REVIEW ARTICLE



Words matter: a systematic review of communication in non-native aquatic species literature

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Abstract

How scientists communicate can influence public viewpoints on invasive species. In the scientific literature, some invasion biologists adopt neutral language, while others use more loaded language, for example by emphasizing the devastating impacts of invasive species and outlining consequences for policy and practice. An evaluation of the use of language in the invasion biology literature does not exist, preventing us from understanding which frames are used and whether there are correlations between message framing in scientific papers and local environmental impacts associated with invasive species. Thus, we conducted a systematic literature review of 278 peer-reviewed articles published from 2008–2018 to understand communication styles adopted by social and natural scientists while reporting on aquatic non-native species research. Species-centered frames (45%) and human-centered frames (55%) were adopted to nearly equal degrees. Negative valence was dominant in that 81.3% of articles highlighted the negative risks and impacts of invasive species. Additionally, the use of terminology was found to broadly align with the stage of invasion, in that "invasive" was most commonly used except when the research was conducted at

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early stages of invasion, when "non-native" was most commonly used. Terminology use therefore enables readers of scientific papers to infer the status and severity of ongoing invasions. Given that science communication within the peer-reviewed literature affects public understanding of research outcomes, these findings provide an important point of reflection for researchers.

Keywords

invasive species, message framing, science communication, spatial analysis, terminology

Introduction

Biological invasions pose escalating threats to natural ecosystems, economies, and human well-being on a global scale (Pyšek et al. 2020), although impacts vary by taxon, ecosystem and region (Wolter and Röhr 2010). There is a longstanding debate in invasion science of how to appropriately communicate about invasive species so as to shape public understanding of the issue (Brown and Sax 2004, 2005; Cassey et al. 2005; Verbrugge et al. 2016; Clarke et al. 2020). Several papers (Larson et al. 2005; Janovsky and Larson 2019), have analyzed the use of militaristic language (i.e., referring to a "battle" or "war" against invasive species), which seeks to emphasize the urgency of responding to the risks of invasive species. Although not necessarily supporting militaristic language, several researchers agree that within published literature, scientists should advocate for the control of non-native species, even if it remains uncertain whether the species has negative impacts (Lodge and Shrader-Frechette 2003; Larson 2007). By contrast, other researchers believe objectivity is most important, and have asserted that value-laden terms such as "battle" introduce bias that diminishes trust in science (Lackey 2007; Keulartz and van der Weele 2008). Further, when management decisions associated with non-native species are reported in the popular press, reporters often present counterarguments (Kueffer and Larson 2014) that condemn such decisions, accusing them of being arbitrary and xenophobic (Comaroff and Comaroff 2010; Verbrugge et al. 2016; Sagoff 2017). This reporting outcome is problematic because it creates controversy after management decisions are implemented and erodes support for the scientific process. In short, the way scientific results are communicated strongly affects public understanding of research outcomes and is thus important to study (Nisbet and Scheufele 2009; Fischhoff 2013).

Investigations of language use in literature can yield insight into the reasons "why" different framings are used across the social and natural sciences. It is possible that loaded language, such as militaristic framing, is a response to the degree of risk associated with invasive species (Otieno et al. 2014), whereas less provocative scientific communication styles may be adopted when the likelihood of invasions is lower, or when a management approach shifts from eradication to resilience (Druschke et al. 2016). Another possibility is that scientists may adopt vivid language to engage and capture the attention of readers (Simberloff 2006), without considering potential consequences of their language use. Militaristic framing remains common in news coverage (Clarke et al. 2020), lending support to the idea that such vivid language is believed

to be appealing to the public. Evaluating the reasons why researchers across different fields of study communicate in specific ways highlights disciplinary norms of language use and the potential consequences that ensue from such word choices.

There are three fundamental facets of invasive species communication. First, scientific results – among all other forms of information – are interpreted through message frames (Nisbet and Mooney 2007). While framing underpins long-standing debates among invasion biologists over the merits of dramatic vs. less dramatic language, a comprehensive assessment of message framing related to aquatic non-native species has yet to be conducted. Message framing is defined as a phenomenon that occurs as people develop an understanding of a concept and communicate their interpretation (Chong and Druckman 2007). Although frames are often expressed and processed subconsciously, they can be intentionally invoked to make concepts comprehensible to a specific audience or to persuade people to change their behavior (Lakoff 2010). For example, framing of environmentalism has become particularly important to shape how information is exchanged because this topical area is increasingly politicized (Druckman 2017) and interpreted using incomplete knowledge and heuristics (Preston et al. 2015). Different opinions on the dangers of biological invasions and the role of scientists (Young and Larson 2011) have resulted in divergent message frames used in both academic literature and environmental outreach. For instance, narratives that position organisms as active agents of change are particularly adept at cultivating higher risk perceptions and greater willingness to take action (Hart and Larson 2014). Although past work has identified common frames used to discuss non-native species (e.g., Clarke et al. 2020), it has not quantified patterns in frame use and investigated the possible reasons why particular language is chosen.

A second fundamental facet of communication is valence – defined as the positive, neutral, or negative tone adopted – which is considered highly influential in shaping judgment and behavior (Russell 2003). Articles written with a positive valence may celebrate biodiversity brought about by new species (Keulartz and van der Weele 2008; Schlaepfer 2018) or highlight learning opportunities provided by non-native species (Larson 2010). Ostensibly neutral valences position humans as passive observers as nature takes its course (Kueffer and Larson 2014; Shackleton et al. 2019), while negative valences highlight the problems posed by invasive species and may frame them as being inherently "bad" and management efforts as "waging war" against biological invasions. Previous research on the effects of valence is mixed, in that positively positioned information has been more persuasive (Muchnik et al. 2013) and encouraged trustworthiness (Lim and Van Der Heide 2014), whereas negative comments have caused reactance or unpleasant motivational arousal (East et al. 2008). Further, repeated exposure to communication campaigns can lead to message fatigue, a negative response to the messages based on perceived overexposure, redundancy, tedium, and a feeling of being burned out (So et al. 2017). The risk of message fatigue can be mitigated by using messages that take a more positive approach (Guan and Monahan 2017). However, there are competing arguments that negative information is more memorable (Baumeister 2001) and helps contribute to higher risk perceptions (Otieno et al. 2014). Although there are divergent opinions among scientists on whether it is their role to advocate for particular management outcomes (Young and Larson 2011), the way scientists communicate, even if opting to be as objective as possible, influences public understanding of research results (Nisbet and Scheufele 2009; Fischhoff 2013). Thus, considering how valence is used in peer-reviewed literature is an important point for research and reflection.

Lastly, terminology and the associated definitions of key concepts are central to non-native species communication. Debate among scientists regarding the precise uses of various terms, including "invasive," has been ongoing for decades (Colautti and MacIsaac 2004; Copp et al. 2005; Blackburn et al. 2011). For instance, many terms are used to describe a species that exists outside of the region in which it evolved. These terms include non-native, foreign, nonindigenous, alien, invasive, and exotic. Some of these terms are technically incorrect and others can easily be misinterpreted, thus impeding collaboration among scientists and stakeholder understanding of invasive species prevention and management (Richardson et al. 2000). Invasion science is generally replete with value-laden differences in communication strategies (Kapitza et al. 2019), and consistency in the conceptualization of key terms will increase the likelihood that all relevant perspectives are considered, mutual acceptability is increased, and misunderstandings are avoided (Colautti and Richardson 2009; Iannone et al. 2021).

Conceptual model that guided this study

Messaging frames, valence and terminology used in the invasion science literature may be influenced by a variety of factors (Fig. 1). Included among these factors are: (1) the disciplinary approach, (2) the study focus, (3) the stage of invasion describing the study population, (4) the transportation vector addressed, and (5) the biodiversity context in which the study is based. Empirical insights into the relationships across these characteristics will illuminate the underlying reasons why different communication strategies are used throughout the aquatic invasive species literature.

