitten crab, actually belong to the Japanese mitten crab (*Eriocheir japonica*), representing the first indication of its presence in European waters. All other COI sequences from Europe could unambiguously be assigned to the Chinese mitten crab. In some Northern German populations of Chinese mitten crabs, genetic diversity was surprisingly high, due to seven unique haplotypes encoding several amino acid substitutions. This diversity may reflect a cryptic introduction from an unsampled native location, or rapid adaptation in the invaded range. Based on the genetic diversity shared between native and introduced range, Feiyunjiang, a tributary of the Yangtze River, emerges as a plausible source population for the original introduction of Chinese mitten crabs to Europe. This study highlights the complex and dynamic invasion processes of mitten crabs in Europe. We urge to further monitor mitten crab invasions using genetic tools.

Keywords

Species invasion, amino acid substitution, barcoding, COI, source population, rapid adaptation

Introduction

Species invasions have altered the global ecological landscape dramatically in the past centuries. Their impacts are exemplified by the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853), one of the taxa included in the list of the “world’s 100 worst invasive alien species” (Lowe et al. 2000). Native to Russia, China, Korea and Taiwan, it has been introduced to Europe at the beginning of the 20th century and subsequently to the United States via the ballast water of large shipping vessels (Panning and Peters 1933; Clark et al. 1998; Rudnick et al. 2000; 2003; Herborg et al. 2003, 2005; Dittel and Epifanio 2009). The economic and ecological effects of its invasion are staggering. While declining in abundance in its native range, where it is considered a delicacy and farmed extensively (Yuan 2005), it has become an unprecedented nuisance in its introduced range. During past mass occurrences, when thousands of crabs migrated from their adult inland freshwater habitats to marine spawning grounds, fishermen lost nets and even abandoned certain fishing grounds (Rudnick et al. 2000). River banks were destabilized due to the extensive burrowing activity of adult crabs (Rudnick et al. 2005). Moreover, ecological communities have the potential to be altered by competition with native crabs and crayfish (Rudnick et al. 2000; Gilbey et al. 2008; Dittel and Epifanio 2009).

Identifying the source or sources of such widespread invasions is an important task for risk assessment and species management. Assigning the geographic sources of invasive populations requires geographic differentiation within the native range. Such differentiation allowed, for example, to pinpoint the source populations of introduced olive populations in Hawaii and Australia as South Africa and western or central Mediterranean, respectively (Besnard et al. 2007). In contrast, if native populations are genetically homogeneous, or the employed genetic markers are not variable enough to detect genetic structure, source populations can only be assigned to broad geographic regions. The European shore crab (*Carcinus maenas*), for example, has invaded the East coast of North America twice (Roman 2006). The source of each invasion could be broadly categorized as Northern and Southern European, respectively, based on slight genetic structure in the native range and ecological differences of the two invading populations. More detailed assignment was hampered by broadly distributed common haplotypes of the studied marker in the native range, possibly caused by anthropogenic reshuffling of native diversity. In the case of another well-dated species invasion, the source of introduction of the North American spiny-cheek crayfish (*Faxonius limosus*) to Poland in 1890 could not be determined because the invaded range is dominated by a haplotype common throughout the native range (Filipová et al. 2011). Similarly, Hänfling et al. (2002) were unable to pinpoint a source population for the mitten crab invasion to Europe due to apparent genetic homogeneity in the native range. This could have been due to small sample sizes (6 to 10 individuals sampled per population) and observed low diversity (5 haplotypes only) of the employed genetic marker, a fragment of the mitochondrial cytochrome oxidase subunit one (COI). Under-sampling the native range is a general problem, not only with regard to the number of populations, but also with number of individu-

als analyzed per population (Muirhead et al. 2008). If only a few individuals are sampled, much of the genetic diversity present at any one site might be missed, obscuring assignment probabilities (Muirhead et al. 2008).

Since the initial assessment by Hänfling et al. (2002), several phylogeographic studies have characterized the genetic population structure of mitten crabs in their large native range (Sui et al. 2009; Wang et al. 2008; Xu et al. 2009). These studies detected five monophyletic lineages (Fig. 1). Some authors refer to these lineages as different species (e.g. Chu et al. 2003; Wang et al. 2008; Naser et al. 2012; Chen et al. 2017), others as subspecies or lineages (e.g. Tang et al. 2003; Xu et al. 2009, Zhang et al. 2012). We do not aim to resolve these taxonomic issues, but use the species names as unambiguous labels throughout the text. These lineages have distinct but partially overlapping ranges (Komai et al. 2006; Xu et al. 2009): the Hepu mitten crab (*Eriocheir hepuensis* Dai, 1991) is present in Southern China from Hepu to Oujiang, the Chinese mitten crab from Tongan in China to Vladivostok in Russia including Korea, the Japanese mitten crab (*Eriocheir japonica* (De Haan, 1835)) is the only lineage present in Japan, but occurs in Russia and Korea as well, *Eriocheir ogasawaraensis* Komai, Yamasaki, Kobayashi, Yamamoto & Watanabe, 2006 is restricted to the Ogasawara Islands and an additional formally undescribed Japanese mitten crab lineage is endemic to Okinawa (Fig. 1) (Wang et al. 2008). Based on combined sequence data for two mitochondrial gene fragments, population structure was significant in Chinese and Japanese mitten crabs, but less so in the Hepu mitten crab (Wang et al. 2008 using cytochrome oxidase subunit two and cytochrome b as genetic markers; Xu et al. 2009 using COI and cytochrome b). The significant population differentiation of both Japanese and Chinese mitten crab provides a working baseline for assigning source populations. To date, Japanese mitten crabs have sporadically occurred outside their native range in the United States only (Benson and Fuller 2019; Jensen and Armstrong 2004), but have not been detected in Europe yet. Chinese mitten crabs, on the other hand, have invaded both Europe and the United States successfully. Population genetic studies of Chinese mitten crabs from the invaded range came to quite discordant conclusions. Some studies found that populations within Europe were admixed (Hänfling et al. 2002; Czerniejewski et al. 2012), while others found significant levels of differentiation (Herborg et al. 2007; Otto 2012). In contrast, only a single COI haplotype of this species has been reported from the United States (Hänfling et al. 2002). While the source populations remained obscure, Hänfling et al. (2002) identified three haplotypes that were shared between the native and invaded ranges as well as a widespread invasive haplotype unknown in the native range, but found in both Europe and the United States. Given the presence of both invasive haplotypes detected in the native range as well as invasive haplotypes not detected in the native range, they concluded that multiple invasions occurred in Europe, and that the USA were likely invaded via Europe.

Invasive populations can become melting pots of novel genetic combinations with unforeseen adaptive potential (Geller et al. 2010). While species invasions are often associated with a loss of genetic diversity in the introduced range, either be-

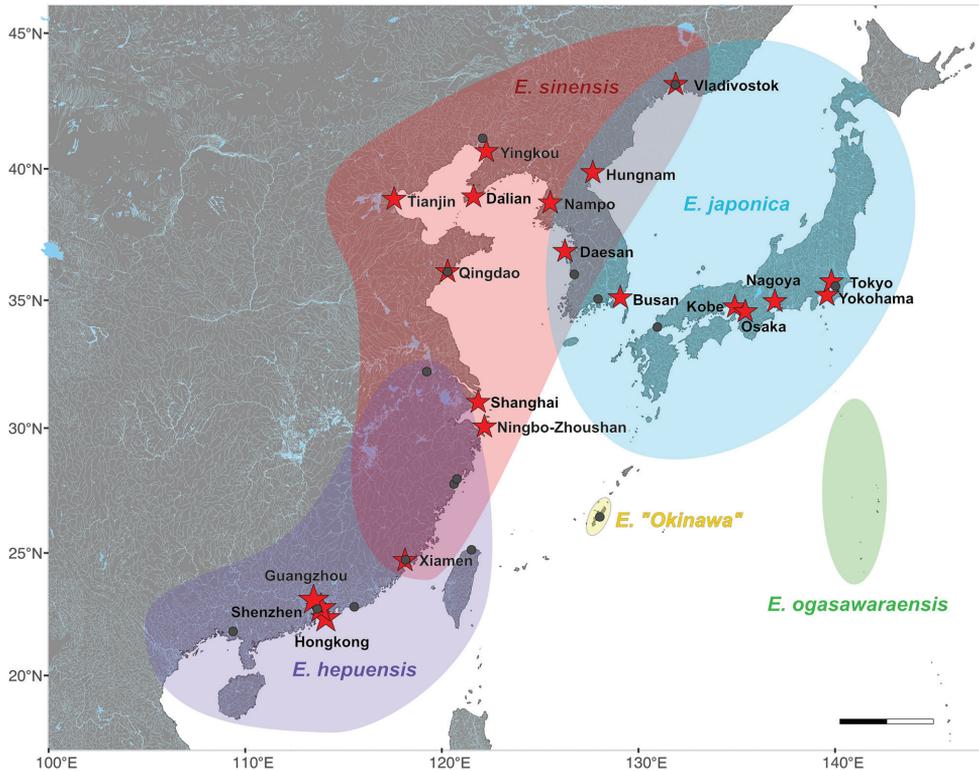


Figure 1. Native distribution of mitten crabs of the genus *Eriocheir*. Map redrawn from Xu et al. (2009), and species ranges interpolated around their sampling sites (marked by grey points) for each lineage to highlight range overlaps. Large shipping ports (marked by red asterisks) are most likely invasion sources.

cause only a few individuals invaded the range, and/or because genetic drift in these small populations subsequently reduces diversity (Nei et al. 1975), multiple invasions can alleviate the effects of these founding events (Dlugosch and Parker 2008). The brown anole lizard (*Anolis sagrei*), for example, has invaded Florida multiple times from geographically distinct source populations. Each source population had a unique genetic makeup, and admixture in the invaded range led to genetic diversity higher than in populations of the native range (Kolbe et al. 2004, 2007). Similar observations have been made for the common ragweed (Genton et al. 2005). Each new invasion might bring in new genetic variation, accompanied by novel ecological and physiological strategies that warrant attention (Geller et al. 2010).

This could be the case for Chinese mitten crabs. Otto (2012) reported on a novel reproductive behavior and physiology in invasive mitten crabs. In contrast to earlier reports (Anger 1991), mitten crabs completed their life cycle in the brackish Baltic Sea, and post-spawning females migrated back into the rivers, and did not die as in other populations. Otto (2012) concluded that this novel behavior might have been caused by a cryptic invasion of mitten crabs with different physiological requirements, in line with an earlier conclusion of cryptic invasions based on genetic data (Hänfling et al.

2002). Both lines of evidence, however, allow for an alternative explanation: invasive mitten crabs could have adapted rapidly to the brackish water conditions of the Baltic Sea, leading to novel genetic, physiological and behavioral diversity.

Rapid adaptation is emerging as a common feature of species invasions (Card et al. 2018; Dlugosch and Parker 2008; Prentis et al. 2008). In animals, hybridization between distinct introduced lineages, allele shifts due to bottlenecks and standing genetic variation are likely agents of rapid adaptation. Selection acts fastest on standing variation, and this process is suggested to be the most common driver of rapid adaptation (Prentis et al. 2008). The Burmese Python (*Python molurus bivittatus*), for example, is native to Southeast Asia and was introduced to Southern Florida in the early 1980s (Card et al. 2018). Despite several freezing periods that caused high python mortality, this species has become a successful invader into North America. Investigations showed that these freezing events resulted in a shift of python physiology caused by changes in allele frequencies in functional genes (Card et al. 2018).

The goal of this study was to re-evaluate the genetic diversity of mitten crabs of the genus *Eriocheir* throughout its native and invaded ranges, and to assess if multiple introductions or rapid adaptation might have caused the recent appearance of biologically distinct mitten crabs in Northern Europe. Given a large body of previous work, we utilized publicly available mitochondrial sequence data. We employed different approaches to assign source populations to the invasive populations in Europe and the United States. First, we reconstructed phylogenetic relationships among *Eriocheir* sequences, grouping thereby invasive individuals into the evolutionary lineages known from the native range. In the next step, we assessed the native distribution of haplotypes also present in the introduced range. Then, we calculated genetic distances for all population pairs, which we use on the one hand to evaluate population genetic structure in the native range, and on the other hand to identify which native populations are most similar to introduced populations. We assume thereby that allele frequencies have not shifted significantly since the invasion, and that enough individuals invaded the new range to mirror native allele frequencies. Bayesian assignment tests have been proposed as a suitable alternative to assign invasive individuals to source populations (Geller et al. 2010). They may not assume that populations are in migration-drift-equilibrium (Aktas 2015), but are limited to detect very recent gene flow between populations within the past few generations (Herborg et al. 2007). Given that the initial invasion occurred around 30 generations ago (generation times taken from Dittel and Epifanio 2009), neither the assumptions of genetic distance measures nor assignment tests are likely to be met. Thus, neither approach provides an ideal fit to the pattern of mitten crab invasion, but our inferences are reinforced when several approaches point to the same source populations. Lastly, we assessed the potential for adaptive evolution in the investigated mitochondrial DNA fragment by identifying amino acid substitution patterns in the introduced haplotypes. Mitochondrial genes are not known to be directly involved in osmoregulations or other adaptations to low-salinity conditions, but the amino acid sequence is highly conserved, being under strong purifying se-

lection (Meiklejohn et al. 2007; Pentinsaari et al. 2016). Any changes in the amino acid sequence we detect are at least unusual and warrant further investigation. Based on our findings, we form several hypotheses regarding the mitten crab invasions that should be followed up using expanded geographic sampling, genomic approaches and historical collections.

Methods

Data preparation

We downloaded all available sequences for the genus *Eriocheir* and for two outgroup species, *Neoeriocheir leptognathus* and *Platyeriocheir formosa* (acc. nos. AF316537, AF317326; Tang et al. 2003), from NCBI GenBank (<http://www.ncbi.nlm.nih.gov/Genbank>), and sequences of the Chinese mitten crab from the Barcode of Life Data System (BOLD, <http://www.barcodinglife.org>), ignoring sequences already available in GenBank. An initial screening showed that most sequences were mitochondrial. Thus, we mapped all sequences to a complete mitochondrial genome sequence of a Chinese mitten crab (GenBank acc. KY041629) in Geneious v. 9.1.8 (Kearse et al. 2012). We chose the genetic locus for subsequent analyses for which data existed from both the native and introduced range, a fragment of the gene for the cytochrome c oxidase subunit one (COI).

For the phylogenetic reconstruction, we included COI sequences of all *Eriocheir* species and two outgroups. Most GenBank data consisted of haplotypes, not the actual sequences for each sampled individual. For the population genetic analyses, we reconstructed original haplotype frequencies by replicating haplotype sequences according to the data reported in the publications, attaching locality information to these sequences, and excluding sequences without sampling site information.

Phylogenetic reconstruction

The first step was to assert the species affinities of all sequences within a phylogenetic framework. For this, we used all sequences available in GenBank and BOLD. We built a maximum likelihood tree with the PHYML (Guindon et al. 2010) plugin in Geneious v. 9.1.8 (Kearse et al. 2012) under the General Time Reversible substitution model, estimating the transition/transversion ratio, the proportion of invariable sites and the gamma distribution parameter. The number of substitution rate categories was set to four. Branch support was calculated from 100 bootstrap replicates. The goal of this phylogenetic reconstruction was not to understand interspecific evolutionary relationships, but to ensure the lineage affinities of the sequences given the recent taxonomic confusion (Chu et al. 2003; Tang et al. 2003; Wang et al. 2008; Xu et al. 2009), and similar morphology of the species (Naser et al. 2012).

Population genetic analyses: The search for invasion sources

All population genetic analyses were conducted in R version 3.3.3 (R Development Core Team 2018). We constructed a parsimony network of all haplotypes for each species that was found in Europe or the United States with the function ‘haplotype’ in the R package ‘haplotypes’ (Aktas 2015), highlighting native and introduced populations. We visualized the geographic haplotype distribution in the native and introduced range by adapting available scripts for haplotype networks. We then identified haplotypes found in both the native and introduced range and evaluated their distribution in the native range to identify possible sources for invasion.

For Chinese mitten crabs, the species with the most complex invasion history, we conducted further population genetic analyses to understand invasion patterns and get additional support for probable source populations. We compared haplotype and nucleotide diversity for all sampling sites, which we also refer to as populations. We wrote our own function to calculate haplotype diversity of each population based on the formula of Nei and Tajima (1981), and used the function ‘nuc.div’ of the ‘pegas’ package (Paradis 2010) to calculate nucleotide diversity (Nei 1987). We assessed if diversity indices were significantly different between native and introduced populations using standard ANOVA. We calculated Tajima’s D , which may indicate population size changes or selective sweeps, using the function ‘tajima.test’ of the ‘pegas’ package (Paradis 2010). Significant deviations from zero were estimated based on a beta distribution (Tajima 1989).

We calculated genetic differentiation between all population pairs as Φ_{st} and Jost’s D with the functions ‘pairwiseTest’ of the package ‘strataG’ (Archer et al. 2016), and the function ‘pairwise_D’ of the ‘mmod’ package (Winter 2012), respectively. Φ_{st} is a derivative of the classical fixation index F_{st} , developed for mitochondrial haplotype data (Excoffier et al. 1992). We estimated significant deviations from zero (no differentiation between population pairs) by comparison with an empirical distribution of Φ_{st} values based on 1000 permutations. Jost’s D provides a more accurate measure of population differentiation when genetic diversity is high and the number of unique alleles per population is large (Jost 2008). Significance was assessed by bootstrapping populations across 1000 replicates. Each measure of differentiation resulted in a large number of pairwise comparisons, which are difficult to interpret. Therefore, we visualized overall population similarity with Metric Multidimensional Scaling, using the function ‘cmdscale’, and with hierarchical cluster analysis, using the function ‘hclust’. Both functions are part of the R package ‘stats’. The analyses of population structure served on the one hand to assess how differentiated the native populations were, and therefore to indicate how narrowly we might be able to pinpoint the sources of introduction. On the other hand, we identified the native populations that were most similar to introduced populations as candidate source populations for the invasions.

We tested whether the native populations were sufficiently diverse to confidently assign individuals from the invaded range using the R package ‘assignPOP’ (Chen et al. 2018), which employs supervised machine learning to evaluate the discrimina-

tory power of genetic or non-genetic data by resampling cross-validation. This means that individuals from each population were randomly divided into training and test sets, and assignment tests were repeated through resampling training individuals 100 times. We used the R function ‘assign.MC’ to conduct Monte Carlo cross validation using 80% of individuals from each population as training data. The proportion of correctly assigned individuals provides an estimate of assignment accuracy, which we calculated with the function ‘accuracy.MC’. In case of sufficiently high discriminatory power, we would assign individuals from the invaded range to native populations using the function ‘assign.X’.

Amino acid substitutions: Indication of the potential for adaptation

We extracted the DNA sequence alignment for the haplotypes, which was generated during the construction of the parsimony network (function ‘haplotype’ of package ‘haplotypes’ and function ‘write.dna’ of package ‘APE’) (Aktas 2015; Paradis et al. 2004) and imported it into Geneious v. 9.1.8 (Kearse et al. 2012). We mapped it to the complete mitochondrial sequence of a Chinese mitten crab from China (GenBank acc. KY041629), and translated the DNA sequences to their amino acid sequence. This translation allowed us to identify amino acid substitutions. We assessed the directionality of change from the most parsimonious ancestral haplotype using the parsimony network constructed earlier.

Results

Data sources

On September 25, 2018, we downloaded a total of 1020 sequences for the genus *Eriocheir* from GenBank, including 11 complete mitochondrial genome sequences. From these, we extracted 106 COI sequences after aligning all sequences to the complete mitochondrial genome sequence of a Chinese mitten crab from China (Li et al. 2016). In addition, we included seven COI sequences of Chinese mitten crabs from BOLD that were not available in GenBank. Prior to analyses, we removed sequence AF317334 of a Hepu mitten crab because it had unusually many substitutions towards one end of the sequence (a sign of poor sequence quality), and discarded sequence CBCC039-11 from BOLD because it was shorter than the other sequences, but otherwise of the same haplotype and sampling site as CBCC049-11. None of the remaining sequences translated any stop codons, which would have indicated sequencing errors or the presence of nuclear pseudogenes.

The final alignment for the phylogenetic reconstruction contained 141 COI sequences (553 bp long) deposited in GenBank and BOLD under the names of the

following species: 2 sequences of *E. ogaswaraensis* (Xu et al. 2009), 45 sequences of Japanese mitten crabs (Tang et al. 2003; Xu et al. 2009), 6 sequences of Hepu mitten crabs (Chu et al. 2003; Tang et al. 2003; Naser et al. 2012; Wang et al. 2016), 82 sequences of Chinese mitten crabs (Hänfling et al. 2002; Chu et al. 2003; Tang et al. 2003; Sun et al. 2005; Xu et al. 2009; Czerniejewski et al. 2012; Otto 2012; Zhang et al. 2015; Raupach et al. 2015; Li et al. 2016; Wang et al. 2016) and two sequences of the outgroup species.

For the population genetic analyses, we removed the following sequences without sampling site information: AF105247, FJ455507, NC_011597, and FJ455505. The final COI dataset for population genetic analyses contained 455 sequences of Chinese mitten crabs from 45 populations belonging to 20 haplotypes and 38 COI sequences of Japanese mitten crabs from 8 populations belonging to 14 haplotypes. We reconstructed the population haplotype frequencies only for these two species because we wanted to infer the invasion sources of European and US invasions. The population-specific sequence information for Japanese and Chinese mitten crabs are summarized in Suppl. material 1: Tables S1 and S2, respectively.

Phylogenetic reconstruction

Our phylogenetic reconstruction recovered five main lineages of the genus *Eriocheir*, in agreement with previous phylogenetic studies (Xu et al. 2009; Naser et al. 2012) (Suppl. material 2: Fig. S1). For legibility, we provide the phylogenetic reconstruction with haplotypes only, highlighting their occurrence in the invaded range (Fig. 2). Haplotypes from invasive individuals of *Eriocheir* belonged to Japanese mitten crabs (Europe), Chinese mitten crabs (Europe and North America) and Hepu mitten crabs (Western Asia). The invasion of Hepu mitten crabs into Iraq has been discussed in detail elsewhere (Naser et al. 2012), and we focus our analyses on Chinese and Japanese mitten crab lineages.

The occurrence of Japanese mitten crabs outside of their native range has not been reported previously. The phylogenetic reconstruction recovered that five European individuals identified as Chinese mitten crab actually grouped with Japanese mitten crabs (Fig. 2). One crab was collected in Germany in 2009 (Raupach et al. 2015), one in Poland in 2015 by Dagmara Wojcik-Fudalewska (BOLD acc. OZ-IMP066-15), and three individuals caught in Holland and obtained from a seafood retailer were studied by Cristian Bernardi of the Università degli Studi di Milano in 2011 (BOLD acc. CBCC038-11, CBCC039-11, CBCC040-11). Sequences of the Polish and Dutch individuals were only available in BOLD (<http://www.boldsystems.org>), not in NCBI GenBank, and have, to our knowledge, not been part of scientifically published studies. The origin of the latter was indicated in BOLD ambiguously as “Italy, Holland” without geographic coordinates but clarified in direct communication with C. Bernardi.

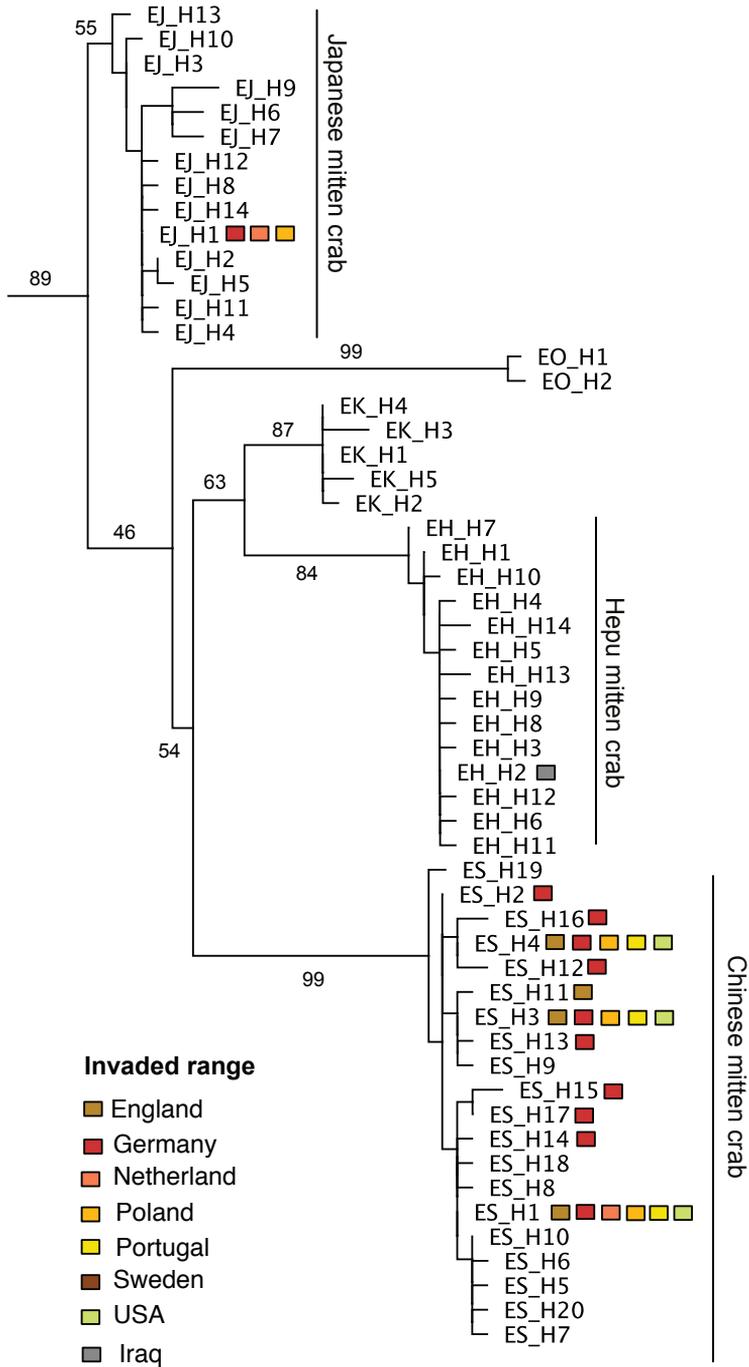


Figure 2. Maximum likelihood tree of mitten crab COI haplotypes. Haplotypes found in the invaded range are color-coded based on the country of collection. Numbers on branches represent bootstrap support. Branches without numbers have less than 50% bootstrap support. We omitted the outgroups from the figure to save space.

Population genetic analyses

We identified 14 haplotypes for Japanese mitten crabs, labelled H1 to H14 (Fig. 3B). The most common haplotype, H1, occurred in all populations but Shimonoseki, Japan. The invasive individuals found in Germany, Poland and Holland also had this haplotype, thus limiting our ability to assign a more detailed source population to invasive Japanese mitten crabs, and not meriting further population genetic analyses.

We identified a total of 20 haplotypes for Chinese mitten crabs (Fig. 3A). Of these, nine haplotypes were found only in the native range, seven haplotypes only in Europe and four haplotypes in both native and introduced ranges. The geographic distribution maps visualize that haplotypes found only in the native region (blue colors) are common in central and northern Asia (Fig. 4A). Most of Europe is dominated by three of the four haplotypes shared between the native and introduced region (H1, H2, H4), which are more or less common in the native range: H1 was widespread in both the native and introduced range, thus providing little detail about the source of invasion (Fig. 4). H2 has a widespread distribution in its native range, found in two northern locations, Dalian City and Wuhu, and two central locations, Liaohe and an unspecified part of the Yangtze River (Fig. 4A, Suppl. material 2: Fig. S2).

In the introduced range, H2 was found in two individuals only, one sampled in the Weser river near Oldenburg and the other in the Elbe river in Brandenburg, suggestive of its overall low frequency in the introduced range (Fig. 4D, Suppl. material 2: Fig. S2). H3 was reported from several central Chinese locations (Feiyunjiang, Hangzhou, Nantong, Yancheng, and Xhenjiang), and was widespread in Europe (Fig. 4, Suppl. material 2: Fig. S3). H4 was also widespread in Europe but reported in the native range only from Feiyunjiang (Fig. 4, Suppl. material 2: Fig. S4). In summary, three widespread invasive haplotypes were found in Feiyunjiang, making it a plausible main source for the invasion.

Several Northern German populations are genetically distinct: Aukrug, Eckernförde, Eider, Finkenwerder, Flemhude, Schlei, and Soholmer Au (marked with an asterisk in Fig. 4C, D). These populations contained most of the haplotypes not detected in the native range, which we colored in green (compare Fig. 4A and C). In contrast to the European populations, the documented diversity in the US populations is lower. The large, established populations of the West coast seem to consist of a single haplotype, H4 (Fig. 4B), while a single individual sampled from an unestablished population on the East coast of the United States has a different haplotype (H3).

Overall haplotype diversity for Chinese mitten crabs was 0.832, and ranged from 0 to 0.805 per population (Table 1). Overall nucleotide diversity was 0.00384, and ranged from 0 to 0.00475 (Table 1). Introduced populations did not have lower haplotype diversity than native populations ($Df = 1$, F -value = 0.46, p -value = 0.505), nor did the nucleotide diversity between native and introduced populations differ ($Df = 1$, F -value = 0.453, p -value = 0.508). Tajima's D ranged from -1.159 to 2.315 per population (Table 1), and was not significantly different from zero in all but one population

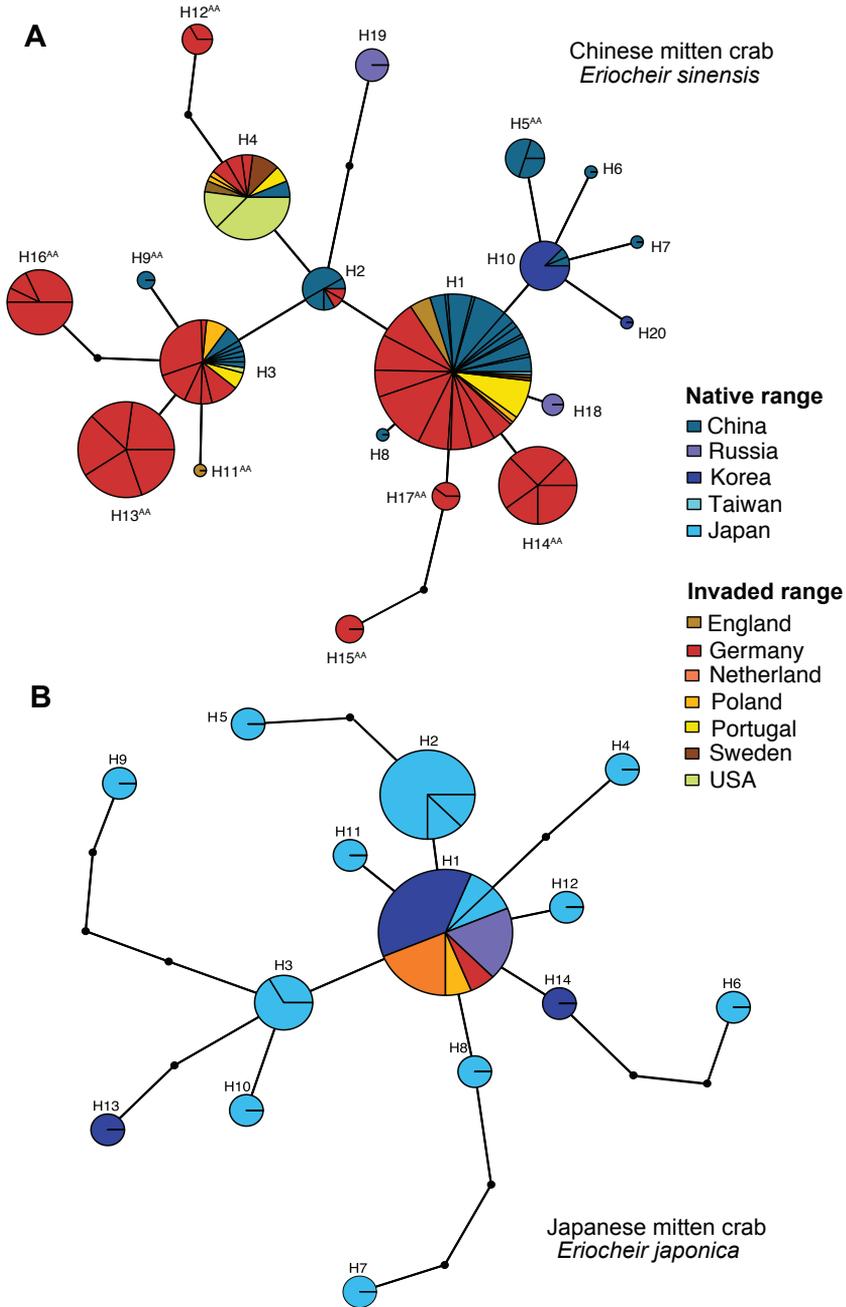


Figure 3. Parsimony networks for Chinese and Japanese mitten crabs. Each circle represents a haplotype, and the size of the circle is proportional to the abundance of this haplotype **A** Chinese mitten crab: The smallest circle (e.g. H8) contains a single sequence, while the largest circle (H1) contains 161 sequences **B** Japanese mitten crab: The smallest circle (e.g. H4) contains a single sequence, while the largest circle (H1) contains 18 sequences. The colors represent sampling sites: blue colors are native sites, yellow-orange-red colors are European sites and green colors are US sites.

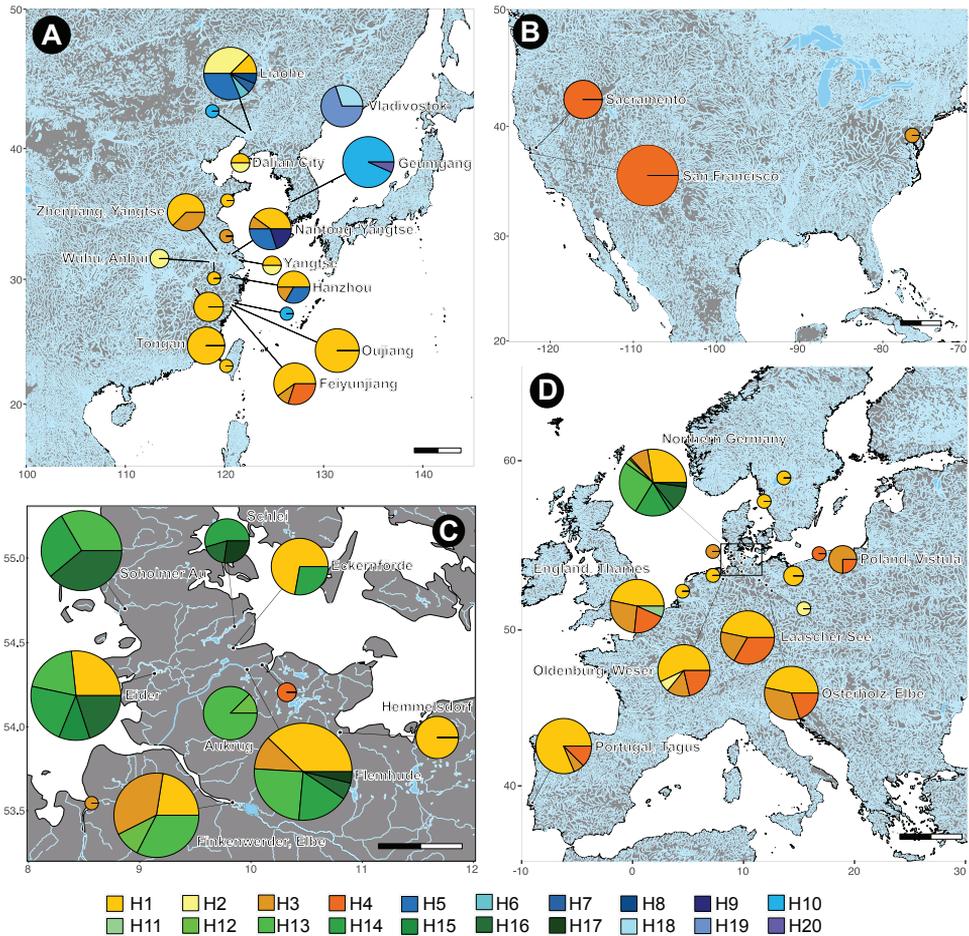


Figure 4. Geographic distribution of COI haplotype frequencies of Chinese mitten crabs. The distribution is shown in the native range (A), the United States (B), Northern Germany (C) and Europe in general (D). Haplotypes only found in the native range are colored in blue tones, haplotypes only found in the introduced range are colored in green tones, and haplotypes found in both the native and introduced range are colored in yellow and orange tones. The smallest pie chart in each graphic represents a single individual. All populations with five or more sampled individuals are named. Additionally, in a we also point out the populations of Dalian City, Wuhu and Yangtze, as they contain the otherwise rare but invasive haplotype H2. Scale bars: 250 km (A, B, D). 25 km (c).

(Soholmer Au, $D = 2.315$, p -value = 0.021). In some cases, this could be the result of low sample size, which reduces the power to detect deviations from the null expectation.

A total of 9 haplotypes were private. They were distributed among four native sites (Liaohu: H6, H7, H8; Nantong: H9; Vladivostok: H18, H19; Geumgang: H20) and two introduced sites (Thames: H11; Eider: H15). Estimates of population differentiation among native populations with five or more sampled individuals revealed significant population structure across the native range (Suppl. material 1: Table S3).

Table 1. Results of population genetic analyses of Chinese mitten crab (*Eriocheir sinensis*) populations from sampling sites with more than five sampled individuals.

Sampling site	n	h	Haplotype diversity	Nucleotide diversity	Tajima's D	D p-value
Native range						
Feiyunjiang, China	10	3	0.600	0.002	0.473	0.636
Hangzhou, China	6	3	0.733	0.003	-0.057	0.954
Liaohe, China	16	6	0.783	0.003	0.095	0.924
Nantong, Yangtze, China	10	4	0.778	0.004	1.032	0.302
Oujiang, China	11	1	0.000	0.000	NA	NA
Tongan, China	8	1	0.000	0.000	NA	NA
Zhenjiang, Yangtze, China	8	2	0.536	0.002	1.449	0.147
Vladivostok, Russia	10	2	0.467	0.003	1.229	0.219
Geumgang, China	15	2	0.133	0.000	-1.159	0.246
Invaded range						
Thames, England	15	4	0.714	0.003	0.666	0.505
Aukrug, Germany	16	2	0.233	0.002	-0.744	0.457
Eckernfoerde, Germany	18	2	0.425	0.001	0.870	0.385
Eider, Germany	45	5	0.805	0.005	1.925	0.054
Finkenwerder, Elbe	40	4	0.729	0.003	0.758	0.448
Flemhude, Germany	53	6	0.766	0.004	1.171	0.241
Hemmelsdorf, Germany	10	1	0.000	0.000	NA	NA
Laascher See, Germany	15	3	0.676	0.002	1.386	0.166
Oldenburg, Weser, Germany	14	4	0.648	0.002	0.683	0.495
Osterholz, Elbe, Germany	15	3	0.676	0.002	1.386	0.166
Schlei, Germany	11	3	0.655	0.004	0.821	0.412
Soholmer Au, Germany	36	3	0.679	0.005	2.315	0.021
Tagus, Portugal	16	3	0.342	0.001	-0.708	0.479
Sacramento, USA	7	1	0.000	0.000	NA	NA
San Francisco, USA	18	1	0.000	0.000	NA	NA

Abbreviations: n: number of available sequences/individuals sampled; h: number of haplotypes.

Jost's D ranged from 0 to 0.215 and Φ_{st} from 0 to 1 (Suppl. material 1: Table S3). The overall pattern of relative differentiation was very similar between the two measures (Suppl. material 1: Table S3). Vladivostok in Russia and Geumgang in South Korea were significantly differentiated from all other native populations, and from each other. Some populations were undifferentiated with either distance measure, and clustered closely: 1) Oujiang and Tongan, both monotypic for haplotype H1, and 2) Hangzhou, Nantong and Liaohe.