Characteristics of authors conducting and publishing research on non-native species may also influence the frameworks adopted, and, in turn, their strategy for communicating scientific results. Indeed, previous research has indicated that communication is influenced by the professional background of scientists and worldviews that emerge from different disciplines (Hakkarainen et al. 2020). For instance, the use of militaristic frames in studies of invasive species was shown to be absent among coastal restoration managers because their management goals did not include eradication (Druschke et al. 2016). Another study assessed the use of militaristic language in work with invasive species across several influential journals and found that applied journals tended to use less militaristic language than basic science journals (Janovsky and Larson 2019). These professional backgrounds, including disciplinary approaches adopted in the study, may translate into different communication strategies.

The objectives or goals of a scientific article, referred to in this paper as "study focus," can also affect its communication style. Previous research on non-native species



Figure 1. Illustration of relationships explored in this study, including five explanatory variables (i.e., study discipline, study focus, stages of invasion, transportation vector, and biodiversity context) that influenced three facets of invasive species communication (i.e., message frame, valence, terminology).

has been motivated by a variety of concerns that can be categorized into four areas of inquiry. First, many studies have sought to assess the risk of invasive species transport or determine the most effective prevention methods (Byers et al. 2013; Davidson et al. 2016; June-Wells et al. 2013). Second, researchers have monitored and detected aquatic invasive species through a variety of research methods, including environmental DNA (eDNA), citizen science, and remote sensing (Larson et al. 2020), with eDNA studies increasing in popularity (Rees et al. 2014; Klymus et al. 2017). Third, researchers have expressed a goal of understanding non-native species, including their relationships with other species and impacts on ecosystems (Lawrence et al. 2014). Finally, the extant literature has determined the effectiveness and suitability of management or control strategies (Sembera et al. 2018). These key goals in scholarship have indicated that study focus is often closely linked to the stage of invasion most relevant to the study. For instance, studies focused on assessing the risk of invasion or evaluating prevention techniques are typically undertaken in response to a population of non-native species at the transport stage of invasion. In contrast, researchers tend to embark on studies evaluating control options for non-native species when a population is at the establishment or spread stage of invasion. Consequently, communication style adopted by an article reporting research results may be related to the research focus.

Previous research has underscored the importance of recognizing stages of invasion to unify approaches to understanding invasions and the ways they are discussed (Blackburn et al. 2011). Researchers have argued for bridging language gaps between disciplines and standardizing language use across stage of invasion (Colautti and MacIsaac 2004). Each population of a species can be classified as existing along a gradient from "transportation" to "spread", with designated terminology to be used at each stage (Robinson et al. 2016). At the "transportation" stage of invasion, whereby species move to a new location, the neutral term "non-native" is most appropriate, given the uncertainty of the species survival and impacts. The terms "introduced" and "established" directly correspond to the second and third stages of invasion: "introduction", involving the arrival and release of species in a new location, and "establishment" when the introduced species survives and reproduces. Finally, when species "spread" aggressively beyond their established range or begin causing negative ecological or economic impacts, they are dubbed "invasive" (Lockwood et al. 2013). These terms and stages are tied to particular locations; for instance, a species may be at the "introduced" stage in one lake, while in a different lake, a different population of the same species is at the "spread" stage. Thus, language use may be related to differences in the abundance of species at each stage of invasion across a region.

Transportation vectors, defined as the mechanism by which species are carried along a pathway, may affect the way that researchers communicate about non-native species in the literature. For instance, intentional vectors, such as biocontrol, fish stocking (Gozlan 2008), and the aquarium trade (Padilla and Williams 2004), may result in more positively valanced language given the benefits of introducing these species (Carey et al. 2011). By contrast, unintentional vectors, such as ballast water (Bailey 2015) and recreational equipment (Clarke Murray et al. 2011) may result in more negatively valenced language that highlights the need for humans to be aware of their unintentional impacts (Lauber et al. 2020).

Finally, scientists develop their communication styles in the specific social and ecological environment in which their study sites and own experiences are situated. There is spatial variation in the fraction of local species richness from non-native species, the degree of impacts attributable to these organisms and the corresponding policy efforts. Researchers are personally exposed to variation in the strength and impacts of nonnative species, which may affect their language in scientific studies. Specifically, the use of strong language may be a response to the degree of risk associated with invasive species in the region given the relationship between risk perceptions and message framing (Van't Riet et al. 2016). Whereas concerns about objectivity may be less pressing when risks are higher, it may be easier to adopt a less alarming viewpoint and communication style when a researcher works in a context with lower risk. As such, an argument could be made that stronger language is necessary to induce change. Finally, many invasive species managers report being limited by funding (Beaury et al. 2020) with the understanding that the capacity to enact and enforce policies varies by region (Peters and Lodge 2009), leading to further spatial differences in communication approaches.

Study Objectives

We conducted a systematic review of aquatic non-native species literature to explore the message frames, valence, and terminology used in research, as well as the reasons why these communication strategies were adopted. Aquatic invasive species cause significant ecological impacts (Gallardo et al. 2016) inflicting costs of at least US\$345 billion annually (Cuthbert et al. 2021), but concurrently contain many species that serve important human needs, such as recreational fishing (Carey et al. 2011; Moore 2012; Fabrizio et al. 2021), making them an ideal context for understanding both positive and negative perceptions. We limited our review to the United States to minimize cultural difference in language use and focus our scope on the role of study characteristics and geographical factors. Given that the vast majority of news articles discussing non-native species comment on management actions (Clarke et al. 2020), we sought peer-reviewed articles that pertained to management, thereby generating implications directly relevant to public messaging, such as communicating management plans, raising awareness of risk, and influencing recreationist behavior. This systematic literature review was guided by the following objectives: 1) Characterize invasive species communication across message frames, valence and terminology in peer-reviewed articles published on non-native species management in the United States from 2008–2018; 2) Define the effects of study discipline, study focus, stage of invasion, and transportation vector on message frames; 3) Quantify the effects of study discipline, study focus, stage of invasion, and transportation vector on valence; and 4) Analyze the relationships among study discipline, study focus, stage of invasion, transportation vector, and terminology. We seek to provide insights into communication and message framing in research conducted by scientists from multiple disciplines that are advancing the study of biological invasions.

Methods

Search criteria and article identification

This systematic literature review (Gough et al. 2012) involved an examination of peerreviewed articles discussing aquatic non-native species from a variety of disciplinary perspectives (Fig. 2). We selected Thomson Reuters Web of Science and Scopus da-



Figure 2. Flow diagram detailing the article search and screening process for a systematic review of aquatic non-native species management.

tabases because of their common use in systematic reviews (Mongeon and Paul-Hus 2016), and searched them on July 3, 2018 using a search string that included seven keywords commonly used to report invasive species research (Colautti and MacIsaac 2004), as well as additional terms to target aquatic species and ecosystems and research that addressed management implications. Specifically, the sets of keywords were:

- invasive species AND (management OR conservation) AND aquatic;
- non-native species AND (management OR conservation) AND aquatic;
- introduced species AND (management OR conservation) AND aquatic;
- alien species AND (management OR conservation) AND aquatic;
- exotic species AND (management OR conservation) AND aquatic;
- non-indigenous species AND (management OR conservation) AND aquatic;
- nuisance species AND (management OR conservation) AND aquatic.

In addition to searching keywords in the topic (TS), the search strings specified the language to be English and the country (CU) to be the United States. We limited articles to English-language studies from the United States (including Puerto Rico) given the focus on communication; accounting for cultural differences or variation across languages was outside the scope of this study. Additionally, we used a 10.5-year time from January 2008 through July 2018. The 10.5 year timeframe was chosen to provide a snapshot of recent articles published after considerations around language were brought to light (e.g., Brown and Sax 2004).