We used these pairwise genetic distances to identify which introduced populations were genetically similar to native populations, representing potential sources of the invasion. In general, populations dominated by the same haplotype cluster together. The two monotypic Chinese populations, Oujiang and Tongan, cluster together with the German populations from Hemmelsdorf, Tagus and Eckernfoerde (Fig. 5). A second large cluster consists of several non-native populations from Germany and England and the Chinese population from Feiyunjiang and Zhenjiang (Fig. 5). These populations are undifferentiated with regard to Jost's D, which ranged from 0 to 0.003, and Φ_{st} , which ranged from 0 to 0.035. The Northern German populations Aukrug, Eckernfoerde, Schlei and Soholmer Au are significantly differentiated from

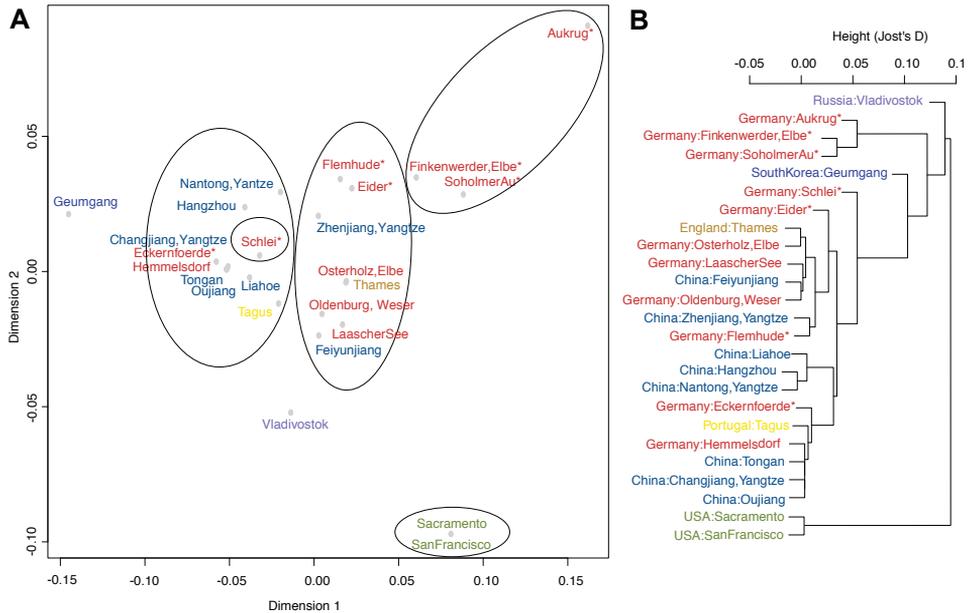


Figure 5. Visualization of genetic population similarity based on pairwise genetic differences calculated as Jost's D values. **A** multi-dimensional scaling plot **B** hierarchical cluster analysis dendrogram. Asterisks denote Northern German populations that are dominated by haplotypes not found in the native range.

each other and all other populations. In the MDS plot of the first two coordinate axes (Fig. 5A), the Schlei population appears to lie within the first large cluster, but it is differentiated well by the third axis (Suppl. material 2: Fig. S5). Similarly, the US populations are differentiated from all other populations. In the introduced range at large, Jost's D ranged from 0 to 0.234, and Φ_{st} from 0 to 1. Within Europe, Jost's D ranged from 0 to 0.234, and Φ_{st} from 0 to 0.691, making European populations much more differentiated than native populations.

The Monte Carlo cross validation procedure revealed little power to discriminate between source populations with assignment tests. The assignment accuracy averaged across replicates was 0.032. Thus, we did not attempt to assign invasive individuals to any particular native population with this method.

Amino acid substitutions

Amino acid substitutions took place in eight COI haplotypes: H5, H9, and H12 to H17 (Suppl. material 2: Fig. S6). Of these, the haplotypes H5 and H9 were only found in China, while H12 to H17 were the haplotypes only detected in Northern Germany. Based on the parsimony network, most substitutions occurred convergently. Only H15 evolved directly from H17. We can further infer the directionality of these substitutions from the haplotype network. It stands out that both proline and threonine evolved repeatedly in this small fragment of the COI gene.

Discussion

The Japanese mitten crab entered the European stage more than a decade ago

To our knowledge, we provide the first report of Japanese mitten crabs (*Eriocheir japonica*) outside their native range. Our phylogenetic reconstruction placed five sequences identified as Chinese mitten crabs clearly within the Japanese mitten crab lineage. The sequences were collected in Holland, Germany and Poland between 2009 and 2015. The German individual was collected inland in the Rhine river, and may not have necessarily migrated successfully to the North Sea for reproduction. The Dutch and Polish individuals were collected closer to the North and Baltic Sea, suggestive of an established, reproducing population of Japanese mitten crabs in Europe for the past ten years or more.

At first, it seems surprising that this invasion of Japanese mitten crabs has remained cryptic for at least a decade, but the morphological similarity between Chinese and Japanese mitten crabs did not make it obvious (Fig. 6) (Guo et al. 1997; Jensen and Armstrong 2004; Naser et al. 2012). Moreover, all European sequences of Japanese mitten crabs were generated as part of sequencing efforts of between one and a few *Eriocheir* specimens (see BOLD records), diluting the meaning of the high genetic dissimilarity to other Chinese mitten crab sequences. Raupach et al. (2015), for example, actually discussed the high intraspecific genetic diversity of mitten crab sequences in their large barcoding study from German waters. They noted that the observed high genetic distances in their sample of Chinese mitten crabs were caused by a single specimen, which we assigned to the Japanese mitten crab based on its COI sequence. Morphological species identification placed this individual clearly as a Chinese mitten crab (Raupach et al. 2015). Similarly, according to the shape of the interocular carapace rim, the Dutch individuals would be identified as Chinese mitten crabs (Guo et al. 1997), but their COI sequences belong to Japanese mitten crabs. Interestingly, the Barcoding of Life Data System itself recognized that the five sequences in question clustered with Japanese mitten crabs, not with Chinese mitten crabs (http://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:AAA8754). This discordance between morphology and mitochondrial sequence data may be due to the taxonomic confusion among *Eriocheir* species (Costa et al. 2007).

Cryptic morphology is a general problem in biological invasions that can only be resolved with molecular data. Bastrop and Blank (2006), for example, used mitochondrial sequence data to show that in addition to the invasive polychaete *Marenzelleria neglecta*, two more species of the genus had invaded the Baltic Sea unnoticed. The invasive populations of the virile crayfish consist completely of a lineage not yet identified in the native North American range (Filipová et al. 2010). The cosmopolitan reed *Phragmites australis* represents an unusual case of cryptic invasion, where a non-native haplotype is currently replacing the native genetic diversity in North America (Saltonstall 2002). The hypothesis of cryptic morphology is therefore clearly appealing

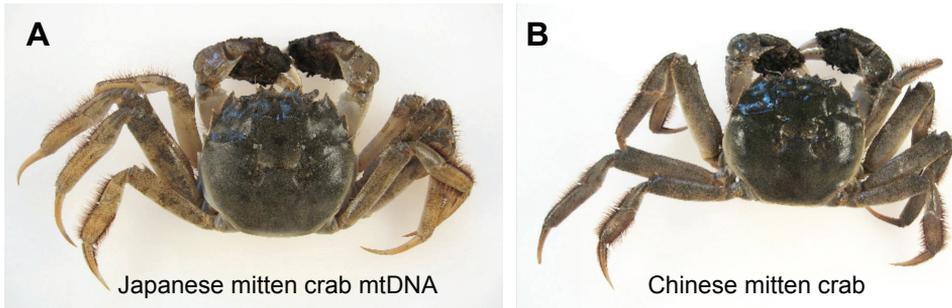


Figure 6. Mitten crabs collected in Holland in 2011. **A** Individual carrying mitochondrial DNA (mtDNA) of the Japanese mitten crab, morphologically identified as Chinese mitten crab, (BOLD accession: CBCC040-11, museum catalogue no: Università degli Studi di Milano, Ispezione degli Alimenti di Origine Animale MALAC:00040) **B** Chinese mitten crab (BOLD accession: CBCC037-11, museum catalogue no: Università degli Studi di Milano, Ispezione degli Alimenti di Origine Animale MALAC:00037). Photographs by Cristian Bernardi published under CC BY-NC 3.0 license.

and plausible. However, it is puzzling that all individuals identified as Japanese mitten crab at the sequence level were morphologically undoubtedly identified as Chinese mitten crabs. This discordance could hint at hybridization and subsequent introgression between Chinese and Japanese mitten crabs, resulting in morphological Chinese mitten crab hybrids carrying Japanese mitten crab mitochondrial genomes. This hybridization could have taken place either in the native or the invaded range. In such a case, pure Japanese mitten crabs do not necessarily have to form a stable population in Europe. Rather, their mitochondrial genomes would occur in some proportion of individuals with predominantly Chinese mitten crab genomes. Interspecific hybridization of another global invader has been confirmed for the Shore crab genus *Carcinus* using a combination of mitochondrial sequence data and nuclear microsatellite data (Darling 2011). To understand the current distribution of Japanese mitten crabs, possible hybrids between Japanese and Chinese mitten crabs or introgressed individuals in Europe, future systematic sampling, mitochondrial and nuclear sequencing of mitten crabs is highly warranted.

Significant genetic structure in the native and introduced range of Chinese mitten crabs

Much work has been conducted on the phylogeography of mitten crabs in their native range (Hänfling et al. 2002; Wang et al. 2008; Sui et al. 2009; Xu et al. 2009; Zhang et al. 2012; Zhang et al. 2014). Our re-analysis was therefore only aimed at assessing how useful the COI marker alone is to differentiate between populations, a prerequisite for identifying source populations with certainty (Geller et al. 2010), and to compare native and introduced diversity. We found that native populations are weakly but

significantly differentiated, but this differentiation does not align with geography or river system, as noted previously (Sui et al. 2009). One reason might be the exchange of crabs for commercial farming. Their extended planktonic larval period could also contribute to weak levels of genetic differentiation. This somewhat "chaotic" distribution of haplotypes makes it impossible to extrapolate the geographic distribution of genetic diversity, and precludes the assignment of broader regions as source regions. We can only discuss specific sampling sites as being more or less likely sources of introduction, as the genetic makeup of even the closest neighbor of any one site can be very different, e.g. in the case of Feiyunjiang and Oujiang. A more extensive sampling with regard to number of individuals and populations is certainly desirable to understand the patterns of diversity in the native range better. The fact that most populations were differentiated provides nonetheless a working baseline to assign source populations.

Most of the native populations had positive Tajima's *D* values, albeit not significantly different from zero, which is generally interpreted as populations being in mutation-drift equilibrium. It suggests that populations did not expand, shrink, or undergo recent selective sweeps at the mitochondrial genome. This pattern of genetic stability is anticipated for native populations. That we find the same pattern in most introduced populations is unexpected. We would expect to find negative Tajima's *D* values, indicative of recent bottlenecks. It seems unlikely that the introduced populations are already at equilibrium. Instead, an invasion of sufficient number of individuals that brought over a substantial amount of the native diversity could explain the observed pattern, either in a single or in multiple introduction events. In concordance with this idea, genetic diversity is not significantly lower in invasive populations, as would be expected when few individuals invade a new range.

The most distinct feature of the introduced range is the presence of seven haplotypes that have not been sampled in the native range. These haplotypes appear restricted to Northern Germany. Their distribution dominates the population structure in Europe, which divides populations with and without those unique alleles. We recovered more population structure than identified by Hänfling et al. (2002), and echo the findings of Otto (2012), who generated and analyzed the Northern German COI data initially. She did not take the other known data into account, however, thus limiting her conclusions.

Plausible source populations of the Chinese mitten crab invasion

Hänfling et al. (2002) conducted the first search for source populations of the European and US invasion. Using COI sequence data, they identified five haplotypes that occurred in both China (three populations sampled) and Europe (five populations sampled). They did not find significant population structure in either the native or introduced range, but observed a significant differentiation between those two. This was due to a haplotype common in all European and US populations, but absent in the native range. They used the presence of this haplotype as evidence for multiple introduc-

tions into Europe, and a secondary introduction of the United States from Europe. We identified this haplotype (H4) in one site in the native range, in Feiyunjiang. The other two haplotypes from Feiyunjiang (H1 and H3) are also common in Europe and the US, making this location the most plausible source of the invasion of those sampled so far. Feiyunjiang is located between the large ports of Shanghai and Xiamen (compare Figs 1–5), which are suitable donor locations, each of the many departing commercial vessels from their ports acting as potential invasion vectors. The results of the analysis of pairwise population differentiation are concordant with these findings. Feiyunjiang clusters with several of the introduced populations, and is not significantly differentiated from them. The last haplotype found in both native and introduced range is H2. It was not detected in Feiyunjiang, but was present in Dalian City, Wuhu, part of the Yangtze River and Liaohe. Any of these locations could therefore be the source population of a second independent invasion into Europe. Unfortunately, the first three sites are only represented by two individuals each, making detailed comparisons of haplotype compositions between these native and invaded sites impossible. Alternatively to a second independent introduction event from a different location, this haplotype might occur in low frequencies in Feiyunjiang, but was not recovered there due to small sample sizes. In this case, the colonization of Europe by all the above-mentioned haplotypes could have been due to a single successful invasion event. Given the low frequency of the haplotype H2 in the invaded range, this is clearly a possibility. In general, the large native range is under-sampled with regard to the number of individuals and number of populations (Muirhead et al. 2008; Geller et al. 2010). This becomes especially important given the weak and chaotic population structure across the native range, which limits our power to predict the region of origin. It is, however, highly unlikely that the northern range of Chinese mitten crabs, e.g. Russia and South Korea, where we recovered only haplotypes absent from Europe and the United States, was the source of the invasion.

The US populations of Chinese mitten crabs have been speculated to be secondarily introduced from Europe (Hänfling et al. 2002). Based on our analyses, this remains a possibility, as the West coast populations are of a single haplotype, which was only found in Feiyunjiang in the native range, but is widespread in Europe. However, whatever led to the successful invasion of Europe from Feiyunjiang (or another native population with similar haplotype composition) might also have led to the successful invasion of the United States. The low genetic diversity of these populations certainly argues for the invasion of few individuals. In contrast, the genetic diversity of European invaders is indicative of the invasion of several individuals. A single sequence available for a Chinese mitten crab from the East coast of the United States from an unestablished population (Benson and Fuller 2019) is genetically distinct from the monotypic West coast population, advocating for an independent invasion of the East coast. The East coast haplotype (H3) is present in Europe, but is also relatively widespread in China, making both a secondary invasion from Europe, or a direct invasion from Asia, equally likely. A secondary invasion from the West coast of the United States is, however, highly unlikely.

The analyses of genetic distances between populations echo on the one hand some of the results obtained by the comparison of haplotype identity between native and invaded ranges, and highlight, on the other hand, some of the difficulties associated with population genetic analyses of non-equilibrium scenarios pervasive during invasions. We found two clusters of mixed native and introduced populations: the first cluster contained populations from across Europe and Feiyunjiang. In line with the results of haplotype identity, Feiyunjiang is therefore a plausible source population. The second cluster contains populations monotypic for the most widespread haplotype. In this case, the native populations of Tongan and Oujiang have the same genetic makeup as the introduced populations of Hemmelsdorf, Eckernförde and Tagus, but this similarity may well be due to small populations and strong drift in the introduced populations, which could have eradicated much of the genetic diversity. Thus the second cluster of native and introduced populations cannot be interpreted as a separate introduction.

The distribution and origin of the uniquely Northern German haplotypes

The restricted distribution of the haplotypes H12 to H17 in northern Germany could either reflect a snapshot taken during an ongoing expansion or ecological restrictions. The most recent samples included in our analyses are those Northern German samples with unique haplotypes collected between 2008 and 2010 (Otto 2012). No sites outside of Northern Germany were sampled, which means these haplotypes may have already spread throughout the remaining European range. One indication of a recent origin or arrival of these haplotypes can be gained via a comparison with other studies that included sites close by. Hänfling et al. (2002) included a site in the Elbe river near Osterholz, from which they collected 15 crabs between 1999 and 2000 (see suppl. material 1: table S1). This site is downstream from the Finkenwerder site sampled by Otto (2012). None of the crabs collected in the Elbe between 1999 and 2000 had any of these uniquely Northern German haplotypes that were common between 2008 and 2010. Herborg et al. (2007) analyzed six microsatellite markers for six European populations, including the same Osterholz site. Otto (2012) also analyzed her samples with microsatellite markers, including four of the markers analyzed by Herborg et al. (2007). The raw data are not available (as is so often the case for microsatellite data), but at the four common microsatellite loci, the Finkenwerder samples from 2008–2010 show higher allelic richness ($A = 9.2\text{--}10.2$) than the Osterholz samples collected ten years earlier ($A = 4.3\text{--}9.1$) across all four loci. While this is suggestive of a recent addition of genetic diversity, it does not preclude a restricted distribution of those haplotypes either, as Chinese mitten crabs commonly show genetic structure within the same river systems (Herborg et al. 2007; Sui et al. 2009). A broad sampling of current mitten crab genetic diversity in the invaded range would clarify how widely distributed those haplotypes really are.

The origin of the haplotypes only found in Northern Germany remains mysterious. If we interpret the absence of those haplotypes in the Osterholz samples, and the presence of two of these haplotypes in the Finkenwerder samples ten years later as the recent and simultaneous addition of these haplotypes to Europe, the most plausible scenario is a cryptic invasion from an unsampled native site. The source of such a cryptic invasion might be located in the northern range of Chinese mitten crabs. Overall, the number of analyzed native populations was rather small given the large range of Chinese mitten crabs (Fig. 4A). The available data did not include some large ports, such as Tianjin, Nampo, Daesan and Hungnam, all located in the northern range of Chinese mitten crabs (Fig. 1), which could be suitable donor areas. Based on the known distribution of Chinese mitten crabs, the very large ports around Hong Kong can be excluded as the invasion source because Hepu mitten crabs, not Chinese mitten crabs, occur in southern China (Wang et al. 2008; Xu et al. 2009) (Fig. 1).

Under a scenario of multiple invasions, the amino acid substitutions we found in all of the uniquely Northern German haplotypes evolved in the native range, and were introduced during the cryptic invasion. Whether these haplotypes confer indeed a selective advantage cannot be answered with certainty. They may carry, in fact, neutral or slightly deleterious mutations but have been swept to high frequencies during a recent strong selection event at a linked region of the genome (Smith and Haigh 1974). The contemporaneous discovery of a novel physiology and behavior in Northern German mitten crabs, which allows them to complete the larval cycle in the brackish Baltic Sea (Otto 2012), may not be a coincidence. The expected range expansion caused by this novel ecology has already been documented by the recent and widespread occurrence of ovigerous females in the Eastern Baltic Sea (Ojaveer et al. 2007). Similarly, the occurrence of mitten crabs throughout much of the freshwater system of Sweden was hard to explain, invoking long-distance migration of crabs from their North Sea spawning grounds (Drotz et al. 2010). We suspect that these crabs belong to the same physiological type as the crabs investigated by Otto (2012), and are able to complete their larval cycle in the Baltic Sea.

Alternative hypotheses to a novel introduction can explain the origin of these uniquely Northern German haplotypes. In our opinion, the second most likely explanation is that the unique haplotypes evolved in the introduced range. Given that all of these haplotypes had one to three AAS, these haplotypes might have evolved rapidly in the introduced range in response to novel ecological conditions. Moreover, all of these uniquely Northern German haplotypes are closest related to a haplotype that was already present in Northern Europe (Fig. 3A). An argument against this hypothesis is that we would have expected to find some temporal sequence of haplotype evolution, with at least a few of the haplotypes occurring in earlier samples. Another hypothesis is that these haplotypes have been in Europe since the initial introduction, but either only recently increased in abundance, or were always restricted to Northern Germany, which had not been sampled before 2008. While genetic structure among sites or sampling years of the same river system exists (Herborg et al. 2007; Sui et al. 2009), crabs

have to migrate along rivers to get to their marine mating and breeding grounds. Thus some mixing of haplotypes should occur along the river. Given the small sample sizes of older sites, a recent expansion of these haplotypes from standing variation is clearly possible. It is not obvious, however, why these haplotypes would have remained at very low frequencies for about 100 years, since their introduction.

Lastly, we cannot ignore the fact that all sequences with uniquely Northern German haplotypes were collected by Otto (2012). If she had fallen prey to sequencing errors, we might expect randomly changed bases throughout each sequence, leading to many haplotypes present only once, and/or to the presence of stop codons. The fact that she sequenced many individuals with the same haplotype, and these translate to functional amino-acid sequences, makes sequencing error an unlikely source of these haplotypes. Furthermore, DNA fragments with uncertain base calls were sequenced in both directions (Otto 2012), which should remove possible sequencing artifacts caused by faulty sequencing chemistry (Wares, pers. comm.).

At this point, we cannot determine if the high and unique haplotype diversity of Northern Germany is due to novel, potentially adaptive mutations that occurred after introduction, or due to multiple invasions. To clarify the origin of the unique haplotypes, we propose three approaches. Firstly, a more extensive sampling of the native range should identify if these haplotypes are present in the native range. Such sampling has already taken place (Tang et al. 2003; Sun et al. 2005), but we were unable to incorporate these data into our study because they used genetic markers not yet applied to the introduced populations. Thus we propose that future genotyping efforts of invasive specimens should include these genetic markers as well. Secondly, population genetic analyses of invasive mitten crabs from museum collections could identify the temporal pattern of haplotype appearance. A sudden appearance of all unique haplotypes during the invasion history would hint at a new invasion event, while a stepwise appearance of novel haplotypes would be consistent with their evolution within the introduced range. Such pattern would, however, also be consistent with multiple additional invasion events, each introducing one or a few novel haplotypes. Lastly, population genomic analyses of native and invasive mitten crabs might reveal if the potentially adaptive haplotypes arose within invasive populations, in which case most of the genome of introduced Chinese mitten crabs should be undifferentiated and only small regions be highly differentiated. In contrast, a second introduction should show a more or less even differentiation across the genome. These efforts are aided by the recent publication of the nuclear genome of Chinese mitten crabs (Song et al. 2016) as well as the complete mitogenomes of several mitten crab species (Liu et al. 2015; Li et al. 2016).

Conclusions

This study uncovered complex population genetic pattern of invasive mitten crabs. Some of our findings are unambiguous, such as the presence of the mitochondrial genome of a second mitten crab species, the Japanese mitten crab, in Europe, suggest-

ing either a cryptic invasion of this species or previous hybridization between Chinese and Japanese mitten crabs. This new European addition was only revealed by our data synthesis, which included barcoding data collected from various entities of a few individuals. The genetic diversity within European populations of Chinese mitten crabs remains puzzling, including the presence of several amino acid substitutions in haplotypes found only in Northern Germany. Taken together with the contemporaneous occurrence of a novel physiology and behavior in the same populations, it is possible that carriers of this haplotype have an adaptive advantage. Given the negative impacts of mitten crabs as an invasive species, we can only urge to monitor these invasive populations closely, using genetic tools such as the commonly used barcoding locus COI (Darling and Blum 2007). Simultaneously, genomic and historical data could greatly enhance our understanding of the invasion process. We show that mitten crabs in Europe are melting pots of genetic diversity (Geller et al. 2010), making them prime targets to study cryptic invasions and possibly also rapid adaptations.

Acknowledgements

We would like to thank Dr. John Wares (UGA) for his improvements to the manuscripts, and his ongoing support for CES' work. We would also like to thank the reviewers Charles Fransen and Milan Koch, as well as the Neobiota subject editor Adam Petrušek. This study was supported by a grant from the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, project number 01UQ1711).

References

- Aktas C (2015) haplotypes: Haplotype Inference and Statistical analysis of genetic Variation.
- Anger K (1991) Effects of temperature and salinity on the larval development of the Chinese mitten crab *Eriocheir sinensis* (Decapoda: Grapsidae). *Marine Ecology Progress Series* 72: 103–110. <https://doi.org/10.3354/meps072103>
- Archer FI, Adams PE, Schneiders BB (2017) strataG: An R package for manipulating, summarizing and analysing population genetic data. *Molecular Ecology Resources* 17: 5–11. <https://doi.org/10.1111/1755-0998.12559>
- Bastrop R, Blank M (2006) Multiple Invasions – A Polychaete genus enters the Baltic Sea. *Biological Invasions* 8: 1195–1200. <https://doi.org/10.1007/s10530-005-6186-6>
- Benson AJ, Fuller PL (2019) *Eriocheir sinensis*: U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL. <https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=182>
- Besnard G, Henry P, Wille L, Cooke D, Chapuis E (2007) On the origin of the invasive olives (*Olea europaea* L., Oleaceae). *Heredity* 99: 608–619. <https://doi.org/10.1038/sj.hdy.6801037>

- Bouma S, Soes D (2010) A risk analysis of the Chinese mitten crab in The Netherlands.
- Card DC, Perry BW, Adams RH, Schield DR, Young AS, Andrew AL, Jezkova T, Pasquesi GIM, Hales NR, Walsh MR, Rochford MR, Mazzotti FJ, Hart KM, Hunter ME, Castoe TA (2018) Novel ecological and climatic conditions drive rapid adaptation in invasive Florida Burmese pythons. *Molecular Ecology* 27: 4744–4757. <https://doi.org/10.1111/mec.14885>
- Chen K-Y, Marschall EA, Sovic MG, Fries AC, Gibbs HL, Ludsin SA (2018) assignPOP: An R package for population assignment using genetic, non-genetic, or integrated data in a machine-learning framework. *Methods in Ecology and Evolution* 9: 439–446. <https://doi.org/10.1111/2041-210X.12897>
- Chen X, Wang J, Huang L, Yue W, Zou J, Yuan C, Lu G, Wang C (2017) Evolutionary relationship of three mitten crabs (*Eriocheir* sp) revealed by mitogenome and 5S ribosomal DNA analysis. *Aquaculture and Fisheries* 2: 256–261. <https://doi.org/10.1016/j.aaf.2017.10.004>
- Chu K, Ho H, Li C, Chan T (2003) Molecular phylogenetics of the mitten crab species in *Eriocheir*, sensu lato (Brachyura: Grapsidae). *Journal of Crustacean Biology* 23: 738–746. <https://doi.org/10.1651/C-2347>
- Clark PF, Rainbow PS, Robbins RS, Smith B, Yeomans WE, Thomas M, Dobson G (1998) The alien Chinese mitten crab, *Eriocheir sinensis* (Crustacea: Decapoda: Brachyura), in the Thames catchment. *Journal of the Marine Biological Association of the United Kingdom* 78: 1215–1221. <https://doi.org/10.1017/S002531540004443X>
- Costa, FO, DeWaard JR, Boutillier J, Ratnasingham S, Dooh RT, Hajibabaei M, Hebert PD (2007). Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 272–295. <https://doi.org/10.1139/f07-008>
- Czerniejewski P, Skuza L, Drotz MK, Berggren M (2012) Molecular connectedness between self and non self-sustainable populations of Chinese mitten crab (*Eriocheir sinensis*, H. Milne Edwards, 1853) with focus to the Swedish Lake Vänern and the Oder and Vistula River in Poland. *Hereditas* 149: 55–61. <https://doi.org/10.1111/j.1601-5223.2012.02246.x>
- Darling JA, Blum MJ (2007) DNA-based methods for monitoring invasive species: a review and prospectus. *Biological Invasions* 9: 751–765. <https://doi.org/10.1007/s10530-006-9079-4>
- Darling JA (2011) Interspecific Hybridization and Mitochondrial introgression in invasive *Carcinus* shore crabs. *PLOS ONE* 6: e17828. <https://doi.org/10.1371/journal.pone.0017828>
- Dittel AI, Epifanio CE (2009) Invasion biology of the Chinese mitten crab *Eriocheir sinensis*: a brief review. *Journal of Experimental Marine Biology and Ecology* 374: 79–92. <https://doi.org/10.1016/j.jembe.2009.04.012>
- Glugosch K, Parker I (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431–449. <https://doi.org/10.1111/j.1365-294X.2007.03538.x>
- Drotz MK, Berggren M, Lundberg S, Lundin K, von Proschwitz T (2010) Invasion routes, current and historical distribution of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853) in Sweden. *Aquatic Invasions* 5: 387–396. <https://doi.org/10.3391/ai.2010.5.4.08>

- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–91.
- Filipová L, Holdich DM, Lesobre J, Grandjean F, Petrusek A (2010). Cryptic diversity within the invasive virile crayfish *Orconectes virilis* (Hagen, 1870) species complex: new lineages recorded in both native and introduced ranges. *Biological Invasions* 12: 983–989.
- Filipová L, Lieb DA, Grandjean F, Petrusek A (2011) Haplotype variation in the spiny-cheek crayfish *Orconectes limosus*: colonization of Europe and genetic diversity of native stocks. *Journal of the North American Benthological Society* 30: 871–881. <https://doi.org/10.1007/s10530-009-9526-0>
- Geller JB, Darling JA, Carlton JT (2010) Genetic perspectives on marine biological invasions. *Annual Review of Marine Science* 2: 367–393. <https://doi.org/10.1146/annurev.marine.010908.163745>
- Genton B, Shykoff J, Giraud T (2005) High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. *Molecular Ecology* 14: 4275–4285. <https://doi.org/10.1111/j.1365-294X.2005.02750.x>
- Gilbey V, Attrill MJ, Coleman RA (2008) Juvenile Chinese mitten crabs (*Eriocheir sinensis*) in the Thames estuary: distribution, movement and possible interactions with the native crab *Carcinus maenas*. *Biological Invasions* 10: 67–77. <https://doi.org/10.1007/s10530-007-9110-4>
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate Maximum-Likelihood Phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321. <https://doi.org/10.1093/sysbio/syq010>
- Guo J, Ng N, Dai A, Ng PK (1997) The taxonomy of three commercially important species of mitten crabs of the genus *Eriocheir* De Haan, 1835 (Crustacea: Decapoda: Brachyura: Grapsidae). *Raffles Bulletin of Zoology* 45: 445–476.
- Hänfling B, Carvalho GR, Brandl R (2002) mt-DNA sequences and possible invasion pathways of the Chinese mitten crab. *Marine Ecology Progress Series* 238: 307–310. <https://doi.org/10.3354/meps238307>
- Herborg L-M, Rushton S, Clare A, Bentley M (2003) Spread of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards) in continental Europe: analysis of a historical data set. *Migrations and Dispersal of Marine Organisms*. Springer, pp 21–28. https://doi.org/10.1007/978-94-017-2276-6_3
- Herborg L-M, Rushton S, Clare A, Bentley M (2005) The invasion of the Chinese mitten crab (*Eriocheir sinensis*) in the United Kingdom and its comparison to continental Europe. *Biological Invasions* 7: 959–968. <https://doi.org/10.1007/s10530-004-2999-y>
- Herborg L-M, Weetman D, Van Oosterhout C, Hänfling B (2007) Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis*. *Molecular Ecology* 16: 231–242. <https://doi.org/10.1111/j.1365-294X.2006.03133.x>
- Jensen GC, Armstrong DA (2004) The occurrence of the Japanese mitten crab, *Eriocheir japonica* (De Haan), on the west coast of North America. *California Fish and Game* 90: 94–99.
- Jost L (2008) GST and its relatives do not measure differentiation. *Molecular Ecology* 17: 4015–4026. <https://doi.org/10.1111/j.1365-294X.2008.03887.x>

- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data (version 8.0.3). *Bioinformatics* 28: 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2007) Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. *Conservation Biology* 21: 1612–1625. <https://doi.org/10.1111/j.1523-1739.2007.00826.x>
- Kolbe JJ, Glor RE, Rodríguez Schettino L, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature* 431: 177. <https://doi.org/10.1038/nature02807>
- Komai T, Yamasaki I, Kobayashi S, Yamamoto T, Watanabe S (2006) *Eriocheir ogasawaraensis* Komai, a new species of mitten crab (Crustacea: Decapoda: Brachyura: Varunidae) from the Ogasawara Islands, Japan, with notes on the systematics of *Eriocheir* De Haan, 1835. *Zootaxa* 1168: 1–20. <https://doi.org/10.11646/zootaxa.1168.1.1>
- Li G, Xu J, Wang H, Chen X, Xu Y, Zheng Y, Mao X, Yang S (2016) Identification and analysis of the complete mitochondrial genome sequence of *Eriocheir sinensis*. *Mitochondrial DNA Part A* 27: 3923–3924. <https://doi.org/10.3109/19401736.2014.987267>
- 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.
- Meiklejohn CD, Montooth KL, Rand DM (2007) Positive and negative selection on the mitochondrial genome. *Trends in Genetics*, 23: 259–263. <https://doi.org/10.1016/j.tig.2007.03.008>
- Muirhead JR, Gray DK, Kelly DW, Ellis SM, Heath DD, MacIsaac HJ (2008) Identifying the source of species invasions: sampling intensity vs. genetic diversity. *Molecular Ecology* 17: 1020–1035. <https://doi.org/10.1111/j.1365-294X.2008.03669.x>
- Naser MD, Page TJ, Ng N, Apel M, Yasser AG, Bishop JM, Ng PK, Clark PF (2012) Invasive records of *Eriocheir hepuensis* Dai, 1991 (Crustacea: Brachyura: Grapsoidea: Varunidae): Implications and taxonomic considerations. *BioInvasions Records* 1: 71–86. <https://doi.org/10.3391/bir.2012.1.1.15>
- Nei M (1987) *Molecular evolutionary genetics*. Columbia University Press, New York. <https://doi.org/10.7312/nei-92038>
- Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution* 29: 1–10. <https://doi.org/10.1111/j.1558-5646.1975.tb00807.x>
- Nei M, Tajima F (1981) Genetic drift and estimation of effective population size. *Genetics* 98: 625–640.
- Ojaveer H, Gollasch S, Jaanus A, Kotta J, Laine AO, Minde A, Normant M, Panov VE (2007) Chinese mitten crab *Eriocheir sinensis* in the Baltic Sea—a supply-side invader? *Biological Invasions* 9: 409–418. <https://doi.org/10.1007/s10530-006-9047-z>
- Otto T (2012) *Reproduction Biology and Population Genetics of the alien Chinese mitten crab (Eriocheir sinensis) in Schleswig-Holstein*. Dissertation, Christian-Albrechts University.

- Panning A, Peters N (1933) Die chinesische Wollhandkrabbe (*Eriocheir sinensis* H. Milne Edwards) in Deutschland. Zoologischer Anzeiger (Suppl. material) 104: 1–180.
- Paradis E (2010) PEGAS: an R package for population genetics with an integrated-modular approach. *Bioinformatics* 26: 419–420. <https://doi.org/10.1093/bioinformatics/btp696>
- Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290. <https://doi.org/10.1093/bioinformatics/btg412>
- Pentinsaari M, Salmel, H, Mutanen M, Roslin T (2016) Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Scientific Reports* 6: 35275. <https://doi.org/10.1038/srep35275>
- Prentis PJ, Wilson JR, Dormontt EE, Richardson DM, Lowe AJ (2008) Adaptive evolution in invasive species. *Trends in Plant Science* 13: 288–294. <https://doi.org/10.1016/j.tplants.2008.03.004>
- R Development Core Team (2018) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>
- Raupach MJ, Barco A, Steinke D, Beermann J, Laakmann S, Mohrbeck I, Neumann H, Kihara TC, Pointner K, Radulovici A, Segelken-Voigt A, Wesse C, Knebelberger T (2015) The application of DNA barcodes for the identification of marine crustaceans from the North Sea and adjacent regions. *PLOS ONE* 10: e0139421. <https://doi.org/10.1371/journal.pone.0139421>
- Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceedings of the Royal Society of London B: Biological Sciences* 273: 2453–2459. <https://doi.org/10.1098/rspb.2006.3597>
- Rudnick DA, Halat KM, Resh VH (2000) Distribution, ecology and potential impacts of the Chinese mitten crab (*Eriocheir sinensis*) in San Francisco Bay. UC Berkeley: University of California Water Resources Center.
- Rudnick DA, Hieb K, Grimmer KF, Resh VH (2003) Patterns and processes of biological invasion: the Chinese mitten crab in San Francisco Bay. *Basic and Applied Ecology* 4: 249–262. <https://doi.org/10.1078/1439-1791-00152>
- Rudnick DA, Chan V, Resh VH (2005) Morphology and impacts of the burrows of the Chinese mitten crab, *Eriocheir sinensis* H. Milne Edwards (Decapoda, Grapsoidea), in south San Francisco Bay, California, USA. *Crustaceana* 78: 787. <https://doi.org/10.1163/156854005774445500>
- Saltonstall K (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences USA* 99: 2445. <https://doi.org/10.1073/pnas.032477999>
- Song L, Bian C, Luo Y, Wang L, You X, Li J, Qiu Y, Ma X, Zhu Z, Ma L, Wang Z, Lei Y, Qiang J, Li H, Yu J, Wong A, Xu J, Shi Q, Xu P (2016) Draft genome of the Chinese mitten crab, *Eriocheir sinensis*. *GigaScience* 5: 5. <https://doi.org/10.1186/s13742-016-0112-y>
- Smith JM, Haigh J (1974) The hitch-hiking effect of a favourable gene. *Genetical Research*, 23: 23–35. <https://doi.org/10.1017/S0016672300014634>
- Sui L, Zhang F, Wang X, Bossier P, Sorgeloos P, Hänfling B (2009) Genetic diversity and population structure of the Chinese mitten crab *Eriocheir sinensis* in its native range. *Marine Biology* 156: 1573–1583. <https://doi.org/10.1007/s00227-009-1193-2>

- Sun H, Zhou K, Song D (2005) Mitochondrial genome of the Chinese mitten crab *Eriocheir japonica sinensis* (Brachyura: Thoracotremata: Grapsoidea) reveals a novel gene order and two target regions of gene rearrangements. *Gene* 349: 207–217. <https://doi.org/10.1016/j.gene.2004.12.036>
- Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 595–595.
- Tang B, Zhou K, Song D, Yang G, Dai A (2003) Molecular systematics of the Asian mitten crabs, genus *Eriocheir* (Crustacea: Brachyura). *Molecular Phylogenetics and Evolution* 29: 309–316. [https://doi.org/10.1016/S1055-7903\(03\)00112-X](https://doi.org/10.1016/S1055-7903(03)00112-X)
- Wang C, Li C, Li S (2008) Mitochondrial DNA-inferred population structure and demographic history of the mitten crab (*Eriocheir sensu stricto*) found along the coast of mainland China. *Molecular Ecology* 17: 3515–3527. <https://doi.org/10.1111/j.1365-294X.2008.03850.x>
- Wang J, Huang L, Cheng Q, Lu G, Wang C (2016) Complete mitochondrial genomes of three mitten crabs, *Eriocheir sinensis*, *E. hepuensis*, and *E. japonica*. *Mitochondrial DNA Part A* 27: 1175–1176. <https://doi.org/10.3109/19401736.2014.936425>
- Winter DJ (2012) mmod: an R library for the calculation of population differentiation statistics. *Molecular Ecology Resources* 12: 1158–1160. <https://doi.org/10.1111/j.1755-0998.2012.03174.x>
- Xu J, Chan T-Y, Tsang LM, Chu KH (2009) Phylogeography of the mitten crab *Eriocheir sensu stricto* in East Asia: Pleistocene isolation, population expansion and secondary contact. *Molecular Phylogenetics and Evolution* 52: 45–56. <https://doi.org/10.1016/j.ympev.2009.02.007>
- Yuan X (2005) China national fishery statistic and analysis in 2004. *Fishery and Wealth Guide* 13: 13–16.
- Zhang D, Ding G, Ge B, Zhang H, Tang B, Yang G (2014) Comparative phylogeography of two marine species of crustacean: Recent divergence and expansion due to environmental changes. *Gene* 550: 141–147. <https://doi.org/10.1016/j.gene.2014.08.006>
- Zhang D, Ding G, Ge B, Zhang H, Zhou C, Tang B (2012) The redivision of geographic population and genetic structure of *Eriocheir* in the West-Pacific Ocean. *Gene* 503: 126–130. <https://doi.org/10.1016/j.gene.2012.04.070>
- Zhang H-B, Jiang S-H, Xuan F-J, Li C-F, Zhang D-Z, Zhou C-L, Tang B-P (2015) The complete mitochondrial genome of *Eriocheir japonica sinensis* (Decapoda: Varunidae) and its phylogenetic analysis. *Biochemical Systematics and Ecology* 62: 241–248 <https://doi.org/10.1016/j.bse.2015.09.008>

Supplementary material 1

Tables S1–S3

Authors: Sarah Hayer, Dirk Brandis, Günther B. Hartl, Christine Ewers-Saucedo

Data type: species data

Explanation note: Table S1. *Eriocheir japonica*. Table S2. *Eriocheir sinensis*. Table S3. Pairwise population.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/neobiota.50.34881.suppl1>

Supplementary material 2

Figures S1–S6

Authors: Sarah Hayer, Dirk Brandis, Günther B. Hartl, Christine Ewers-Saucedo

Data type: multimedia

Explanation note: Figure S1. Maximum likelihood tree of mitten crab COI sequences from GenBank and BOLD. Figure S2. Geographic distribution of COI haplotype H2 of the Chinese mitten crab (*Eriocheir sinensis*). Figure S3. Geographic distribution of COI haplotype H3 of the Chinese mitten crab (*Eriocheir sinensis*). Figure S4. Geographic distribution of COI haplotype H4 of the Chinese mitten crab (*Eriocheir sinensis*). Figure S5. Multidimensional scaling plot of the first and third axes based on Jost's D distances between sampling locations. Figure S6. Codons of the COI sequence of Chinese mitten crabs that translate to amino acid substitutions (AAS).

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/neobiota.50.34881.suppl2>

Long term monitoring of recruitment dynamics determines eradication feasibility for an introduced coastal weed

John K. Scott^{1,2}, Kathryn L. Batchelor¹, Bruce L. Webber^{1,2,3}

1 CSIRO Health & Biosecurity, 147 Underwood Ave, Floreat, Western Australia 6014, Australia **2** School of Biological Sciences, University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia **3** Western Australian Biodiversity Science Institute, 133 St Georges Terrace, Perth, Western Australia 6000, Australia

Corresponding author: Bruce L. Webber (Bruce.Webber@csiro.au)

Academic editor: Belinda Gallardo | Received 2 April 2019 | Accepted 16 July 2019 | Published 2 September 2019

Citation: Scott JK, Batchelor KL, Webber BL (2019) Long term monitoring of recruitment dynamics determines eradication feasibility for an introduced coastal weed. NeoBiota 50: 31–53. <https://doi.org/10.3897/neobiota.50.35070>

Abstract

Bitou bush (*Chrysanthemoides monilifera* subsp. *rotundata*) is a Weed of National Significance in Australia and has impacted a significant portion of the eastern coastline. Its discovery in Western Australia was, therefore, a cause for concern. Assessment and control of the isolated and well-defined population began in 2012. To assess the feasibility of eradication in Western Australia as a management outcome for bitou bush, we applied a rigorous data-driven quantification and prediction process to the control program. Between 2012 and 2018 we surveyed over 253 ha of land and removed 1766 bitou bush plants. Approximately 97 person-days were spent over the six years of survey. We measured the seed bank viability for five years starting in 2013, with the 2017 survey results indicating a decline of mean viable seeds/m² from 39.3 ± 11.4 to 5.7 ± 2.2 . In 2018 we found only ten plants and no newly recruited seedlings in the population. No spread to other areas has been recorded. Soil core studies indicate that the soil seed bank is unlikely to persist beyond eight years. Eradication of the population in Western Australia, defined as five years without plants being detected, therefore remains a realistic management goal. The information generated from the documentation of this eradication program provides invaluable insight for weed eradication attempts more generally: novel detection methods can be effective in making surveys more efficient, all survey methods are not entirely accurate and large plants can escape detection, bitou bush seeds persist in the soil but become effectively undetectable at low densities, and migration of seed was unquantifiable, possibly compromising delimitation. Continued monitoring of the Western Australian population will determine how much of a risk these factors represent to eradication as the outcome of this management program.

Keywords

Control effort, management, monitoring, population decline, seed bank, seed viability, survey, weed control, weed eradication.

Introduction

The management of invasive alien plants costs billions of dollars and represents a significant challenge for a range of stakeholder groups, including agriculture, conservation and tourism (Pejchar and Mooney 2009; Shackleton et al. 2019). Depending on the characteristics and context of the invasion, the suitability of management approach and the likelihood of different outcomes can vary substantially (Blackburn et al. 2011). If the situation is appropriate then a desirable goal is the enduring eradication of the target population, but the reality is that this end point is particularly difficult to achieve (Wilson et al. 2017).

Eradication means the elimination of every single individual of a species from a defined area to a level beyond which recolonization is unlikely to occur (Myers et al. 1998). In the case of plants, eradication can be a reasonable management goal if the area of spread is small, if the target species is easily identified among other vegetation, if the infestation is detected early enough, and if sufficient resources are available for the full eradication program, especially in the final stages when target abundance is very low (Wilson et al. 2017). Unfortunately most species targeted by eradication programs have different characteristics, and eradication is only feasible in a limited number of circumstances (Wilson et al. 2017). It is, therefore, particularly important to share lessons learnt from eradication attempts if we are to improve outcomes for these management programs.

Soil seed bank longevity is often a key determinant of eradication success or failure, but is rarely measured. Panetta (2007) estimated that weed eradication programs often required 10 or more years to achieve their objective, or at least attempt the objective of complete mortality of any remaining seed bank. Very few weeds have been successfully eradicated world-wide, largely due to the seed bank outlasting monitoring and control efforts. For example, in Australia Panetta (2004) mentions that *Eupatorium serotinum* in southern Queensland required 18 years, to eradicate despite covering a relatively small area. However, in Western Australia, the annual chenopod herb, *Bassia scoparia*, was eradicated following a ten year monitoring and control program (Dodd and Randall 2002). The success of this program was largely due to the weed having a short-lived seed bank, mostly one year, with up to three years possible (Dodd and Randall 2002).

We have previously reported on the discovery of one of Australia's Weeds of National Significance, *Chrysanthemoides monilifera* (L.) T. Norl. subsp. *rotundata* (DC.) T. Norl. (Asteraceae; hereafter, bitou bush), as a well-established population in Kwinana, a major port and industrial area near Perth, Western Australia (Weiss et al. 1998, Scott and Batchelor 2014). Our initial surveys in 2012 documented a restricted extent for this bitou bush incursion, aside from the potential for seed export from the site (Scott and Batchelor 2014). The Kwinana bitou bush population has existed, as verified from

aerial photography, since at least 1995 (Scott et al. 2019). The Kwinana population is unusual because the nearest populations of bitou bush are found more than 3,000 km to the east as an introduction in eastern Australia, or more than 8,000 km to the west as a native species of southern Africa (Scott and Batchelor 2014). This specific context makes it feasible to consider eradication as a management goal for bitou bush in WA, rather than local extirpation where there is a possibility of recolonization (Wilson et al. 2017).

Here we document the changing dynamics of the bitou bush infestation in Western Australia as an eradication program is implemented for its control. To address knowledge gaps in past eradication programs and to inform future control programs, we included information on both plants and the seed bank. We use this to make an ecologically informed assessment of the feasibility of eradication of bitou bush from WA, including for how long the infested area should be monitored. More generally, we use the example of bitou bush to illustrate how plant population biology data can be used to provide greater context, and thus improve the likelihood of success for the popular but rarely achieved objective of eradication for plant invasion management programs.

Materials and methods

Population surveys

The focal population was located in the Kwinana region of Perth, Western Australia – an industrial port area with mixed land-use, including fragments of low-quality native vegetation, and high levels of industrial goods traffic on and off-shore (32°12.652'S, 115°46.018'E). Scott and Batchelor (2014) provides details on the context of the focal population, including local climate, landscape and features that influence delimitation and detection. Initial surveys of the population started in September 2012 (Scott and Batchelor 2014), continued through 2013 and were repeated each year in March and April from 2014 to 2018 (Figures 1, 2). Additional site visits were made in spring (October–November) of each year, mainly to observe if there were newly germinated plants. The main infestation area only was surveyed in 2014 (the main or “core” infestation is defined in Scott and Batchelor (2014) Figure 1b). Port and industrial activity (e.g. construction of paved areas for heavy equipment, roads, new industries) meant that surveyed areas were not identical each year (Figure 1).

Surveys were done on foot, with personnel making traverses 5–10 m apart (depending on the density and height of vegetation). Particular interest was paid to areas under obvious bird perches (trees, fences, buildings and lights posts), likely locations for new seedlings resulting from bird dispersal of the fleshy coated seeds of bitou bush (Gosper 1999). Surveys were timed for March and April (i.e. end of summer) when the lush green of the bitou bush foliage is easily distinguished from surrounding vegetation. At this time the annual and perennial grasses and other annual vegetation has died back following the dry hot summer that is typical of the Mediterranean climate of this region. Records were kept of the search effort, measured in person days (where a

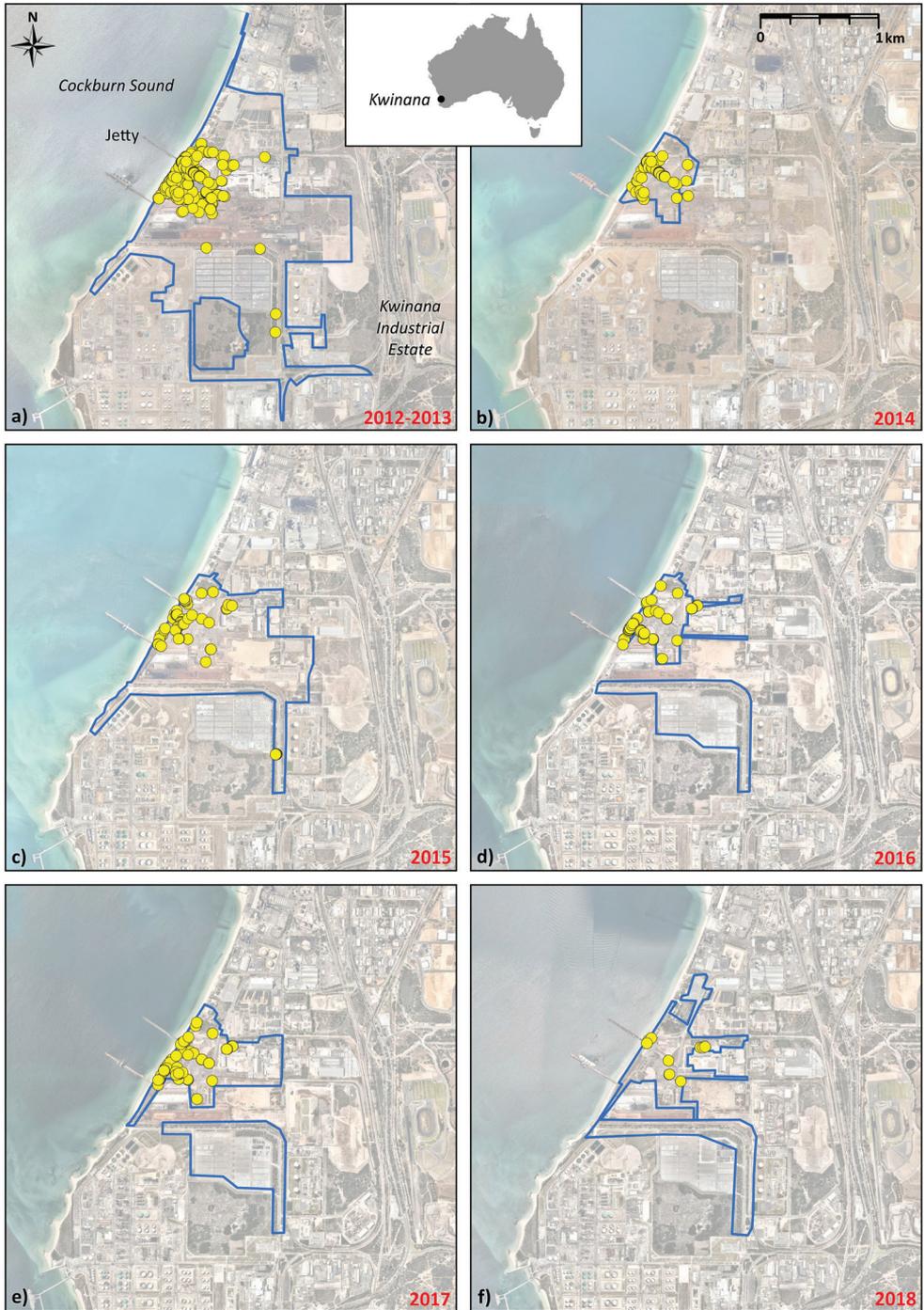


Figure 1. Location of each bitou bush (yellow dot) and outline of the survey area (blue) for each year (between 2012 and 2018) in the Kwinana Industrial Estate, Western Australia. Background images generated by Nearmap were taken in February of the designated year.



Figure 2. Bitou bush soil-core plant locations in the Kwinana Industrial Estate, Western Australia. Three zones were sampled: Kwinana Bulk Terminal (KBT) beach (red), Foreshore Rd (blue) and Horse Beach (green), in addition to a selection of other scattered plants. The background aerial photograph dates from 8 Jan 2012 (reproduced with permission of Western Australian Land Information Authority; CL05 – 2013).

day is considered to be 8 AM to 4 PM). A total of 97 person days have been expended on the survey (see Suppl. material 1).

The location of each bitou bush was mapped using a differential GPS (Hemisphere R100 Series Receiver utilizing the Australian Maritime Safety Authority DGPS beacon system for differential correction). All spatial information was managed within ArcInfo 10.3 (<https://www.esri.com/>).

Buffer zones

As the majority of plants discovered were found within a 500 m radius of a dilapidated wooden jetty, this area was defined as the main infestation (Scott and Batchelor 2014). A buffer zone of a further 500 m was calculated in ArcMap 10.3 based on the maximum observed dispersal distance (as shown in figure 3 in Scott and Batchelor (2014)). Any reproductive plants detected in this buffer zone would determine the need to expand the buffer zone until such point as the population was delimited, or at a point where the population was determined to be beyond the scope of eradication. To provide further confidence in survey scope decisions, additional transect areas were surveyed beyond the defined monitoring regions, either along the coast or based on potential areas of seed dispersal by birds. These outer areas were surveyed by vehicle where the landscape was open, or on foot where there was dense vegetation or lack of vehicle access. Further details of the buffer zones and initial surveys are provided in Scott and Batchelor (2014).

Aerial photography

To check for the possibility of missed plants in ground surveys, the survey area was examined using recently obtained very high resolution aerial photography (5.8–7.5 cm per pixel) in 2016 and 2018. These photos were available from Nearmap (<https://www.nearmap.com.au/>) via the University of Western Australia. Potential bitou bush plants that may have been overlooked by the ground surveys were identified from the photos and ground-truthed during additional surveys in 2016 and 2018.

Plant measurements

For each bitou bush plant we measured: size (maximum and minimum plant canopy diameter (m), maximum height (m), basal stem diameter (mm)), and reproductive output (number of capitula in flower, number of capitula with seed). A capitulum is an inflorescence consisting of a head of closely packed stalkless flowers typical of the Asteraceae. We noted the habitat type and whether there were proximate perch points for birds (e.g. trees, fences, other structures). Plants were classed as seedlings if the cotyledons were still attached and green. Seedlings were counted rather than measured for size.

Plant removals

We killed the plants once measurements were taken. This was mostly achieved by uprooting the plant by hand. Some larger plants (~40) were removed with the assistance of earth-moving machinery and/or killed by herbicide (arranged by the landowners). Follow up surveys confirmed the death of all plants sprayed with herbicide or that had been hand-weeded.

Bitou bush is relatively shallow rooted, but care was required to make sure all stem material was removed from the soil, as the plant is capable of regrowth from stems cut at ground level or by layering from buried stem sections (Weiss et al. 2008). Some layered plants required multiple annual visits to confirm that all layered stem sections had been found. Regrowing layered sections were not counted as new individuals. All uprooted plants were left *in situ* to mark their original location so any regrowth could be readily found in future surveys.

Most of the new plants found in 2013–2017 were seedlings germinating from the soil seed bank under the canopy area of plants removed in 2012. These new plants were all small enough to be removed by hand when detected. However, seedlings can be small and hidden in other vegetation and consequently not detected in the year of germination, but readily detected when larger in the following year. Even so, one or two plants per year remained previously undetected (e.g. hidden in bushes or in difficult to access parts of the port infrastructure) and only detected when flowering occurred. All these plants were removed by hand.

Seed bank studies

We selected 15 plants within three locations in the main infestation area (Beach, Foreshore Road, Horse Beach) as sites for detailed seed bank studies (locations shown in Figure 2). All selected plants had evidence of flowering and seed production in 2012 and were > 3 m in canopy diameter. In 2013, 10 soil cores (10 cm wide by 15 cm deep) were taken from underneath the canopy and 10 soil cores were taken 1 – 2 m from the perimeter of the plant canopy. Each soil core was sieved on site, outside of the canopy area, to minimise seed bank disturbance. Initially, large leaf litter was removed with a >1 cm sieve, then fine sand was removed using a < 3 mm mesh sieve. The remaining material from each soil core was taken in a paper bag to the laboratory where it was sorted under 10× magnification (Magilamp) to partition debris from seeds and seed fragments. Seeds were then stored in vials for further quantitative processing.

We dissected intact seeds found in each soil core to assess their viability. We counted seed fragments (the seed coat naturally splits into three portions on germination or decay) and noted whether these seed parts had evidence of rodent damage (i.e. gnawing on the seed coat) or other evidence of seed predation (holes in the seed). We counted seed fragments because this provided confirmatory evidence that the soil core

was taken under the original canopy (note that the plants were killed in 2012 and the canopy extent was known). Seed viability was assessed by the condition of endosperm in intact seed: firm, pale green/white in a viable seed, or dried, blackened or decayed in an unviable seed. Each soil core was assessed separately and aggregated by plant number for analysis.

We used the same method to measure the seed bank in 2014 and 2015, although the number of sampling sites decreased for various reasons: vehicle traffic destroyed one sample site at Horse Beach and five were lost by the removal of top soil along Foreshore Road and Horse Beach. Soil cores 1 m from the perimeter of the plants were not taken after 2013 owing to no viable seeds being found in any of these cores (totals in 100 cores: 6 seed fragments, 4 intact but dead seeds). In 2016 and 2017, the number of soil cores was increased to 20 per sample site. We increased the sampling effort in this way to compensate for the decreased frequency of viable seeds found previously in soil cores during 2014 and 2015. Sampling the seed bank was not carried out in 2018 because the target sampling areas were now highly disturbed (estimated at between 1 to 11% of the target area was cored and the rest trampled) and so few seeds were present that an unrealistic sample size of cores would be required to detect their presence, if there were seeds left at all. Instead specific attention was paid on the annual survey in the second half of 2018 to record seedlings present.

Soil cores were also collected from six plants on the fringe of the infestation to test whether isolated plants had a history of reproduction. Two methods were used: either 10 soil cores were taken under the canopy, as described above, or the collection and sieving of two or up to five samples of a 25 × 25 cm square of leaf litter and a 25 × 25 cm square of soil to 5 cm depth, also taken under the canopy.

Results

Annual surveys

Initial surveys in July 2012 covered three land holdings and identified 117 plants (Suppl. material 1: Table S1). Of these plants 138 were >1 m in canopy diameter, and the largest plant was over 11 m in diameter. The more detailed survey between September and December 2012 found 903 plants, of which most were seedlings and small plants <1 m in diameter, over an area that covered 253 hectares and 6 landholdings (Figure 1a). In 2013 the area within the main infestation was re-surveyed, capturing a further 365 plants, mostly seedlings located within the canopy area of plants removed in 2012. It required 59 person days, spread over two years (due to staff availability; Suppl. material 1: Table S1) to survey, locate and kill all plants, which is why the results for 2012–2013 are combined.

In 2014 the main infestation area was re-surveyed (Figure 1b) and subsequently the main and buffer area was re-surveyed each year (Figure 1 c–e) yielding in total

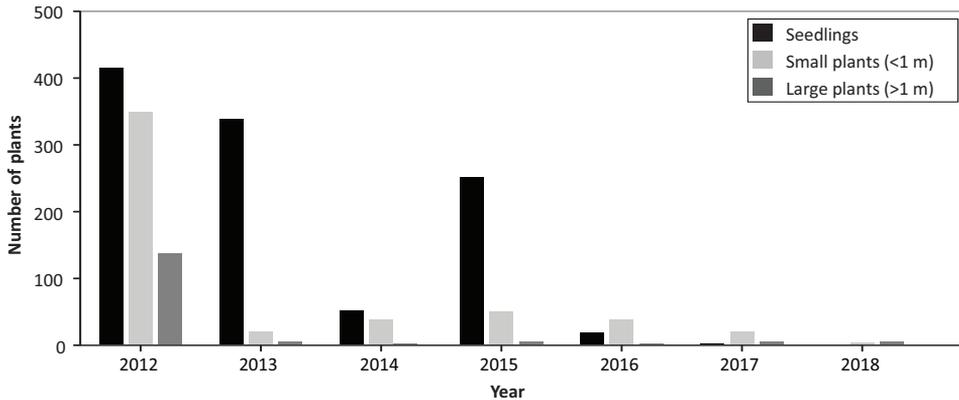


Figure 3. Comparison of number of bitou bush plants and seedlings found at Kwinana between 2012 and 2018. Plant size categories refer to maximum canopy diameter.

123 plants and 273 seedling plants over the four year period. Only 10 plants were found in 2018. Most plants found each year were small or newly emerged seedlings, but large plants that were greater than a year old were found, indicating that they were missed in surveys from previous years (Figure 3).

Of the 1756 individuals, all but five were found in the main infestation area (i.e. within 500 m of the putative population centre; Figure 1). Of these five, two plants were found within the first 500 m buffer from the main infestation: (i) a large (4 m canopy diameter) flowering plant in a nature strip, some 225 m from the nearest plant, and (ii) a small seedling in a reticulated garden bed, 284 m from the nearest flowering plant. Finding these two plants required expansion of the survey area a further 500 m. One plant was found in this third buffer zone (i.e. 1000 to 1500 m from the population centre), but as this plant was in a nature corridor (area where original vegetation including trees had been retained), and therefore an obvious dispersal route due to birds, the survey was extended along the nature corridor. This survey found two more plants. The discovery of these last two plants required a further redesign of the delimitation area so another 500 m and 1 km buffer specifically around these two plants was delimited and surveyed (see Scott and Batchelor (2014) for maps showing the sequentially increasing buffer areas).

The five individuals outside the main infestation area were found in the 2012 survey. In 2015, four plants were found growing in close proximity to where one of the five plants was killed in 2012 (location of plant number 624, on the southern end of the distribution; Figure 3 in Scott and Batchelor (2014)). Upon closer inspection, all four plants had regenerated from the layering of stem material removed in 2012. None of the four plants had any evidence of seed production based on the absence of seed coat fragments in the soil. A large number of seedlings were found within the main infestation area in 2015, indicating conditions were more favourable for seed germination relative to other years (Figure 3). See also Suppl. material for results of live versus dead plants.

Survey effort and area

The number of plants found per unit of effort steadily decreased over the sampling period, which up to this point in the program has come to a total of 97 person days of surveys (Suppl. material 1). Extrapolating from the declining plant detection records indicates that there should be few plants found beyond 2019 (Figure 4a). The number of plants also decreased with the area searched each year (Figure 4b), similarly suggesting that few plants will be found after 2019 if there are no further additions to the soil seed bank.

The average size of plants found each year steadily decreased from 2012 to 2016 (Figure 5). In 2017, unseasonal summer rain (> 100 mm in February) and mild summer temperatures were likely responsible for the unusually high increase in the annual growth (diameter and height) of plants found (Figure 5). Over all survey years from

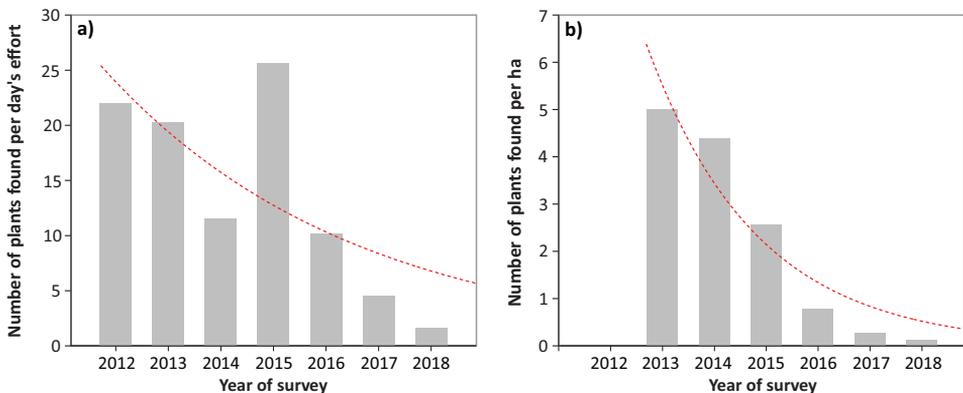


Figure 4. Number of bitou bush found with exponential decay trends represented (a) per person day effort ($Y = 29.54^{(-0.21X)}$, $R^2 = 0.54$, $df = 6$, $p < 0.05$) and (b) per ha searched ($Y = 14.16^{(-0.47X)}$, $R^2 = 0.91$, $df = 5$, $p < 0.01$).

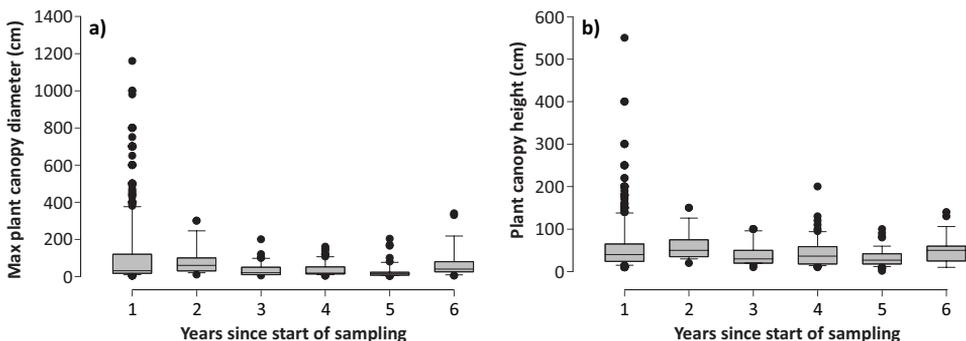


Figure 5. Canopy diameter (a) and height (b) for bitou bush plants in the Kwinana population between 2012 and 2017. Box plots depict median values with 10th, 25th, 75th and 90th percentiles. Results from 2018 are excluded as there were few individuals recorded and not all were measured.

2012 to 2017, nearly 70% of all small plants (canopy area < 1 m diameter) and seedlings were found directly underneath or within 1 m of the location of a large plant (a plant with canopy diameter > 1 m; Figure 6). Of the remaining 30%, approximately 25% were found more than 1 m from a previously removed large plant and 6% were > 10 m from a previously removed large plant, indicating likely vector dispersal (Figure 6). The maximum distance between a seedling and large plant was 284 m. These measurements do not factor in the distance between the large plants found south of the main infestation area, as they were all either large or layered plants that had not produced seed (based on seed bank assessment).

Capitula in flower or seed

The timing of plant surveys to coincide with optimal detection meant that bitou bush was not usually in the peak of flowering. Bitou bush produces flowers throughout the year (Scott 1996), with a peak in June to September. We were unable to determine the flowering phenology and reproductive output of plants because the primary objective was to kill plants as soon as found, preventing further seed set.

Aerial photography surveys

Examination of aerial photography in 2016 occurred after the majority of foot surveys had been completed. We focused on areas to the north of the study area that had eye-height vegetation difficult to survey. Plants that could potentially be bitou bush were identified and located *in situ*. All were *Schinus terebinthifolia* Raddi. In 2018 a survey of aerial photography was made of the entire survey area (Figure 1f) and adjacent areas

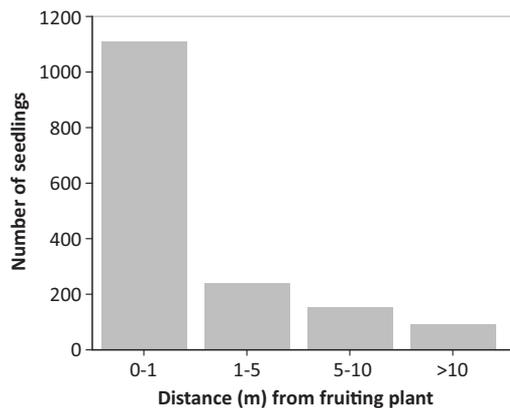


Figure 6. Distance of seedlings from the centre of the nearest large bitou bush canopy (> 1 m in maximum diameter). Average distance over the population 7.7 ± 0.7 m. Maximum distance was 284 m.

(369 ha in total). This virtual survey systematically grid checked photos of the study area over two hours. Ten potential bitou bush were identified. Ground searches found that none were bitou bush, being instead *Acacia*, *Eucalyptus* or *Schinus* plants.

Plant removals

All plants discovered during the six surveys between 2012 and 2018 – a total of 1766 individuals - were killed as part of the control program (Suppl. material 1: Table S1). Seedlings that most likely germinated in the 12 months preceding the annual surveys comprised 61% of the plants. Many plants were still quite small (i.e. < 1 m in maximum canopy diameter), but these were often observed with flowers, despite probably germinating between 12 and 24 months preceding the survey. Overall 9% and 4% (61) of plants respectively had canopy diameters greater >1 m and > 3m. Most of the plants were weeded by hand, though a number were sprayed by herbicide (arranged by landholders) or removed by heavy machinery.

Seed bank studies

Seeds were consistently found in soil cores taken under the plant canopy (Figure 7). However, no intact seeds were found in the soil cores that were taken 1 m from the perimeter of the plant canopy in 2013, indicating that most seeds remained within the canopy area or vectors transport the seeds beyond this short distance (potential vectors are listed in Suppl. material 1: Table S2). Of the nine original plants that were able to be sampled for seed bank studies throughout the project, the viable under-canopy seed bank averaged 39.3 ± 11.4 seeds.m⁻² in 2013, decreasing to 5.7 ± 2.2 in 2017 (Figure 7).

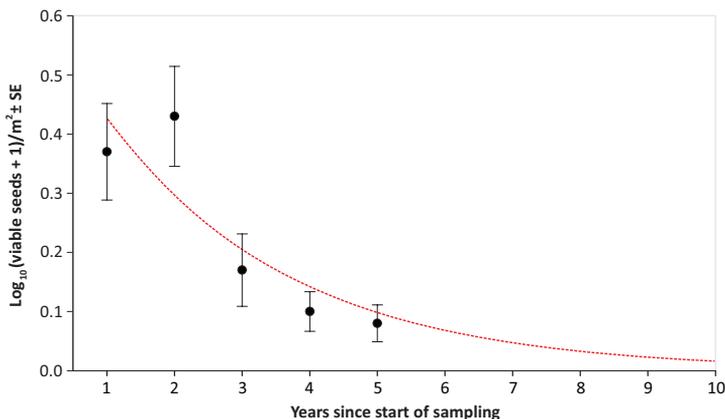


Figure 7. Density of viable seeds in the bitou bush seed bank at Kwinana between 2013 and 2017. Exponential decay trend (dotted line): $Y = -0.72e^{-0.41X}$, $R^2 = 0.76$, $df = 4$, $p < 0.05$.

Discussion

Source of the infestation

Bitou bush was possibly originally introduced into Australia in ship ballast, but was planted in the 1940s to 1960s for dune stabilisation in New South Wales (NSW) (Weiss et al. 2008). It is not known for certain how the Kwinana population arrived in Western Australia, but based on plant size (Scott 1996) and the presence of bitou plants in aerial photography (Scott et al. 2019) it is certain that they were present at Kwinana in 1995 (17 years before detection in 2012). The Kwinana area has well established transport linkages to NSW, through the use of the port area by Broken Hill Propriety Co. Ltd until April 1995 (BHP Steel International Group – Rod and Bar Products Division 1995). Another of BHP steel's sites at the time was Port Kembla, NSW, an area with an abundant bitou bush infestation (Love 1986). We hypothesise that that transport of goods between Port Kembla and Kwinana enabled the introduction of bitou bush seed. The other alternative is for plants to have arrived from southern Africa by ship and somehow been transported to shore, but at this stage we have not identified a putative vector.

It might be expected that the foci of the invasion would result in larger plants clustered together. But this is not the case, except for two of the largest plants near the old jetty. It is possible that a single point of introduction occurred and that some of the initial plants that germinated were planted as part of gardens that are now abandoned (bitou bush was used as a dune stabilizing plant). Molecular studies will be the best way to elucidate the origins of the Kwinana population, and may provide useful biosecurity guidance depending on the putative source population(s).

The most likely dispersal vectors for bitou bush seed in the population were foxes, mice, doves and parrots (Suppl. material 1: Table S2). No birds were observed on the bushes during the entire period of surveys, but bees were seen visiting flowers. Elsewhere in Australia and southern Africa, seed dispersal is mainly by birds and some mammals ingesting fruits and carrying seeds to new locations (Gosper 1999; Meek 1998; Weiss et al. 2008).

Current versus past presence of bitou bush

While it is possible that some plants could have disappeared from the population as part of site works, the documented history of little change to the landscape, in particular in the main infestation area, indicates that we have located every plant to have ever been present, both in space and time (excluding seeds and small seedlings of course). This conclusion is supported by the aerial photography which did not show additional plants in the past. It is also reasonable to suggest that all plants that have ever germinated and survived past the seedling stage are included in this study. The level of temporal and spatial population data collected here opens the possibility of modelling the invasion process once we have a better knowledge of the breeding system and number of introductions, as indicated by a study of the genetic diversity.

Bitou bush missed and subsequently found in surveys

The reduction in the number of new plants found in the most recent survey in 2018 is encouraging, but the discovery of large plants clearly missed in earlier surveys is of concern to all involved in the control program. The 2018 survey discovery of two bitou bush plants completely contained within the canopy of a large *Acacia cyclops* emphasised the role of detectability in eradication programs (Cacho et al. 2006; Regan et al. 2006). These similarly sized bitou bush plants probably germinated in 2013–2014, had grown underneath the acacia canopy and were not visible before about August 2017 (the area was surveyed each year; date of detectability within the acacia canopy based on aerial photographs). These discoveries show that despite experience and strategically planned and comprehensive surveying, plants can be difficult to find amongst other vegetation with similar leaf and flower colour (e.g. *Acacia saligna* and *Nicotiana glauca*). It also seems possible that a highly favourable climate over summer (e.g. the summer of 2016–2017) facilitated the growth within a year of previously undetectable small plants into larger flowering individuals.

Seed bank studies

The exponential decay of viability in the sampled seeds over 5 years shows a similar pattern to most seeds after a disturbance (e.g. soil cultivation); a peak in germination followed by steadily declining viability over time (Long et al. 2014). There was no significant decrease in seed viability in 2015–2017. However, extrapolation of the trend line indicates that the seed bank would not be completely depleted until at least 2020 or perhaps longer (Figure 7), depending on a number of caveats and assumptions.

The same pattern of seed distribution seen at Kwinana (i.e. large numbers of seed under the canopy, none 1 m away from the canopy) was observed over 12 sites in the native habitat of *C. monilifera* in South Africa (Scott 1996). The most striking difference between the seed bank at Kwinana population and the seed bank in South Africa (Scott 1996) is the absence of specialised seed predators at Kwinana. One of these (*Mesoclanis* sp.; Diptera) is now used as a biological control agent in eastern Australia (Edwards et al. 2009). In contrast, the South African populations of bitou bush had a low level of rodent predation, whereas this occurs in the Kwinana population at a frequency of about 1 to 3 seeds per m².

Seed production by isolated plants

No seed fragments were found in the soil in samples taken underneath the six plants on the outskirts of the infestation (yellow dots; except 791 and 792; Figure 2), despite these plants having flowered prior to the survey period. However, in 2018 seeds were found on two plants found growing together, hidden inside an *Acacia* plant (791 and

792 in Figure 2). No seed or seed fragments were found in the soil underneath these plants, indicating that seeds had not been produced in previous years. When found in April 2018, these two plants were in flower (which is why they were detected) and seed production had commenced. Mature seed still attached to the plant and covered with the dried remains of the fruit had 100% germination (sample of 10 seeds). All seeds on these plants were collected, but that does not exclude the possibility of seed dispersal by vectors such as birds. One other plant in 2018 had a few seeds, but a nearby plant for potential cross-pollination was not found.

Taken together, these observations provide circumstantial support for bitou bush being an obligate outcrossing species, perhaps with a very low level of selfing. Gross et al. (2017) performed cross pollination experiments on six populations from eastern Australia and found that in all populations bitou bush was an obligate outcrossing species. This implies that invasion would be more difficult than if the species was self-fertile (Baker's law; Baker 1955), although there are many examples of colonists with obligate outcrossing and the ideal of a "law" is questioned (Pannell et al. 2015). The key to this issue in the case of bitou bush is dispersal vectors. If seed and fruit size are a sufficiently small "package" at consumption so that more than one seed is deposited in the vector's droppings, and/or if there are perch sites that are frequently visited, then the requirement for outcrossing is unlikely to be a constraining factor because the chances are high that more than one plant will be growing in the same location.

Seed germination affected by allelopathy

While there has been a steady decline in the seed bank, a spike in seedlings in 2015 (Figure 3), indicates a possible wearing-off of allelopathic effects that prevent germination under the parent bush. Ens et al. (2009) found bitou bush roots and leaves to be phytotoxic and allelopathic when applied to five native species, and the root extracts are likely to have a long residence time in soil, particularly soils with little humic matter such as in the sand dunes. Structured germination trials would be required to confirm that allelopathy plays a role in germination inhibition in bitou bush.

Seed bank longevity

There was an average of 39.3 viable seeds per m² under bitou bush canopies in at the beginning of the management program in 2013. The number of plants that were large enough to be reproductive came to 151, representing a total canopy area of 1670 m² at the Kwinana site. Multiplying the seed density by the canopy area gives a total of 65,629 seeds in 2012. Using an exponential decline function (Figure 7) and extrapolating forward, the first year with zero seeds is 2020. There are a lot of assumptions in this calculation, including no further input into the seed bank, and an absence of

bird dispersed seed. Indeed, while most seed production ceased in 2012, seeds were produced in 2018 on three plants, and there is a remote possibility of seeds entering the seed bank that year. Even so, the density of seeds is now so low that sampling via soil cores to assess seed bank viability has become unfeasible. Extrapolating from earlier core data (Figure 7) indicates that there is potentially a long tail of viable seeds (at low density), for at least another three to four years.

Seed viability over time for bitou bush is known to be variable. In this study, seed collected in 2012 and stored in the laboratory did not germinate in 2018 (unpublished results, bearing in mind the difficulty of extrapolating results generated in laboratory conditions to field situations). In eastern Australia, bitou bush seeds have remained viable in the soil for up to seven years (Kristine French, pers. comm.). However, surveys, such as currently practiced, will detect germination from the seed bank either as seedlings in the year of germination or as plants in the following year when they are more obvious. Taken together, these results indicate that seed longevity data from lab trials or natural populations (native or introduced) elsewhere may not apply to the Mediterranean environment of the Kwinana population. More complex modelling of seed bank decline trajectories and their uncertainties may produce a more robust understanding of required monitoring timelines for bitou bush at Kwinana. With the information currently available, a more realistic estimate is that the Western Australian population could persist via a viable seed bank until at least 2024. As such, monitoring and active management until this time is essential for achieving eradication.

Has delimitation been achieved?

Knowledge of the spatial and temporal extent of a weed's incursion is critical to any eradication effort (Panetta and Lawes 2005). Very few weeds have been eradicated from Australia, with most cases failing because the spread of infestations to areas well outside the target area. While it is not possible to say with absolute certainty that delimitation of bitou bush has been achieved in Kwinana, as there are many avenues where soil potentially containing seed could have moved offsite (Scott and Batchelor 2014), it is very encouraging that no bitou bush have been found in Western Australia outside of the known infestation area (Figure 1) in the seven years since first detection. The conclusion is reinforced by the non-detection of bitou bush in widespread monitoring in Western Australia for the other subspecies in Australia (boneseed *Chrysanthemoides monilifera* subsp. *monilifera*; see Figure 2 in Scott and Batchelor (2014)).

Feasibility of eradication

Bitou bush is subject to localised eradication to implement broader containment at the southern and northern extremes of its range in eastern Australia. These management programs have achieved mixed results. At the southern end in Victoria, bitou bush has been eradicated from two locations, Kew and Frankston (Adair and Butler 2010),

although the current view on this is that local eradication may not have been achieved (Royal Botanic Gardens Victoria 2017). Two further populations, Daveys Bay and Mallacoota, are undergoing eradication, with Mallacoota well advanced (in 2010). An extra challenge for the Mallacoota population is the hybridization with the sibling species, *C. m.* subsp. *monilifera* (boneseed; Adair and Butler 2010). The Victorian eradication is complemented by a “containment line” in southern New South Wales (Cherry et al. 2008).

Queensland has had a long running eradication and containment program against bitou bush in the northern extent of its range, starting in 1982. After 10 years of control effort the 700 ha infestation was reduced to a few small infestations. By 2007 few scattered plants remained which are removed in annual surveys. Effectively this is a containment program to limit spread to the north (Wilson et al. 2017), which will need to run forever given the large population of bitou bush just to the south in New South Wales (Cherry et al. 2008). Recently Behrendorff et al. (2019) described progress since 1982 towards eradication of bitou bush on K’gari-Fraser Island in south east Queensland, where the plant is at the limits of its northern expansion. While the seed bank was not measured, counts of seedlings recorded each year at one fixed location indicated a potential seed survival of six years. However, a delimitation survey was not described and bitou bush plants were still being found after 20 years of survey.

Aside from Behrendorff et al. (2019) there is little published quantitative data from these eradication programs (a general problem of eradication projects noted also in New Zealand; Howell 2012), which makes it difficult to adopt lessons learnt or to take on improvements to techniques, or even to estimate how long eradication will take (and consequently the cost). The lack of such data to inform areas for improving management was raised as a priority by Wilson et al. (2017). In 2018 bitou bush is still managed as an eradication target in Queensland (Queensland Department of Agriculture and Fisheries 2018) and in Victoria (Royal Botanic Gardens Victoria 2017).

Indicators that eradication could be achieved in Western Australia, at least locally in Kwinana, are the decline to zero observed seedlings in 2018 and substantial areas where plants have not been seen for three years (Figure 1 d,e,f). Likewise, the decline in plants detected per hectare and per day of sampling effort also indicate that eradication could be achievable within a few years. The overall survey area was about 250 ha, well within the maximum feasible survey area (1000 ha) for which Panetta and Timmins (2004) and Rejmanek and Pitcairn (2002) indicate eradication could be achieved. Supporting this are the soil seed bank studies indicating that the number of viable seeds is approaching zero. Also supporting the feasibility of eradication is bitou’s reproductive system, where it appears that cross pollination is necessary (Gross et al. 2017). This means that isolated plants can become large enough for detection, often by the presence of flowers, without the risk of contributing to the seed bank.