In the first stage of screening, we read 665 titles and abstracts to determine whether the following criteria were met: 1) conducted in the United States; 2) speaks to management of non-native species; 3) studies an aquatic ecosystem. The 445 articles that met the first stage of screening criteria were advanced to the second stage of screening. During the second stage of screening, we read the full article, and articles that did not meet the following criteria were excluded: 1) conducted in the United States, 2) study objectives pertain to management of non-native species; 3) the study ecosystem is aquatic; 4) peer-reviewed article that is article-length and not a book. The final pool included 278 articles, distributed across the 10.5-year window used for the review (Fig. 3). Screening and management of the articles was conducted using EPPI Reviewer 4 software (Thomas et al. 2010).

Coding process

To provide an overview of the types of studies included in the review, we recorded key characteristics of each study, including location of the study site, species studied, journal outlet, and affiliation of the lead author. Our systematic review unearthed published studies that were conducted across the United States (Fig. 4). Species of study were grouped into the broad categories of plants (37%) and animals (45%), with 17% featuring both plants and animals. In line with the study objectives, we coded each article for the seven features in our conceptual model (Fig. 1).



Figure 3. Publication year of 278 articles published from January 2008 through July 2018 that assessed non-native aquatic species management in the United States.

First, we coded each article for three facets of communication: message frame, valence and terminology. Message frame was categorized as either human-centered or species-centered (Table 1). Specifically, two independent coders identified the message frame adopted in the introduction section of each article, using the following definitions: "Human-centered frames" were those that focused on the human drivers or causes of species introductions or centered human responsibility for taking action, whereas "species-centered frames" were those that did not discuss human influences on species introductions but focused on the species themselves as the drivers, at times anthropomorphizing the species. These codes were mutually exclusive, in that whenever human influence was mentioned, the article was classified as human-centered. To assess agreement between coders, we used Cohen's Kappa (\varkappa) a measure of interrater reliability (McHugh 2012), which indicated substantial agreement ($\varkappa = 0.760$; percent agreement = 89%). For each article with an initial disagreement on code (n = 31), the coders discussed the article until an agreement was reached.

Each article was next categorized according to its positive, negative or neutral valence. Specifically, the introduction section was coded as expressing positive valence when the benefits of a study species were discussed or predicted, whereas negative valence was indicated when the study species was described as problematic or its negative effects were detailed. The article was coded as having neutral valence if positive and negative impacts were both described, or no effects at all. Again, two independent coders identified the valence; interrater reliability indicated substantial agreement (x = 0.620; percent agreement = 88%), and when there was disagreement on valence (n = 33), the article was discussed until agreement was reached. Terminology was assessed quantitatively. The text of each article, excluding the references, was searched for seven common terms used to refer to aquatic non-native species (i.e., alien, exotic,



Figure 4. Geographic locations of study sites across 278 articles that reported on findings from aquatic non-native species research. Each point represents one study and shows its location in relation to other studies across **A** the contiguous United States **B** Alaska and **C** Hawaii.

introduced, invasive, non-indigenous, nuisance, non-native), and the number of times each term appeared in the article was tallied.

Second, data reflecting four explanatory variables - study discipline, study focus, stage of invasion, and transportation vector - were extracted from each article. Study discipline was classified by identifying whether the disciplinary orientation and methods used were in line with the biological sciences, social sciences or an interdisciplinary approach. Data drawn from plants, animals or ecosystems were classified as "biological sciences", whereas data drawn from humans (e.g., methods involving surveys or interviews) were classified as "social sciences". Study focus was derived from the stated objective of the paper and categorized as: "prevention" when objectives related to risk assessments or analysis of prevention measures; "monitoring" when objectives dealt with detecting or identifying non-native species; "understanding" when objectives pertained to analyzing the impacts or ecological characteristics of a species; and "control" when objectives related to the evaluation of management or control methods. The stage of invasion was identified based on the description of the study population provided in the introduction or methods of the paper. In some cases, the stage of invasion was explicitly stated; when it was not stated, articles were coded as "transportation" if the species was in the process of moving to a new location, "introduction" if the species had been released at a new location, "establishment" if the species had survived at the new location or "spread" if the species had spread beyond the initial point of introduction (Blackburn et al. 2011). Articles that could not be classified as occuring

	Definition	Example
Message frame		
Human-centered	Research focused on the human drivers or causes	Zebra mussels are spread by recreational boaters
	of species introductions or centered on human	
	responsibilities for taking action	
Species-centered	Research focused on the species themselves as	Zebra mussels filter water and reduce food availability
	drivers, at times anthropomorphizing the species; no	lower in the food web
	discussion of human influences	
Valence		
Positive	Benefits of the study species are discussed or predicted	Zebra mussels filter algae and make water clearer
Neutral	Both positive and negative impacts, or no effects at	Zebra mussels make water clearer, but also reduce food
	all, are described	availability for desirable species in the food web
Negative	A study species is described as problematic or its	Zebra mussels make water clearer but also reduce food
	negative effects are detailed	availability for desirable species in the food web

Table 1. Message frames and valences that were coded from peer-reviewed articles about non-native aquatic species management.

at one particular stage or for which stage of invasion was entirely irrelevant were coded as a fifth category. Finally, transportation vector was classified as natural, humanintentional and/or human-unintentional (Lockwood et al. 2013). Specifically, a vector was coded as "natural" if the study population was transported by dispersal patterns not directly mediated by humans, "human-intentional" if invasive species were transported deliberately by humans (e.g., stocking, biocontrol, aquaculture), and "humanunintentional" if the study population was transported accidentally by humans (e.g., ballast water, recreational equipment). Full details on the coding approach are available in the supplementary information.

Finally, we collected information on biodiversity context. We defined biodiversity context as watershed-level estimates of the percent of aquatic species classified as non-native where the study was conducted. We determined native and non-native species occurrence within watersheds of the contiguous United States using the NatureServe Central Database, the United States Geological Society (USGS) Non-indigenous Aquatic Species Database, the Early Detection and Distribution Mapping System (EDDMapS) and the USGS Biodiversity Serving Our Nation (BISON) database. These databases contained native and non-native species occurrences (defined as a species introduced from outside its native range) that were sourced from the literature, museums, databases, monitoring programs, state and federal agencies, professional communications, online reporting forms, and hotline reports. Occurrence records were geo-referenced to watersheds according to USGS hydrological unit code 8 (HUC 8) using ArcGIS (v. 10.3.1).

Analysis

Quantitative analyses were performed to define relationships between language use and the selected characteristics in the included articles. First, predictors of message frame were assessed using multinomial logistic regression with study discipline (i.e., biological science, social science and interdisciplinary), study focus (i.e., prevention, monitoring, understanding, or control), invasion stage (i.e., transportation, introduction, establishment, or spread) and transportation vector (i.e., natural, unintentional, intentional, both, all, or not mentioned) as fixed effects. The model did not exhibit large over-dispersion (residual deviance = 243, with 226 degrees of freedom). Second, predictors of valence (i.e., biological, interdisciplinary or social) were assessed using multinomial logistic regression with the same fixed effects used in the message frame model. Because only one study was coded as positively valanced, that study was excluded from analysis. Thus, the dependent variable was a binary categorical variable; studies were either negative or neutral. This model also did not exhibit large over-dispersion (residual deviance 212 on 224 degrees of freedom). Finally, the use of terminology was modeled as a function of four explanatory variables (i.e., study focus, study discipline, stage of invasion, and transportation vector) using multivariate redundancy analysis (RDA) in the R package 'vegan' (Oksanen et al. 2020). Because most papers did not use all terms, we used the Hellinger distance function to account for the many zeros in the dataset (Legendre and Gallagher 2001). The correlation biplot was based on the covariance matrix and omitted the reference levels of the explanatory variables to avoid collinearity (Zuur et al. 2007). To test the hypothesis that the four variables explained a larger degree of variation than a random contribution, an ANOVA-like permutation test for RDA was performed (Oksanen et al. 2020). All analysis was conducted in the R programming language version 4.1.2.