The main counter indication against feasibility of eradication is the continued detection each year of a few large plants, especially the detection in 2018 of two reproductive individuals (791 and 792, Figure 2), which potentially resets the clock of seed persistence. Detectability is a critical element of an eradication project (Panetta and Timmins 2004). Monitoring over the next couple of years will establish if detectability

is the main risk. It is evident that small plants (or seedlings) are not always detected during the surveys, but are detected when emerging through the surrounding vegetation. Plants also come into flower within a year (Weiss et al. 2008). Early flowering has been noted in other eradication targets (Panetta 2015), resulting in the need to change survey frequency. Annual surveys in March or April still remain the best way of detecting these plants before seed set. Finally, a contra-indication for eradication continues to be the inability to detect small plants in aerial surveys. In addition, the eradication effort is made complicated by the multitude of landholders, unmanaged land where the vegetation is dense, and the inability to use fire as a management tool because of the proximity of petro chemical plants. Fire forms an important part of bitou bush management in eastern Australia (Lindenmayer et al. 2015).

Eradication program feasibility insight

The area of occupancy for bitou bush in Western Australia was small, and delimitation was defined early and has not changed despite the possibility of seed being moved by soil (Scott and Batchelor 2014). The ongoing delimitation of this population continues to be a critical element defining the eradication attempt and making possible the planning for surveys (Panetta and Lawes 2005). Initially we thought the target species would be easily detected among other vegetation, but that proved to be overly optimistic. A range of survey techniques, including aerial photography, was required to achieve necessary detection probabilities. The soil seed bank confounds the detection process because it is out of sight, but must be measured to establish that decline is occurring. Ongoing population recruitment via seedlings does not give this information, as germination is dependent on a wide range of factors that vary between years (Long et al. 2014). Fortunately, the infestation was not far from the main city, which reduced the costs of the management program. It remains to be seen if sufficient resources are available for the full eradication program, especially in the final stages when additional resources and care is required because the target abundance is very low (Wilson et al. 2017).

Buddenhagen and Tye (2015) list five barriers to achieving eradication based on a review of work carried out on the Galapagos Islands. In the following the barriers are assessed against the situation with bitou bush. Barrier 1 (insufficient effort) does not appear to be the case (Figure 4). There is a considerable literature on control of bitou bush so Barrier 2 (no control technique) is not likely. Barrier 3 (permission) is particularly relevant given the range of tenure at Kwinana (Scott and Batchelor 2014) and access to highly secure areas is an ongoing annual challenge, complicated by change in land ownership and changes in personnel. Barrier 4 is size and time. Both continue to be relevant, especially now that the plant (and seeds in the soil) have become rare at the study area and the danger of a false sense of confidence threatens ongoing investment to pursue the eradication program to an appropriate end point. Barrier 5 (available resources) will continue to be an issue as it is for any management strategy. Lastly, Barrier 6 (commitment and understanding) is possibly the most important and least

quantifiable, and one where the strength of the collaboration between land managers and those leading the eradication program is critical.

How will we know that eradication has been achieved?

Of course we can never be certain that eradication has been achieved. Surveillance will need to continue once there are no plants at the infestation site and it will take some years before eradication can be confidently declared (even if uncertain). Various time periods have been proposed such as three years, based on a review of all eradication programs in New Zealand (Howell 2012) to five years (Rejmanek and Pitcairn 2002). Another reasonable suggestion is to monitor for the same duration as the seed bank longevity (in this case six to seven years; Dodd et al. 2015). The New Zealand experience is that eradication is more difficult than initially anticipated, so it would be wise not to under-estimate the resources and duration required (Howell 2012). Economic criteria have been proposed to define where monitoring stops, when the expected costs outweigh the expected benefits (Regan et al. 2006; Moore et al. 2011), but can this be meaningfully applied to weeds where the main impact is on the environment? Once we have zero plants present in annual surveys then we can realistically start to estimate the duration to eradication, for example, using methods such as developed in Rout et al. (2009).

Reflecting on one of the few successful weed eradications, *Bassia scoparia* in Western Australia, the plant was detected for eight years after the program began. Surveys concluded at each site if the plant was not detected for three years (Dodd and Randall 2002). However, *B. scoparia* was known to have short lived seeds, in the order of 1–3 years, with the soil seed bank largely exhausted after 12 months (Dodd and Randall 2002). Bitou bush in Western Australia can hope for a similar level of success, if the commitment to management of this population is maintained.

Acknowledgements

The authors would like to acknowledge all the personnel at Fremantle Port Authority that have assisted with the physical removal and observations of plants over the study period. Craig Wilson from the Kwinana Town Council made the initial discovery, recognised that it was a new weed invasion and has promoted awareness to local landholders and the general community. The initial surveys were conducted by Craig Wilson, Luke McMillan from Perth NRM and Matthew Oswald from the Fremantle Port Authority. We thank the following for their help with access during subsequent surveys: Melissa Manns, Matthew Oswald and Denis Doak, Fremantle Ports; Paul Johnson, HIs melt, Rio Tinto Limited; Ellen Sherman, LandCorp; Kate Brown, BP Refinery; John Tarcoe, Cockburn Cement; Reg Williamson, SteelMains (Kwinana Steel Pipe Manufacturing Facility); Kwinana Council; the site manager and security staff at CB&I Kentz Joint Venture (CKJV); and Jon Dodd and Dave Stewart, Western

Australian Department of Primary Industry and Resource Development. We thank Crystal Jones for technical assistance and Noboru Ota for GIS advice. We thank Dane Panetta, John Wilson, Karen Bell and Ben Gooden for their comments on manuscript drafts. This work was funded by CSIRO Health & Biosecurity, Perth NRM and Fremantle Port Authority.

References

- Adair RJ, Butler KL (2010) Hybridisation within *Chrysanthemoides monilifera* and implications for the eradication of bitou bush from Victoria, Australia. Proceedings of the 17th Australasian Weeds Conference, 26–30 September, 2010, Christchurch, 378–381.
- Baker HG (1955) Self-compatibility and establishment after “long distance” dispersal. *Evolution* 9: 347–348. <https://doi.org/10.2307/2405656>
- Behrendorff L, Harris SM, Muirhead IF (2019) Towards eradication: The history and management of bitou bush on K’gari-Fraser Island, Australia. *Ecological Management & Restoration* Jan 2019: 1–9. <https://doi.org/10.1111/emr.12349>
- BHP Steel International Group – Rod and Bar Products Division (1995) A place in history: BHP Steel, Rod and Bar Products Division, Kwinana Market Mill April 1956 – April 1995. BHP, Kwinana.
- Blackburn TM, Pysek P, Bacher S, Carlton JT, Duncan RP, Jarosik V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution* 26: 333–339. <https://doi.org/10.1016/j.tree.2011.03.023>
- Buddenhagen CE, Tye A (2015) Lessons from successful plant eradications in Galapagos: commitment is crucial. *Biological Invasions* 17: 2893–2912. <https://doi.org/10.1007/s10530-015-0919-y>
- Cacho OJ, Spring D, Pheloung P, Hester S (2006) Evaluating the feasibility of eradicating an invasion. *Biological Invasions* 8: 903–917. <https://doi.org/10.1007/s10530-005-4733-9>
- Cherry H, Willsher L, Whyte B (2008) Bitou bush and boneseed eradication and containment in Australia. *Plant Protection Quarterly* 23: 38–40.
- Dodd AJ, Ainsworth N, Burgman MA, McCarthy MA (2015) Plant extirpation at the site scale: implications for eradication programmes. *Diversity and Distributions* 21: 151–162. <https://doi.org/10.1111/ddi.12262>
- Dodd J, Randall RP (2002) Eradication of Kochia (*Bassia scoparia* (L.) A.J. Scott, Chenopodiaceae) in Western Australia. In: Spafford-Jacob J, Dodd J, Moore JH (Eds) Proceedings of the 13th Australian Weeds Conference, Perth, Western Australia, 8–13 September, 2002, 301–303.
- Edwards PB, Adair RJ, Holtkamp RH, Wanjura WJ, Bruzzese AS, Forrester RI (2009) Impact of the biological control agent *Mesoclanis polana* (Tephritidae) on bitou bush (*Chrysanthemoides monilifera* subsp. *rotundata*) in eastern Australia. *Bulletin of Entomological Research* 99: 51–63. <https://doi.org/10.1017/S000748530800610X>
- Ens EJ, French K, Bremner JB (2009) Evidence for allelopathy as a mechanism of community composition change by an invasive exotic shrub, *Chrysanthemoides monilifera* spp. *rotundata*. *Plant and Soil* 316: 125–137. <https://doi.org/10.1007/s11104-008-9765-3>

- Gosper CR (1999) Plant food resources of birds in coastal dune communities in New South Wales. *Corella* 23: 53–62.
- Gross CL, Whitehead JD, de Souza CS, Mackay D (2017) Unsuccessful introduced biocontrol agents can act as pollinators of invasive weeds: Bitou Bush (*Chrysanthemoides monilifera* ssp. *rotundata*) as an example. *Ecology and Evolution* 7: 8643–8656. <https://doi.org/10.1002/ece3.3441>
- Howell CJ (2012) Progress toward environmental weed eradication in New Zealand. *Invasive Plant Science and Management* 5: 249–258. <https://doi.org/10.1614/IPSM-D-11-00001.1>
- Lindenmayer DB, Wood J, MacGregor C, Buckley YM, Dexter N, Fortescue M, Hobbs RJ, Catford JA (2015) A long-term experimental case study of the ecological effectiveness and cost effectiveness of invasive plant management in achieving conservation goals: Bitou bush control in Booderee National Park in eastern Australia. *PLoS ONE* 10(6): e0128482. <https://doi.org/10.1371/journal.pone.0128482>
- Long RL, Gorecki MJ, Renton M, Scott JK, Colville L, Goggin DE, Commander LE, Westcott DA, Cherry H, Finch-Savage WE (2014) Seed persistence: The journey from dispersal to germination. *Biological Reviews* 90: 31–59. <https://doi.org/10.1111/brv.12095>
- Love A (1986) Distribution of bitou bush along the N.S.W. coast. In: Love A, Dyason R (Eds) *Bitou bush and boneseed: Proceedings of a Conference on Chrysanthemoides monilifera*. NSW National Parks and Wildlife Service and NSW Department of Agriculture, Sydney, 8–9 August 1984, Port Macquarie, NSW, 53–64.
- Meek PD (1998) ‘Weed seeds and whoopsie daisies’: viability of bitou bush *Chrysanthemoides monilifera* seeds in fox (*Vulpes vulpes*) scats. *Plant Protection Quarterly* 13: 21–24.
- Moore JL, Runge MC, Webber BL, Wilson JR (2011) Contain or eradicate? Optimizing the management goal for Australian acacia invasions in the face of uncertainty. *Diversity and Distributions* 17: 1047–1059. <https://doi.org/10.1111/j.1472-4642.2011.00809.x>
- Myers JH, Savoie A, van Randen E (1998) Eradication and pest management. *Annual Review of Entomology* 43: 471–491. <https://doi.org/10.1146/annurev.ento.43.1.471>
- Panetta FD (2004) Seed banks: the bane of the weed eradicator. In: Sindel BM, Johnson SB (Eds) *Proceedings of the 14th Australian Weeds Conference, Wagga Wagga, New South Wales, 6–9 September 2004*, 523–526.
- Panetta FD (2007) Evaluation of weed eradications programs: containment and extirpation. *Diversity and Distributions* 13: 33–41.
- Panetta FD (2015) Weed eradication feasibility: lessons of the 21st century. *Weed Research* 55: 226–238. <https://doi.org/10.1111/wre.12136>
- Panetta FD, Lawes R (2005) Evaluation of weed eradication programs: the delimitation of extent. *Diversity and Distributions* 11: 435–442. <https://doi.org/10.1111/j.1366-9516.2005.00179.x>
- Panetta FD, Timmins SM (2004) Evaluating the feasibility of terrestrial weed eradications. *Plant Protection Quarterly* 19: 5–11.
- Pannell JR, Auld JR, Brandvain Y, Burd M, Busch JW, Cheptou PO, Conner JK, Goldberg EE, Grant AG, Grossenbacher DL, Hovick SM, Igc B, Kalisz S, Petanidou T, Randle AM, Rubio de Casas R, Pauw A, Vamosi JC, Winn AA (2015) The scope of Baker’s law. *New Phytologist* 208: 656–667. <https://doi.org/10.1111/nph.13539>

- Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution* 24: 497–504. <https://doi.org/10.1016/j.tree.2009.03.016>
- Queensland Department of Agriculture and Fisheries (2018) Bitou bush *Chrysanthemoides monilifera* sub-species *rotundata*. https://www.daf.qld.gov.au/__data/assets/pdf_file/0018/61344/IPA-Bitou-Bush-PP10.pdf [accessed 29 April 2018]
- Regan TJ, McCarthy MA, Baxter PWJ, Panetta FD, Possingham HP (2006) Optimal eradication: when to stop looking for an invasive plant. *Ecology Letters* 9: 759–766. <https://doi.org/10.1111/j.1461-0248.2006.00920.x>
- Rejmanek M, Pitcairn MJ (2002) When is eradication of exotic pest plants a realistic goal? In: Vietch CR, Clout MN (Eds) *Turning the tide: The eradication of island invasives*. IUCN SSC Invasive species Specialist Group IUCN, Gland, 249–253.
- Rout TM, Salomon Y, McCarthy MA (2009) Using sighting records to declare eradication of an invasive species. *Journal of Applied Ecology* 46: 110–117. <https://doi.org/10.1111/j.1365-2664.2008.01586.x>
- Royal Botanic Gardens Victoria (2017) *Chrysanthemoides monilifera* subsp. *rotundata* (DC.) Norl. Bitou Bush. <https://vicflora.rbg.vic.gov.au/flora/taxon/256a3a83-1950-4ed0-b1e3-f4637adc1d74> [accessed 30 April 2018]
- Scott JK (1996) Population ecology of *Chrysanthemoides monilifera* in South Africa: Implications for its control in Australia. *Journal of Applied Ecology* 33: 1496–1508. <https://doi.org/10.2307/2404788>
- Scott JK, Batchelor KL (2014) Management of *Chrysanthemoides monilifera* subsp. *rotundata* in Western Australia. *Invasive Plant Science and Management* 7: 190–196. <https://doi.org/10.1614/IPSM-D-13-00052.1>
- Scott JK, Batchelor KL, Jucker T, Webber BL (2019) Aerial photography and dendrochronology as tools for recreating invasion histories: do they work for bitou bush, *Chrysanthemoides monilifera* subsp. *rotundata*? *Biological Invasions* 21(9): 2983–2996. <https://doi.org/10.1007/s10530-019-02026-5>
- Shackleton RT, Shackleton CM, Kull CA (2019) The role of invasive alien species in shaping local livelihoods and human well-being: A review. *Journal of Environmental Management* 229: 145–157. <https://doi.org/10.1016/j.jenvman.2018.05.007>
- Weiss PW, Adair RJ, Edwards PB (1998) *Chrysanthemoides monilifera* (L.) T. Norl. In: Panetta FD, Groves RH, Shepherd RCH (Eds) *The biology of Australian weeds Volume 2*. R.G. and F.J. Richardson, Melbourne, 49–61.
- Weiss PW, Adair RJ, Edwards PB, Winkler MA, Downey PO (2008) *Chrysanthemoides monilifera* subsp. *monilifera* (L.) T.Norl. and subsp. *rotundata* (DC.) T.Norl. *Plant Protection Quarterly* 23: 3–14.
- Wilson JR, Panetta FD, Lindgren C (2017) *Detecting and responding to alien plant incursions*. Cambridge University Press, Cambridge. <https://doi.org/10.1017/CBO9781316155318>

Supplementary material 1

Supplementary tables

Authors: John K. Scott, Kathryn L. Batchelor, Bruce L. Webber

Data type: species data

Explanation note: **Table S1.** Number of individuals and average diameter of plant canopy (note this excludes seedlings) at Kwinana. Survey effort reflects the number of people days (i.e. 6 days could equal 2 people \times 3 days, or a combination thereof).

Table S2. Potential frugivores, granivores and/or seed dispersal agents amongst vertebrate animals observed on the Kwinana Industrial Estate in bitou bush infested areas. Daytime observations only.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/neobiota.50.35070.suppl1>

Quantifying the potential impact of the European wasp (*Vespula germanica*) on ecosystem services in Western Australia

David C. Cook¹

¹The University of Western Australia, Department of Primary Industries and Regional Development PO Box 1231, Bunbury WA 6231, Australia

Corresponding author: David C. Cook (david.cook2@dpird.wa.gov.au)

Academic editor: Alain Roques | Received 21 June 2019 | Accepted 17 August 2019 | Published 20 September 2019

Citation: Cook DC (2019) Quantifying the potential impact of the European wasp (*Vespula germanica*) on ecosystem services in Western Australia. NeoBiota 50: 55–74. <https://doi.org/10.3897/neobiota.50.37573>

Abstract

This paper estimates the ecosystem services return on investment in government control of the introduced European wasp (*Vespula germanica*) in the state of Western Australia. The predictive model used accounts for uncertainties in the spread and impact of *V. germanica* on provisioning ecosystem services, represented by pollination, apiculture and viticulture, and cultural ecosystem services represented by households. Results produced by simulating a 20-year period suggest government expenditure on management will generate net benefits of AU\$3.2–6.3 million per year, most of which will accrue to producers of pollination-dependent crops. This provides justification for investment from the government's agriculture portfolio to manage an insect often thought of as an urban pest.

Keywords

Benefit cost analysis, economic impact assessment, ecosystem service impact, European wasp, German wasp, pest management, pollination services, *Vespula germanica*, yellowjacket

Introduction

European wasp (*Vespula germanica*) is an agricultural, environmental, and urban pest first introduced to the Australian state of Western Australia (WA) in the 1970s. To support the investment of public funds on the control of this pest, this paper estimates ecosystem service benefits attributable to ongoing WA government management activities. Ecosystem services are benefits provided by ecosystems, including provisioning services like pollina-

tion and food, cultural services such as outdoor recreation, and regulating services such as flood mitigation (Costanza et al. 1997; Millennium Ecosystem Assessment 2005). This analysis focuses on avoided disruptions to provisioning services in the form of pollination, apiculture and viticulture, and cultural services in the form of household recreation.

Since the 1940s, *V. germanica* has spread from its native range in Europe and the Mediterranean region to North America, Chile, South Africa, New Zealand, and Australia where it has become invasive (Centre for Agricultural Bioscience Information 2017). The first detection in Australia occurred in Sydney, New South Wales in 1954 when hibernating queens were discovered on timber imported from New Zealand and destroyed (Chadwick and Nikitin 1969; Lefoe et al. 2001). The wasp then became established on the island state of Tasmania in the late 1950s (Bashford 2001). However, nests were not discovered on mainland Australia until the late 1970s (Smithers and Holloway 1977, 1978). By the early 1980s, *V. germanica* had spread to Victoria and South Australia (East 1984; Crosland 1991).

The first WA detection occurred in 1977 when six nests were discovered in the Fremantle port area and eradicated (Crosland 1991). Further introductions occurred in the early 1980s, and the wasp has now been reported every year since 1984 (Tennant et al. 2011). Over this period, WA's Department of Agriculture and Food, now the Department of Primary Industries and Regional Development (DPIRD), has used targeted and passive surveillance techniques to detect and destroy wasp nests. Over 700 *V. germanica* nests have been destroyed across the south-west of the state, largely concentrated around the greater metropolitan Perth area (Tennant et al. 2011).

As with most government departments, DPIRD activities are highly scrutinised because of opportunity costs created with every funding decision. There is a tendency to consider state government money invested in *V. germanica* control as only creating social benefits in urban areas at the expense of agricultural and developmental opportunities (The Advertiser 2015; Williams 2015). While removing a public nuisance and human health concern is perceptible to the public, other more subtle benefits to pollination services, fruit growers, and beekeepers have not garnered the same amounts of attention when it comes to funding decisions. This paper shines a light on some of the less-visible benefits of *V. germanica* control by estimating their monetary value over time.

The premise of the paper is that without DPIRD's activities the population of wasps and their colonies are likely to grow rapidly. Mild winter temperatures and the sandy soil of the Swan Coastal Plain on which Perth is located make the area well suited to nest building. Overwintering nests can reach large sizes by the following summer and produce thousands of new queens (Tennant et al. 2011). This would reduce pollination services to horticulture and broadacre crops as *V. germanica* attack wild European honeybee (*Apis mellifera*) hives. Attacks on managed hives will also affect apiculture, and wasps feeding on fruit will affect industries like viticulture (Clapperton et al. 1989; MacIntyre and Hellstrom 2015). These agricultural impacts are considered alongside household costs, which will rise due to the need to remove nests and avoid disruption to recreational activities.

This paper estimates the difference in ecosystem service costs under two scenarios, one in which *V. germanica* management in its current form is ongoing and the other in which all government efforts to manage the wasp are halted. A bioeconomic model is

used to estimate damages under both scenarios over a 20-year period and, thus, damages avoided by ongoing *V. germanica* management. Despite being relatively simple, the model suffices to provide indicative benefits of the management policy. Benefit estimates are then compared to the costs to government of providing management services to indicate the return on investment. All monetary values are stated in Australian dollars.

Materials and methods

Cost and revenue implications

To predict ecosystem service effects resulting from *Vespula germanica* spread over time under management and nil management scenarios, impacts on three provisioning ecosystem services and one cultural ecosystem service are considered.

I. Pollination impacts

Apis mellifera was introduced into Australia soon after the arrival of the first Europeans and has become widespread (Cunningham et al. 2002; Paton 1995). In WA, *A. mellifera* has no competitors of comparable efficiency, so insect-pollinated crops receive a high level of service from feral *A. mellifera*. *Vespula germanica* has the propensity to severely deplete honeybee colonies, placing feral or unmanaged hives at particular risk (Clapperton et al. 1989). This imposes a cost on pollinator-reliant crops, particularly horticultural crops. The crops used to predict the cost of pollination disruption from *V. germanica* and their pollination reliance appear in Table 1.

II. Apiculture

Managed *A. mellifera* hives are affected by ‘raiding’ behaviour of expanding *V. germanica* populations. There are approximately 28,500 managed hives in WA producing over 1,600 tonnes of honey worth \$4.9 million per year (Australian Honey Bee Industry Council 2014). Beekeepers in areas where *V. germanica* are present must perform management actions to prevent managed hives from being destroyed. These include replenishing bee stocks that have been raided and feeding replacement bees on a sugar mixture to bring them to a productive level of health as rapidly as possible (MacIntyre and Hellstrom 2015).

III. Viticulture

Vespula germanica damage grapes and introduce foreign yeasts that can interfere with the fermentation process (Bashford 2001). Damage caused by wasps hollowing out fruit can be particularly severe, with yield losses of 10–15% having been reported in vineyards in the states of South Australia (Goodall and Smith 2001) and Victoria (Thomas 1993). In areas affected by *V. germanica*, additional costs are imposed on vineyard managers due to the need to bait to control wasp numbers and reduce damage to fruit.

Table 1. Insect-pollinated crops in Western Australia.

Crop	Area [†] (ha)	Volume [†] (T)	Gross Value [†] (\$ million)	Pollinator reliance [§] (%)
Almond	210	145	1.5	100
Avocado	8506	24621	118.4	100
Blueberry	23	81	1.8	100
Canola	1093647	1327849	730.0	15
Citrus	1436	13282	27.0	30
Cucumber	238	4028	17.8	100
Lupin	331493	457262	158.4	10
Mango	840	1424	8.1	50
Melons	591	16076	20.4	100
Pome fruit	2981	38802	98.4	50
Pumpkin	1114	18774	16.9	90
Stone fruit	298	8039	26.1	70
Strawberry	194	5112	42.5	40
TOTAL	1441571	1915495	1267.3	

[†]ABS (2018b); [‡]ABS (2018c); [§]Cunningham et al. (2002).

IV. Households

Vespula germanica is a serious household pest in warmer climates where breeding and nest construction continue throughout the year, resulting in large summer colonies containing many thousands of individuals (Tennant et al. 2011). Health statistics related to wasp stings are lacking. Although no fatalities attributable to the insect reportedly occurred in the period 1979 to 1998 (McGain et al. 2000), the nuisance value associated with large colonies near homes is assumed large enough to motivate householders to invest in private pest management services in the absence of government-provided services. The cost of such services is assumed to be \$200–250 per nest in the nil management scenario (FUMAPEST Pest Control 2018), and zero under the management scenario in which the WA government incurs the cost.

Uncertainty and spread prediction

Vespula germanica impacts over time are approximated using a Monte Carlo simulation model. The main purpose of the model is to provide the benefit component of a benefit cost analysis to inform DPIRD managers of likely returns to investment in *V. germanica* management activities. However, the model also required sufficient detail to gain traction with these managers, and to produce spread scenarios they considered plausible given their experiences with the pest.

The Monte Carlo model simulates a 20-year period. Uncertain parameters are entered as distributions and a Latin hypercube sampling algorithm used to sample from each using the *@Risk* software package (Palisade Software, Ithaca, New York). Parameter distribution types used in the model include: (i) PERT, a type of beta distribution specified using minimum, most likely (i.e. skewness), and maximum values; (ii) uni-

Table 2. Pollination parameters.

Parameter	Nil management	Management
Biological		
Infestation growth, ω_i (unitless) [†]	0.33–0.83	0.22–0.33
Maximum proportion affected, I_i^{\max} (%) [‡]	Uniform(20,30)	Uniform(20,30)
Minimum proportion affected, I_i^{\min} (%) [‡]	0.01	0.01
Proportion of I_i^{\max} affected at t^{θ_i} , θ_i (%) [‡]	15–100	15–100
Time taken for θ_i to be affected (yr) [‡]	Uniform(10,20)	Uniform(20,30)
Economic		
Demand elasticity, η_i^{\S}	Uniform(-1.1,-1)	Uniform(-1.1,-1)
Discount rate, ν (%) [‡]	Pert(2,5,7)	Pert(2,5,7)
Increased variable cost, V_{it}	0	0
Inflation rate, ι (%) ^{††}	Pert(1.5,2,2.5)	Pert(1.5,2,2.5)
Price of per unit, P_{it} (\$/T) ^{‡‡}	Almond 10300 Avocado 4800 Blueberry 22700 Canola 500 Citrus 2000 Cucumber 4400 Lupin 300 Macadamia nut 5100 Mango 5700 Melons 1300 Pome fruit 2500 Pumpkin 900 Stone fruit 3200 Strawberry 8300	Almond 10300 Avocado 4800 Blueberry 22700 Canola 500 Citrus 2000 Cucumber 4400 Lupin 300 Macadamia nut 5100 Mango 5700 Melons 1300 Pome fruit 2500 Pumpkin 900 Stone fruit 3200 Strawberry 8300
Yield loss despite control, Y_{it} (%) ^{§§}	Uniform(8,10)	Uniform(8,10)

[†] See eq. A2 and explanation. The infestation growth constant ω is determined by specifying a proportion of a stock infested, θ , and the amount of time taken for this proportion to be reached (t^θ). Assume it will take *V. germanica* 10–20 years to achieve an infestation rate of Uniform(10%,20%) in the nil management scenario, and 20–30 years in the management scenario.

[‡] Based on pollinator reliance figures in Free (1993) and Cunningham et al. (2002).

[§] Ulubasoglu et al. (2011).

[‡] Commonwealth of Australia (2006).

^{††} ABS (2018a).

^{‡‡} ABS (2018b; 2018c).

^{§§} Clapperton et al. (1989). New Zealand data on the percentage of hives destroyed is used as a proxy for the loss in pollination services from wild (or unmanaged) *A. mellifera* hives depleted by expanding *V. germanica* populations.

form, a rectangular distribution bounded by minimum and maximum values; and (iii) discrete, a distribution containing several discrete outcomes and their probabilities of occurrence. Biological and economic parameter values appear in Tables 2–5.

To describe changes in *V. germanica* impacts across multiple regions, the logistic model of Schaefer (1957) is modified to so that the length of time taken to affect horti-

culture pollination, apiculture, viticulture, and households can be specified. This model is combined with a measure of the marginal damage cost to simulate losses related to *V. germanica* over a 20-year period under nil management and management scenarios.

The model assumes that the proportion of a sector i (i.e. horticulture, apiculture, viticulture, households) affected in period t (S_{it}) increases over time following the logistic equation:

$$S_{it} = S_i^{\max} \frac{I_i^{\max}}{1 + \left(\frac{I_i^{\max}}{I_i^{\min}} - 1\right) e^{-\omega_i t}} \quad (1)$$

Here, S_i^{\max} is the total size of sector i affected (i.e. in number of ha for horticulture and viticulture, the number of hives for apiculture and the number of residences for households); I_i^{\max} is the maximum proportion of sector i affected; I_i^{\min} is the minimum proportion of sector i affected, and; ω_i is the rate at which *V. germanica* moves from I_i^{\min} to I_i^{\max} . In the absence of information about ω , a hypothetical impact growth rate is used determined by the number of time periods taken for *V. germanica* to affect a given proportion, θ_i , of S_i^{\max} such that:

$$\omega_i = -t^{\theta_i} \ln \left[\frac{I_i^{\max} - \theta_i}{\theta_i \left(\frac{I_i^{\max}}{I_i^{\min}} - 1\right)} \right] \quad (2)$$

Here, θ_i is a specified proportion of S_i^{\max} affected and t^{θ_i} is the number of periods (years) taken for *V. germanica* to reach θ_i . The values and distributions assigned to each parameter in each sector are provided in Tables 2–5.

Validation of the model for all sectors in both scenarios is not possible due to a lack of data. No data exists for a nil management scenario as *V. germanica* has been managed since it was first detected in WA, but data relevant to the management scenario are available from DPIRD for the past 20 years (1999–2018). These data include all reported and detected instances of wasps responded to by DPIRD over time, and given the majority of activity has occurred in the Perth metropolitan area they are used as a proxy for numbers of households affected. This allowed a rudimentary validation of the model to be undertaken as it applied to the household sector using visual assessment and deviance measures.

Visual assessment involved a graphical display of the data and model simulation output being shown to two experts involved in the DPIRD management project. They were presented with a diagram similar to Figure 1 and asked to comment on the apparent fit of the model. Both experts were reasonably comfortable that most of the data points fell within the range produced by the model and that those points that fell outside the model range were due to extenuating circumstances (i.e. chiefly the period 2003–2005 when several nests were missed, enabling a build-up that persisted over several years). However, one expert expressed concern at the upward trajectory of households affected by wasps and the negative perception on the management team's performance.

Table 3. Apiculture parameters.

Parameter	Nil management	Management
Biological		
Infestation growth, ω_i (unitless) [†]	0.31–0.61	0.2–0.31
Maximum proportion affected, I_i^{\max} (%) [†]	Uniform(8,10)	Uniform(8,10)
Minimum proportion affected, I_i^{\min} (%) [†]	0.01	0.01
Proportion of I_i^{\max} affected at t^{θ_i} , θ_i (%) [†]	5	5
Time taken for θ_i to be affected (yr) [†]	Uniform(10,20)	Uniform(20,30)
Economic		
Demand elasticity, η^{\ddagger}	Uniform(-1.1,-1)	-0.28
Discount rate, ν (%) [§]	Pert(2,5,7)	Pert(2,5,7)
Increased variable cost, V_{it} (\$/hive) [§]	Pert(25,30,50)	Pert(25,30,50)
Inflation rate, ι (%) ^{††}	Pert(1.5,2,2.5)	Pert(1.5,2,2.5)
Price of per unit, P_{it} (\$/hive) ^{‡‡}	170	170
Yield loss despite control, Y_{it} (%) [§]	0–10	0–10

[†] See eq. A2 and explanation. The infestation growth constant ω is determined by specifying a proportion of a stock infested, θ , and the amount of time taken for this proportion to be reached (t^θ). The maximum proportion of hives affected is Uniform(8%, 10%) (Clapperton et al. 1989). Assume it will take *V. germanica* 10–20 years to achieve an infestation rate of 5% in the nil management scenario, and 20–30 years in the management scenario.

[‡] Ulubasoglu et al. (2011).

[§] Commonwealth of Australia (2006).

[§] MacIntyre and Hellstrom (2015). Wasp management costs are based on survey response data indicating a positively skewed distribution, Pert(\$0,\$5,\$25). Bee replacement costs consist of a 1kg pack of bees plus a queen (i.e. \$60) and 25 kg of sugar to build replacement bees up (i.e. \$25 per hive). Yield losses are taken directly from survey response data (i.e. RiskDiscrete[(0,0.01,0.02,0.03,0.04,0.05,0.06,0.07,0.08,0.09,0.1),(0.27,0.11,0.1,0.15,0.03,0.11,0.07,0.02,0.03,0.04,0.07)]). Costs consist of (i) wasp management costs, and (ii) bee replacement costs. Monetary values have been converted to Australian dollars using an exchange rate of 0.93.

^{††} ABS (2018a).

^{‡‡} Australian Honey Bee Industry Council (2014).

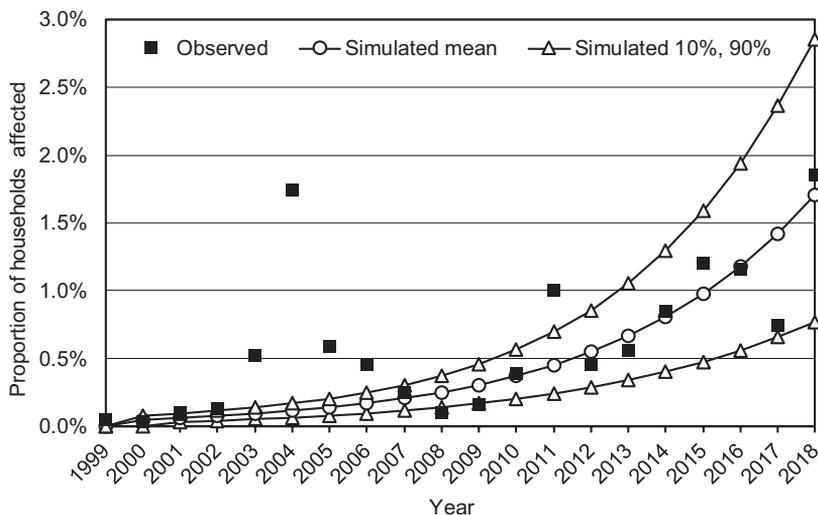


Figure 1. Visual validation plotting simulated and observed data of the proportion of households affected by *V. germanica* over the past 20 years.

Table 4. Viticulture parameters.

Parameter	Nil management	Management
Biological		
Infestation growth, ω_i (unitless) [†]	0.3–0.6	0.2–0.3
Maximum proportion affected, I_i^{\max} (%) [†]	Uniform(10,15)	Uniform(10,15)
Minimum proportion affected, I_i^{\min} (%) [†]	0.01	0.01
Proportion of I_i^{\max} affected at t^{θ_i} , θ_i , (%) [†]	Uniform(5,9)	Uniform(5,9)
Time taken for θ_i to be affected (yr) [†]	Uniform(10,20)	Uniform(20,30)
Economic		
Demand elasticity, η_i^{\dagger}	Uniform(-1.1,-1)	Uniform(-1.1,-1)
Discount rate, ν (%) [§]	Pert(2,5,7)	Pert(2,5,7)
Increased variable cost, V_{it} (\$/ha) [¶]	145	145
Inflation rate, ι (%) ^{††}	Pert(1.5,2,2.5)	Pert(1.5,2,2.5)
Price of per unit, P_{it} (\$/T) ^{**}	2500	2500
Yield loss despite control, Y_{it} (%)	0	0

[†] See eq. A2 and explanation. The infestation growth constant ω is determined by specifying a proportion of a stock infested, θ , and the amount of time taken for this proportion to be reached (t^{θ}). Maximum infestation of 10–15% is approximated using anecdotal information from Goodall and Smith (2001) on yield losses in South Australian vineyards. Assume it will take *V. germanica* 10–20 years to achieve an infestation rate of 5% in the nil management scenario, and 20–30 years in the management scenario.

[‡] Ulubasoglu et al. (2011).

[§] Commonwealth of Australia (2006).

[¶] Assumes vineyard managers use baiting to control wasp numbers. Bait costs of \$95 for a pack of 5 are based on Wine Tasmania (2018) and assumes bait stations are deployed at a density of 5 stations per ha per year. Assuming it takes 1 hour of labour per affected ha per year to deploy and manage bait stations, labour cost are approximately \$50 per ha per year.

^{††} ABS (2018a).

^{**} ABS (2018b) and ABS (2018c).

Statistical validation of the model is problematic as it is stochastic, producing a distribution for comparison to each observation. Moreover, only a single set of observed time-series data is available to compare the model output against, which introduces an autocorrelation problem. As a simple deviance measure test, the mean absolute error (MAE) and mean absolute percentage error (MAPE) between observed and model output were calculated using the mean of the simulated data. The MAE was 0.14%, indicating predicted values for the proportion of households affected were an average of 0.14% from observed values. The MAPE was 8.3%, indicating prediction error is, on average, 8.3% of the observed value. As a rule of thumb, a 10% MAPE is an approximate maximum limit for model acceptance (Kleijnen 1987; Mayer and Butler 1993).

Damage costs over time

The model estimates the ecosystem services damage (d) caused by *V. germanica* under nil management (d^{NM}) and on-going management (d^M) scenarios. The nil management scenario is constructed as a counterfactual to which a management policy can be compared to determine the reduction in damages attributable to the policy over time.

Table 5. Households parameters.

Parameter	Nil management	Management
Biological		
Infestation growth, ω_i (unitless) [†]	0.41–0.82	0.27–0.41
Maximum proportion affected, I_i^{\max} (%) [‡]	1	1
Minimum proportion affected, I_i^{\min} (%) [‡]	0.01	0.01
Proportion of I_i^{\max} affected at t^{θ} , θ_i , (%) [‡]	0.9	0.9
Time taken for θ_i to be affected (yr) [‡]	Uniform(10,20)	Uniform(20,30)
Economic		
Demand elasticity, η	na	na
Discount rate, ν (%) [‡]	Pert(2,5,7)	Pert(2,5,7)
Increased variable cost, V_{it} (\$/household) [§]	Uniform(200,250)	Uniform(200,250)
Inflation rate, ι (%) [‡]	Pert(1.5,2,2.5)	Pert(1.5,2,2.5)
Price of per unit, P_{it} (\$/T)	na	na
Yield loss despite control, Y_{it} (%)	na	na

See eq. A2 and explanation. The infestation growth constant ω is determined by specifying a proportion of a stock infested, θ , and the amount of time taken for this proportion to be reached (t^{θ}). Maximum infestation is approximated using data from Crosland (1991) showing the number of wasp nests destroyed in Hobart and converting this to a percentage of households using population data from McLennan (1997). Assume it will take *V. germanica* 10–20 years to achieve an infestation rate of 0.9% in the nil management scenario, and 20–30 years in the management scenario. The number of households in the Perth metropolitan area is assumed to be 818,100 (ABS 2017). Commercial structures are omitted.

[‡] Commonwealth of Australia (2006).

[§] FUMAPEST Pest Control (2018).

[†] ABS (2018a).

The difference between d^{NM} and d^{M} is simulated over 20 years. The ecosystem services damage cost of *V. germanica* in sector i in time period t under a nil management policy (d_{it}^{NM}) is calculated as:

$$d_{it}^{\text{NM}} = \sum_{i=1}^n S_{it}^{\text{NM}} (Y_{it} P_{it} N_{it} + V_{it} N_{it}) \quad (3)$$

where: n is the number of sectors affected by *V. germanica*; S_{it}^{NM} is the proportion of sector i affected by *V. germanica* in period t under a nil management policy scenario; Y_{it} is the mean change in yield in sector i attributable to *V. germanica* in year t ; P_{it} is the world price of product produced in sector i in year t ; N_{it} is the number of “units” (i.e. ha, hives, residences) in sector i potentially affected by *V. germanica* in year t , and; V_{it} is the increase in variable cost per unit induced by *V. germanica* in sector i in year t .

The ecosystem services damage cost of *V. germanica* in a region i in time period t under an ongoing management policy (d_{it}^{M}) is calculated as:

$$d_{it}^{\text{M}} = \sum_{i=1}^n S_{it}^{\text{M}} (Y_{it} P_{it} N_{it} + V_{it} N_{it}) \quad (4)$$

where: S_{it}^{M} is the proportion of sector i affected by *V. germanica* in period t if an ongoing management policy is in place.

For each sector that experiences yield effects from *V. germanica*, an estimate of price, P_{it} , is given for the first time step of the model (i.e. P_{i0} , corresponding to the year 2018). This is the initial price per unit for an affected product, but its price will change over time given that the demand for agricultural products is elastic (i.e. price increases with relative scarcity, and vice versa). The price in periods after t_0 will be partially influenced by the impact of *V. germanica* on production.

This price effect assumes the markets for affected products are protected, preventing perfect substitution of externally produced goods for those damaged by *V. germanica*. If WA markets were unregulated and open to free trade with suppliers from other states and overseas, and if the WA industries contributed a relatively small amount to global production, local prices of affected agricultural products would remain unchanged in response to *V. germanica* spread and impact (e.g. James and Anderson 1998). However, WA is protected by state and national phytosanitary measures and large distances separate its markets from external suppliers. Hence, reductions in local supplies tend to raise local prices.

Predicted yield loss, $Y_{it}N_{it}$, is used as a proxy for the *V. germanica*-induced reduction in sectoral output. This is combined with the lagged per unit price, P_{t-1} , to calculate

$$P_t = P_{t-1} \left[1 - \left(\frac{Y_{it}A_{it}}{G_{it}\eta} \right) \right].$$

Here, G_{it} is the gross value of production divided by 100 and η is the elasticity of demand for the affected commodity (i.e. the ratio of percentage change in quantity demanded over the percentage change in price).

Returning to equations 3 and 4, d^{NM} and d^M accrue over time and are subject to discounting. Discounting has an erosive effect on monetary values that increases with time, meaning that the present value of one unit of damage caused in the present is worth more than the same amount of damage caused in the future.

Applying an exponential discount rate, the present value of benefits anticipated from an on-going management policy in time period t (PVB_t^M) is estimated by summing $d_{it}^{NM} - d_{it}^M$ across all affected sectors (n) in WA:

$$PVB_t^M = \sum_{i=1}^n \left[\frac{d_{it}^{NM} - d_{it}^M}{(1+v)^t} \right] \quad (5)$$

where v is the discount rate.

The net present value of the *V. germanica* management policy (NPV_t^M) is calculated summing the difference between the present value of costs (PVC_t^M) and PVB_t^M over m time periods:

$$NPV_t^M = \sum_{t=1}^m (PVB_t^M - PVC_t^M). \quad (6)$$

The benefit cost ratio for the on-going *V. germanica* management option (BCR^M) is calculated by dividing the summed PVB_t^M over m time periods by the summed PVC_t^M over m time periods. Note that PVB_t^M represents gross (as opposed to net) benefits (i.e. $PVB_t^M = NPV_t^M + PVC_t^M$).

$$BCR_t^M = \sum_{t=1}^m \left[\frac{PVB_t^M}{PVC_t^M} \right]. \quad (7)$$

In the results section to follow, all costs and benefits are stated in Australian dollars. NPV^M and BCR^M are given for a range of PVC^M between \$230,000 and \$250,000 per annum over a period of 20 years. This range approximates the total amount spent by DPIRD in the past several years, and is indexed to the inflation rate. This means that PVC^M is fixed in real terms and nominal costs (C^M) increase at the inflation rate (i) over time (i.e. $C_t^M = \frac{PVC_t^M}{(1-i)^t}$).

Results

Ecosystem services damage predicted by the model under the nil management scenario (i.e. d^{NM} , eq. 3) and on-going management scenario (i.e. d^M , eq. 4) for each sector are shown in the box-whisker plots in panels A–D of Figure 2, while panel D shows aggregated ecosystem service damages under both scenarios. In each panel, damages over time under the nil management scenario increase initially as *Vespula germanica* spreads, but then begin to decrease due to the effects of discounting (Epanchin-Niell and Liebhold 2015). Noting the scale differences in the y-axes of panels A–D, the largest component of damages under the nil management scenario in panel E is pollination impacts, accounting for approximately 85% in each year simulated. Household damages in panel D, attributable to necessary purchases of pest removal services, are also substantial, making up approximately 13% of total. Household damages under the management scenario are zero as nest destruction costs are paid by DPIRD.

The uncertainty in model predictions is evident in the width of the boxes and length of whiskers in Figure 2. Aggregated damage costs in panel D peak in year 12 under the nil management scenario with a mean value of \$6.9 million, but vary between \$5.0–9.0 million in 80% of model iterations, and between \$4.6–9.7 in 90% of iterations. The present value of benefit created by DPIRD management efforts is represented by the vertical distance between the two scenarios, which in year 12 is estimated to be between \$4.9–8.8 million (80%) and \$4.5–9.5 million (90%).

The benefits and costs of *V. germanica* management are compared in Figure 3 where hollow boxes represent the present value of benefit (i.e. PVB^M , eq. 5), filled boxes represent net present value (i.e. NPV^M) and bold horizontal lines indicate the present value of costs (i.e. PVC^M). The mean annual present value of benefit and net present value between years 1–10 is \$3.7 million (S.D. \$1.4 million) and \$3.4 million (S.D. \$1.4 million), respectively. Between years 1–20, the mean present value of benefit and net pre-

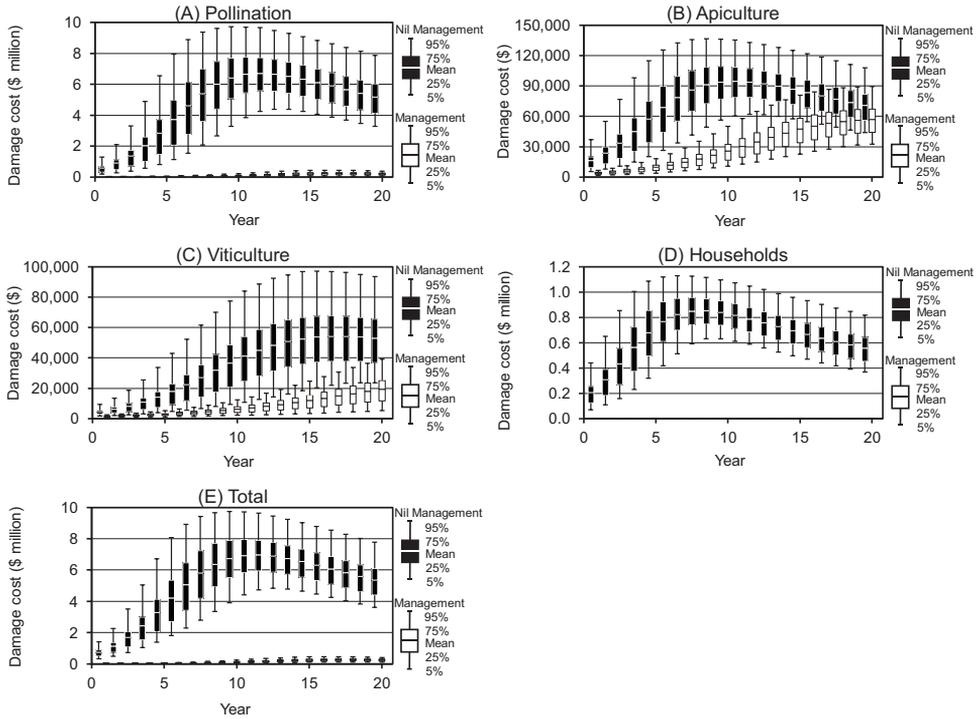


Figure 2. Predicted damage cost per year associated with *V. germanica* impacts in WA over 20 years. Panels **A, B, C** and **D** show pollination, apiculture, viticulture and household damage costs, respectively, under both scenarios, while panel **E** shows the summed damage costs across all sectors under both scenarios. Box whisker plots indicate 5th, 25th, mean, 75th and 95th percentile values, with shaded boxes representing the nil management scenario and hollow boxes the management scenario.

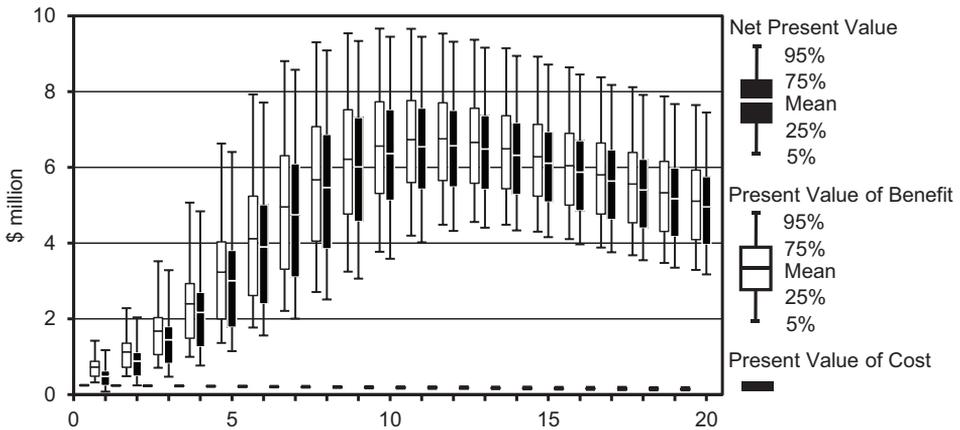


Figure 3. Net present value of *V. germanica* management in WA over 20 years. The box whisker plot indicates 5th, 25th, mean, 75th and 95th percentile values.

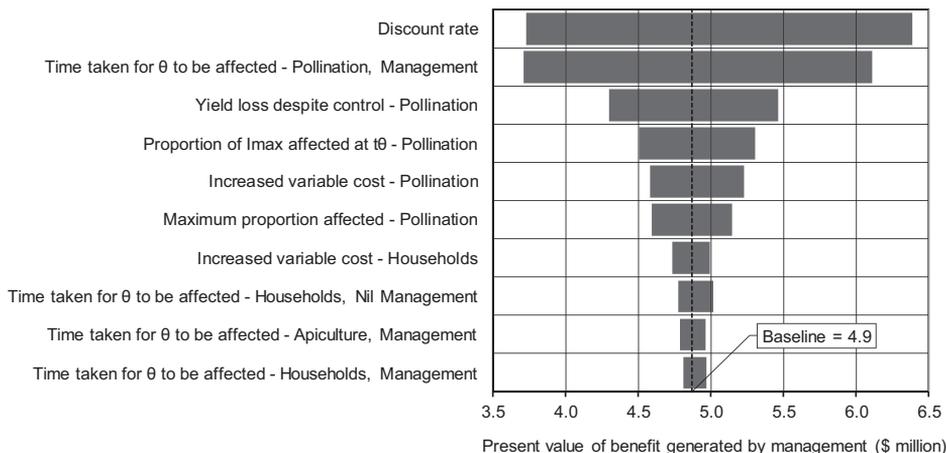


Figure 4. Sensitivity analysis illustrating how the mean net benefit of *V. germanica* management in WA 20 years is affected by changes in input parameters.

sent value increase to \$4.9 million (S.D. \$1.2 million) and \$4.6 million (S.D. \$1.2 million), respectively. This results in a benefit cost ratio for the management of *V. germanica* (i.e. BCR^M , eq. 7) over 10 years of 15.4, and over 20 years of 19.9. Based on the mean present value of benefit, the results imply each dollar invested in ongoing *V. germanica* management is most likely to prevent \$19.90 in damage costs over 20 years.

However, there is considerable uncertainty in the model predictions that could lead to a substantially better or worse return on investment than indicated by the mean. Over 10 years, 80% of model iterations produced a present value of benefit of \$2.1–5.6 million, suggesting a benefit cost ratio between 8.3 and 22.5. Moreover, the uncertainty in model predictions increases as the length of the simulation period increases. Over 20 years, the estimated present value of benefit varies between \$6.5–26.2 million, resulting in a benefit cost ratio between 13.8–26.2.

Despite this uncertainty, results of a parameter sensitivity analysis indicate that the return to investment in management remain positive even under worst-case scenarios. To gauge the effect of the parameters on model output, each parameter is sampled across its specified range while holding all other parameters constant in Figure 4. Here the 10 parameters producing the most change are ranked from top to bottom according to their strength of influence on the present value of benefit generated.

Results are most sensitive to changes in the discount rate, which is specified as $Pert(2\%,5\%,7\%)$. It is inversely related to the present value of benefit. Lowering the discount rate from its most likely value of 5% to 2% (a change of -60%) increases the present value of benefit by approximately 31% (from \$4.9 million to \$6.4 million), and increasing it to 7% (a change of 40%) lowers the present value of benefit by approximately 24% (to \$3.7 million). Determining an appropriate discount rate is one of the most controversial and important issues in benefit cost analysis since as it has a major impact on the viability of many public projects (Abelson and Dalton 2018).

Yet there is no definitive answer as to what rate should be applied in different circumstances. This is not critical here since the present value of benefits exceed the present value of costs across the range of discount rates considered (i.e. costs do not exceed \$250,000 per year in real terms).

Results are also highly sensitive to the time taken for the indicative proportion θ_i to be affected under the management scenario. This is also inversely related to the present value of benefit, producing a $\pm 24\%$ change when increased or decreased 20% from the mean value (25 years). As it relates to the effectiveness of DPIRD activities in slowing the spread of *V. germanica*, the time taken for θ_i to be affected under the management scenario is a key assumption. Citing the DPIRD time series data used to validate the model, the range 20–30 years is a reasonable approximation for this parameter. Even when at 20 years, the model still produces a present value of benefit of \$3.7 million.

Other parameters with relatively high sensitivities mostly relate to the pollination sector, including yield loss despite control, increase in variable costs, maximum proportion affected (I_i^{\max}) and the indicative proportion θ_i . This reflects the large size of pollination sector impacts compared with those in the household, viticulture and apiculture sectors.

Discussion

The model used in this analysis takes into account multiple ecosystem services and conveys the uncertain future benefits of invasive species controls to decision-makers in relatively simple terms. As the impacts of invasive species change with respect to time, location, and other variables in ways that are difficult to predict, policy-makers need to be informed by predictive (ex ante) analyses that are explicit about the uncertain future effects of decisions made in the present (Regan et al. 2002). At the same time, as they are typically time-pressured, policy-makers require model outputs that condense complex spread and impact information into easily understood metrics. The model presented here adds to the literature by simultaneously fulfilling both of these requirements.

Research concerning economic impacts of invasive species has increased in recent decades, but most has involved ex post impact assessments and management evaluation (Naylor 2000; Born et al. 2005; Cook et al. 2013). Research involving predictive models has tended to rely on partial budgeting techniques or deterministic models that ignore uncertainties in species behaviour and environmental interactions (de Wit et al. 2001; MacLeod et al. 2004; Bolda et al. 2010).

Several ex ante studies have used complex, spatially explicit approaches and stochastic simulations to characterise uncertainty in spread patterns over time combining environmental variables and invasive species behaviours (Rafoss 2003; Yemshanov et al. 2009; Leung et al. 2014). Others have integrated established ecological models with economic management frameworks (Sharov and Liebhold 1998; Barbier 2001; Cacho et al. 2008; Hyder et al. 2008; Carrasco et al. 2010) or have used metapopulation models to predict future impacts (Albers et al. 2010; Sanchirico et al. 2010).

Economic modelling has seldom been used as part of an invasive species ecosystems service impact assessment. Cook et al. (2007) used a reaction diffusion model to estimate future spread and impact of varroa bee mite (*Varroa destructor*) on a single ecosystem service (pollination) in Australia. Other examples include several ex post studies involving the weed leafy spurge (*Euphorbia esula*) that have estimated impacts on provisioning and cultural services in the northern Great Plains region of the United States (Leistritz et al. 1992; Leitch et al. 1996; Leistritz et al. 2004). Changes in grazing land output were used to estimate effects on producer and local agribusiness income, and reductions in environmental output were used to estimate changes in outdoor recreation expenditures.

The future ecosystem service impact predicted in this analysis hint at large returns to investment in the ongoing management of *V. germanica* in WA, particularly in terms of provisioning ecosystem services to private producers of pollination-dependent crops. This justifies the WA government's use of DPIRD resources in managing the pest rather than another department since the impacts of the wasp are mainly agricultural. Funding is relatively low (i.e. \$200,000–250,000 per year) when compared to the gross value of crops affected (i.e. \$1.3 billion, see Table 1), in part due to pollination benefits being historically omitted from funding decisions.

If the pollination sector is removed from the model, the household sector becomes the largest beneficiary of management activities and the 20-year benefit cost ratio falls from 13.8–26.2 to 3.0–4.3. This might suggest the state's demand for wasp nest removal could be met by private pest controllers in the Perth metropolitan area rather than government. The main beneficiaries are spatially concentrated in this area and benefits to the apiculture and viticulture sectors are small in comparison. Hence, the positive flow-on effects beyond the household sector would be minimal.

However, if pollination services are included in policy decisions, the situation changes considerably. Beneficiaries of management are now spatially diffuse, consisting of various industry groups, community groups and institutions. This would make it logistically challenging and prohibitively costly to bring all affected parties together to negotiate wasp management plans and control targets and monitoring with private pest control operators. Therefore, government intervention is necessary to ensure an adequate level of management services are provided to all affected groups.

If cultural ecosystem service impacts of *V. germanica* related to biodiversity are also included in policy decisions, the need for government intervention becomes even stronger because biological diversity is a public good. Public goods are non-rivalrous in consumption (i.e. enjoyment of biodiversity by one person does not affect the quantity available for another) and have benefits that are non-exclusive (i.e. one person cannot prevent another from enjoying the benefits of biodiversity). As such, these goods cannot be provided to a socially desirable level by private providers who are unable to charge for the full benefits their services create, nor prevent people from enjoying benefits they have not paid for.

To the author's knowledge, no research is currently available concerning the potential for *V. germanica* to affect biodiversity in WA, but experience elsewhere suggests

damage could be considerable. For instance, the introduction of the wasp to Tasmania has resulted in severe local reductions of invertebrates (Spradbery and Maywald 1992; Potter-Craven et al. 2018). In New Zealand, prey biomass captured by *V. germanica* is equivalent to that of the entire insectivorous bird fauna (Harris 1991; Toft and Beggs 1995; Toft and Rees 1998; Matthews et al. 2000). Given the status of south-west WA as a biodiversity hotspot there is a pressing need to study potential impacts on species unique to the region (Myers et al. 2000).

Conclusion

The model presented in this paper estimates the return on government investment in continued *V. germanica* management in WA in terms of provisioning and cultural ecosystem services. Results suggest that the combined ecosystem service benefits of ongoing management over the next 20 years are likely be \$3.4–6.5 million per year. With annual costs of management being \$200,000–250,000, this indicates a net benefit of \$3.2–6.3 million per year. The largest beneficiaries are producers of crops depended on insect pollination. These benefits have a tendency to be overlooked due to the reputation of *V. germanica* as an urban nuisance, rather than an agricultural pest. If pollination benefits are ignored, households are indeed the largest beneficiaries of wasp control and there may be grounds for turning management over to the private sector. However, if pollination impacts are as large as the results of this analysis suggest, negotiation costs and information constraints are likely to prevent private controllers from providing sufficient management services. If cultural service benefits of *V. germanica* management are also considered, such as prevented damage to unique species in the south west of WA, the case for government provision is also strengthened.

Acknowledgements

Thank you to Catherine Webb and Marc Widmer from the Department of Primary Industries and Regional Development for information generously provided. Thanks also to two anonymous reviewers for comments and suggestions that greatly improved the paper.

References

- Abelson P, Dalton T (2018) Choosing the social discount rate for Australia. *Australian Economic Review* 51: 52–67. <https://doi.org/10.1111/1467-8462.12254>
- ABS (2017) Census 2016 QuickStats. Australian Bureau of Statistics, Belconnen, A.C.T.
- ABS (2018a) 6401.0 – Consumer Price Index, Australia, Mar 2018. Australian Bureau of Statistics, Canberra.

- ABS (2018b) Agricultural Commodities, Australia, 2015–16. Australian Bureau of Statistics, Canberra.
- ABS (2018c) Value of Agricultural Commodities Produced, Australia, 2015–16. Australian Bureau of Statistics, Canberra.
- Albers HJ, Fischer C, Sanchirico JN (2010) Invasive species management in a spatially heterogeneous world: effects of uniform policies. *Resource and Energy Economics* 32: 483–499. <https://doi.org/10.1016/j.reseneeco.2010.04.001>
- Australian Honey Bee Industry Council (2014) Submission to senate inquiry on the future of the beekeeping and pollination service industries in Australia. Australian Honey Bee Industry Council Inc., Raceview, Queensland, 24 pp.
- Barbier EB (2001) A note on the economics of biological invasions. *Ecological Economics* 39: 197–202. [https://doi.org/10.1016/S0921-8009\(01\)00239-7](https://doi.org/10.1016/S0921-8009(01)00239-7)
- Bashford R (2001) The spread and impact of the introduced vespine wasps *Vespula germanica* (F.) and *Vespula vulgaris* (L.) (Hymenoptera: Vespidae: Vespinae) in Tasmania. *The Australian Entomologist* 28: 1.
- Bolda MP, Goodhue RE, Zalom FG (2010) Spotted wing drosophila: potential economic impact of a newly established pest. *Agricultural and Resource Economics Update* 13: 5–8.
- Born W, Rauschmayer F, Bräuer I (2005) Economic evaluation of biological invasions – a survey. *Ecological Economics* 55: 321–336. <https://doi.org/10.1016/j.ecolecon.2005.08.014>
- Cacho OJ, Wise RM, Hester SM, Sinden JA (2008) Bioeconomic modeling for control of weeds in natural environments. *Ecological Economics* 65: 559–568. <https://doi.org/10.1016/j.ecolecon.2007.08.006>
- Carrasco LR, Baker R, MacLeod A, Knight JD, Mumford JD (2010) Optimal and robust control of invasive alien species spreading in homogeneous landscapes. *Journal of the Royal Society Interface* 7: 529–540. <https://doi.org/10.1098/rsif.2009.0266>
- Centre for Agricultural Bioscience Information (2017) Crop Protection Compendium. CAB International, Cayman Islands.
- Chadwick CE, Nikitin MI (1969) Some insects and other invertebrates intercepted in quarantine in New South Wales: Part 2. Arthropoda (excluding Coleoptera), and Mollusca. *Journal of the Entomological Society of Australia* 6: 37–56.
- Clapperton B, Alspach P, Moller H, Matheson A (1989) The impact of common and German wasps (Hymenoptera: Vespidae) on the New Zealand beekeeping industry. *New Zealand Journal of Zoology* 16: 325–332. <https://doi.org/10.1080/03014223.1989.10422897>
- Commonwealth of Australia (2006) Handbook of Cost-Benefit Analysis. Department of Finance and Administration, Canberra, 180 pp.
- Cook D, Liu S, Edwards J, Villalta O, Aurambout J-P, Kriticos D, Drenth A, De Barro P (2013) An assessment of the benefits of yellow Sigatoka (*Mycosphaerella musicola*) control in the Queensland Northern Banana Pest Quarantine Area. *NeoBiota* 18: 67–81. <https://doi.org/10.3897/neobiota.18.3863>
- Cook DC, Thomas MB, Cunningham SA, Anderson DL, De Barro PJ (2007) Predicting the economic impact of an invasive species on an ecosystem service. *Ecological Applications* 17: 1832–1840. <https://doi.org/10.1890/06-1632.1>

- Costanza R, D'arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'neill RV, Paruelo J, Raskin RG, Sutton P, van der Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature* 387: 253–260. <https://doi.org/10.1038/387253a0>
- Crosland MWJ (1991) The spread of the social wasp, *Vespula germanica*, in Australia. *New Zealand Journal of Zoology* 18: 375–387. <https://doi.org/10.1080/03014223.1991.10422843>
- Cunningham SA, FitzGibbon F, Heard TA (2002) The future of pollinators for Australian agriculture. *Australian Journal of Agricultural Research* 53: 893–900. <https://doi.org/10.1071/AR01186>
- de Wit MP, Crookes DJ, Van Wilgen BW (2001) Conflicts of interest in environmental management: estimating the costs and benefits of a tree invasion. *Biological Invasions* 3: 167–178. <https://doi.org/10.1023/A:1014563702261>
- East RW (1984) Biology and control of European wasps *Vespula germanica* in Victoria. In: Bailey P, Swincer D (Eds) *Proceedings of the Fourth Australian Applied Entomological Research Conference: Pest Control: Recent Advances and Future Prospects*. Department of Agriculture, South Australia, Adelaide, 21–26.
- Epanchin-Niell RS, Hastings A (2010) Controlling established invaders: integrating economics and spread dynamics to determine optimal management. *Ecology Letters* 13: 528–541. <https://doi.org/10.1111/j.1461-0248.2010.01440.x>
- Epanchin-Niell RS, Liebhold AM (2015) Benefits of invasion prevention: effect of time lags, spread rates, and damage persistence. *Ecological Economics* 116: 146–153. <https://doi.org/10.1016/j.ecolecon.2015.04.014>
- Free JB (1993) *Insect Pollination of Crops*. Academic Press, London, 684 pp.
- FUMAPEST Pest Control (2018) *Pest Control – European Wasps – Paper Wasps*. FUMAPEST Pest Control. <https://www.termite.com.au/wasps-pest-control.html> [Accessed on: 2018-11-13]
- Goodall S, Smith DL (2001) The European wasp in metropolitan Adelaide: it's ecology, spread and impacts. *South Australian Geographical Journal* 100: 25.
- Harris RJ (1991) Diet of the wasps *Vespula vulgaris* and *V. germanica* in honeydew beech forest of the South Island, New Zealand. *New Zealand Journal of Zoology* 18: 159–169. <https://doi.org/10.1080/03014223.1991.10757963>
- Hyder A, Leung B, Miao ZW (2008) Integrating data, biology, and decision models for invasive species management: application to leafy spurge (*Euphorbia esula*). *Ecology and Society* 13: 1–12. <https://doi.org/10.5751/ES-02485-130212>
- James S, Anderson K (1998) On the need for more economic assessment of quarantine policies. *The Australian Journal of Agricultural and Resource Economics* 42: 425–444. <https://doi.org/10.1111/1467-8489.00061>
- Kleijnen JPC (1987) *Statistical Tools for Simulation Practitioners*. Marcel Dekker, New York, 448 pp.
- Lefoe G, Ward D, Honan P, Darby S, Butler K (2001) *Minimising the impact of European wasps on the grape and wine industry*. Final report to Grape and Wine Research & Development Corporation. Department of Natural Resources and Environment, Keith Turnbull Research Institute, Frankston, 38 pp.

- Leistritz F, Bangsund D, Hodur N (2004) Assessing the economic impact of invasive weeds: The case of leafy spurge (*Euphorbia esula*). *Weed Technology* 18: 1392–1395. [https://doi.org/10.1614/0890-037X\(2004\)018\[1392:ATEIOI\]2.0.CO;2](https://doi.org/10.1614/0890-037X(2004)018[1392:ATEIOI]2.0.CO;2)
- Leistritz FL, Thompson F, Leitch JA (1992) Economic impact of leafy spurge (*Euphorbia esula*) in North Dakota. *Weed Science* 40: 275–280. <https://doi.org/10.1017/S0043174500057349>
- Leitch JA, Leistritz FL, Bangsund DA (1996) Economic effect of leafy spurge in the upper Great Plains: Methods, models, and results. *Impact Assessment* 14: 419–433. <https://doi.org/10.1080/07349165.1996.9725915>
- Leung B, Springborn MR, Turner JA, Brockerhoff EG (2014) Pathway-level risk analysis: the net present value of an invasive species policy in the US. *Frontiers in Ecology and the Environment* 12: 273–279. <https://doi.org/10.1890/130311>
- MacIntyre P, Hellstrom J (2015) An evaluation of the costs of pest wasps (*Vespula* species) in New Zealand. Department of Conservation and Ministry for Primary Industries, Wellington, 44 pp.
- MacLeod A, Head J, Gaunt A (2004) An assessment of the potential economic impact of *Thrips palmi* on horticulture in England and the significance of a successful eradication campaign. *Crop Protection* 23: 601–610. <https://doi.org/10.1016/j.cropro.2003.11.010>
- Matthews RW, Goodisman MA, Austin AD, Bashford R (2000) The introduced English wasp *Vespula vulgaris* (L.) (Hymenoptera: Vespidae) newly recorded invading native forests in Tasmania. *Australian Journal of Entomology* 39: 177–179. <https://doi.org/10.1046/j.1440-6055.2000.00173.x>
- Mayer DG, Butler DG (1993) Statistical validation. *Ecological modelling* 68: 21–32. [https://doi.org/10.1016/0304-3800\(93\)90105-2](https://doi.org/10.1016/0304-3800(93)90105-2)
- McGain F, Harrison J, Winkel KD (2000) Wasp sting mortality in Australia. *Medical journal of Australia* 173: 198–200.
- McLennan W (1997) 4102.0 Australian Social Trends 1996. Australian Bureau of Statistics, Belconnen, ACT, 187 pp.
- Millennium Ecosystem Assessment (2005) Ecosystems and Human Well-being: Health Synthesis. Island Press, Washington, DC, 53 pp.
- Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853. <https://doi.org/10.1038/35002501>
- Naylor RL (2000) The economics of alien species invasions. In: Mooney HA, Hobbs RJ (Eds) *Invasive Species in a Changing World*. Island Press, Washington, DC, 241–259.
- Paton DC (1995) Overview of Feral and Managed Honey Bees in Australia: Distribution, Abundance, Extent of Interactions with Native Biota, Evidence of Impacts and Future Research: Report to the Australian Nature Conservation Agency. University of Adelaide, Adelaide, 74 pp.
- Potter-Craven J, Kirkpatrick JB, McQuillan PB, Bell P (2018) The effects of introduced vespid wasps (*Vespula germanica* and *V. vulgaris*) on threatened native butterfly (*Oreixenica ptunarra*) populations in Tasmania. *Journal of Insect Conservation* 22: 1–12. <https://doi.org/10.1007/s10841-018-0081-9>
- Rafoss T (2003) Spatial stochastic simulation offers potential as a quantitative method for pest risk analysis. *Risk Analysis* 23: 651–661. <https://doi.org/10.1111/1539-6924.00344>

- Regan HM, Colyvan M, Burgman MA (2002) A taxonomy and treatment of uncertainty for ecology and conservation biology. *Ecological applications* 12: 618–628. [https://doi.org/10.1890/1051-0761\(2002\)012\[0618:ATATOU\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2002)012[0618:ATATOU]2.0.CO;2)
- Sanchirico J, Albers H, Fischer C, Coleman C (2010) Spatial management of invasive species: pathways and policy options. *Environmental and Resource Economics* 45: 517–535. <https://doi.org/10.1007/s10640-009-9326-0>
- Schaefer MB (1957) Some considerations of population dynamics and economics in relation to the management of the commercial marine fisheries. *Journal of the Fisheries Research Board of Canada* 14: 669–681. <https://doi.org/10.1139/f57-025>
- Sharov AA, Liebhold AM (1998) Bioeconomics of managing the spread of exotic pest species with barrier zones. *Ecological Applications* 8: 833–845. [https://doi.org/10.1890/1051-0761\(1998\)008\[0833:BOMTSO\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1998)008[0833:BOMTSO]2.0.CO;2)
- Smithers CN, Holloway GA (1977) Recent specimens of *Vespula* (*Paravespula*) *germanica* (Fabricius) (Hymenoptera: Vespidae) taken in Sydney. *The Australian Entomologist* 4: 75.
- Smithers CN, Holloway GA (1978) Establishment of *Vespula germanica* (Fabricius) (Hymenoptera: Vespidae) in New South Wales. *The Australian Entomologist* 5: 55.
- Spradbery J, Maywald G (1992) The distribution of the European or German wasp, *Vespula germanica* (F) (Hymenoptera, Vespidae), in Australia – past, present and future. *Australian Journal of Zoology* 40: 495–510. <https://doi.org/10.1071/ZO9920495>
- Tennant P, Davis P, Widmer M, Hood G (2011) Final Report CRC 30133: Urban surveillance for Emergency Plant Pests (EPPs). Cooperative Research Centre for National Plant Biosecurity, Canberra, 17 pp.
- The Advertiser (2015) Wasp cash ‘is a waste’. *The Advertiser*, 1st October. News Limited Australia, Adelaide, 10.
- Thomas G (1993) Wineloving wasps. *Australian Horticulture*, September: 28–30.
- Toft RJ, Beggs JR (1995) Seasonality of crane flies (Diptera: Tipulidae) in South Island beech forest in relation to the abundance of *Vespula* wasps (Hymenoptera: Vespidae). *New Zealand Entomologist* 18: 37–43. <https://doi.org/10.1080/00779962.1995.9722000>
- Toft RJ, Rees JS (1998) Reducing predation of orb-web spiders by controlling common wasps (*Vespula vulgaris*) in a New Zealand beech forest. *Ecological Entomology* 23: 90–95. <https://doi.org/10.1046/j.1365-2311.1998.00100.x>
- Ulubasoglu M, Mallick D, Wadud M, Hone P, Haszler H (2011) How price affects the demand for food in Australia – an analysis of domestic demand elasticities for rural marketing and policy. Rural Industries Research and Development Corporation, Canberra, 41 pp.
- Williams T (2015) Sting in the fight against wasps. *The Advertiser*, 29th September. News Limited, Adelaide, 6.
- Wine Tasmania (2018) European Wasp Baits. http://winetasmania.com.au/products/european_wasp_baits [Accessed on: 2018-11-15]
- Yemshanov D, Koch FH, McKenney DW, Downing MC, Sapio F (2009) Mapping invasive species risks with stochastic models: A cross-border United States–Canada application for *Sirex noctilio* Fabricius. *Risk Analysis* 29: 868–864. <https://doi.org/10.1111/j.1539-6924.2009.01203.x>

Seed-bank dynamics of native and invasive *Impatiens* species during a five-year field experiment under various environmental conditions

Hana Skálová¹, Lenka Moravcová¹, Jan Čuda¹, Petr Pyšek^{1,2}

1 Institute of Botany, The Czech Academy of Sciences, Zámek 1, CZ-252 43 Průhonice, Czech Republic **2** Department of Ecology, Faculty of Science, Charles University, Viničná 7, CZ-128 44 Prague, Czech Republic

Corresponding author: Hana Skálová (hana.skalova@ibot.cas.cz)

Academic editor: Harald Auge | Received 25 March 2019 | Accepted 16 July 2019 | Published 26 September 2019

Citation: Skálová H, Moravcová L, Čuda J, Pyšek P (2019) Seed-bank dynamics of native and invasive *Impatiens* species during a five-year field experiment under various environmental conditions. NeoBiota 50: 75–95. <https://doi.org/10.3897/neobiota.50.34827>

Abstract

Despite recent evidence on the important role of seed banks associated with plant invasions, and a large body of literature on invasive annual *Impatiens* species, little is known about the seed bank characteristics of *Impatiens* species. To bridge this gap, we conducted a five-year field experiment where we buried seeds of two invasive species (*I. glandulifera* and *I. parviflora*) and one native species (*I. noli-tangere*) across four localities in the Czech Republic, harbouring all three *Impatiens* species and differing in the environmental conditions. We found that the three *Impatiens* species differed in the characteristics of their seed banks. Both invasive species had a high seed germination rate of almost 100% in the first year after seed burial, while <50% of seeds of the native *I. noli-tangere* germinated during this year. In *I. parviflora* all seeds germinated in the first year after seed burial and later decomposed, i.e. the species had a transient seed bank. For *I. glandulifera*, the most invasive species, the survival of seeds differed among localities. At the first and second localities, the seeds decomposed in the first year after seed burial; in the third locality the seeds germinated in the second year; and in the fourth one, the seeds still germinated in the fourth year. The native *I. noli-tangere* formed a short-term persistent seed bank across all localities. Germinating or dormant seeds were found in the third year after burial in all localities, and in one locality the seeds persisted until the fifth year. The germination and dormancy in *I. noli-tangere* were constrained by low minimum temperatures during winter. In addition, germination was highest at intermediate soil moisture, and the most dormant seeds were recorded in soils with intermediate nitrogen concentration. The germination of *I. glandulifera* was slightly limited by low soil nitrogen. However, no such effect was found in *I. parviflora*. We suggest that in the invasive *Impatiens* species seed resistance to environmental factors and high germination at least partly explain their wide distribution.

Keywords

balsam, *Impatiens glandulifera*, *Impatiens noli-tangere*, *Impatiens parviflora*, plant invasions, seed dormancy, seed germination, soil environment

Introduction

Factors driving invasion success of alien plant species are a significant research topic in ecology and invasion biology, and this research has considerable applied relevance (Vilà et al. 2011, Pyšek et al. 2012a, Simberloff et al. 2013). Of these factors, species traits have been a research focus, and thus many studies have predicted a set of key traits associated with successful invaders, including traits related to reproduction (for a review see Pyšek and Richardson 2007, van Kleunen et al. 2010). Successful invaders are typically characterised by massive seed production (Moravcová et al. 2010, 2015) and early and/or rapid germination (Schlaepfer et al. 2010, Wilsey et al. 2015, Gioria and Pyšek 2017), which allows them to germinate and establish seedlings when competition from resident plants is low (Gioria et al. 2018).

Some studies pointed to the important role of soil seed banks in species invasiveness, as the formation of a seed bank represents a mechanism by which a species can persist in the invaded localities (Thuiller et al. 2008, Gioria et al. 2011, 2012). Persistent soil seed banks allow species to survive in the soil when conditions for germination or reproduction are unfavourable. In addition, seed banks maintain population genetic diversity, and thus improve the ability of alien species to respond to novel site conditions encountered in the introduced range by spreading the environmental risks over time (Gioria et al. 2012). The role of seed banks in population persistence has to be taken into account during eradication, because a few viable seeds in the soil may allow re-invasions (Fletcher et al. 2015, Leary et al. 2018, Moravcová et al. 2018).

The capacity to form a persistent soil seed bank was revealed as the most powerful trait explaining the large ecological amplitude of Central European species in their native range, as well as the naturalisation success of these species in North America (Pyšek et al. 2015). Successful invasive species often have larger and longer-persisting seed banks when compared to their native congeners or confamilials (Pyke 1990, Honig et al. 1992, Van Clef and Stiles 2001) or non-invasive species (Radford and Cousens 2000, Phillips and Murray 2012). Furthermore, the timing of germination from soil seed banks is supposed to be an important factor influencing plant invasions (Gioria et al. 2018). Unfortunately, the data on seed banks of individual species are still rather scarce and often disconnected from other traits, regardless of the fact that the seed bank characteristics contribute to understanding the invasion success of plant species, especially of annuals that are strictly dependent on population recovery from seed. Studies that included seed banks characteristics as explanatory variable suggest that it

is an important trait contributing to the invasion success of species (Pyšek et al. 2015). In addition, the seed bank characteristics should be taken into account for eradication plans, while monitoring of the eradicated sites should be longer than seed bank longevity to prevent population recovery.

As other life stages of plants, seed bank dynamics are influenced by environmental factors. Surprisingly, only a few studies have addressed this topic. The existing studies demonstrated the role of habitat (Hesse et al. 2007), water and temperature (Schafer and Kotanen 2003), precipitation (Bebawi et al. 2015), soil type (Abedi et al. 2014, Bebawi et al. 2015), soil pH (Basto et al. 2015 and Pakeman et al. 2012) and soil nutrients (Moravcová et al. 2018 and Pakeman et al. 2012).

We used a group of central European annual species belonging to the genus *Impatiens*, i.e. the native *I. noli-tangere* and the invasive *I. glandulifera* and *I. parviflora*, which co-occur in the same localities and have been assessed previously for traits associated with invasiveness. The invasive *I. glandulifera* is characterised by rapid and high germination (Perglová et al. 2009), a high biomass throughout the species life cycle (Skálová et al. 2012, 2013), and its competitiveness (Čuda et al. 2015). The invasive *I. parviflora* invests more resources into reproduction than *I. glandulifera* or the native *I. noli-tangere* (Skálová et al. 2013, Minden and Gorschlüter 2016). All three species show among-population variation in germination and frost resistance reflecting conditions from localities where the seeds were collected (Skálová et al. 2011), as well as differences in seedling but not adult traits (Skálová et al. 2012, 2013). This indicates that development in the juvenile stages is crucial for these annual species to reach the single reproductive event in their life cycle (Čuda et al. 2016). One response to compensate for possible failure before reaching reproduction is to store seeds in the soil seed bank.

Seed banks are a part of the life cycle of *Impatiens* species that are poorly understood. Information about their seed banks is mostly based on indirect observations, for example, the occurrence of seedlings after thwarting seed production due to the removal of plants before fruiting, or it was tested in the laboratory or in an experimental garden (see Perglová et al. 2009), where ecological conditions differ from field sites. The garden experiment indicated no persistent soil seed bank in two invasive species *I. parviflora* and *I. glandulifera*, but a short-term persistent seed bank in the native *I. noli-tangere*. However, together with seed persistence the timing of germination from the seed bank could be another important factor influencing invasiveness (Gioria et al. 2018).

To improve the seed bank knowledge for *Impatiens* species, we established a burial experiment in which we studied germination, dormancy and seed persistence of *I. glandulifera*, *I. noli-tangere* and *I. parviflora* for five years in natural populations in the Czech Republic, harbouring all three *Impatiens* species and differing in the environmental conditions. We assessed whether seed bank dynamics and seed persistence in the field (i) differ among the invasive and native species, (ii) differ across and within individual localities, (iii) and whether they are influenced by environmental factors.