Lastly, we tested whether language use in articles was associated with the biodiversity context in which the study was conducted. Comparisons of the percent of non-native species and types of message frames and valence were assessed using Wilcoxon rank sum tests with continuity correction and the relationship between percent non-native species and the overall article frequency of invasive species terminology (number of occurrences of the words: invasive, introduced, exotic, non-native, alien, nonindigenous, nuisance) was evaluated using simple linear regression.

Results

The articles included in this systematic review exhibited diverse patterns in message framing, valence and terminology. An approximately equal number of articles were classified as using species-centered language (45.0%) versus human-centered language (55.0%). Valence was predominately negative (81.3%) across articles, with only one study framed positively (0.4%), and the remainder framed neutrally (18.3%). Finally, the term "invasive" was used most often in the published literature; 95.3% of the articles included this term on at least one occasion. Many articles also included the terms "introduced" (70.5%), "non-native" (57.9%), "nuisance" (29.9%), "exotic" (27.7%), "non-indigenous" (23.4%), and "alien" (10.4%).

Examining study discipline, we found that biological sciences (84.5%) was dominant, with a minority of studies drawing on environmental social science (12.6%) and interdisciplinary methods (2.9%). Study focus was split among prevention (25.2%), monitoring (9.4%), understanding species impacts (31.3%), and control of the species (27.0%). A majority of articles (61.5%) were conducted during the spread stage of invasion, with fewer results published on the transport (5.4%), introduction (10.8%) or establishment (14.0%) stages. Stages of invasion were not relevant for several articles (8.3%); this category was excluded from further analysis. Intentional and unintentional spread were each discussed in approximately one quarter (24.1%) of the articles. Many studies (37.1%) did not report transportation vector, 9.0% covered multiple types of vectors, and only 5.8% focused on natural dispersal rather than human causes.

Both transportation vector ($\chi^2(5) = 38.600$; p<.001) and study focus $\chi^2(3) = 15.616$; p<.001) significantly predicted message frames. Message frame, transportation vector and study focus showed strong associations within the published literature ($\chi^2(13) = 89.756$; p<.001). Specifically, species-centered frames were used more frequently when the study

Variable	В	Standard error	Z	р	Exp(B)
Intercept	0.272	0.954	.286	0.775	1.313
Study discipline ¹					
Interdisciplinary	-0.315	1.168	-0.270	0.787	0.730
Social sciences	0.381	0.832	0.457	0.647	1.463
Study focus ²					
Monitoring	-0.920	0.626	-1.469	0.142	0.398
Understanding	-1.187	0.488	-2.433	0.015	0.305
Control	-1.886	0.496	-3.804	<0.001	0.152
Stage of invasion ³					
Introduction	0.074	1.034	0.072	0.943	1.077
Establishment	-0.287	0.967	-0.297	0.766	0.750
Spread	Spread -0.340		-0.384	0.701	0.712
Transportation vector ⁴					
Natural	0.999	0.591	1.690	0.091	2.716
Human (unintentional)	2.159	0.479	4.503	<0.001	8.660
Human (intentional)	2.014	0.400	5.043	<0.001	7.494
Human (Both)	1.616	0.780	2.071	0.038	5.033
All	2.198	1.156	1.902	0.057	9.005

Table 2. Predictors of human-centered (reference level) vs. species-centered framing in peer-reviewed articles focused on non-native aquatic species management. Significant results are highlighted in bold.

¹Biological sciences served as the reference level; ²Prevention served as the reference level; ³Transportation served as the reference level; ⁴Vector not mentioned served as the reference level; Note: Results: $\gamma^2(13) = 89.756$; p < .001; Nagelkerke's Pseudo R² = 0.416.

focus was "understanding" impacts or "control", whereas human-centered frames were used more frequently when the study focus was "prevention" (Table 2).

We found a strong relationship between frame use and transportation vector. Human-centered frames were more common when human vectors were emphasized; when no vectors were emphasized, the species-centered frame dominated (Fig. 5). Likewise, species-centered messaging became more common with increasing stages of invasion, though this was not a statistically significant result of the logistic regression. Additionally, species-centered frames were more likely to be used in research conducted in watersheds containing proportionally more non-native species (Fig. 6A; W = 3929.5, p = 0.027, Wilcox test).

Negative valence was used more often for studies that focused on preventing the spread of invasive species or the evaluation of control options, in contrast to moni-



Figure 5. Comparison of **A** negative (red) vs. neutral (black) valence, and **B** human-centered (blue) vs. species-centered (green) message frames according to four study attributes including study discipline, study focus, stages of invasion, and transportation vector. Width of each column indicates the proportion of studies falling into each category. Comparisons between negative vs. neutral valence and human vs. species centered frames are likewise indicated proportionally in each graph.

toring studies (Fig. 5). This result was supported by the logistic regression model ($\chi^2(13) = 29.238$; p=.006; Nagelkerke's Pseudo R² = 0.181), in which study focus was a significant predictor ($\chi^2(3) = 10.660$; p=.014). That is, a neutral valence was



Figure 6. Relationship between non-native species richness (% of total species) in watershed of the study site and language use within the study, including message frame **A** and valence **B**.

more likely to be adopted when the study focus was monitoring or understanding the species, in contrast to studies with a focus on risk assessment that used predominantly negative valences (Table 3). Stage of invasion, transportation vector and study discipline had no influence on valence. Though the stage of invasion was not a significant predictor in the logistic regression model, there was a pattern in which negative language was used proportionally more often in studies examining establishment and spread, compared to transport and introduction (Fig. 5). Finally, we found no evidence that articles were more likely to portray non-native species negatively when conducted in watersheds containing more non-native species (Fig. 6B; W = 1235.5, p = 0.099, Wilcox test).

Relationships between terminology and the four predictor variables were assessed through RDA, where the first two axes explained 13% of the variation in terminology use ($F_{13,224}$ = 3.3, p = 0.001, Fig. 7). Of the total variation explained, stages of invasion (39%) and study focus (31%) contributed the most to explaining patterns in terminology (Table 4). As shown in the correlation triplot (Fig. 7), studies that looked at the "establishment" stage of invasion and had the study focus to "understand" used the term "non-native" more often and the term "invasive" less often. By comparison, studies that had the study focus to analyze "control" measures or that looked at the stage of "spread" were more likely to use the terms "invasive" and less likely to use the term "non-native." Use of the term "introduced" correlated with intentional human introductions and the term "non-indigenous" with unintentional human introductions. Studies that looked at the "introduced" and "non-indigenous" more commonly than studies addressing other stages of invasion.

Variable	В	Standard error	Z	р	Exp(B)
itercept 2.394		1.191	2.010	0.044	10.959
Study discipline ¹					
Interdisciplinary	14.243	956.232	0.015	0.988	1533180
Social sciences	0.130	1.111	0.117	0.907	1.139
Study focus ²					
Monitoring	-1.926	0.731	-2.637	0.008	0.146
Understanding	-1.462	0.642	-2.275	0.023	0.232
Control	-0.719	0.679	-1.059	0.290	0.487
Stages of invasion ³					
Introduction	0.499	1.305	0.382	0.702	1.647
Establishment	-0.970	1.182	-0.821	0.412	0.379
Spread	-0.075	1.138	-0.066	0.948	0.928
Transportation vector ⁴					
Natural	0.120	0.667	0.180	0.857	1.128
Human (unintentional)	0.364	0.551	0.660	0.509	1.439
Human (intentional)	0.228	0.430	0.528	0.597	1.255
Human (Both)	1.308	1.144	1.143	0.253	3.698
All	0.387	1.192	0.324	0.746	1.472

Table 3. Predictors of negative (reference level) vs. neutral valence in peer-reviewed articles regarding non-native aquatic species management. Significant results are highlighted in bold.

¹Biological sciences served as the reference level; ²Prevention served as the reference level; ³Transportation served as the reference level; ⁴Vector not mentioned served as the reference level; Note: Results: ($\chi^2(13) = 29.238$; p=.006; Nagelkerke's Pseudo R² = 0.181).