Methods

Study species

The three *Impatiens* species have similar life histories and reproductive characteristics (Coombe 1956, Beerling and Perrins 1993, Hatcher 2003, Adamowski 2008). *Impatiens noli-tangere* L., a native species to central Europe, is recorded from 39 habitat types in the Czech Republic (Sádlo et al. 2007), mainly from damp forests, clearings and riparian habitats (Slavík 1997). *Impatiens glandulifera* Royle, a highly invasive species which occurs predominantly along rivers, has also colonised forest clearings and margins, wet ditches, forest roads and ruderal sites (Čuda et al. 2017). In the Czech Republic, the first record of cultivation is from 1846 and that of occurrence outside cultivation from 1896 (Slavík 1997). The species is recorded from 16 habitat types (Sádlo et al. 2007, Pyšek et al. 2012b, Pahl et al. 2013), but this number is expected to increase due to its ongoing spread. *Impatiens parviflora* DC., an invasive species, is characterised by broad ecological amplitude, being recorded from 45 habitat types in the Czech Republic (Sádlo et al. 2007, Pyšek et al. 2012c). In this country it was first recorded in 1844 in a botanical garden in Prague, and outside cultivation around 1870 (Slavík 1997). It often grows as a dominant species in nitrophilous herbaceous vegetation of shady mesic sites, in alluvial forests, oak-hornbeam forests, ravine forests, and spruce or *Robinia pseudoacacia* plantations (Pyšek et al. 2012b), and its current spread is slower, indicating limits of distribution.

The presence of *Impatiens* species depends on some degrees of disturbance, and thus they often occur in early-successional herbaceous communities. They coexist in some localities where the spatial pattern of the occurrence is driven by canopy closure and water availability (Čuda et al. 2014). Seeds of the species differ in the stratification demands and germination rates (Perglová et al. 2009, Skálová et al. 2011). Seeds of *I. glandulifera* only require a short period of cold-wet stratification after which most of them germinate, while *I. parviflora* requires a long period of stratification, which is followed by germination of a high percentage of seed, and *I. noli-tangere* has the longest stratification period but the lowest germination. Both seedling and adult plants of the three species suffer from frost damage (Coombe 1956, Beerling and Perrins 1993, Perrins et al. 1993, Hatcher 2003, Skálová et al. 2011), and this may be one of the factors limiting their spread. The temperature regime of the seed source explained differences in frost sensitivity among populations of *I. glandulifera* and *I. parviflora*, while in *I. noli-tangere* and *I. parviflora* the temperature influenced the time of germination (Skálová et al. 2011). In addition, populations of *I. glandulifera* differ in flowering phenology along latitudinal gradients (Kollmann and Bañuelos 2004). Finally, for all three species differences among populations were observed for seedling height, biomass and root/shoot ratio, with stronger differentiation in the invasive species (Skálová et al. 2012).

Table 1. Characteristics describing the four localities for burial of *Impatiens* seeds. The climatic profiles for each locality were taken from Tolasz (2007).

Locality	Prevailing Habitat	GPS coordinates	Altitude [m a.s.l.]	Mean annual temperature [°C]	Mean annual precipitation [mm]	Average/maximum/minimum temperature in December – half/May [°C]	Average/maximum/minimum temperature in August–October [°C]	Average soil moisture [%]	Average soil N [%]	Average soil C [%]	Average soil C/N ratio
Čelina	mixed forest	49°43.87'N, 14°20.67'E	300	8	500	3.7/16.7/-4.0	13.2/31.5/1.5	49.2	0.31	3.95	13.06
Potštejn	alluvial forest	50°04.25'N, 16°19.42'E	340	7	650	4.3/16.6/0.1	13.8/34.0/0.7	49.7	0.15	1.92	12.46
Třebsín	mixed forest	49°51.57'N, 14°27.93'E	280	8	550	4.0/14.9/-1.7	13.0/33.6/0.5	50.9	0.80	13.54	15.62
Volyně	alluvial forest	49°08.50'N, 13°53.73'E	460	7	500	3.5/17.6/-5.0	13.4/22.5/0.5	63.1	0.57	6.83	12.01

Seed burial experiment

Seeds of *I. noli-tangere* and *I. parviflora* were collected in July 2008, and of *I. glandulifera* in August 2008. Four localities, that harboured all three *Impatiens* species and differed in temperature, precipitation and soil nutrients, were selected in the Czech Republic (Table 1) to investigate site-specific differences in the seed banks of the species (Čuda et al. 2014). Seeds from the same localities were used in previous experiments (Skálová et al. 2011, 2012, 2013), thus consistently including maternal effects (Roach and Wulff 1987, Galloway 2005). For each species, a mixed sample of seeds from at least 100 randomly chosen individuals across each locality was collected in the summer of 2008. In the localities Čelina, Třebsín and Volyně, at least 3,000 seeds per species were collected; in Potštejn only about 2,000 seeds of *I. noli-tangere* and *I. parviflora* were collected due to the low seed production. After collection, the seeds were dry stored at room temperature. Polyamide bags (fabric Norin, 6 × 6 cm) containing 50 seeds for each species were buried in late November 2008. In each locality only locally collected seeds were buried. As we found differentiation among species with *I. glandulifera* occurring in less shaded sites than *I. noli-tangere* and *I. parviflora* (Čuda et al. 2014), the bags were buried within the respective populations. In Čelina, Třebsín and Volyně, 54 bags for each species were buried, respectively (i.e. three replicates within each of two sites retrieved across nine time points), while in Potštejn 36 bags were buried due to the seed shortage (i.e. three replicates within each of two sites retrieved across six time points). The bags with seeds of individual species were joined to triplets (one bag per species) for each replicate, site and time point, and sewed to the end of an about 1 m long string; the second end of the string was used to make it easier to find the seed bags in the field and to enable their careful removal from the soil. In addition each triplet was equipped with an iron ring for proper localisation of

the bags using a metal detector to minimise soil disturbance during excavation. Along 3-m transects the bags were horizontally placed into the upper soil layer, at a depth of 5 cm (Suppl. material 1: Fig. S1). The turf was carefully removed and placed back after burial of the bags. The ends of each transect were marked by wooden and iron sticks. The seeds were exhumed in the following years in late March and late May (2009–2012), and in late March 2013.

After exhumation, the bags were washed and the seeds separated as (i) germinated, (ii) decayed in the soil (i.e. this category consisted of dead non-germinated seeds) or (iii–v) supposedly viable. A seed was considered germinated if at least the tip of the radicle was visible. The supposedly viable seeds were placed in Petri dishes filled with heat-sterilised river sand which was kept wet using tap water. The dishes were placed into a growth chamber with 12/12 day/night regime of 15/5 °C (this corresponds to the mean early-spring temperature in the Czech Republic); seed germination was checked every second day. Germinating seeds were considered as (iii) non-dormant, while seeds that did not germinate within one month were stained with tetrazolium to further differentiate between (iv) dormant and (v) dead seeds. Species- and site-specific dynamics of the soil seed bank were described as the total numbers of germinating seeds (i+iii) and that of dormant seeds (iv).

Environmental variables

Since seed bank depletion is influenced by water and temperature, and at least partly moderated by soil microbial activity (Schafer and Kotanen 2003), we measured soil moisture and temperature within 5 m of the burial sites. Temperature was measured using IBTN sensors (Thermochron Generic F5ROHS DS1921G-F5#) at the two sites per each of the four localities from December 2008 until the end of April 2009. The sensors were placed at the soil surface, covered with a thin layer of litter, and temperatures were recorded every 2 hours. Due to technical problems, we could not obtain the data from one temperature sensor in Potštejn. During both exhumations in 2009, we measured soil moisture using a moisture meter HH2 device with probe Theta Probe ML29 (Delta-T Devices, UK). The measurement was done at six places within 3 m around the burial sites. These values were averaged for each site.

Seed longevity and seed bank abundance are influenced by soil conditions (Pakeman et al. 2012, Abedi et al. 2014, Basto et al. 2015, Moravcová et al. 2018), and thus we included soil data from neighbouring plots collected during previous studies (Čuda et al. 2014). Total C and N were measured in soil samples taken close to the plots (i.e. up to 1 m) at each locality in 2008 and 2009, using Carlo Erba NC 2500 analyser. For the analyses, we included data for 1–3 plots that were up to 30 m from each of the burial sites, as well as those with similar environments. The soil samples were taken from the upper horizon (0–5 cm), which corresponds to the burial depth.

Data analysis

The depletion pattern of the soil seed-bank of *Impatiens* species was tested using generalised linear mixed-effect models with binomial error structure and logit link function. The proportion of germinating and dormant seeds in each bag were used as the response variables. The predictor variables (i.e. *Impatiens* species, year and month of exhumation, locality and site, and two-way interactions of these variables) were fixed effects within the models. Plot ID was used as a random factor to account for repeated measurements within the same plot over time (i.e. temporal pseudoreplications). The number of seeds in each bag ($n = 50$) was set as the “weights” argument within the `glmer` function and represented the number of trials used to generate each proportion (i.e. numbers of germinating or dormant seeds in each of the 50 bags). The year was treated as a continuous variable because we expected a gradual decrease in seed viability with time. We also examined the effect of environmental factors on *Impatiens* seed germination and dormancy from the first seed burial until the first exhumation in March 2009.

Due to the strong correlation between three measures of soil temperature (minimum, maximum and mean, Suppl. material 2: Fig. S2) we decided to use only minimum temperature that is most important for seed germination and dormancy, because frost may kill germinating seeds (Čuda et al. 2015). Similarly, soil carbon and nitrogen were highly correlated, and the latter variable was selected. Thus, we tested the effect of winter minimum temperature in the upper soil layer, soil nitrogen and soil moisture on seed germination and dormancy. Furthermore, since environmental data was only available at the site level, we used the means for seed germination and dormancy as response variables (i.e. average of three bags with 50 seeds per site).

Models were built separately for each species, and the square function of these variables was included to account for possible non-linear relationships. Locality was set as a random factor to reflect similarity within each locality (i.e. spatial pseudoreplication). Finally, we fitted separate models for each significant environmental variable, its square function and species (five models in total) to visualise the individual species-environment relationships. Maximal models were simplified by backward elimination of non-significant terms and retention of significant ones. The deletion of terms was validated step-by-step by comparing the significances between the original and the simplified models (Crawley 2013). The differences within significant terms were post-hoc tested by Tukey HSD pairwise comparison of estimated marginal means (Lenth 2018). Apart from R base packages, we used *lme4* for fitting generalised mixed-effect models by `glmer` function (Bates et al. 2015), and *emmeans* for subsequent multiple comparisons among significant terms (Lenth 2018). Package *effects* was used to calculate the effects of significant environmental variables in the models for germination and dormancy. Graphs were plotted using the packages *dplyr* (Wickham et al. 2018) and *ggplot2* (Wickham 2016). All computations were done in programme R 3.5.1 (R Core Team 2018).

Results

Seed germination

Germination was highest in the first spring after burial of the seed bags (i.e. March 2009) and differed significantly among the *Impatiens* species (Fig. 1, Table 2, Suppl. material 4: Table S1). During this time, germination was much higher for the invasive *I. glandulifera* and *I. parviflora*, with almost all seeds germinating (95%), compared to the native *I. noli-tangere*, where less than half of the seeds germinated (42%). Germination

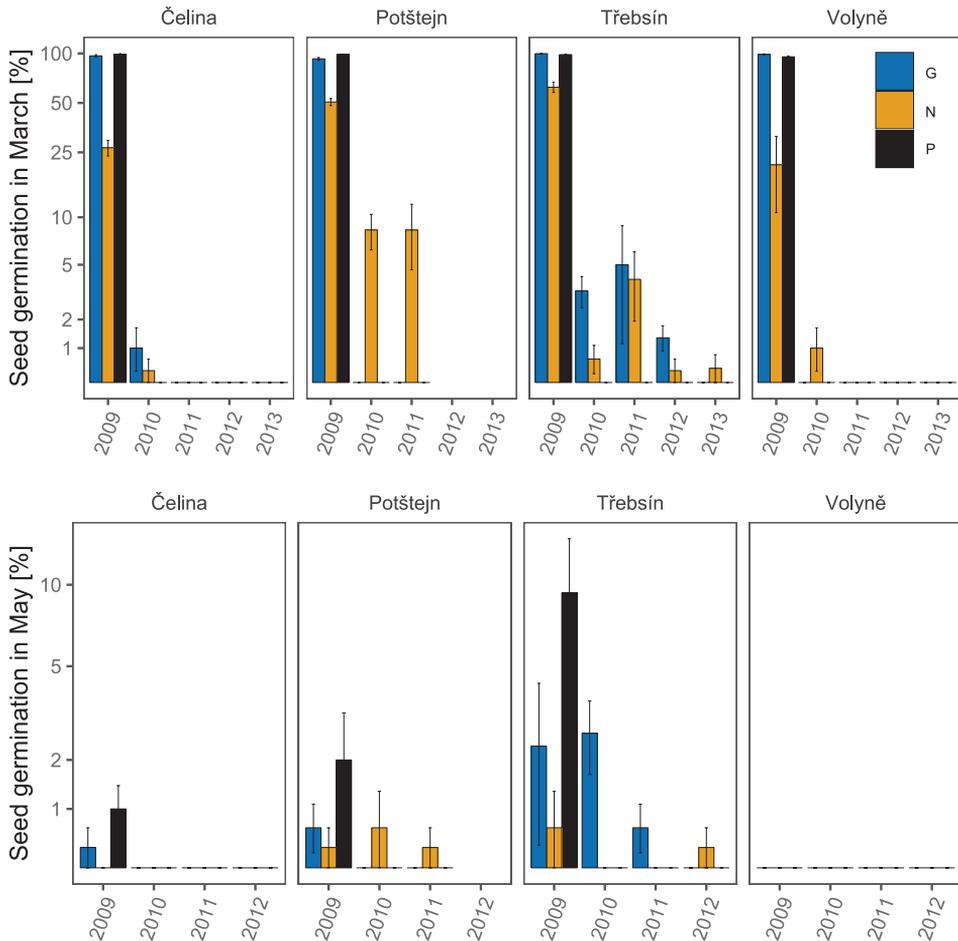


Figure 1. Proportions of germinated seeds in three *Impatiens* species across four localities over five years in March (A) and May (B). The bars depict the average for particular combinations; vertical lines represent the standard error of the mean and horizontal lines denote zero germination. Blue bars depict *I. glandulifera* (G), yellow *I. noli-tangere* (N) and those in black *I. parviflora* (P). Each bar is based on n = 6 bags comprising 50 seeds per bag (six bars is based on n = 4–5 due to lost samples). Note that response variable and error bars are displayed in a logarithmic scale. Seeds were only buried for three years at Potštejn due to seed shortage.

Table 2. The effect of selected factors and their two-way interactions on *Impatiens* germination. Summary of generalised linear mixed-effect model, where minimal adequate model is presented and significant values ($p < 0.05$) are in bold. Degrees of freedom, statistics of likelihood-ratio tests and p values are shown.

Factor	df	χ^2	P
Species	2	37.39	< 0.001
Year	1	746.28	< 0.001
Month	1	730.31	< 0.001
Locality	3	21.81	< 0.001
Site	1	1.05	0.161
Species \times year	2	173.19	< 0.001
Species \times locality	6	7.16	< 0.001
Year \times month	1	200.02	< 0.001
Year \times locality	3	15.40	< 0.001
Year \times site	1	3.81	0.012
Month \times locality	3	5.75	0.002
Month \times site	1	6.81	0.048
Locality \times site	3	8.02	< 0.001

ability dropped rapidly during May of the same year, and decreased further in the following years. Importantly, we found no germination of *I. parviflora* seeds following the first year of burial. For the other species, germination was higher in March than in May of the following years. The difference between these two months was most obvious in the first year, but still remained significant over time ($P < 0.001$, $F = 29.5$).

Germination also differed across localities (Fig. 1, Table 2). In Třebšín, a high percentage of seeds germinated and this was true for the first as well as the subsequent years. All species maintained their germinability for the longest duration at this locality. Seeds of *I. parviflora* were germinable for one year, *I. glandulifera* for four years, and the native *I. noli-tangere* for all five years. Importantly, *I. glandulifera* was germinable for more than one year only at this locality, and to a small extent in Čelina in the second year, while *I. noli-tangere* was germinable in all four localities, while the germination rate was very low in Čelina and Volyně where the seeds were germinable only for two years. Lastly, we found no significant effect of site, i.e. regardless of whether the seeds were buried under *I. glandulifera* stands or mixed stands of *I. noli-tangere* and *I. parviflora*.

Seed dormancy

The results for dormancy corresponded well with those for germination. We found significant effects of species, year of exhumation, and locality, as well as non-significant effects of site (Table 3). Unlike seed germination, there was no difference between the months of seed exhumation (i.e. March and May). We did not record any dormant seeds for *I. parviflora*, while we found dormant seeds for up to four years in *I. glandulifera* and *I. noli-tangere* (Fig. 2, Suppl. material 4: Table S1).

Dormancy decreased with time, however the decline between the first and second year was not as rapid as for germination. The sites varied considerably in dormancy and the pattern was similar for the results of germination after the first year. The most dor-

Table 3. The effect of selected factors and their two-way interactions on *Impatiens* seed dormancy. Summary of generalised linear mixed-effect model, where minimal adequate model is presented, significant values are in bold.

Factor	df	χ^2	P
Species	2	50.18	< 0.001
Year	1	84.40	< 0.001
Month	1	1.13	0.296
Locality	3	44.09	< 0.001
Site	1	0.50	0.471
Species × month	2	4.49	0.010
Species × locality	6	4.76	< 0.001
Year × site	1	5.08	0.024

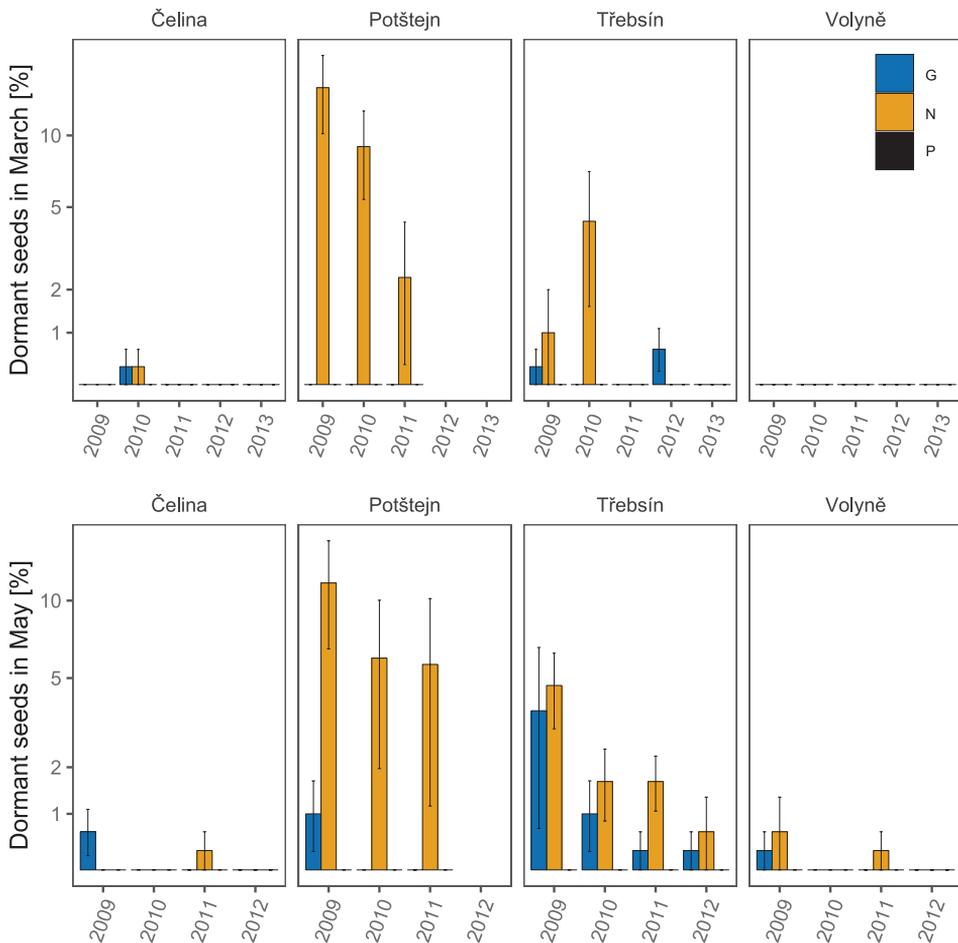


Figure 2. Proportions of dormant seeds in three *Impatiens* species across four localities over five years in March (A) and May (B). For details see Figure 1.

mant seeds over the four-year period for *I. glandulifera* were found in Třebšín, while, in the other localities, dormant seeds were only found up to the second year. *Impatiens noli-tangere* had the highest dormancy in Potštejn followed by Třebšín.

Environmental effects on seed banks

The effect of environmental factors on seed germination and dormancy in 2009 was most pronounced for the native *I. noli-tangere* (Fig. 3, Table 4). Seed germination and dormancy across sites increased with growing winter minimum temperature (see Suppl. material 3: Fig. S3 for detailed temperature course across localities). Seed germination exhibited a unimodal relationship with soil moisture, with the highest percentages recorded in moderately wet sites (i.e. soil moisture of 60%). The same relationship was recorded between seed dormancy and soil nitrogen concentration, with dormancy being low under intermediate nitrogen levels. In *I. glandulifera*, there was a slight positive effect of soil nutrient content on seed germination. However, we did not find any significant effect of environmental conditions on germination in *I. parviflora*.

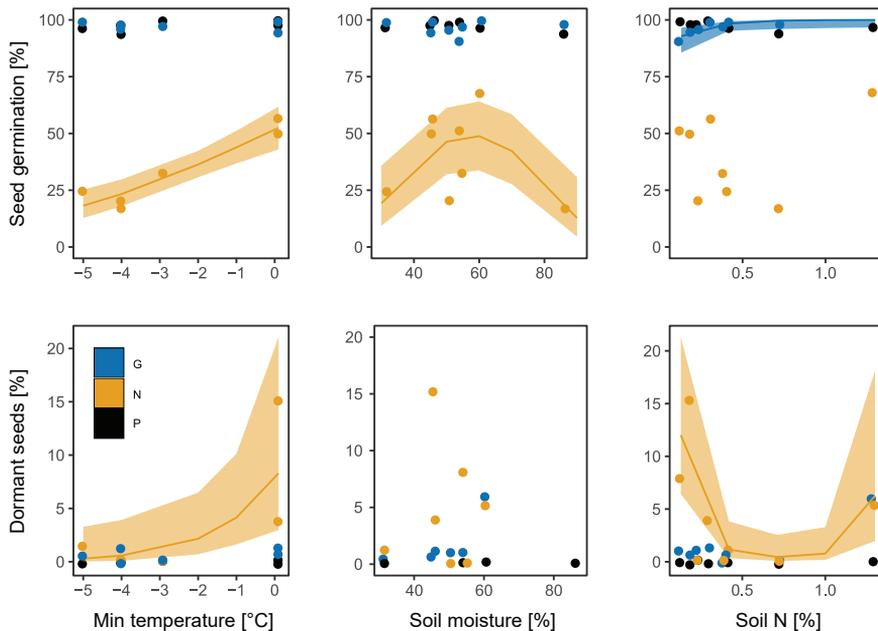


Figure 3. The effects of winter minimum temperature, soil moisture, and soil nitrogen concentration on seed germination in March 2009 and dormancy in May 2009. Blue circles depict *I. glandulifera* (G), yellow *I. noli-tangere* (N) and those in black *I. parviflora* (P). Lines in the same colours show a significant relationship fitted with generalised linear mixed effect models, shading indicates 95% confidence intervals (yellow in *I. noli-tangere* and blue in *I. glandulifera*). Models for each species are based on 8 replicates (i.e. 2 sites at each of the 4 localities). The mean of three replicates per locality and site were used for seed germination and dormancy.

Table 4. The effect of environmental factors and their square functions on *Impatiens* germination and dormancy for March 2009. The minimal models for each species are presented, terms that were excluded within the model simplification are marked with -, and significant values are in bold. Dormancy of *I. parviflora* seeds were not tested (n.t.) because there were no dormant seeds across all plots.

Species	Factor	df	Germination March		Dormancy May	
			χ^2	p	χ^2	p
<i>I. glandulifera</i>	winter minimum temperature	1	0.49	0.484	0.17	0.684
	soil moisture	1	0.48	0.491	0.22	0.638
	soil N	1	4.30	0.038	2.07	0.150
<i>I. parviflora</i>	winter minimum temperature	1	1.83	0.176	n.t.	n.t.
	soil moisture	1	1.59	0.208	n.t.	n.t.
	soil N	1	2.29	0.129	n.t.	n.t.
<i>I. noli-tangere</i>	winter minimum temperature	1	25.12	< 0.001	5.94	0.015
	soil moisture	1	15.70	< 0.001	1.07	0.300
	soil moisture ²	1	16.18	< 0.001	–	–
	soil N	1	1.53	0.215	9.54	0.002
	soil N ²	1	–	–	8.99	0.003

Discussion

Species- and site-specific germination

We found significant differences in seed germination between the native and invasive *Impatiens* species, as well as across the four localities. The high germination rate for the invasive species is in agreement with findings from a garden experiment (Perglová et al. 2009). On the other hand, the germination of the native *I. noli-tangere* was considerably higher in the four localities compared to the common garden (8%; Perglová et al. 2009). This indicates the key role of local conditions, and it also highlights the limitations of garden experiments. Our results also support previous findings which suggest that high germination success of alien invasive species, compared to their native congeners, promotes invasiveness (Pyšek and Richardson 2007).

Seed-bank dynamics and invasive species management

While seed persistence is suggested to be associated with invasiveness (e.g. Pyke 1990, Radford and Cousens 2000, van Clef and Stiles 2001, Gioria et al. 2012, Pyšek et al. 2015), we found that the most persistent seed bank was associated with the native *I. noli-tangere*. In our study system, this might indicate that early massive germination, that occurred shortly after seed burial (i.e. during early spring), of the two invasive *Impatiens* species may be another factor contributing to their invasion success, more than seed persistence (cf. Gioria et al. 2018), provided that the timing coincides with favourable environmental conditions.

In *I. parviflora* we recorded germination only in the first year after seed burial, while no dormant seeds persisted throughout the experiment. Consequently, due to

the absence of seeds after spring germination, as well as a modest root system (Slavík 1997), mechanical eradication could be an effective treatment for this species. Despite its weak impact on native vegetation (Hejda 2012, Diekmann et al. 2016, Florianová and Münzbergová 2017) and general recommendation for tolerance (Pergl et al. 2016), eradication is being applied in some areas, typically in communities with greater conservation values, such as thermophilous oak forests or steppes on rocks (Pyšek et al. 2012b, Florianová and Münzbergová 2017). These actions have been reported from Poland (Adamowski and Keczynski 1999), Hungary (Csontos 1986) and more recently also from protected areas of the Czech Republic (Pěkníková 2008). Transient soil seed banks (sensu Thompson et al. 1997), high sensitivity to frost (Skálová et al. 2011) and low competitive ability (Čuda et al. 2015) together represent a rather sensitive stage in population development that is probably partly compensated by a wide ecological amplitude (Pyšek et al. 2012c), high investment into reproduction (Skálová et al. 2013), high seed production compared to the native congener (Daumann 1967), and properly timed and synchronised germination (Skálová et al. 2011, Perglová et al. 2009).

The highly invasive *I. glandulifera* forms short-term persistent soil seed banks (sensu Thompson et al. 1997) at suitable localities. We found germinating and dormant seeds of *I. glandulifera* in the second year after seed burial at two of the four localities, while in Třebšín, germinating and dormant seeds were found still after four years. We are not aware of any data on the seed bank of *I. glandulifera* reported from the field conditions prior to our study. Until now, only indirect evidence was used to infer the seed-bank dynamics of this species, for example, the occurrence of seedlings after the removal of plants before fruiting. Beerling and Perrins (1993) observed seedlings for up to 18 months after plant removal; this indicates a two-year persistence of seed viability in this species. Even longer persistent seed banks were indicated by an eradication study with plants being found after four years (Saegesser et al. 2016). Our field experiment supports these indirect observations and also reveals that *I. glandulifera* seeds are able to survive in the soil much longer than previously thought. On the other hand, a four-year soil seed bank is the maximum survival period that was reached by a small proportion of seeds at one locality. Although the proportion of germinating seeds was very low following the first year of seed burial, the absolute number of seeds should be rather high due to abundant seed production (Daumann 1967, Beerling and Perrins 1993), a pattern similar to that found in *Heracleum mantegazzianum* (Moravcová et al. 2018). This suggests that in *I. glandulifera* the seed bank allows population recovery after disturbance. This includes damage to young plants by spring frost, which is highly probable due to early germination in that species (Perglová et al. 2009), and considerable frost sensitivity of the seedlings (Skálová et al. 2011). In addition, the seed bank must be taken into account during eradication actions, and sites should be monitored for at least four years following any control treatment. Nevertheless, systematic eradication has the potential to be very effective in small areas (Saegesser et al. 2016).

The native *I. noli-tangere* was characterised by low seed germination in the first year, but a higher proportion of germinating and dormant seeds in the following years

compared to its invasive congeners. This strategy will likely lead to better population persistence and recovering ability, which could, however, be limited by lower seed production (Daumann 1967). In all localities, the germinating seeds were found in the second year after burial and in one site even in the fifth year. Dormant seeds were found in all localities until the third year and in one locality in the fourth year. Thus, this study revealed that the native *I. noli-tangere* forms short-term persistent soil seed banks (sensu Thompson et al. 1997). Despite limited data on the comparison of seed banks of native and invasive species, similar trends, i.e. lower germination in the first year but longer soil seed-bank persistence of native species compared with non-native congeners, were observed by van Clef and Stiles (2001).

Soil environment and seed-bank persistence

The seeds of the native *I. noli-tangere* were most responsive to the soil environmental factors. For *I. glandulifera*, the effect was low and for *I. parviflora* we did not detect any effect. The significant effect of environmental conditions confirms that not only species-specific attributes, but also environmental factors, determine seed longevity in the soil (Abedi et al. 2014, Long et al. 2015). This holds true for the native and partly also for the two invasive species. Sub-zero temperatures strongly decreased seed germination and dormancy of *I. noli-tangere*, while no such effect was detected for the invasive species. This contradicts the results of an earlier study that found the invasive species to be more sensitive to frost (Skálová et al. 2011). Seeds of *I. noli-tangere* germinated better under intermediate soil moisture. This may be explained by increased fungal activity (Schafer and Kotanen 2003, 2004) and also anoxic conditions in very wet soils which disrupt metabolic processes and thus cause high seed mortality (Bekker et al. 1998). On the other hand, the low germination rate may be due to the negative response of this species to low soil moisture (Skálová et al. 2013). Seed germination of *I. glandulifera* was greater at sites with high soil nitrogen concentration. These field results are in accordance with previous laboratory experiments by Andrews et al. (2009) who observed a substantial increase in seed germination of *I. glandulifera* under increased nitrate availability. However, we cannot exclude the positive effect due to a higher seed mass that was observed under high soil nitrogen concentration for *I. parviflora* (Acharya et al. 2017), and a positive correlation between seed mass and germination capacity.

The absence of environmental effects may explain the wide ecological amplitude of *I. parviflora* that has been recorded from 45 of 88 habitat types in the Czech Republic (Pyšek et al. 2012c). The low effects of environmental factors together with a high performance under various conditions (Skálová et al. 2012, 2013), will likely facilitate the further spread of *I. glandulifera* into more habitat types (currently reported in 16 habitats); small populations were recently recorded outside traditional riverine habitats of this species (Čuda et al. 2017). The wide environmental tolerance of the two alien *Impatiens* species probably facilitates their invasion success.

Acknowledgements

Our thanks are due to Vendula Havlíčková, Šárka Dvořáčková, Zuzana Sixtová, and Michal Pyšek for technical assistance. We are grateful to Desika Moodley for the language revision of the final text. Our thanks are also due to the editors and reviewers, especially to Johannes Kollmann, who provided us with valuable suggestions that improved the manuscript. The study was supported by project grants GAČR 17-10280S, GAČR 19-20405S, long-term research development project RVO 67985939 (The Czech Academy of Sciences). PP was supported by EXPRO grant no. 19-28807X (Czech Science Foundation).

References

- Abedi M, Bartelheimer M, Poschlod P (2014) Effects of substrate type, moisture and its interactions on soil seed survival of three *Rumex* species. *Plant and Soil* 374: 485–495. <https://doi.org/10.1007/s11104-013-1903-x>
- Acharya KP, De Frenne P, Brunet J, Chabrerie O, Cousins SAO, Diekmann M, Hermy M, Kolb A, Lemke I, Plue J, Verheyen K, Graae BJ (2017) Latitudinal variation of life-history traits of an exotic and a native *Impatiens* species in Europe. *Acta Oecologica* 81: 40–47. <https://doi.org/10.1016/j.actao.2017.05.002>
- Adamowski W (2008) Balsams on the offensive: the role of planting in the invasion of *Impatiens* species. In: Tokarska-Guzik B, Brock JH, Brundu G, Child L, Daehler CC, Pyšek P (Eds) *Plant Invasions: Human Perception, Ecological Impacts and Management*. Backhuys Publishers, Leiden, 57–70.
- Adamowski W, Keczynski A (1999) Success of early eradication: the case of *Impatiens parviflora* in Białowieża National Park (NE Poland). In: Brundu G, Brock J, Camarda I, Child L, Wade M (Eds) *Proceedings of the 5th International Conference on the Ecology of Invasive Alien Plants*. Backhuys Publishers, La Maddalena, 3–5.
- Andrews M, Maule HG, Hodge S, Cherrill A, Raven A (2009) Seed dormancy, nitrogen nutrition and shade acclimation of *Impatiens glandulifera*: implications for successful invasion of deciduous woodland. *Plant Ecology and Diversity* 2: 145–153. <https://doi.org/10.1080/17550870903186256>
- Basto S, Thompson K, Rees M (2015) The effect of soil pH on persistence of seed of grassland species. *Plant Ecology* 216: 1163–1175. <https://doi.org/10.1007/s11258-015-0499-z>
- Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bebawi FF, Campbell SD, Mayer RJ (2015) Seed bank longevity and age to reproductive maturity of *Calotropis procera* (Aiton) WT Aiton in the dry tropics of northern Queensland. *Rangeland Journal* 37: 239–247. <https://doi.org/10.1071/RJ14130>
- Beerling DJ, Perrins DM (1993) Biological Flora of British Isles. *Impatiens glandulifera* Royle (*Impatiens Roylei* Walp.). *Journal of Ecology* 81: 367–381. <https://doi.org/10.2307/2261507>

- Bekker RM, Oomes MJM, Bakker JP (1998) The impact of groundwater level on soil seed bank survival. *Seed Science Research* 8: 399–404. <https://doi.org/10.1017/S0960258500004323>
- Coombe DE (1956) Biological Flora of British Isles. *Impatiens parviflora* DC. *Journal of Ecology* 44: 701–714. <https://doi.org/10.2307/2256857>
- Crawley MJ (2013) *The R Book-Second Edition*. Wiley. <https://doi.org/10.1007/s007690000247>
- Csontos P (1986) Dispersal and establishment of *Impatiens parviflora*, an introduced plant in a hardwood forest. *Abstracta Botanica* 10: 341–348.
- Čuda J, Skálová H, Janovský Z, Pyšek P (2014) Habitat requirements, short-term population dynamics and coexistence of native and invasive *Impatiens* species: a field study. *Biological Invasions* 16: 177–190. <https://doi.org/10.1007/s10530-013-0512-1>
- Čuda J, Skálová H, Janovský Z, Pyšek P (2015) Competition among native and invasive *Impatiens* species: the roles of environmental factors, population density and life stage. *AoB Plants* 7: plv033. <https://doi.org/10.1093/aobpla/plv033>
- Čuda J, Skálová H, Janovský Z, Pyšek P (2016) Juvenile biological traits of *Impatiens* species are more strongly associated with naturalization in temperate climate than their adult traits. *Perspectives in Plant Ecology, Evolution and Systematics* 20: 1–10. <https://doi.org/10.1016/j.ppees.2016.02.007>
- Čuda J, Rumlerová Z, Brůna J, Skálová H, Pyšek P (2017) Floods affect the abundance of invasive *Impatiens glandulifera* and its spread from river corridors? *Diversity and Distributions* 23: 342–354. <https://doi.org/10.1111/ddi.12524>
- Daumann E (1967) Zur Bestäubungs- und Verbreitungsökologie dreier *Impatiens*-Arten. *Preslia* 39: 43–58.
- Diekmann M, Effertz H, Baranowski M, Dupre C (2016) Weak effects on plant diversity of two invasive *Impatiens* species. *Plant Ecology* 217: 1503–1514. <https://doi.org/10.1007/s11258-016-0663-0>
- Fletcher CS, Westcott DA, Murphy HT, Grice AC, Clarkson JR (2015) Managing breaches of containment and eradication of invasive plant populations. *Journal of Applied Ecology* 52: 59–68. <https://doi.org/10.1111/1365-2664.12361>
- Florjanová A, Münzbergová Z (2017) Invasive *Impatiens parviflora* has negative impact on native vegetation in oak-hornbeam forests. *Flora* 226: 10–16. <https://doi.org/10.1016/j.flora.2016.11.006>
- Galloway LF (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist* 166: 93–99. <https://doi.org/10.1111/j.1469-8137.2004.01314.x>
- Geng YP, Pan XY, Xu CY (2007) Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. *Biological Invasions* 9: 245–256. <https://doi.org/10.1007/s10530-006-9029-1>
- Gioria M, Pyšek P (2017) Early bird catches the worm: germination as a critical step in plant invasion. *Biological Invasions* 19: 1055–1080. <https://doi.org/10.1007/s10530-016-1349-1>
- Gioria M, Dieterich B, Osborne BA (2011) Battle of the giants: primary and secondary invasions by large herbaceous species. *Biology and Environment* 111: 177–193. <https://doi.org/10.3318/BIOE.2011.14>

- Gioria M, Pyšek P, Moravcová L (2012) Soil seed banks in plant invasions: promoting species invasiveness and long-term impact on plant community dynamics. *Preslia* 84: 327–350.
- Gioria M, Pyšek P, Osborne BA (2018) Timing is everything: does early and late germination favor invasions by herbaceous alien plants? *Journal of Plant Ecology* 11: 4–16. <https://doi.org/10.1093/jpe/rtw105>
- Hatcher PE (2003) Biological Flora of British Isles. *Impatiens noli-tangere* L. *Journal of Ecology* 91: 147–167. <https://doi.org/10.1046/j.1365-2745.2003.00741.x>
- Hejda M (2012) What is the impact of *Impatiens parviflora* on diversity and composition of herbal layer communities of temperate forests? *PLOS One* 7: e39571. <https://doi.org/10.1371/journal.pone.0039571>
- Hesse E, Rees M, Mueller-Schaerer H (2007) Seed bank persistence of clonal weeds in contrasting habitats: implications for control. *Plant Ecology* 190: 233–243. <https://doi.org/10.1007/s11258-006-9203-7>
- Honig MA, Cowling RM, Richardson DM (1992) The invasive potential of Australian banksias in South African fynbos: a comparison of the reproductive potential of *Banksia ericifolia* and *Leucadendron laureolum*. *Australian Journal of Ecology* 17: 305–314. <https://doi.org/10.1111/j.1442-9993.1992.tb00812.x>
- Kollmann J, Bañuelos MJ (2004) Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Diversity and Distributions* 10: 377–385. <https://doi.org/10.1111/j.1366-9516.2004.00126.x>
- Leary J, Mahnken B, Wada C, Burnett K (2018) Interpreting life-history traits of miconia (*Miconia calvescens*) through management over space and time in the east Maui watershed, Hawaii (USA) *Invasive Plant Science and Management* 11: 191–200. <https://doi.org/10.1017/inp.2018.26>
- Lenth R (2018) Emmeans: Estimated marginal means, aka least-square means. R package version 1.2.1. <https://CRAN.R-project.org/package=emmeans>
- Long RL, Gorecki MJ, Renton M, Scott JK, Colville L, Goggin DE, Commander LE, Westcott DA, Cherry H, Finch-Savage EF (2015) The ecophysiology of seed persistence: mechanistic view of the journey to germination or demise. *Biological Reviews* 90: 31–59. <https://doi.org/10.1111/brv.12095>
- Minden V, Gorschlüter J (2016) Comparison of native and non-native *Impatiens* species across experimental light and nutrient gradients. *Plant Ecology and Evolution* 149: 59–72. <https://doi.org/10.5091/plecevo.2016.1118>
- Moravcová L, Pyšek P, Jarošík V, Pergl J (2015) Getting the right traits: reproductive and dispersal characteristics predict the invasiveness of herbaceous plant species. *PLOS One* 10: e0123634. <https://doi.org/10.1371/journal.pone.0123634>
- Moravcová L, Pyšek P, Jarošík V, Havlíčková V, Zákavský P (2010) Reproductive characteristics of neophytes in the Czech Republic: traits of invasive and non-invasive species. *Preslia* 82: 365–390.
- Moravcová L, Pyšek P, Krinke L, Müllerová J, Perglová I, Pergl J (2018) Long-term survival in soil of seed of the invasive herbaceous plant *Heracleum mantegazzianum*. *Preslia* 90: 225–234. <https://doi.org/10.23855/preslia.2018.225>

- Pakeman RJ, Small JL, Torvell L (2012) Edaphic factors influence the longevity of seeds in the soil. *Plant Ecology* 213: 57–65. <https://doi.org/10.1007/s11258-011-0006-0>
- Pahl AT, Kollmann J, Mayer A, Haider S (2013) No evidence for local adaptation in an invasive alien plant: field and greenhouse experiments tracing a colonization sequence. *Annals of Botany* 112: 1921–1930. <https://doi.org/10.1093/aob/mct246>
- Pěkníková J (2008) Netykavka malokvětá v porostech bučin v CHKO Kokořínsko (Ústí nad Labem): <http://www.npcs.cz/netykavka-malokveta-pod-pravcickou-branou>
- Pergl J, Sádlo J, Petrussek A, Laštůvka Z, Musil J, Perglová I, Šanda R, Šefrová H, Šíma J, Vohralík V, Pyšek P (2016) Black, grey and watch lists of alien species in the Czech Republic based on environmental impacts and management strategy. *NeoBiota* 28: 1–37. <https://doi.org/10.3897/neobiota.28.4824>
- Perglová I, Pergl J, Skálová H, Moravcová L, Jarošík V, Pyšek P (2009) Differences in germination and seedling establishment of alien and native *Impatiens* species. *Preslia* 81: 357–375.
- Perrins J, Fitter A, Williamson M (1993) Population biology and rates of invasion of three introduced *Impatiens* species in the British Isles. *Journal of Biogeography* 20: 33–44. <https://doi.org/10.2307/2845737>
- Phillips ML, Murray BR (2012) Invasiveness in exotic plant species is linked to high seed survival in the soil. *Evolutionary Ecology Research* 14: 83–94.
- Pyke DA (1990) Comparative demography of co-occurring introduced and native tussock grasses: persistence and potential expansion. *Oecologia* 82: 537–543. <https://doi.org/10.1007/BF00319798>
- Pyšek P, Richardson DM (2007) Traits associated with invasiveness in alien plants: Where do we stand? In: Nentwig W (Ed.) *Biol invasions*, Ecological Studies 193, Springer-Verlag, Berlin/Heidelberg, 97–126. https://doi.org/10.1007/978-3-540-36920-2_7
- Pyšek P, Chytrý M, Pergl J, Sádlo J, Wild J (2012b) Plant invasions in the Czech Republic: current state, introduction dynamics, invasive species and invaded habitats. *Preslia* 84: 575–629.
- Pyšek P, Jarošík V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M (2012a) 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. <https://doi.org/10.1111/j.1365-2486.2011.02636.x>
- Pyšek P, Manceur AM, Alba C, McGregor KF, Pergl J, Štajerová K, Chytrý M, Danihelka J, Kartesz J, Klimešová J, Lučanová M (2015) Naturalization of central European plants in North America: species traits, habitats, propagule pressure, residence time. *Ecology* 96: 762–774. <https://doi.org/10.1890/14-1005.1>
- Pyšek P, Danihelka J, Sádlo J, Chrtek Jr J, Chytrý M, Jarošík V, Kaplan Z, Krahulec F, Moravcová L, Pergl J, Štajerová K, Tichý L (2012c) Catalogue of alien plants of the Czech Republic (2nd edn): checklist update, taxonomic diversity and invasion patterns. *Preslia* 84: 155–255.
- R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>
- Radford IJ, Cousens RD (2000) Invasiveness and comparative life history traits of exotic and indigenous *Senecio* species in Australia. *Oecologia* 125: 531–542. <https://doi.org/10.1007/s004420000474>

- Rejmánek M, Richardson DM, Pyšek P (2005) Plant invasions and invasibility of plant communities. In: Van der Maarel E (Ed.) *Vegetation Ecology*. Blackwell, Oxford, 332–355.
- Roach DA, Wulff RD (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics* 18: 209–235. <https://doi.org/10.1146/annurev.es.18.110187.001233>
- Sádlo J, Chytrý M, Pyšek P (2007) Regional species pools of vascular plants in habitats of the Czech Republic. *Preslia* 79: 303–321.
- Saegesser J, Fischer D, Fischer K (2016) Long-term control of *Impatiens glandulifera* in a Swiss Forest: does zero-tolerance pay off? Biological invasions: interactions with environmental change. In: Ries C, Krippel Y (Eds) *Book of abstracts. Neobiota 2016 – 9th International Conference on Biological Invasions*. Fondation fauneflore, Vianden, 69.
- Schlaepfer DR, Glättli M, Fischer M, van Kleunen M (2010) A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species. *New Phytologist* 185: 1087–1099. <https://doi.org/10.1111/j.1469-8137.2009.03114.x>
- Schafer M, Kotanen PM (2003) The influence of soil moisture on losses of buried seeds to fungi. *Acta Oecologica* 24: 255–263. <https://doi.org/10.1016/j.actao.2003.09.001>
- Schafer M, Kotanen PM (2004) Impacts of naturally-occurring soil fungi on seeds of meadow plants. *Plant Ecology* 175: 19–35. <https://doi.org/10.1023/B:VEGE.0000048096.00772.23>
- Simberloff D, Martin JL, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M, Pyšek P (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology and Evolution* 28: 58–66. <https://doi.org/10.1016/j.tree.2012.07.013>
- Skálová H, Moravcová L, Pyšek P (2011) Germination dynamics and seedling frost resistance of invasive and native *Impatiens* species reflect local climatic conditions. *Perspectives in Plant Ecology, Evolution and Systematics* 13: 173–180. <https://doi.org/10.1016/j.ppees.2011.03.005>
- Skálová H, Havlíčková V, Pyšek P (2012) Seedling traits, plasticity and local differentiation as strategies of invasive species of *Impatiens* in central Europe. *Annals of Botany* 110: 1429–1438. <https://doi.org/10.1093/aob/mcr316>
- Skálová H, Jarošík V, Dvořáčková Š, Pyšek P (2013) Effect of intra- and interspecific competition on the performance of native and invasive species of *Impatiens* under varying levels of shade and moisture. *PLOS One* 8: e62842. <https://doi.org/10.1371/journal.pone.0062842>
- Slavík B (1997) *Impatiens* L. In: Slavík B, Chrtek Jr J, Tomšovic P (Eds) *Květena České republiky 5*. Academia, Praha, 230–240. [Flora of the Czech Republic]
- Thompson K, Bekker JP, Bekker RM (1997) *The soil seed bank of northwest Europe: methodology, density and longevity*. Cambridge University Press, Cambridge.
- Tolasz R, Míková T, Valeriánová A, Voženílek V (2007) *Climate Atlas of Czechia*. Czech Hydrometeorological Institute, Praha/Olomouc.
- Thuiller W, Albert C, Araujo MB, Berry PM, Cabeza M, Guisan A, Hickler T, Midgley GF, Paterson J, Schurr FM, Sykes MT, Zimmermann NE (2008) Predicting global change impacts on plant species distributions: future challenges. *Perspectives in Plant Ecology, Evolution and Systematics* 9: 137–152. <https://doi.org/10.1016/j.ppees.2007.09.004>

- Van Clef M, Stiles EW (2001) Seed longevity in three pairs of native and non-native congeners: assessing invasive potential. *Northeastern Naturalist* 8: 301–310. <https://doi.org/10.2307/3858486>
- Van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* 13: 235–245. <https://doi.org/10.1111/j.1461-0248.2009.01418.x>
- 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. <https://doi.org/10.1111/j.1461-0248.2011.01628.x>
- Wickham H (2016) *ggplot2: elegant graphics for data analysis*. Springer-Verlag, New York.
- Wickham H, François R, Henry L, Müller K (2018) *dplyr: a grammar of data manipulation*. R package version 0.7.6. <https://CRAN.R-project.org/package=dplyr>
- Wilsey BJ, Barber K, Martin LM (2015) Exotic grassland species have stronger priority effects than natives regardless of whether they are cultivated or wild genotypes. *New Phytologist* 205: 928–937. <https://doi.org/10.1111/nph.13028>

Supplementary material 1

Figure S1. Seed burial in the field

Authors: Hana Skálová, Lenka Moravcová, Jan Čuda, Petr Pyšek

Data type: multimedia

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/neobiota.50.34827.suppl1>

Supplementary material 2

Figure S2. Correlation matrix of selected environmental variables on seed germination in March 2009 and seed dormancy in May 2009

Authors: Hana Skálová, Lenka Moravcová, Jan Čuda, Petr Pyšek

Data type: statistical data

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/neobiota.50.34827.suppl2>

Supplementary material 3

Figure S3. Daily soil temperatures across the four localities since seed burial in November 2008 until April 2009

Authors: Hana Skálová, Lenka Moravcová, Jan Čuda, Petr Pyšek

Data type: multimedia

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/neobiota.50.34827.suppl3>

Supplementary material 4

Table S1. Changes in germination and dormancy percentage for three *Impatiens* species over time

Authors: Hana Skálová, Lenka Moravcová, Jan Čuda, Petr Pyšek

Data type: statistical data

Explanation note: Changes in germination and dormancy percentage (mean \pm standard error) for three *Impatiens* species (i.e. G = *I. glandulifera*, N = *I. noli-tangere* and P = *I. parviflora*) over time.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/neobiota.50.34827.suppl4>

Using verified citizen science as a tool for monitoring the European hornet (*Vespa crabro*) in the island of Sardinia (Italy)

Michelina Pusceddu¹, Ignazio Floris¹, Roberto Mannu¹,
Arturo Cocco¹, Alberto Satta¹

¹ Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università di Sassari, viale Italia 39, 07100 Sassari, Italy

Corresponding author: *Alberto Satta* (albsatta@uniss.it)

Academic editor: *Sven Bacher* | Received 21 June 2019 | Accepted 17 August 2019 | Published 2 October 2019

Citation: Pusceddu M, Floris I, Mannu R, Cocco A, Satta A (2019) Using verified citizen science as a tool for monitoring the European hornet (*Vespa crabro*) in the island of Sardinia (Italy). NeoBiota 50: 97–108. <https://doi.org/10.3897/neobiota.50.37587>

Abstract

The European hornet, *Vespa crabro* L. (Hymenoptera: Vespidae), is a eusocial insect native to Eurasia that was accidentally introduced in the island of Sardinia (Italy) in 2010. This alien generalist predator could impact on native insect species through predation or competition by modifying interspecific relations in sensitive island ecosystems. As part of the Interreg project ALIEM, the present work regarded the first monitoring activity of the European hornet conducted in Sardinia by means of verified citizen science. The main goals of this study were to define the distribution area of the wasp in 2018 and evaluate the profile and the performance of citizen scientists. Our results showed that *V. crabro* is mainly located in north-eastern Sardinia and that the hotspot was probably the commercial port of Olbia. Furthermore, data provided by contributors were very accurate and none of the parameters considered to define the participants (age, gender, education level, job category) was a true predictor of a volunteer's ability to recognise the wasp. In conclusion, this small-scale study suggests that citizen scientists could be a valuable aid to monitor already-established alien species and could be part of a surveillance network for early detection of other potentially invasive alien species not yet introduced in Sardinia, such as the yellow-legged hornet, *Vespa velutina*, which is already present in northern Italy.

Keywords

Alien species; biological invasion; data quality; European hornet; public information.

Introduction

The European hornet, *Vespa crabro* L. (Hymenoptera: Vespidae), is a social insect native to Eurasia (Carpenter and Kojima 1997), being distributed from Japan to the Iberian peninsula. Due to its predation ability, *V. crabro* was voluntarily introduced and successfully established in North America in the mid-nineteenth century as a predator of forest-damaging caterpillars (Shaw and Weidhaas 1956). This species was accidentally introduced to Central America in 2010, even though it does not seem to have established (Landolt et al. 2010). The European hornet constructs large paper nests mainly in hollow trees, but it is also able to colonise abandoned beehives, hollow walls and other building cavities (Matsuura and Yamane 1990). Several subspecies of the European hornet are distributed around the world. Amongst these, *V. crabro crabro* is widely spread in Italy (Carpenter and Kojima 1997; Dubatolov et al. 2003; Fernández 2004).

In Sardinia, the second largest island in the Mediterranean basin, the presence of *V. crabro* was first reported by Costa (1883), but specimens were not kept in any entomological collection and this report was not confirmed in the following years. In fact, no specimens of *V. crabro* from Sardinia were reported in the studies of Guiglia (1948, 1972) on Italian and European wasps. Subsequently, its absence in the island was noted in the checklist of the Italian fauna (Stoch 2003). Conversely, this species is present in other Mediterranean islands, such as Corsica and Sicily. The presence of *V. crabro* in Sardinia was ascertained only in August 2010, based on several findings of this species in Cannigione (municipality of Arzachena) (41°05'07"N, 9°25'52"E). The collected specimens were identified and stored in the entomological collections of the University of Sassari and University of Cagliari (Italy).

Surveillance and monitoring of alien species over large areas require a large amount of funding and personnel. Unfortunately, these resources are often scarce, thus resulting in a lack of information about current and potential invasions (Delaney et al. 2008; Crall et al. 2010). As a consequence, invasive species continue to spread throughout the world, resulting in economic and environmental damage (Mack et al. 2000; Pimentel et al. 2005; Stohlgren and Schnase 2006; Beggs et al. 2011; Lester and Beggs 2019).

Monitoring by “citizen scientists” can be an alternative means to solve this problem, because it requires fewer resources (Lodge et al. 2006). Although volunteer-based monitoring reduces research costs, the decrease in the accuracy of data collected by citizen scientists in comparison to those collected by few experts must be carefully considered (Ericsson and Wallin 1999; Genet and Sargent 2003). Nevertheless, the great sampling power derived by a high number of volunteers could offset the greater variability in collected data (Schmeller et al. 2009). Two types of citizen science methods have been recently proposed as follows: “*direct*”, without data verification and “*verified*”, in which all observations collected by citizen scientists are verified by “true researchers” (Gardiner et al. 2012). Previous studies showed that citizen scientists could identify large organisms very well (Bloniarz and Ryan 1996), whereas they often misidentified inconspicuous species (Mumby et al. 1995; Brandon et al. 2003; Genet and Sargent 2003). In general, insects are difficult to identify because of their small size

and great variation in morphological features, even within the same taxonomic group. Few studies have assessed the accuracy of the identification of insects made by citizen scientists (Gardiner et al. 2012; Ratnieks et al. 2016; Roy et al. 2016). Previous citizen science studies evaluated different parameters such as age, education, experience, science literacy and attitudes (Crall et al. 2011), as well as size of the species monitored (Delaney et al. 2008), as predictors of volunteer's ability to collect data. However, no studies have considered whether there are predictors of voluntary participation that could be used to facilitate the recruitment of citizens in citizen science programmes.

In this study, as a part of the Interreg ALIEM project "Action pour Limiter les risques de diffusion des espèces Introduites Envahissantes en Méditerranée" (<http://interreg-maritime.eu/web/aliem>), the monitoring of the European hornet was undertaken for the first time in Sardinia (Italy) in 2018. The project involves institutional partners from five border territories of France and Italy and aims to develop adequate tools for early detection of invasive pests and a cross-border observatory for flora and fauna. Considering that *V. crabro* is a large insect that can be easily noticed and that other similar hornet species are not present in Sardinia, we monitored its distribution in the island by using *verified* citizen science, as already done for other insect species in other regions of Italy (Mason et al. 2015). Our main goal was to determine the area of spread of the European hornet in Sardinia after its introduction in 2010. Furthermore, in order to better define the specific profile of the citizen scientist, we analysed the features of citizens (age, gender, education level and job) who joined our monitoring programme. Finally, this study assessed the general accuracy of the data collected by volunteers in relation to the features considered.

Materials and methods

Volunteer recruitment

The promotion of the monitoring and reporting activities was performed in 2017 with a publicity campaign conducted throughout Sardinia. The participants were recruited through the following channels: 1) advertisements posted online; 2) leaflets distributed in public meeting places (e.g. bars); 3) public seminars and meetings; 4) articles published in local newspapers and 5) informative documents sent via email to municipalities, research centres, environmental organisations and institutions located in Sardinia. Citizens were asked to report *V. crabro* nests and/or adults and were invited to place a home-made funnel-type trap in their own property (e.g. farm) to monitor *V. crabro* adults. The trap contained 33 centilitres of beer as bait and had to be checked by citizens every two weeks.

The protocol guidelines for citizens were disseminated online and on paper and consisted of the following activities: 1) supply of information about the project to raise public awareness of the importance of monitoring and to encourage participation in the programme; and 2) release of a guide and a form to be filled in with personal infor-

mation and European hornet report data to recruited participants. The identification guide outlined the main morphological characteristics of *V. crabro* compared to those of the German yellow jacket, *Vespula germanica*, the most common wasp in Sardinian environments and the yellow-legged hornet, *Vespa velutina*, not reported in the island so far, but already present in some Italian regions (Liguria and Piedmont). The latter species was included in the guide in order to create a prevention surveillance network because of its negative impact in the areas of new introduction (Choi et al. 2012).

Hornet data collection

All volunteers were asked to report nests or individuals of the European hornet found in 2018, by including information about *date*, *place* (geographic coordinates) and *habitat type* (urban or rural). Participants were also encouraged to provide other information, such as *environmental sub-category* (e.g. forest, agricultural, apiary, inside a building and garden) and *hornet behaviour* (e.g. in flight, predating bees or other preys and eating fruit). In order to verify the accuracy of the report, citizens were required to provide photos, videos or hornet samples as evidence. Alternatively, the citizen report was validated through site inspection by researchers.

Participant evaluation

Personal details of participants (i.e. age, gender, education level and job) were classified as follows: age *below* or *above* the median age of participants; *male* or *female*; *middle school*, *high school* or *university* education level; and, finally, *outdoor*, *office* or *institutional* job. The different job types were selected according to their likelihood of finding and/or reporting the presence of the European hornet. In particular, outdoor workers are likely to come into contact with local flora and/or fauna and thus detect and report the disturbance or damage caused by the European hornet. Office workers are less likely to be in contact with nature and recognise the presence of the hornet. Finally, institutional employees, who work for the government and might work indoors and/or outdoors (mixed category), have the common characteristic of being likely to report the presence of *V. crabro* due to a sense of duty towards citizens. In order to measure the accuracy of the data collected by volunteers, all reports were verified by researchers and analysed as a whole and within the categories chosen in this study. Citizens were also asked to specify the channel through which they became aware of the monitoring programme.

Statistical analyses

Differences in the frequency of correct and incorrect reports (i.e. data accuracy) were analysed separately for each predictor (age, gender, education level and job) using chi-

squared tests (two-way tests). A chi-squared test was also used to measure the proportional difference in identification accuracy between nests and single-individual reports. To reduce the chances of a type I error, continuity correction was used for the chi-squared test because the sample size was less than 200 (Sokal and Rohlf 1981). A binomial exact test was used to test for differences in the frequency of reports from urban and rural areas. All statistical analyses were conducted using R software (R Core Team 2018).

Results

From May to November 2018, the European hornet was reported 125 times, out of which 41 (32.8%) referred to nests and 84 (67.2%) to single adults. The number of correct reports was 101 (80.8%). *Vespa crabro* was found in a large area of north-eastern Sardinia covering approximately 6000 km² and corresponding to 20% of the total regional area. Correct records were collected between 40°21'44"N, 8°48'50"E (Fig. 1). All the incorrect reports ($n = 24$) were recorded outside this area. Most reports were concentrated in the surroundings of the commercial port of Olbia (Fig. 2). The total number of reports received from rural areas ($n = 67$) was significantly higher than that of urban areas ($n = 34$) (Binomial test, $P < 0.01$).

The accuracy of recognition was greater for hornet's nests than individuals (95.1% vs. 73.8%, respectively) ($\chi = 6.75$, $df = 1$, $P = 0.009$). The most common error made by citizen scientists was the misidentification of *V. crabro* with other species belonging to the genera *Scolia* and *Eumenus*. The report accuracy was not affected by the four selected predictors considered (age: $\chi = 0.322$, $df = 1$, $P = 0.569$, Fig. 2A; gender: $\chi = 0.007$, $df = 1$, $P = 0.930$, Fig. 2B; education level: $\chi = 0.667$, $df = 2$, $P = 0.716$, Fig. 2C; job category: $\chi = 1.616$, $df = 2$, $P = 0.445$, Fig. 2D).

The average profile of the citizen scientists who voluntarily participated in the monitoring programme can be summarised as follows: male (68% of reports), mean age 46.5 ± 0.9 years old, with an outdoor work (55% of cases) and high-school level of education (46%).

The most effective channel for spreading the information about the monitoring programme amongst citizens was *word of mouth* (from us or amongst citizens), with 56% of reports, followed by *online notices* (42.4% of cases) and, finally, *newspaper advertising*, with only 1.6% of reports.

Discussion

Based on the monitoring activity of *V. crabro* carried out in Sardinia during 2018 by a citizen science programme, the distribution of the European hornet is restricted to the north-east of the island. In addition, considering the location of the majority of reports, the hotspot for introduction of this wasp is likely associated with the Olbia sea-

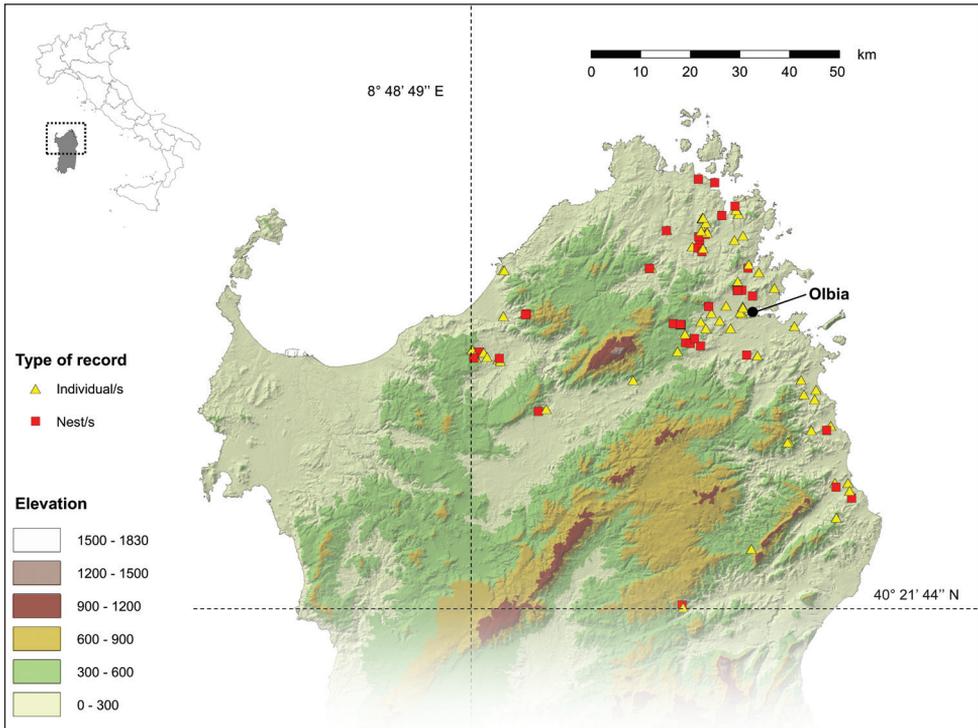


Figure 1. Distribution of the *Vespa crabro* in Sardinia (Italy).

port, which represents a point of continuous commercial traffic (e.g. timber transport) between Sardinia and the Italian peninsula and other European and extra-European countries. In fact, the introduction of potentially invasive species in new areas takes place mainly through human and goods traffic (Spradbery and Maywald 1992; Hulme 2009; Beggs et al. 2011; Lester and Beggs 2019). For example, fecundated queens could have been easily introduced through timber import (Beggs et al. 2011; Lester and Beggs 2019).

The present hornet distribution in Sardinia and the fact that *V. crabro* had not been confirmed before 2010 suggest that the report of Costa (1883) was probably incorrect. An alternative, but less probable, hypothesis could involve the extinction of the species in the island and its subsequent re-introduction. Future genetic studies could shed light on the introduction pattern of *V. crabro* in Sardinia.

In order to establish in a new region, the introduced queens have to find a nest, adapt to the new environment (e.g. climate), find food, resist new pathogens, escape predators and overcome native competitors (Lester and Beggs 2019). The high fertility of a single queen is one of the main characteristics that make social Vespidae a highly successful invasive species and difficult to eradicate all over the world (Beggs et al. 2011). Therefore, nest foundation is clearly a key moment in the adaptation process, which acts by selecting the most suitable characteristics to promote invader dispersal.

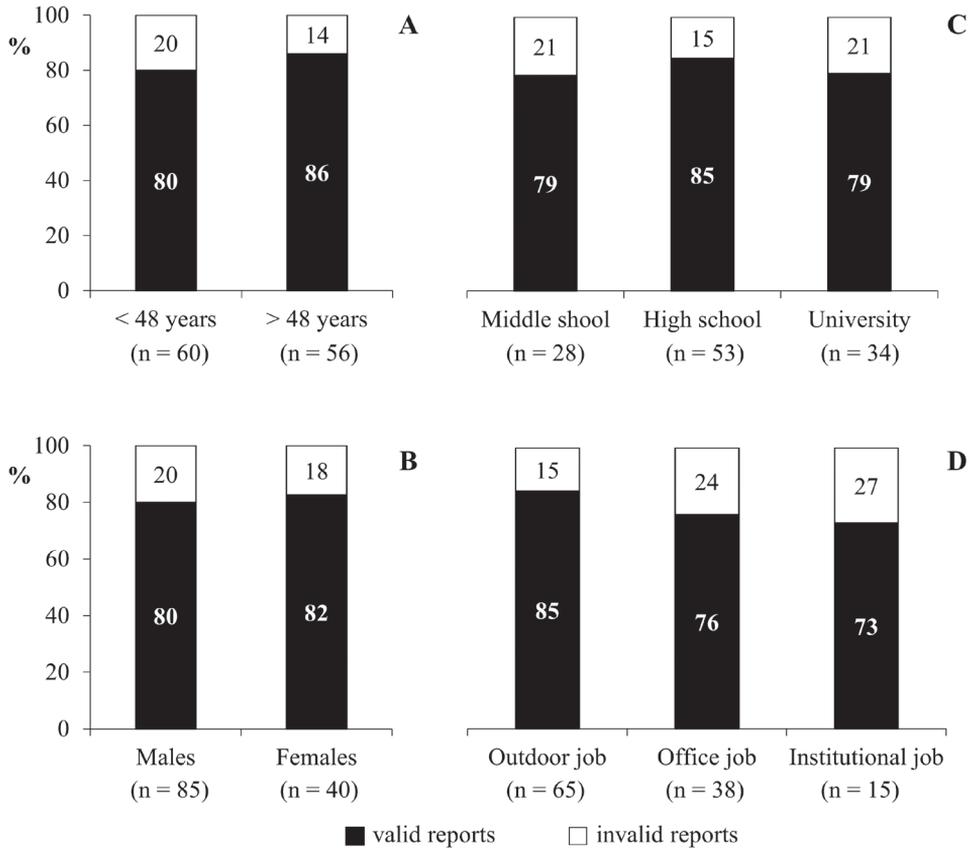


Figure 2. Percentage incidence of valid and invalid reports in: **A** citizens below and above the median age of participants (48 years) **B** male and female volunteers **C** participants with a middle school, high school and university education level **D** participants with an outdoor, office and institutional job. No significant differences were observed for any of the comparisons made.

The response of a species to a new environment can vary greatly and a non-harmful species in its native region can exhibit invasive behaviour in areas of new introduction (Monceau et al. 2015a). In fact, the introduction of alien generalist predators may impact the food web, by modifying the interspecific relationships through predation and/or competition (Snyder and Evans 2006; Bourdeau et al. 2011). This is the case for *V. germanica*, which is a harmless species to honeybees in its native region (Pusceddu et al. 2018), but becomes a threat in areas where it is introduced (Lester and Beggs 2019). Despite having a preference for bees as food source (Cini et al. 2018), *V. crabro* is known as a mild predator of *Apis mellifera* in Europe (Morse and Nowogrodzki 1990), being subjected to protection measures in Germany since 1987 (Monceau et al. 2015b). This probably depends on the long process of arms-race co-evolution that involves prey and predator (Baracchi et al. 2010; Pusceddu et al. 2017). However, specific studies on impacts of the European hornet as alien species are missing. In addi-

tion, the congeneric species *V. velutina* and *V. orientalis*, which share a similar seasonal phenology (Monceau et al. 2015b) and food preferences (Cini et al. 2018) with *V. crabro*, have not yet been reported in Sardinia.

The lack of native competitors, the favourable climate and the availability of nesting sites (e.g. ancient olive groves) could facilitate the spread of *V. crabro* throughout the island. This paper provides basic information on the spread of *V. crabro* in an introduced area, but further studies are needed to understand its expansion speed and its economic and ecological impact on sensitive insular ecosystems.

The highest number of reports of *V. crabro* in September and October (28 and 26, respectively) is in line with the seasonal biology of this species (Matsuura and Yamane 1991). In fact, nest foundation by a mated queen occurs in spring, with the colony growing from summer until the end of autumn, when new queens emerge, mate and overwinter, while males and the colony die (Matsuura and Yamane 1991). As a result, citizens become more aware of the presence of hornets when colonies are in full expansion. In addition, the potential impact of the European hornet on human activities partly explains the high interest of volunteers.

A higher number of reports was received from rural areas as compared with that received from urban areas. This is an interesting result considering that citizens' reports tend to arrive most frequently from the most populated areas. However, this result can be explained considering that the hornet shows a preference for nesting in the countryside (e.g. hollow trees) (Matsuura and Yamane 1991).

This pilot study highlights the fact that citizen scientists can be a valuable aid to integrate data on large areas (Lye et al. 2012; Roy et al. 2015). In fact, citizen identification was highly accurate (about 80%), which is considered as an acceptable accuracy percentage in ecological studies (Cohn 2008). Our results are even more encouraging, considering that citizen training was very limited. However, it is important to highlight that the high proportion of correct reports was also due to the absence of other hornet species, which could be misidentified with *V. crabro* by the citizens, in Sardinia. Despite this, participants were more accurate in identifying nests than individuals, as a consequence of misidentification of hornet adults with similar vespidae species. Therefore, the most common identification errors observed in the present study will be used to improve the future monitoring protocols by including *Scolia* spp. and *Eumenus* spp. pictures in the next identification guide.

The participation of volunteers from various areas of the island, including those where the hornet was not present, highlighted that the publicity campaign was effective throughout the whole island. Volunteers represent a large free work force and often have more access to private land compared to scientists, which is a major advantage in relation to the early detection of alien species (Lepczyk 2005), but their recruitment is sometimes difficult. Furthermore, volunteers are reluctant to perform routine procedures and observations repeated in time (Delaney et al. 2008), as found in our study, where only two out of 125 participants reported data from trap captures.

This paper also outlined the profile of the citizen willing to participate in the public monitoring of *V. crabro*. Participants were mainly middle-aged males with a medium

education level and working in contact with nature. This result was not influenced by the demographic trend of the Sardinian population, as the number of middle-aged males and females in Sardinia in 2018 was very similar (49.9 % vs 50.1 %) (http://dati.istat.it/Index.aspx?DataSetCode=DCIS_POPRES1#). It was also shown that volunteer success in identifying the European hornet did not depend on the parameters investigated in this study (age, gender, education level and job). Although no significant differences were observed amongst job categories, outdoor workers showed a higher proportion of valid reports, indicating that a better predictor for identification accuracy could be citizen occupation. This suggests that working in contact with nature provides citizens with a direct experience with the European hornet, thus facilitating its correct identification. Finally, despite the small scale of this study, our results suggest that the public monitoring network of *V. crabro* in Sardinia could play a key role in the protection of insular ecosystems and it could also represent a valuable early-detection tool for other alien vespid species, such as *V. velutina*, already present in northern Italy (Demichelis et al. 2014; Liyo et al. 2019).

Acknowledgements

The Interreg ALIEM project “Action pour Limiter les risques de diffusion des espèces Introduites Envahissantes en Méditerranée” (PC IFM 2014–2020) financially supported the present study.

The authors are also grateful to Ana Helena Dias Francesconi for revising the manuscript.

References

- Baracchi D, Cusseau G, Pradella D, Turillazzi S (2010) Defence reactions of *Apis mellifera ligustica* against attacks from the European hornet *Vespa crabro*. *Ethology Ecology & Evolution* 22: 281–294. <https://doi.org/10.1080/03949370.2010.502323>
- Beggs JR, Brockerhoff EG, Corley JC, Kenis M, Masciocchi M, Muller F, Rome Q, Villemant C (2011) Ecological effects and management of invasive alien Vespidae. *BioControl* 56: 505–526. <https://doi.org/10.1007/s10526-011-9389-z>
- Bloniarz DV, Ryan HDP (1996) The use of volunteer initiatives in conducting urban forest resource inventories. *Journal of Arboriculture* 22: 75–82.
- Bourdeau PE, Pangle KL, Peacor SD (2011) The invasive predator *Bythotrephes* induces changes in the vertical distribution of native copepods in Lake Michigan. *Biological Invasions* 13: 2533–2545. <https://doi.org/10.1007/s10530-011-0073-0>
- Brandon A, Spyreas G, Molano-Flores B, Carroll C, Ellis J (2003) Can volunteers provide reliable data for forest vegetation surveys? *Natural Areas Journal* 23: 254–262.
- Carpenter JM, Kojima J (1997) Checklist of the species in the subfamily Vespinae (Insecta: Hymenoptera: Vespidae). *Natural history bulletin of Ibaraki University* 1: 51–92.

- Choi MB, Martin SJ, Lee JW (2012) Distribution, spread, and impact of the invasive hornet *Vespa velutina* in South Korea. *Journal of Asia-Pacific Entomology* 15: 473–477. <https://doi.org/10.1016/j.aspen.2011.11.004>
- Cini A, Cappa F, Petrocelli I, Pepiciello I, Bortolotti L, Cervo R (2018) Competition between the native and the introduced hornets *Vespa crabro* and *Vespa velutina*: a comparison of potentially relevant life-history traits. *Ecological Entomology* 43: 351–362. <https://doi.org/10.1111/een.12507>
- Cohn JP (2008) Citizen science: can volunteers do real research? *BioScience* 58: 192–197. <https://doi.org/10.1641/B580303>
- Costa A (1883) Notizie ed osservazioni sulla Geo-Fauna sarda Memoria Seconda. Risultamento di ricerche fatte in Sardegna nella primavera del 1882. (Results of research conducted in Sardinia during spring in 1882 [in Italian] *Atti della Reale Accademia delle Scienze Fisiche e Matematiche* 1(2): 1–109.
- Crall AW, Newman GJ, Jarnevich CS, Stohlgren TJ, Waller DM, Graham J (2010) Improving and integrating data on invasive species collected by citizen scientists. *Biological Invasions* 12: 3419–3428. <https://doi.org/10.1007/s10530-010-9740-9>
- Crall AW, Newman GJ, Stohlgren TJ, Holfelder KA, Graham J, Waller DM (2011) Assessing citizen science data quality: an invasive species case study. *Conservation Letters* 4: 433–442. <https://doi.org/10.1111/j.1755-263X.2011.00196.x>
- Delaney DG, Sperling CD, Adams CS, Leung B (2008) Marine invasive species: validation of citizen science and implications for national monitoring networks. *Biological Invasions* 10: 117–128. <https://doi.org/10.1007/s10530-007-9114-0>
- Demichelis S, Manino A, Minuto G, Mariotti M, Porporato M (2014) Social wasp trapping in north west Italy: comparison of different bait-traps and first detection of *Vespa velutina*. *Bulletin of Insectology* 67: 307–317.
- Dubatolov V, Kojima J, Carpenter JM, Lvovsky A (2003) Subspecies of *Vespa crabro* in two different papers by Birula in 1925. *Entomological Science* 6: 215–216. <https://doi.org/10.1046/j.1343-8786.2003.00037.x>
- Ericsson G, Wallin K (1999) Hunter observations as an index of moose *Alces alces* population parameters. *Wildlife Biology* 5: 177–185. <https://doi.org/10.2981/wlb.1999.022>
- Fernández J (2004) Distribution of vespidae species in Europe. *Current Opinion in Allergy and Clinical Immunology* 4: 319–324. <https://doi.org/10.1097/01.all.0000136760.43571.f2>
- Gardiner MM, Allee LL, Brown PMJ, Losey JE, Roy HE, Smyth RR (2012) Lessons from lady beetles: accuracy of monitoring data from US and UK citizen-science programs. *Frontiers in Ecology and the Environment* 10: 471–476. <https://doi.org/10.1890/110185>
- Genet KS, Sargent LG (2003) Evaluation of methods and data quality from a volunteer-based amphibian call survey. *Wildlife Society Bulletin* 31: 703–714.
- Guiglia D (1948) Le Vespe d'Italia. *Memorie della Società Entomologica Italiana* 27(Suppl.): 5–84.
- Guiglia D (1972) Le guêpes sociales (Hymenoptera: Vespidae) d'Europe occidentale et septentrionale. *Faune de l'Europe et du Bassin Méditerranéen*, 6. Masson et C^{ie}, Paris.
- Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46: 10–18. <https://doi.org/10.1111/j.1365-2664.2008.01600.x>

- Landolt PJ, Sierra JM, Unruh TR, Zack RS (2010) A new species of *Vespula*, and first record of *Vespa crabro* L. (Hymenoptera: Vespidae) from Guatemala, Central America. *Zootaxa* 2629: 61–68. <https://doi.org/10.11646/zootaxa.2629.1.4>
- Lepczyk CA (2005) Integrating published data and citizen science to describe bird diversity across a landscape. *Journal of Applied Ecology* 42: 672–677. <https://doi.org/10.1111/j.1365-2664.2005.01059.x>
- Lester PJ, Beggs JR (2019) Invasion success and management strategies for social *Vespula* wasps. *Annual Review of Entomology* 64: 51–71. <https://doi.org/10.1146/annurev-ento-011118-111812>
- Lioy S, Manino A, Porporato M, Laurino D, Romano A, Capello M, Bertolino S (2019) Establishing surveillance areas for tackling the invasion of *Vespa velutina* in outbreaks and over the border of its expanding range. *NeoBiota* 46: 51–69 <https://doi.org/10.3897/neo-biota.46.33099>
- Lodge DM, Williams S, MacIsaac HJ, Hayes KR, Leung B, Reichard S, Mack RN, Moyle PB, Smith M, Andow DA, Carlton JT, McMichael A (2006) Biological invasions: recommendations for U.S. policy and management. *Ecological Applications* 16: 2035–2054. [https://doi.org/10.1890/1051-0761\(2006\)016\[2035:BIRFUP\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[2035:BIRFUP]2.0.CO;2)
- Lye GC, Osborne JL, Park KJ, Goulson D (2012) Using citizen science to monitor *Bombus* populations in the UK: nesting ecology and relative abundance in the urban environment. *Journal of Insect Conservation* 16: 697–707. <https://doi.org/10.1007/s10841-011-9450-3>
- Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689–710. [https://doi.org/10.1890/1051-0761\(2000\)010\[0689:BICEGC\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2)
- Mason F, Roversi PF, Audisio P, Bologna MA, Carpaneto GM, Antonini G, Mancini E, Sabbatini Peverieri G, Mosconi F, Solano E, Maurizi E, Maura M, Chiari S, Sabbatelli S, Bardiani M, Toni I, Redolfi De Zan L, Rossi De Gasperis S, Tini M, Cini A, Zauli A, Nigro G, Bottacci A, Hardersen S, Campanaro A (2015) Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). *Fragmenta Entomologica* 47: 51–52. <https://doi.org/10.4081/fe.2015.134>
- Matsuura M, Yamane S (1990) *Biology of the Vespine Wasps*. Springer Verlag, Berlin. <https://doi.org/10.1007/978-3-642-75230-8>
- Matsuura M, Yamane S (1991) *Vespa* and *Provespa*. In: Ross KG, Matthews RW (Eds) *The Social Biology of Wasps*. Cornell University Press, Ithaca, 232–262. <https://doi.org/10.7591/9781501718670-010>
- Monceau K, Moreau J, Poidatz J, Bonnard O, Thiéry D (2015a) Behavioral syndrome in a native and an invasive hymenoptera species. *Insect Science* 22: 541–548. <https://doi.org/10.1111/1744-7917.12140>
- Monceau K, Maher N, Bonnard O, Thiéry D (2015b) Evaluation of competition between a native and an invasive hornet species: do seasonal phenologies overlap? *Bulletin of Entomological Research* 105: 462–469. <https://doi.org/10.1017/S0007485315000280>
- Morse RA, Nowogrodzki R (1990) *Honey Bee Pests Predators and Diseases* (2nd edn). Cornell University Press, Ithaca.