Figure 7. Redundancy analysis (RDA) of the terminology used in scientific publications (grey rectangles) concerned with invasive species management in the United States from 2008–2018. Eigenvectors (site scores) are scaled to their square-root. In total, 13.3% of variance is explained. Corresponding reference levels and further statistics are listed in Table 4.

Variable	df	Variance	F	р	Eigenvalue using only one explanatory variable	Eigenvalue as %
Study discipline ¹	2	0.002	0.7	0.702	0.000	0.00
Study focus ²	3	0.014	3.5	<0.001	0.019	0.34
Stages of invasion ³	3	0.017	4.2	<0.001	0.023	0.41
Transportation vector ⁴	5	0.013	2.0	0.009	0.010	0.18
Residual	224	0.298				

Table 4. Permutation test and marginal effects of four explanatory variables on terminology use. The total sum of all Eigenvalues is 0.055. Significant results are highlighted in bold.

¹Biological sciences served as the reference level; ²Prevention served as the reference level; ³Transportation served as the reference level; ⁴Vector not mentioned served as the reference level.



Figure 8. Relationship between non-native terminology used in each study and proportion of non-native species at the study site, assessed at the watershed level.

The overall frequency of non-native terminology used in each article was positively related to the percent of non-native species in the watershed where the study was conducted (Fig. 8; F = 5.4, p = 0.022), although considerable variation in this relationship existed.

Discussion

Our study aimed to quantify patterns and drivers of language use in the scientific aquatic non-native species literature in the United States. We discovered considerable variation in communication strategies used by scientists, including message frame, valence, and terminology. We contend that the factors explaining variation in communication patterns can be better understood through knowledge of message framing. Specifically, we observed that species-centered vs. human-centered frames strongly related to transportation vector and study focus, indicating that the role of humans tends to be highlighted when there is greater urgency in preventing the spread of non-native species, whereas the role of the species itself is centered when transportation vectors are not mentioned and the focus is on control. Aligned with previous research (Clarke et al. 2020), we found negative valences to be most common. Additionally, terminology use corresponded with stage of invasion, indicating that researchers are following guidance by past work to use standardized and consistent language, specifically relying on more general terms like "non-native" at earlier stages of invasion, and only classifying species as invasive after accelerating spread or clear impacts are occurring (Colautti and MacIsaac 2004; Blackburn et al. 2011).

We found researchers adopted message framing that aligned with a stated study focus. When an objective pertaining to risk assessment or a focus on prevention was expressed, human-centered frames were more common, corresponding to the important role humans play in curbing the spread of invasive species (Tabak et al. 2017). The importance of self-efficacy (i.e., beliefs that one has the ability to complete an action; Bandura 1977) in enabling people to engage in preventative measures is well-documented in the literature (Niemiec et al. 2017; Landon et al. 2018; Mankad and Loechel 2020), which underscores the importance of human-centered frames that emphasize the role of humans in biological invasions. By contrast, when the focus of research was to understand a species or to analyze control measures, species-centered frames dominated the narrative adopted in reporting results. This finding aligns with past research suggesting that species-centered frames are likely to activate risk perceptions and engagement in preventative behaviors (Hart and Larson 2014). Thus, because past work indicates the ability of both species- and human-centered frames to heighten risk perceptions, more research is needed to understand public responses to these frames and their success in changing behavior in positive ways. Such research (e.g., Clarke et al. 2020; Orth et al. 2020) should focus on analysis of science communication outside of traditional scientific papers or in press releases by scientific organizations because it is unlikely that the public or policy makers are readers of scientific papers.

The finding that negative valences were predominant in scientific papers is not surprising given the focus of the literature review on non-native species management, rather than targeting bodies of work on, for instance, stocking fish for capture fisheries. Accordingly, our selection of keywords (e.g., "invasive") may not always be used in studies of introduced species that are beneficial, although this is very unlikely to be the case given the need to comment on the negative impacts of non-native species even when reporting positive outcomes (e.g., Johnson et al. 2009; Aas et al. 2018). Despite this, we recognize that studies on the positive effects of non-native species may be underrepresented in our search (e.g., Carey et al. 2011). Emphasizing the negative impacts associated with invasive species seems to be perceived by invasion biologists as necessary – or at least helpful – to inform readers and generate support for preventing or controlling invasive species. However, there is a risk associated with an overabun-

dance of negative language: as negative valences are translated into public news media, extreme negativity can lead to feelings of helplessness and disinterest in management initiatives (Clarke et al. 2020). This is particularly worrisome given recent evidence that invasive species can, in some instances, play positive roles for local livelihoods and human well-being (Shackleton et al. 2019), and in other instances, not have measurable ecological or social impacts (e.g., Wolter and Röhr 2010).

The use of terminology broadly aligned with recommendations in previous research to be deliberate about defining concepts and study contexts in invasion biology (Colautti and MacIsaac 2004; Copp et al. 2005). "Invasive" was the most frequently used term across all study attributes except when it was appropriate by definition to use "non-native." Specifically, the use of "non-native" rather than "invasive" aligned with stages of invasion such as establishment, where the species had yet to meet the requirements to be classified as invasive, defined as a species causing negative ecological or social impacts (Blackburn et al. 2011). Terms that were synonymous with "nonnative," including "exotic," "alien," and "non-indigenous" were rarely used. In summary, invasive species researchers have responded to past calls for clarity in research (Richardson et al. 2000; Colautti and MacIsaac 2004; Blackburn et al. 2011), and are using consistent terms aligned with stages of invasion.

Language use showed some evidence of being related to the regional biodiversity context in which the study was conducted. Specifically, in watersheds containing relatively more non-native species, studies were more likely to use species-centered frames. Past work has shown species-centered frames to be more effective in raising stakeholder engagement in preventative behaviors (Hart and Larson 2014), thus the correlation between this framing and increasing dominance of non-native species is notable. Additionally, there was a positive relationship between non-native species richness and overall use of non-native terminology. Researchers may be reflecting the degree of risk perceived in the study region with language that highlights these risks more clearly. Ultimately, higher-risk areas may warrant stronger language to better convey the need for greater management attention and heightened public awareness.

A strikingly small proportion of studies within the biological invasion literature were conducted through an environmental social science lens. Given the role of recreationists in non-native species transport (Johnson et al. 2009; Rothlisberger et al. 2010; Cole et al. 2019; Golebie et al. 2021) and complex and often controversial views about non-native species (Schlaepfer et al. 2011; Russell and Blackburn 2017; Schlaepfer 2018), there is a strong need for more social science research (e.g., Kochalski et al. 2019; Shackleton et al. 2019). The social science studies included in the review exclusively used negative valences, with a strong emphasis on human-centered frames. Use of human-centered frames was logical, given that social science seeks to understand the thoughts, feelings and actions of humans. Negative valences may have dominated given that the studies in our review predominantly investigated boater and angler transport of invasive species, and thus stressed the negative impacts of invasive species that could be averted by human action. Additionally, raising self-efficacy, the awareness of how individuals can play a role in invasive species spread, is an important step in encouraging

people to take action. However, these results reveal an untapped area of inquiry on relationships between humans and non-native species. Several research questions should be addressed: In what ways are invasive species meaningful to humans? What are stakeholder preferences for invasive species management? On what information do people base these beliefs? Which non-native species are perceived as beneficial rather than harmful, and in what socioeconomic or cultural contexts? How can managers nudge recreationists and other people (e.g., aquarium fish holders, see Wolbers and Donnelly 2019) to refrain from further spreading non-native fishes and which messages' frames are most effective in such communication strategies (e.g., Shaw et al. 2021)? Answering these questions will enhance invasive species management practices by deepening knowledge of how people do (or do not) support decision-making outcomes.

Conclusion

In conclusion, our work quantifies how published literature on aquatic non-native species research conveys varied message framing, valence and terminology. We show that authors of peer-reviewed journal articles are effectively using standardized terminology established in past work. For instance, we found limited evidence for inflammatory or exaggerative framings being dominant within peer-reviewed published literature from 2008 to 2018. Additionally, message frames evoked in these articles are correlated with study focus and local biodiversity context, indicating that language use is tailored to contextual conditions. We encourage researchers to be aware of how their language might be influenced by such factors and actively consider whether communication choices match the study goals. Future work should seek to evaluate language use in public-facing communication to identify relationships between public and academic communication, as well as the impacts of communication style on public perceptions of invasion biology research. Understanding the role of science communication more broadly in public understanding of invasion biology and support for management decisions is an important direction for future research.

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Supplementary material I

Codebook

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RESEARCH ARTICLE

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Chew-cards can accurately index invasive rat densities in Mariana Island forests

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Abstract

Rats (*Rattus* spp.) are likely established on 80–90% of the world's islands and represent one of the most damaging and expensive biological invaders. Effective rat control tools exist but require accurate population density estimates or indices to inform treatment timing and effort and to assess treatment efficacy. Capture-mark-recapture data are frequently used to produce robust density estimates, but collecting these data can be expensive, time-consuming, and labor-intensive. We tested a potentially cheaper and easier alternative, chew-cards, as a count-based (quantitative) index of invasive rat densities in tropical forests in the Mariana Islands, an archipelago in the western North Pacific Ocean. We trialed chew-cards in nine forest grids on two Mariana Islands by comparing the proportion of cards chewed to capture-mark-recapture density estimates and manipulated rat densities to test whether the relationship was retained. Chew-card counts were positively correlated with rat capture-mark-recapture density estimates found in the region. Additionally, the correlation between the two sampling methods increased with the number of days chew-cards were deployed. Specifically, when chew-cards were deployed for five nights, a 10% increase in the proportion of cards chewed equated to an estimated increase in rat density of approximately 2.4 individuals per ha ($R^2 = 0.74$). Chew-cards can provide a valid index of rat densities in Mariana Island forests and are a cheaper alternative

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to capture-mark-recapture sampling when relative differences in density are of primary interest. New cost-effective monitoring tools can enhance our understanding and management of invaded islands while stretching limited resources further than some conventional approaches, thus improving invasive species management on islands.

Keywords

Abundance estimation, capture-mark-recapture, Guam, invasive predator control, *Rattus*, Rota, spatial capture-recapture, tropical ecology

Introduction

Invasive species jeopardize worldwide biodiversity (Liu et al. 2020), economies (Hanley and Roberts 2019), and human health (Mazza and Tricarico 2018) and cost billions of dollars annually in ecological damages, economic losses, and management efforts (Cuthbert et al. 2022), an amount that is continually increasing (Diagne et al. 2021). Minimizing costs to control invasive species makes combatting this problem more feasible at larger scales (Jardine and Sanchirico 2018). Primary ways invasive species management costs can be reduced are via effective pathway and vector management (e.g., screening protocols), early detection and rapid response, and improved approaches to mitigation and restoration (Green and Grosholz 2020).

Islands are often the focus of invasive species research and control efforts (Holmes et al. 2019). Housing an estimated quarter of Earth's plant and animal species, islands are biodiversity hotspots and targeting them can maximize conservation funds (Myers et al. 2000). However, isolation—the same feature that supports high endemism and richness (MacArthur and Wilson 1967)—makes islands remarkably susceptible to damage from biological invasions (Whittaker and Fernández-Palacios 2007). In particular, introduced predators can easily exploit native species that have evolved under limited predation pressure (Doherty et al. 2016).

Rats (*Rattus* spp.) are difficult to detect (Yackel Adams et al. 2011; Smart et al. 2021), easily transportable (Gatto-Almeida et al. 2021), highly fecund (Harper and Bunbury 2015; Clapperton et al. 2019), and adaptable (Duron et al. 2019). These an-thropogenic commensal generalists (Dammhahn et al. 2017) are likely established on 80–90% of the world's islands (Towns et al. 2006)—of which at least 78% are documented to support highly threatened vertebrates (Spatz et al. 2017)—and represent one of the most damaging and expensive biological invaders (Harper and Bunbury 2015; Cuthbert et al. 2021). Invasive rats have a myriad of impacts on island ecosystems. As direct predators of many small vertebrates, rats have caused extinctions or severe declines in birds (Bond et al. 2019), reptiles (Donihue et al. 2021), and mammals (Hanna and Cardillo 2014). Cascading effects shift interspecific dynamics, causing further deterioration to island communities and exacerbating destruction caused by rats (Campbell and Atkinson 2002; Kurle et al. 2021). For example, on some islands, invasive rats help sustain populations of co-occurring invasive predator species

at higher trophic levels as an abundant prey source (e.g., cats, stoats, snakes; Savidge 1986, 1987; Murphy and Bradfield 1992). Such multitrophic invasive predator assemblages inflict compounded impacts to the islands they inhabit and make invasive species control more complicated and costly (Stobo-Wilson et al. 2021). Invasive rats can also serve as vectors for novel pathogens to highly susceptible insular faunas (Pickering and Norris 1996).

Trapping (Duron et al. 2020) and rodenticides (Keitt et al. 2015) can reduce rat densities, and both approaches have been employed in successful rodent eradications on hundreds of islands (Howald et al. 2007). However, cost-effective rodenticide treatments or other control efforts first require accurate target population density estimates to inform treatment timing and effort and to assess treatment efficacy (Kim et al. 2020). Capture-mark-recapture data are frequently used to produce robust density estimates (Otis et al. 1978; Williams et al. 2002; Wiewel et al. 2009a; Yackel Adams et al. 2011), but collecting these data can be expensive, time-consuming, and labor-intensive (Wiewel et al. 2009b). Developing cheaper and easier techniques for indexing density (i.e., count-based indices) is thus a priority.

Count-based indices are commonly used as relative measures of abundance or density (McKelvey and Pearson 2001). An effective count-based density index enumerates animal evidence, meets the assumption of constant detection (Anderson 2003), and strongly correlates with true density across all possible densities (Nichols 1992). Ideal indices should also be inexpensive, user-friendly, and applicable at large spatial scales (Williams et al. 2002; Engeman 2005; Engeman and Whisson 2006). However, indices have been criticized because assumptions are often ignored and untested (e.g., constant detection probability; Anderson 2001, 2003; Skalski et al. 2005), and they are frequently used to make inferences or inform management decisions without any preceding testing or calibration (Rosenstock et al. 2002). Nevertheless, indices can represent relative differences in abundance (Engeman 2003) if in situ calibration studies show a positive, monotonic relationship between the index and true density across the range of possible densities in a given region (Nichols 1992). Counts of animal observations (Fagerstone and Biggins 1986), automated-camera photos (Engeman et al. 2006), tracks (Brown et al. 1996), chew-marks (Sweetapple and Nugent 2011), bait-take rates (Byers 1975), hair deposition (Zielinski et al. 2006), physical captures (Village and Myhill 1990), and feces detections (Mills et al. 2005) have all been used to index diverse small mammal populations across the globe with varying successes and limitations.

Chew-track-cards, a tool for indexing rodents, are baited pieces of plastic that retain animal tooth impressions and footprints. Seminal work conducted in Australia and New Zealand determined that chew-track-cards are a cost-effective means of accurately indexing small mammal abundances across multiple species, including rats (Caughley et al. 1998; Sweetapple and Nugent 2011; Kavermann et al. 2013; Ruffell et al. 2015; Burge et al. 2017; Forsyth et al. 2018; Balls 2019). However, to our knowledge, no study since Caughley et al. (1998) has validated chew-track-card counts against measured (i.e., capture-mark-recapture) density estimates; latter studies simply document relationships among multiple small mammal abundance indices (Sweetapple and Nugent 2011; Kavermann et al. 2013; Ruffell et al. 2015; Forsyth et al. 2018; Balls 2019; Nottingham et al. 2021; Brown et al. 2022). Further, indices should be tested and calibrated in other ecosystems, regions, and climates before they are used to approximate population sizes beyond the range of existing studies. For example, rats on temperate islands (e.g., New Zealand) respond differently to baits than rats on tropical islands (Keitt et al. 2015); regionally based differences in foraging behavior may affect chew-track-card interaction frequencies and thus influence their effectiveness in the tropics. Consequently, evaluating chew-track-cards on tropical islands informs the ability to apply a detection tool developed in temperate environments to other ecosystems.