- Mumby PJ, Harborne AR, Raines PS, Ridley JM (1995) A critical assessment of data derived from Coral Cay Conservation volunteers. *Bulletin of Marine Science* 56: 737–751.
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273–288. <https://doi.org/10.1016/j.ecolecon.2004.10.002>
- Pusceddu M, Floris I, Buffa F, Salaris E, Satta A (2017) Agonistic interactions between the honeybee (*Apis mellifera ligustica*) and the European wasp (*Vespula germanica*) reveal context-dependent defense strategies. *PLoS ONE* 12(7): e0180278. <https://doi.org/10.1371/journal.pone.0180278>
- Pusceddu M, Mura A, Floris I, Satta A (2018) Feeding strategies and intraspecific competition in German yellowjacket (*Vespula germanica*). *PLoS ONE* 13(10): e0206301. <https://doi.org/10.1371/journal.pone.0206301>
- R Core Team (2018) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. <http://www.R-project.org>
- Ratnieks FL, Schrell F, Sheppard RC, Brown E, Bristow OE, Garbuzov M (2016) Data reliability in citizen science: learning curve and the effects of training method, volunteer background and experience on identification accuracy of insects visiting ivy flowers. *Methods in Ecology and Evolution* 7:1226–1235. <https://doi.org/10.1111/2041-210X.12581>
- Roy HE, Baxter E, Saunders A, Pocock MJ (2016) Focal plant observations as a standardised method for pollinator monitoring: Opportunities and limitations for mass participation citizen science. *PloS ONE* 11(3): e0150794. <https://doi.org/10.1371/journal.pone.0150794>
- Roy HE, Rorke SL, Beckmann B, Booy O, Botham MS, Brown PM, Harrower C, Noble D, Sewell J, Walker K (2015) The contribution of volunteer recorders to our understanding of biological invasions. *Biological Journal of the Linnean Society* 115: 678–689. <https://doi.org/10.1111/bij.12518>
- Schmeller DS, Henry PY, Julliard R, Gruber B, Clobert J, Dziock F, Lengyel S, Nowicki P, Déri E, Budrys E, Kull T, Tali K, Bauch B, Settele J, Van Swaay C, Kobler A, Babij V, Papastergiadou E, Henle K (2009) Advantages of volunteer-based biodiversity monitoring in Europe. *Conservation Biology* 23: 307–316. <https://doi.org/10.1111/j.1523-1739.2008.01125.x>
- Shaw FR, Weidhaas Jr J (1956) Distribution and habits of the giant hornet in North America. *Journal of Economic Entomology* 49: 275 pp. <https://doi.org/10.1093/jee/49.2.275>
- Snyder WE, Evans EW (2006) Ecological effects of invasive arthropod generalist predators. *Annual Review of Ecology, Evolution, and Systematics* 37: 95–122. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110107>
- Sokal RR, Rohlf FJ (1981) *Biometry* (2nd edn). WH Freeman and Co., New York.
- Spradbery JP, Maywald GF (1992) The distribution of the European or German wasp, *Vespula germanica* (F.) (Hymenoptera: Vespidae), in Australia: past, present and future. *Australian Journal of Zoology* 40: 495–510. <https://doi.org/10.1071/ZO9920495>
- Stoch F (2003) Checklist of the species of the Italian fauna. <http://www.faunaitalia.it/checklist/> [Accessed 7 February 2019]
- Stohlgren TJ, Schnase JL (2006) Risk analysis for biological hazards: what we need to know about invasive species. *Risk Analysis* 26: 163–173. <https://doi.org/10.1111/j.1539-6924.2006.00707.x>

InvasiBES: Understanding and managing the impacts of Invasive alien species on Biodiversity and Ecosystem Services

Belinda Gallardo^{1,2}, Sven Bacher³, Bethany Bradley⁴, Francisco A. Comín¹, Laure Gallien⁵, Jonathan M. Jeschke^{6,7,8}, Cascade J. B. Sorte⁹, Montserrat Vilà¹⁰

1 Department of Biodiversity Conservation, Pyrenean Institute of Ecology (IPE-CSIC), Avda Montañana 1005, 50059 Zaragoza, Spain **2** Department of Zoology, University of Cambridge, Downing St. CB23EJ, Cambridge, UK **3** Department of Biology, University of Fribourg, Chemin du Musée 10, 1700 Fribourg, Switzerland **4** Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003 USA **5** LECA (Laboratoire d'Ecologie Alpine), University of Grenoble Alpes, University of Savoie Mont Blanc, CNRS, 38000 Grenoble, France **6** Freie Universität Berlin, Department of Biology, Chemistry, Pharmacy, Institute of Biology, Königin-Luise-Str. 1-3, 14195 Berlin, Germany **7** Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Germany **8** Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstr. 34, 14195 Berlin, Germany **9** Department of Ecology & Evolutionary Biology, University of California, Irvine, CA 92697 USA **10** Doñana Biological Station (EBD-CSIC), Avda Américo Vespucio 26, Isla de la Cartuja, 41092 Sevilla, España

Corresponding author: *Belinda Gallardo* (belinda@ipe.csic.es; galla82@hotmail.com; bg306@cam.ac.uk)

Academic editor: *Philip Hulme* | Received 7 May 2019 | Accepted 29 August 2019 | Published 14 October 2019

Citation: Gallardo B, Bacher S, Bradley B, Comín FA, Gallien L, Jeschke JM, Sorte CJB, Vilà M (2019) InvasiBES: Understanding and managing the impacts of Invasive alien species on Biodiversity and Ecosystem Services. *NeoBiota* 50: 109–122. <https://doi.org/10.3897/neobiota.50.35466>

Abstract

Invasive Alien Species (IAS) are amongst the most significant drivers of species extinction and ecosystem degradation, causing negative impacts on ecosystem services and human well-being. InvasiBES, a project funded by BiodivERsA-Belmont Forum for 2019–2021, will use data and models across scales, habitats and species to understand and anticipate the multi-faceted impacts of IAS and to provide tools for their management. Using Alien Species Narratives as reference, we will design future intervention scenarios focused on prevention, control and eradication of IAS in Europe and the United States, through a participatory process bringing together the expertise of scientists and stakeholders. We will also adapt current impact assessment protocols to assess both the detrimental and beneficial impacts of IAS on biodiversity and ecosystem services. This information will then be combined with maps of the potential distribution of Invasive Species of Interest in Europe under current and future climate-change scenarios. Likewise, we will

anticipate areas under risk of invasion by range-shifting plants of concern in the US. Finally, focusing on three local-scale studies that cover a range of habitats (freshwater, terrestrial and marine), invasive species (plants and animals) and ecosystem services (supporting, provisioning, regulating and cultural), we will use empirical field data to quantify the real-world impacts of IAS on biodiversity and ecosystem services and calculate indicators of ecosystem recovery after the invader is removed. Spatial planning tools (InVEST) will be used to evaluate the costs and benefits of species-specific intervention scenarios at the regional scale. Data, models and maps, developed throughout the project, will serve to build scenarios and models of biodiversity and ecosystem services that are relevant to underpin management of IAS at multiple scales.

Keywords

alien species, biodiversity, climate change, ecosystem services, management scenarios, Nature's Contribution to People, non-native species, participatory planning, risk assessment, species distribution models.

Introduction

Biological invasions are considered a direct driver of biodiversity loss and have pronounced negative impacts on supporting, provisioning, regulating and cultural services (Vilà and Hulme 2016). Both the numbers and distributions of invasive species are increasing in many parts of the world (Seebens et al. 2017), to the extent that the biogeographic distinctiveness of different regions is becoming blurred (Capinha et al. 2015). The costs of invasive species, currently estimated at €12.5 to 20 billion per year in Europe (Kettunen et al. 2008) and \$120 billion per year in the US (Pimentel et al. 2005), are likely underestimated and will escalate with time (Bradshaw et al. 2016). These costs mostly arise from economic loss in the agriculture, forestry, energy and health sectors, diminished delivery of ecosystem services and cost of controlling and eradicating unwanted species. Past research on biological invasions has mainly focused on the ecological factors determining the species success and distribution, treating ecosystem services only marginally and focusing on particular species, habitats or ecosystem functions, such as nutrient and water cycling. Moreover, current knowledge on the impacts of biological invasions on ecosystem services is strongly biased towards terrestrial habitats and services that have marketable values (agriculture yields, forestry production, human health), whereas aquatic habitats and non-marketable services are largely ignored (Vilà and Hulme 2016). By synthesising knowledge across habitats (terrestrial, freshwater and marine) and scales (continental to local), the project InvasiBES (<http://elabs.ebd.csic.es/web/invasibes>), funded through the 2017–2018 Joint BiodivERsA-Belmont Forum Call on “Biodiversity Scenarios and Ecosystem Services”, aims to provide a comprehensive understanding of the multi-faceted impacts of biological invasions on biodiversity and ecosystem services. Within this general framework, InvasiBES has identified a number of research needs associated with invasive species and ecosystem services.

The challenges posed by biological invasions in a global-change context have prompted a strong policy response at international and national levels (Turbelin et al. 2016). To support new regulations, researchers have developed standard protocols to systematically evaluate and prioritise impacts, including the *Environmental Impact Classification of Alien Taxa*-EICAT (Blackburn et al. 2014; Hawkins et al. 2015) for bi-

odiversity and the *Socio-Economic Impact Classification of Alien Taxa*-SEICAT (Bacher et al. 2017) for human well-being. Such tools are fundamental to evaluate the costs and benefits of plausible intervention scenarios to maintain ecosystem services. This is especially important in cases where invaders are perceived by society as having both positive and negative impacts, depending on the sector under consideration (e.g. invasive plants increase forage production but reduce pollination) or when management options are controversial due to ethical concerns (e.g. culling introduced animals) or for economic reasons (e.g. if cost-effectiveness of management actions is unclear, such as when the species is already widespread). InvasiBES will evaluate the beneficial and detrimental impacts of invasive species for an unbiased evaluation of the costs and benefits for society and ecosystems of intervention scenarios. This knowledge is fundamental for designing pro-active management plans that can effectively address the invasion threat.

Climate change introduces an additional challenge for management because species' ranges are shifting in response to warming temperatures (Walther et al. 2009). Climate change is expected to alter the vectors and pathways of invasion, enabling some species to expand into regions where they previously could not survive and reproduce (Dukes and Mooney 1999). Unprecedented arrivals of new colonisers, as well as range expansions of established invaders, are thus expected. Yet, which species, regions and ecosystem services will be most affected by climate change remains unknown. At the same time, climate change modelling provides a unique opportunity to identify areas under risk, thereby preventing and eradicating range-shifting species before they become widespread and problematic. The intervention scenarios envisioned by InvasiBES will consider the interactions between invasive species and climate change to ensure the most effective protection of biodiversity and ecosystem services in the context of global change.

Continental assessments of invasion risk are useful to guide trans-national policy development. However, the impacts caused by biological invasions on ecosystem services are strongly context-dependent, varying markedly between species and habitats (Kumschick et al. 2015). To support local-scale management, quantifying how ecosystem services differ in invaded *vs.* uninvaded sites across a range of systems is critical. Moreover, local-scale analysis provide a means to explore the degree of ecosystem recovery once the species has been removed, which is a key aspect to risk assessment that is difficult to determine. Focusing on three local-scale studies that cover a range of habitats (freshwater, terrestrial and coastal), invasive species (plants and animals) and ecosystem services (supporting, provisioning, regulating and cultural), InvasiBES will use field data to evaluate the real-world costs and benefits of IAS management.

Objectives

In the framework of the research needs identified above, the InvasiBES objectives are to:

- Develop intervention scenarios of invasive species management in Europe and the US. These intervention scenarios will be representations of possible futures that evaluate the effects of alternative management options and levels of policy implementation.

- Adapt and test impact assessment protocols to consider both the beneficial and detrimental effects of non-native species on biodiversity and ecosystem services.
- Evaluate current and future impacts of key invasive species of interest on biodiversity and ecosystem services in Europe and the US.
- Evaluate the effects of particular invasive species on biodiversity and ecosystem services at the local to regional scale and compare them with previous continental assessments.

Structure

Employing a multi-disciplinary combination of techniques, the objectives of InvasiBES are addressed in five interlinked work packages (Fig. 1).

WP 1. Participatory planning and evaluation of scenarios of invasive species and ecosystem services

The main objective of this work package (WP) is to develop future intervention scenarios of invasive species management together with other scientists and stakeholders through a process of Participatory Scenario Planning (PSP). This is an increasingly used tool that facilitates exploration of the future evolution of complex systems, thereby providing information for decision-making (Palomo et al. 2017). First, we will select a number of Invasive Species of Interest in Europe and the United States that will be the focus of research in the following WPs. In Europe, between 20 and 50 species will be selected to cover various habitats (freshwater, terrestrial, marine), stages of invasion (non-established through to widespread) and impacts (Minimal to Massive, positive and negative). The selection of species will accommodate the research needs of all WPs, which may focus on subsets of the species list, depending on the WP's objectives, data and resources availability. Priority will be given to species already included or expected to be included in the “List of IAS of EU concern”, a list of 49 (+18 candidates) plants and animals whose management has been prioritised by the European Union under Regulation 1143/2014. In the absence of similar official species listings in the US, we will first collate information about the impacts of invasive species on biodiversity and ecosystem services with EICAT and then select 100 high-risk plants and marine organisms.

Intervention scenarios will use as reference the Alien Species Narratives (ASNs), developed by AlienScenarios (<https://alien-scenarios.org/>, Essl et al. 2019), another BiodivERsA-Belmont Forum project closely related to InvasiBES. ASNs are qualitative narratives of plausible futures of global invasive species richness (Lenzner et al. 2019) that will serve as a framework to explore the impacts of different levels of policy implementation. For InvasiBES, we envision two extreme possible intervention situations: under a *worst-case* scenario, invasive species are not managed and, by 2050, they are able to fill their potential climate range modelled in WPs 3 and 4, fostered by increas-

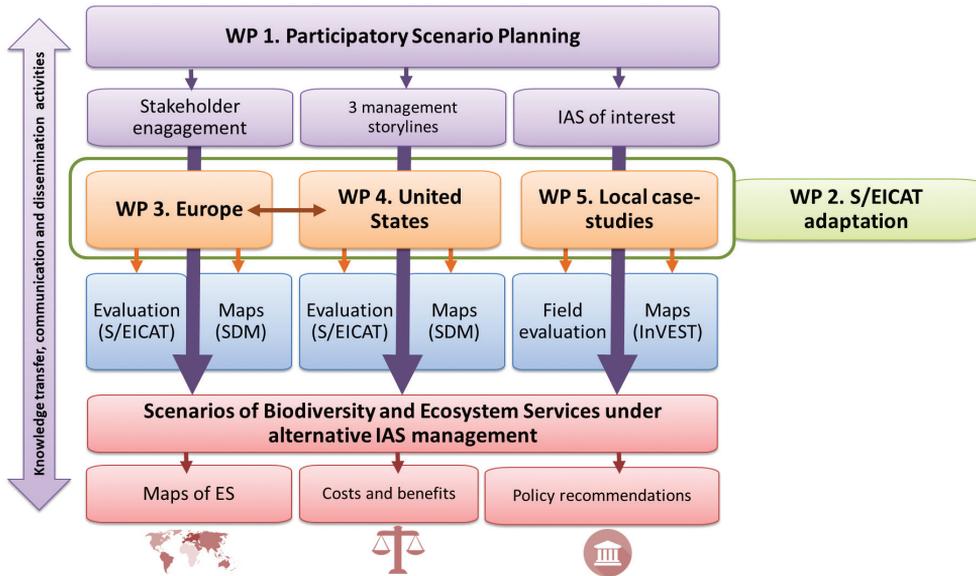


Figure 1. InvasiBES aims to use scenarios and models to understand and manage the impacts of Invasive Alien Species on biodiversity and ecosystem services. First, we will develop intervention scenarios with other scientists and stakeholders and select Invasive Species of Interest for the project (WP 1). Second, we will use standard protocols such as EICAT and SEICAT (S/EICAT) to evaluate the impacts of species on biodiversity and ecosystem services (WP 2). This information will then be combined with species distribution models (SDM) under current and future climate conditions in Europe and the US (WP3-4). Local-scale studies combining field data and scenario generation (through InVEST) will be used to evaluate the real-world costs and benefits of IAS management (WP5). Deliverables of the project include spatially-explicit assessments of the threat posed by invasive species to biodiversity and ecosystem services under a range of climate-change and intervention scenarios.

ing globalisation. Under a *best-case* scenario, international co-ordinated actions prevent the arrival of new invaders – through, for instance, border control and trade regulations – and existing invasive species are managed via eradication techniques and ecological restoration. In between, a number of scenarios can be drawn, ideally through a participatory process involving stakeholders with practical experience in environmental management and ecosystem restoration. In all cases, intervention scenarios will consider all stages of the invasion process and the cost-effectiveness, practicality and acceptability of alternative management options (following Booy et al. 2017).

WP 2. Evaluating the positive and negative impacts of invasive species on biodiversity and ecosystem services

The main objective of this WP is to adapt and validate impact assessment protocols considering both the detrimental and beneficial impacts of invasive species on biodiversity and ecosystem services. The impact assessment protocol, EICAT (Blackburn et al. 2014; Hawkins et al. 2015) – and its adaptation to include socio-economic im-

pacts, SEICAT (Bacher et al. 2017) – will be used to score impacts on biodiversity and ecosystem services of Invasive Species of Interest in five levels, from Minimal Concern to Massive. For example, provisioning ecosystem services are considered in SEICAT through impacts on “*Material and immaterial assets*” (e.g. agriculture, fisheries etc.), supporting ecosystem services are captured in EICAT mechanisms “*Chemical, physical or structural impact on ecosystems*” and cultural ecosystem services through SEICAT “*Social, spiritual and cultural relations*”. EICAT has the advantage that it has been adopted by the IUCN and, thus, our implementation at the continental scale can be used as a proof-of-concept for international adoption of the protocol.

Both EICAT and SEICAT (S/EICAT hereafter) classifications include estimations of uncertainty, but currently consider only detrimental impacts. In this project, we aim to adapt them to also quantify beneficial impacts (e.g. providing food for native species or humans, cultural values as recreational fishing and hunting) that are important to resolve management conflicts. We aim to measure positive impacts at a similar 5-point scale as detrimental impacts (e.g. by quantifying how much native species or human activities benefit from the presence of an invasive species), plus a 3-point scale uncertainty estimation (low-medium-high). Such consistency will facilitate the comparison of detrimental and beneficial impacts. We will test the adaptation using the Invasive Species of Interest selected in WP1 across a wide range of taxa and habitats. We will ensure that the selection of species for testing will include species with presumably detrimental and beneficial impacts.

WP 3. Modelling and mapping the impacts of invasive species on biodiversity and ecosystem services in Europe

Under the framework of this WP, we will model and map the potential impacts of Invasive Species of Interest on biodiversity and ecosystem services in Europe under current and future 2050 climate-change scenarios. Distribution modelling techniques already employed in Gallardo et al. (2017) will be used to correlate the presence of Invasive Species of Interest in Europe with the environmental conditions of their native and introduced range. We will use these models to predict the expansion of species under current and future 2050 scenarios, identifying regions of maximum concern because of their susceptibility towards invasion. SDMs will account for uncertainty in data availability through: i) bias-analyses of occurrence data; ii) modelling approach, through the use of ensemble models combining multiple modelling settings; and iii) climate change forecasts, by exploring multiple IPCC scenarios.

Potential impacts of invasive species will be calculated by integrating species-specific S/EICAT scores with maps of predicted distributions and ecosystem services supply. To that end, we will build on the approach used by Nentwig et al. (2010) to assess the ecological and economic impacts of invasive mammals in Europe. First, we will gather from literature the existing maps reflecting ecosystem services (e.g. pollination, leisure, water purification, all available at European scale through the Joint Research Centre, <https://data.jrc.ec.europa.eu/>) and/or target assets (e.g. human population density in the case

of invaders causing human health problems) that are directly affected by Invasive Species of Interest. Then we will combine distribution maps of species affecting a particular ecosystem service/asset to identify areas with high provision-high risk of invasion. As the impacts of invasive species are highly context-dependent, we will necessarily assume that the potential impacts of selected invaders are similar across Europe, a precautionary principle common in invasion biology. Local case studies (WP5) will better explore the spatio-temporal variability of impacts and allow comparing projections across scales.

Finally, we will use the Non-Native Risk Management (NNRM) scheme of Booy et al. (2017) to translate the general intervention scenarios developed in WP1 into species-specific scenarios. This scheme provides a structured evaluation of management options for current and future invasive species that, similar to S/EICAT, uses semi-quantitative responses and uncertainty scores to assess seven feasibility criteria: effectiveness, practicality, cost, impact, acceptability, opportunity and likelihood of re-invasion. We will finally compare the potential costs and benefits of alternative intervention scenarios in terms of biodiversity and ecosystem services.

Data, maps and models generated in this package will provide spatially-explicit estimations of the threats posed by IAS to biodiversity and ecosystem services in Europe and their potential evolution under a range of climate and management scenarios.

WP 4. Assessing and mapping the impacts of invasive species on biodiversity and ecosystem services in the US

In this WP, we will identify range-shifting invasive plants that have not yet been reported in parts of the US but are projected to expand with climate change. This will help anticipating the threat posed by invasive species to biodiversity and ecosystem services under alternative intervention scenarios. We will first develop a database synthesising all ecological, agricultural, economic and human health impacts reported in the scientific literature and use S/EICAT protocols to evaluate impacts. Then we will capitalise on available occurrence data to model the potential distribution of ca. 100 range-shifting invasive plants. This approach is focused on emergent threats, whereas established species with the potential to become invasive under climate change, often called sleeper species (Crooks 2005), are out of the scope of this project.

WP 5. Impacts of invasive species on biodiversity and ecosystem services at the local scale

This WP aims to advance the empirical understanding of invasive species impacts on biodiversity and ecosystem services and their context-dependency. At the local scale, a number of invaded and uninvaded sites across freshwater, terrestrial and marine habitats will be selected to measure impacts on biodiversity and ecosystem services (Table 1). In addition, physical removal experiments with minimal disturbance to non-target species will be used to follow changes in ecosystem properties after resto-

Table 1. Characteristics of local scale studies foreseen in WP5.

Habitat	Location	Targeted invasive species	Targeted ecosystem services	Control techniques
Freshwater	Lower Ebro River and delta, NE Spain	Freshwater invertebrates (<i>Pomacea</i> spp., <i>Callinectes sapidus</i>)	Supporting (biodiversity maintenance), regulating (water quality), provisioning (food provision) and cultural (aesthetic, recreation)	Physical removal Exclusion experiments
Terrestrial	Grasslands, SE France	Terrestrial plants (<i>Solidago gigantea</i> , <i>Impatiens glandulifera</i>)	Regulating (pollination, biodiversity maintenance), supporting (carbon storage) and provisioning (forage production)	Physical removal experiments
Marine	Marine habitats, California, USA	Marine invertebrates (<i>Watersipora subtorquata</i> , <i>Mexacanthina lugubris</i>)	Supporting (habitat quality and biodiversity maintenance), regulating (carbon sequestration and water quality), provisioning (food production) and cultural (aesthetic, recreation and environmental education)	Physical removal experiments

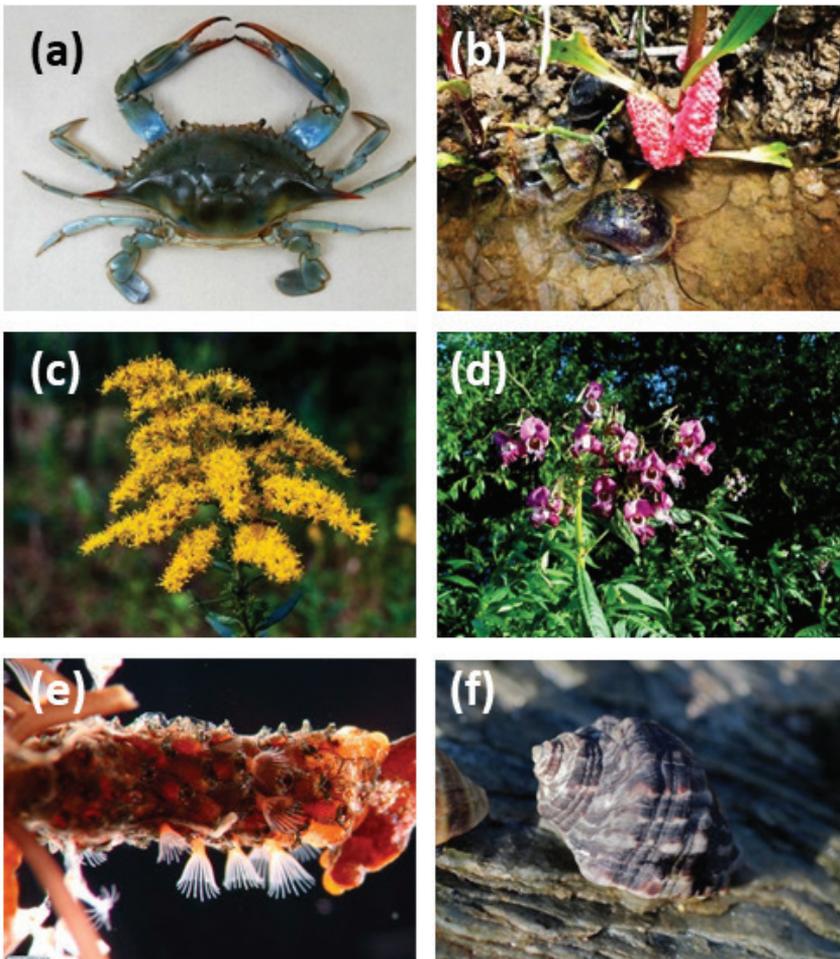


Figure 2. Focal invasive species that will be investigated during InvasiBES in three local case studies planned in the Ebro River (Spain) (a) the blue crab (*Callinectes sapidus*) and (b) apple snail (*Pomacea canaliculata*); the French Alps lowlands (c) the Canadian goldenrod (*Solidago canadensis*) and (d) Himalayan Balsam (*Impatiens glandulifera*); and in marine habitats of California (e) the red-rust bryozoan (*Watersipora subtorquata*) and (f) the dark unicorn sea snail (*Mexacanthina lugubris*). (All photos licensed through CC BY-SA 3.0).

ration. Our aim is to measure biodiversity and ecosystem services under the general assumption that restored sites will fall somewhere in between invaded and uninvaded treatments. This simple assumption has been rarely tested in the field, but plays an important role in differentiating major from massive impacts in S/EICAT protocols. Finally, we will use the software InVEST (<https://naturalcapitalproject.stanford.edu/invest/>) to spatially model the provision of ecosystem services, biodiversity and trade-offs at the local/regional scale (Nelson et al. 2009). All species sampled in WP5 will be modelled in WP3-4, evaluated with S/EICAT (WP2) and considered in the elaboration of management scenarios (WP1). In contrast to the continental evaluations of WPs 3 and 4, case studies will provide real on-the-ground information to test the accuracy of impact assessments like S/EICAT and to quantify the real costs and benefits of alternative management decisions. While case studies share a common work plan for the evaluation of impacts, the specific field methodologies differ depending on the habitat, invasive species and ecosystem services targeted in each case.

Case study 1-Freshwater ecosystems. We will focus on two recent invaders of the Ebro River and Delta (NE, Spain): the apple snail (*Pomacea canaliculata* Lamark, 1928) and the blue crab (*Callinectes sapidus* Rathbun, 1896). The apple snail, accidentally introduced in the area in 2010 (López et al. 2010), consumes vast amounts of rice and can devastate the flora and fauna of natural wetlands, with important impacts on food provisioning, nutrient cycling and primary productivity (EFSA Panel on Plant Health 2014). It is still spreading in the Ebro Delta despite the control and eradication measures implemented in rice paddies and the river channel (physical removal). The blue crab, also present in the Ebro Delta since 2013, is an omnivore able to feed on plants and animals, including apple snails, but it is also an important fishing resource with economic value. Therefore, this species has both negative (reduced biodiversity, changes in habitat structure) and positive (biological control, fishing resource) impacts on ecosystem services. Both species are being managed in parts of the lower Ebro River, which will facilitate the selection of invaded, uninvaded and restored plots under similar environmental and anthropogenic conditions. The work-plan includes 15 sampling stations per treatment (invaded, uninvaded and restored, total N = 45 per species) where we will determine the density and population structure of the invader. We will also take water, sediment and macroinvertebrate samples to calculate indicators of ecosystem services. The sampling campaign will be repeated at least twice (2019 and 2020) with the possibility of a third campaign in 2021. The impacts of the blue crab may be more difficult to assess because it is a highly mobile and territorial species. For this reason, we will consider using enclosures to further investigate changes in ecosystem services caused by the blue crab. With this information, we will finally employ the software InVEST to explore spatially how alternative intervention scenarios may affect ecosystem services at the local scale.

Case study 2-Terrestrial ecosystems. We will focus on two terrestrial plants that are highly invasive in semi-natural meadows and forest edges across Europe: the Canadian goldenrod (*Solidago canadensis* L.) and the Himalayan Balsam (*Impatiens glandulifera* Royle). The impacts on biodiversity and ecosystem services of these two invasive plants will be studied in detail in two nature reserves located in the French Alps

lowlands: the "Reserve du Bout du Lac d'Annecy" and the "Marais de Giez". These two species and two natural reserves are particularly interesting because of their joint conservation and agricultural values. From the conservation perspective, these nature reserves are protecting hyper-sensitive habitats that harbour rare and protected species, which are now threatened by the presence of mono-specific stands of the two plant invaders. From the agricultural perspective, the eradication of the invaders is highly contested by farmers and beekeepers of the region who benefit from the high quantity of pollen they produce. It is thus important to clarify the real positive and/or negative impacts of Canadian goldenrod and Himalayan balsam on regulation (plant diversity and pollination), supporting (carbon storage) and provisioning (forage production) services. These impacts will be quantified from field observations along a gradient of invasion: from non-invaded sites to gradually more invaded sites, up to near-mono-specific stands. Eradication of these plants is not feasible in protected areas with limited application of herbicides. For this reason, we will explore the possibility of conducting laboratory experiments for physical removal.

Case study 3-Marine ecosystems. The choice of marine study species will be based on the results of our literature review, in which we will look for studies reporting distributions, abundances and per capita effects of range-expanding species and the > 250 alien marine species reported in California (<https://www.wildlife.ca.gov/OSPR/Science/Cal-NEMO>). Target species will be chosen based on their current occurrence in southern California and lack of previous studies, despite having high potential for impacts based on their abundance, range size, expected strength of community interactions and functional relationships to other impacting species (Parker et al. 1999; Thomsen et al. 2014). Potential candidates for this study include sub-tidal epibenthic invertebrates in the "fouling" community (such as the bryozoan *Watersipora subtorquata* d'Orbigny, 1852) and consumers, including the intertidal, range-expanding whelk *Mexacanthina lugubris* (G. B. Sowerby I, 1822). We will quantify impacts using field observations across gradients in invader abundance, field physical removal experiments and lab experiments to resolve community interactions and ecosystem dynamics, such as effects on water quality. Sampling sites will be chosen to share similar environmental and disturbance conditions to avoid confounding factors. These marine invasive species are likely to play supporting (habitat quality and biodiversity maintenance), regulation (carbon sequestration and water quality), provisioning (food production) and cultural (aesthetic, recreation and environmental education) roles in local ecosystems.

Outlook

The project InvasiBES is designed to provide direct support to the implementation of national and international regulations of invasive species in Europe and the US, as well as to make progress towards accomplishing the targets of the Convention on Biological Diversity's Strategic Plan for Biodiversity 2011–2020 (Aichi Target #9: "By 2020

[...], priority species are controlled or eradicated and measures are in place to manage pathways to prevent their introduction and establishment”), the UN’s 2030 Agenda for Sustainable Development (Goal #15.8: “By 2020, introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species on land and water ecosystems and control or eradicate the priority species”) and the EU Regulation 1143/2014 on IAS that aims to establish rules to prevent, minimise and mitigate adverse effects of invasive species on biodiversity and related ecosystem services. In particular, *InvasiBES* will contribute towards accomplishing the needs of these international regulations through the development of intervention scenarios that evaluate the cost of inaction and the cost-effectiveness and socio-economic aspects of invasive species management (WP1). By considering multiple scenarios of climate change (WPs 3 and 4), the project will also provide insights into how different levels of commitment to the Paris Agreement on Climate Change may affect the expansion of invasive species and their impacts on biodiversity and ecosystem services. Furthermore, *InvasiBES* will prioritise species listed under EU regulation for analysis (WP3) and so European and member state policy-makers and practitioners are expected to be especially interested in the project and will be invited to engage in participatory scenario planning. In the US, we will collaborate with the Northeast Regional Invasive Species and Climate Change (RISCC, <https://people.umass.edu/riscc/>) Management Network and the North American Invasive Species Management Association (NAISMA, <https://www.naisma.org/>) to support the regulatory listing of high-priority species identified in this project (WP4). At national and local scales, *InvasiBES* will provide the best-available evidence and models to evaluate the costs and benefits of invasive species management (WP5), thereby helping to make decisions that are relevant for the conservation of biodiversity and ecosystem services. Ultimately, knowledge and data produced in the framework of this project will support the implementation of national and international policies, evaluate strategies and actions to improve management of invasive species, mitigate any potential negative effects and, ultimately, promote sustainability.

Acknowledgements

InvasiBES was funded through the 2017–2018 Belmont Forum and BiodivERsA joint call for research proposals, under the BiodivScen ERA-Net COFUND programme and with the following funding organisations: the Spanish Ministry of Science, Innovation and Universities (PCI2018-092986 and PCI2018-092939, MCIU/AEI/FEDER, UE), the Swiss National Science Foundation (31BD30_184114 and 31003A_179491), the US National Science Foundation (ICER-1852326), the German Federal Ministry of Education and Research BMBF (01LC1803A), and the French National Research Agency (ANR-18-EBI4-0001-06). BG is currently supported by the Biosecurity Initiative at St. Catherine’s, BioRISC (<http://www.biorisc.com>) and JMJ is additionally supported by the Deutsche Forschungsgemeinschaft DFG (JE 288/9-2).

References

- Bacher S, Blackburn TM, Essl F, Genovesi P, Heikkilä J, Jeschke JM, Jones G, Keller R, Kenis M, Kueffer C, Martinou A, Nentwig W, Pergl J, Pyšek P, Rabitsch W, Richardson DM, Roy HE, Wolf-Christian S, Scalera R, Vilà M, Wilson JRU, Kumschick S (2017) Socio-economic impact classification of alien taxa (SEICAT). *Methods in Ecology and Evolution* 9: 159–168. <https://doi.org/10.1111/2041-210X.12844>
- Blackburn TM, Essl F, Evans T, Hulme PE, Jeschke JM, Kühn I, Kumschick S, Marková Z, Mrugała A, Nentwig W, Pergl J, Pyšek P, Rabitsch W, Ricciardi A, Richardson DM, Sendek A, Vilà M, Wilson JRU, Winter M, Genovesi P, Bacher S (2014) A Unified Classification of Alien Species Based on the Magnitude of their Environmental Impacts. *PLoS Biol* 12: e1001850. <https://doi.org/10.1371/journal.pbio.1001850>
- Booy O, Mill AC, Roy HE, Hiley A, Moore N, Robertson P, Baker S, Brazier M, Bue M, Bullock R (2017) Risk management to prioritise the eradication of new and emerging invasive non-native species. *Biological Invasions* 19: 2401–2417. <https://doi.org/10.1007/s10530-017-1451-z>
- Bradshaw CJA, Leroy B, Bellard C, Roiz D, Albert C, Fournier A, Barbet-Massin M, Salles J-M, Simard F, Courchamp F (2016) Massive yet grossly underestimated global costs of invasive insects. *Nature Communications* 7: 12986. <https://doi.org/10.1038/ncomms12986>
- Capinha C, Essl F, Seebens H, Moser D, Pereira HM (2015) The dispersal of alien species redefines biogeography in the Anthropocene. *Science* 348: 1248–1251. <https://doi.org/10.1126/science.aaa8913>
- Crooks JA (2005) Lag times and exotic species: The ecology and management of biological invasions in slow-motion. *Ecoscience* 12: 316–329. <https://doi.org/10.2980/i1195-6860-12-3-316.1>
- Dukes JS, Mooney HA (1999) Does global change increase the success of biological invaders? *Trends in Ecology & Evolution* 14: 135–139. [https://doi.org/10.1016/S0169-5347\(98\)01554-7](https://doi.org/10.1016/S0169-5347(98)01554-7)
- EFSA Panel on Plant Health (2014) Scientific Opinion on the environmental risk assessment of the apple snail for the EU. *EFSA Journal* 12: 3641. <https://doi.org/10.2903/j.efsa.2014.3641>
- Essl F, Lenzner B, Courchamp F, Dullinger S, Jeschke JM, Kühn I, Leung B, Moser D, Roura-Pascual N, Seebens H (2019) Introducing AlienScenarios: a project to develop scenarios and models of biological invasions for the 21st century. *NeoBiota* 45: 1–17. <https://doi.org/10.3897/neobiota.45.33366>
- Gallardo B, Aldridge DC, González-Moreno P, Pergl J, Pizarro M, Pyšek P, Thuiller W, Yesson C, Vilà M (2017) Protected areas offer refuge from invasive species spreading under climate change. *Global Change Biology* 23: 5331–5343. <https://doi.org/10.1111/gcb.13798>
- Hawkins CL, Bacher S, Essl F, Hulme PE, Jeschke JM, Kühn I, Kumschick S, Nentwig W, Pergl J, Pyšek P (2015) Framework and guidelines for implementing the proposed IUCN Environmental Impact Classification for Alien Taxa (EICAT). *Diversity and Distributions* 21: 1360–1363. <https://doi.org/10.1111/ddi.12379>

- Kettunen M, Genovesi P, Gollasch S, Pagad S, Starfinger U, ten Brink P, Shine C (2008) Technical support to EU strategy on invasive species (IS) – assessment of the impacts of IS in Europe and the EU. Institute for European Environmental Policy (IEEP), Brussels, 40 pp.
- Kumschick S, Bacher S, Evans T, Markova Z, Pergl J, Pyšek P, Vaes-Petignat S, Veer G, Vilà M, Nentwig W (2015) Comparing impacts of alien plants and animals in Europe using a standard scoring system. *Journal of Applied Ecology* 52: 552–561. <https://doi.org/10.1111/1365-2664.12427>
- Lenzner B, Leclère D, Franklin O, Seebens H, Roura-Pascual N, Dullinger S, Essl F (2019) A framework for global 21st century scenarios and models of biological invasions. *Bioscience* biz070. <https://doi.org/10.1093/biosci/biz070>
- López M, Altaba C, Andree K, López V (2010) First invasion of the apple snail *Pomacea insularum* in Europe. *Tentacle* 18: 26–28.
- Nelson E, Mendoza G, Regetz J, Polasky S, Tallis H, Cameron D, Chan K, Daily GC, Goldstein J, Kareiva PM (2009) Modeling multiple ecosystem services, biodiversity conservation, commodity production, and tradeoffs at landscape scales. *Frontiers in Ecology and the Environment* 7: 4–11. <https://doi.org/10.1890/080023>
- Nentwig W, Kuhnel E, Bacher S (2010) A generic impact-scoring system applied to alien mammals in Europe. *Conservation Biology* 24: 302–311. <https://doi.org/10.1111/j.1523-1739.2009.01289.x>
- Palomo I, Múgica M, Piñeiro C, Martín-López B, Aauri J, Montes C (2017) Envisioning protected areas through participatory scenario planning: navigating coverage and effectiveness challenges ahead. *Parks* 23.1: 29–44. <https://doi.org/10.2305/IUCN.CH.2017.PARKS-23-1IIP.en>
- Parker IM, Simberloff D, Lonsdale W, Goodell K, Wonham M, Kareiva P, Williamson M, Von Holle B, Moyle P, Byers J (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* 1: 3–19. <https://doi.org/10.1023/A:1010034312781>
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273–288. <https://doi.org/10.1016/j.ecolecon.2004.10.002>
- Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jäger H, Kartesz J, Kenis M, Kreft H, Kühn I, Lenzner B, Liebhold A, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, van Kleunen M, Walker K, Weigelt P, Yamanaka T, Essl F (2017) No saturation in the accumulation of alien species worldwide. *Nature Communications* 8: 14435. <https://doi.org/10.1038/ncomms14435>
- Thomsen MS, Byers JE, Schiel DR, Bruno JF, Olden JD, Wernberg T, Silliman BR (2014) Impacts of marine invaders on biodiversity depend on trophic position and functional similarity. *Marine Ecology-Progress Series* 495: 39–47. <https://doi.org/10.3354/meps10566>

- Turbelin AJ, Malamud BD, Francis RA (2016) Mapping the global state of invasive alien species: patterns of invasion and policy responses. *Global Ecology and Biogeography* 26: 78–92. <https://doi.org/10.1111/geb.12517>
- Vilà M, Hulme PE (2016) *Impact of Biological Invasions on Ecosystem Services*. Springer. <https://doi.org/10.1007/978-3-319-45121-3>
- Walther G-R, Roques A, Hulme PE, Sykes MT, Pyšek P, Kühn I, Zobel M, Bacher S, Botta-Dukát Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarošík V, Kenis M, Klotz S, Minchin D, Moora M, Nentwig W, Ott J, Panov VE, Reineking B, Robinet C, Semchenko V, Solarz W, Thuiller W, Vilà M, Vohland K, Settele J (2009) Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution* 24: 686–693. <https://doi.org/10.1016/j.tree.2009.06.008>