The Marianas are a chain of 15 volcanic islands in the western North Pacific Ocean (Fig. 1) that are optimal locations for field validating chew-track-cards in the tropics because they have a range of rat densities (Wiewel et al. 2009a) and are the focus of extensive conservation research and action (e.g., Faegre et al. 2019). We tested the accuracy of chew-track-cards as a count-based index of invasive rat densities, by comparing chew-track-card counts to capture-mark-recapture density estimates and manipulated densities to test whether the relationship was retained. Our methods allowed us to identify prospects and caveats to the use of chew-track-cards on tropical islands, described herein. Our study informs future management efforts by testing a rat density index that, if effective, should reduce costs and improve efficiency for monitoring invasive rat populations in forests in the Marianas and, potentially, similar habitats on other tropical islands.

Methods

Study area

Guam and Rota are the southernmost and larger (Guam = 550 km²; Rota = 85 km²) of the Mariana Islands (Fig. 1). The southern Marianas are characterized as coralline limestone islands and are dominated by forest and grassland habitats. The climate is tropical with seasonal rains during July–October.

Pacific rats (*Rattus exulans*), brown rats (*Rattus norvegicus*), and black rats (*Rattus rattus*) have been established in the Mariana Islands—where bats are the only native mammals—for centuries (Baker 1946; Steadman 1999; Musser and Carleton 2005). Despite their proliferation, rats have had minimal direct impacts on native plants and animals in the Marianas compared to other oceanic islands (Fritts and Rodda 1998). In fact, rats did not become a major conservation concern in the Mariana Islands until they became key prey for an alien apex invader, the brown treesnake (*Boiga irregularis*; Fritts and Rodda 1998).



Figure 1. Nine forest grids sampled via chew-cards and live-trapping for rats (*Rattus* spp.) during June 2018–August 2019 on Guam (G1–4) and Rota (R1–5) in the Mariana Islands. In the inset map, the red rectangle indicates the location of the two islands in the western North Pacific Ocean. In the main map, the blue circles indicate 11×11 grids with 12.5-m intervals between each station (grid area = 1.56 ha), and orange circles indicate 10×10 grids with 10-m intervals between each station (grid area = 0.81 ha).

The brown treesnake was accidentally introduced from its native range in the South Pacific (Shine 1991) to the naturally snake-free island of Guam shortly after World War II (Rodda et al. 1999). By the 1980s, the snakes were widespread and abundant across Guam (Savidge 1987) and caused ecological destruction in their wake (Rodda and Savidge 2007). Most notably, brown treesnakes extirpated most of Guam's forest birds (Savidge 1987; Fritts and Rodda 1998; Wiles et al. 2003). Decades of research and adaptive management have culminated in the potential for landscape-scale brown treesnake suppression in Guam forests (Clark et al. 2018; Siers et al. 2019, 2020). However, synchronous monitoring and control of rats is likely to be important because they are a key prey base for snakes on Guam and may affect the efficacy of some snake control tools (Gragg et al. 2010; Siers et al. 2018).

Our work on Guam occurred during 2018 within a 55-ha plot of homogenous disturbed limestone forest located on Andersen Air Force Base, termed the Habitat Management Unit. An extensive, interagency restoration plan including removal of non-native animals, constructing barriers, native plant recovery, and bird reintroductions exists for the Habitat Management Unit (Siers and Savidge 2017). A fence surrounding the entire site was erected in 2010 to prevent brown treesnake immigration and exclude non-native deer (Rusa marianna) and pigs (Sus scrofa; Siers and Savidge 2017). The Habitat Management Unit has undergone two major periods of experimental lethal snake treatments involving aerial deployment of toxic baits (dead neonatal mice laced with acetaminophen; Dorr et al. 2016; Siers et al. 2019). The first occurred during 2013 and 2014, and the second started during our study in 2018 and is ongoing. Without any rat treatments, we expected rat densities to increase following snake treatments via prey release (Ritchie and Johnson 2009), thereby providing a gradient of rat densities to test chew-track-cards on Guam. However, rat populations remained low. So, in 2019, we conducted additional fieldwork on Rota to test our index method on an island with higher rat densities (Savidge 1987; Wiles et al. 2003; Wiewel et al. 2009a, b). Rota lacks brown treesnakes and, consequently, has more ecologically intact forests with abundant native birds and fruit bats that represent what successfully restored forests may resemble on Guam.

Grid selection and setup

We sampled nine forest grids on Guam (n = 4 grids) and Rota (n = 5 grids; Fig. 1). All four grids on Guam were located within the Habitat Management Unit, hereafter G1, G2, G3, and G4, with selection to maximize spatial coverage as well as avoid threatened and endangered plant species. Of the five grids sampled on Rota, three were part of a concurrent rat study where high populations were anticipated (Page 2020), hereafter R1, R2, and R3 (corresponding to grids 1, 2, and 5 in Page 2020). The other two grids had historically high rat densities, hereafter R4 and R5 (mixed and *Leucaena* forest habitats, respectively, in Wiewel et al. 2009a, b). After sampling each grid once, we manipulated rat densities in G2, G3, and R4 before resampling to increase our sample size without having to establish new grids. We resampled G3 and G2 three months after lethal snake treatments that we anticipated would increase rat density via predator reduction. At R4, we humanely euthanized rats to manually reduce the population size before resampling with cards. To denote this, we appended .1 and .2 to the codes of grids we sampled twice (e.g., first sampling period in G2 = G2.1, second sampling period in G2 = G2.2).

All Guam grids and Rota grids R4 and R5 consisted of 11 × 11 trap stations with 12.5-m intervals between each station (grid area = 1.56 ha). The remaining three grids on Rota (R1–3) were part of a concurrent study (Page 2020) and consisted of 10×10 trap stations with 10-m intervals between each station (grid area = 0.81 ha). For the larger grids, we placed one large folding Sherman live trap (H.B. Sherman Traps, Inc., Tallahassee, FL, USA) at each trap station (n = 121 traps; spacing = 12.5 m) and one wire basket trap (Haguruma and Uni-King, Standard Trading Co., Honolulu, HI, USA) at every other station (n = 36 traps; spacing = 25 m) for a total of 157 live traps per grid (every other station had two traps). We baited traps with a mixture of peanut butter, oats, and food-grade paraffin wax and live-trapped for 10 consecutive nights. For the smaller grids (R1–3), we placed one basket trap at every station (n = 100 traps; spacing = 10 m) and baited traps with a combination of coconut and peanut butter. We live-trapped at these grids for four consecutive nights. Both grid sizes were at least four times the target species' home range estimates (Bondrup-Nielsen 1983), and spacing between stations was less than twice the target species' daily mean maximum distances moved (MMDM) in accordance with best practices (Otis et al. 1978; Wilson and Anderson 1985; Sun et al. 2014). Further, both trap and bait types are proven to be effective in this system (Baker 1946; Wiewel et al. 2009a, b; Page 2020). We accounted for trapping duration and all other sampling differences in our analyses.

Data collection

We conducted capture-mark-recapture trapping of rats ≤ 2 days before (G1, G4, G2.2, G3.2, R1, R2, and R3) or after (G2.1, G3.1, and R5) a five-day card deployment so the cards would reflect the same rat densities estimated with capture-markrecapture methods. We did not deploy live-traps and cards simultaneously to avoid competing baits on the landscape. We set baited, fixed-open traps two days prior to the start of live-trapping to allow the rats to acclimate to their presence (Wiewel et al. 2009a, b) and placed traps on flat ground beneath or adjacent to cover (e.g., vegetation, debris, rocks) to provide shelter from sun and rain. We checked traps every morning and recorded the trap station, the lowest possible taxonomic classification (e.g., Rattus spp.), and marked status (new or recaptured) for each captured individual. For newly captured rats, we determined sex and age via the external genitalia (imperforate vagina = juvenile female; perforated vagina = adult female; undescended testes = juvenile male; descended testes = adult male) and measured mass and headbody length. We double-marked individuals by inserting a numbered, metal ear tag (Style #1005-1, National Band and Tag Company, Newport, KY, USA) into each ear in the distal one-third of the pinna (Wang 2005) before releasing at the capture location. For recaptured individuals, we simply recorded both ear tag numbers before immediately releasing at the capture location (i.e., we did not collect additional mass and length measurements). We closed traps after the morning check to prevent mid-day captures when temperatures were highest to minimize heat-related trap mortalities. In the late afternoon/evening, we set and re-baited all traps and repaired and replaced them as necessary.

We constructed rat indexing cards by cutting 4-mm thick, twin-walled polypropylene sheeting into 90×180 -mm rectangles and aligned the flutes parallel to the short sides of the cards (Fig. 2). We folded cards in half crosswise, cut a shallow slit lengthwise along the center of one half to prevent flutes from pressurizing when baited, and filled flutes with bait (peanut butter-paraffin mixture) to 2–3 cm from each edge (Fig. 2). On a subset of cards (chew-track-cards), we placed 60×75 mm of contact paper in the center of the bottom halves of the cards and applied a 2–3-cm wide strip of black ink onto the plastic surrounding the contact paper (Fig. 2B). We placed additional bait (~ 1 oz) at the top of the contact paper (Fig. 2B). This design was intended to lure rats to walk through the ink and step on the contact paper, leaving visible tracks that could be identified to order (Rodentia [rats], Decapoda [crabs], Squamata [lizards], or Carnivora [cats]). However, we quickly



Figure 2. A chew-card and **B** chew-track-card designs used to index rat (*Rattus* spp.) density in forest habitats on Guam and Rota in the Mariana Islands during June 2018–August 2019. Designs were patterned after Sweetapple and Nugent (2011). Photographs taken by Emma B. Hanslowe during July 2018 in the Habitat Management Unit on Guam.
found this use of tracking ineffective due to the Marianas' wet climate (ink ran or faded) and the inability to distinguish rat tracks from those of non-target species. We therefore stopped alternating chew- (Fig. 2A) and chew-track-cards (Fig. 2B) within grids and, instead, deployed solely chew-cards after completing our fourth grid (G4) in August 2018.

At each station, we stapled the cards to trees approximately one meter off the ground with the baited half up (Fig. 2). We checked the cards each morning and recorded if a card had been chewed. To identify species chews, we cross-referenced our cards with published reference photos and guides (Sweetapple and Nugent 2011; Manaaki Whenua Landcare Research 2020) and cards we placed in captive rat enclosures on Guam. We did not replace, repair, or re-bait cards during the five-day deployment to simulate the cards being left in the field without maintenance, as they would likely be in practice (Sweetapple and Nugent 2011). We removed the cards and all associated materials at the conclusion of the five-day card deployment.

To confirm or refute rat-chew identification, we deployed a RECONYX PC900 HyperFire Professional Covert Camera Trap (RECONYX, Holmen, WI, USA) at six randomly selected cards from each grid, except R2 and R3, for the duration of the fiveday card deployment. We initially programmed the cameras to trigger upon motion detection (for G1, G2.1, G3.1, G4, G3.2) but switched to a time-lapse setting after December 2018 (for grids G2.2, R1, R4.1, R4.2, and R5) to better capture species interactions with the cards. We reviewed all camera-trap photos and cross-referenced our field assessments of rat chews with the photos from the corresponding camera-trap night. We measured daily rainfall via rain gauges at all grids except R2 and R3.

Data analyses

We calculated individual body condition indices by dividing mass by head-body length (Li et al. 2021). We evaluated differences between masses, head-body lengths, and body condition indices between rats trapped on Guam versus Rota with Wilcoxon rank-sum tests ($\alpha = 0.05$).

Density estimation

We used spatially explicit capture-recapture models (Efford 2004) executed in the R package *secr* 4.2.2 (Efford 2020; R Core Team 2020) to estimate rat density because these models produce unbiased density estimates for social species, like rats (Davis 1953), even when study animals' movements and home ranges may violate model assumptions (Efford et al. 2009; López-Bao et al. 2018). We used Akaike's Information Criterion corrected for small sample size (AIC_C) to determine the best-supported models in our candidate sets (Burnham and Anderson 2002) and derived model-averaged density estimates and standard errors from the final model set (Burnham and Anderson 2002). Rationale for all models came from a combination of results from preceding studies, the biology and life history traits of small mammals, and knowledge of our system, described herein.

Capture probabilities can vary by time, behavior, and individual heterogeneity (Otis et al. 1978). Small mammals tend to be wary of new objects (Clapperton 2006; Yackel Adams et al. 2011) and, in the Marianas, have previously exhibited a two-day neophobic behavioral response where capture probabilities during the first two nights were lower than capture probabilities on the remaining occasions, even after a trap acclimation period (Wiewel et al. 2009a, b). Other hypothesized patterns of temporal variation included a time trend where rat capture probabilities changed linearly on the logit-scale over all capture occasions (Cusack 2011) or via daily changes in weather (e.g., rain; Stokes et al. 2001; Wiewel et al. 2009a, b). Behavioral responses are also well documented across taxa and systems and occur when animals become 'trap-happy' or 'trap-shy' (Hammond and Anthony 2006) and are associated with a positive (e.g., food) or negative (e.g., stress) trap experience resulting in unequal initial capture and recapture probabilities (Otis et al. 1978). We based our *a priori* hypotheses regarding individual heterogeneity largely on Wiewel et al. (2009a, b) who found higher capture probabilities for reproductively active (i.e., adult) female small mammals in the Marianas. Lastly, we tested a hypothesis that individuals in lower body condition may be more attracted to our baits, resulting in higher capture probabilities.

We analyzed data from each grid separately. At grids with sufficient data (R4–5), we used a two-step approach to model capture probabilities from which we derived density estimates. First (Step 1), we accounted for all available hypothesized sources of individual heterogeneity in capture probability by including sex, age, and body condition index as predictors. We fit models with additive combinations of temporal covariates, including a two-night neophobic response (neophobia2), a time trend (Time), daily rainfall amount (rain; when available), a behavioral response (behavior), and no temporal variation (.). We did not include neophobia2 with either rain or Time in the same model. We retained the best-supported temporal variation structure(s) to test all possible additive combinations of individual covariates, including sex, age, body condition index, and no individual heterogeneity (Step 2). We failed to collect individual covariate and rain data for Rota grids R1–3, and thus did not have sufficient data for the two-step approach. For these grids, we simply fit all other possible additive combinations of the remaining temporal covariates. We held the spatial parameter (σ) constant (i.e., null) in all models.

Data from grids on Guam were too sparse (< 10 total captures per grid) to use spatially explicit models, so we used simpler closed-capture conditional likelihood models (Huggins 1989, 1991) from Program MARK 6.2 (White and Burnham 1999). We combined encounter histories from all Guam grids, differentiated grids by group, and with the sparse data—were able to fit two simple models: constant capture probability (i.e., a null model) and a model with a behavioral effect (see Suppl. material 1: Table S1). We used the derived model-averaged abundance estimates to calculate density by dividing each estimate by an effective trapping area (ETA; Wilson and Anderson 1985; Efford 2004). We used results from the spatially explicit analysis to inform our choice of boundary strip (full MMDM) for our ETA calculations (see Suppl. material 2: Fig. S1). For grids with no movement metrics, we used the mean MMDM of all other grids from the same island and calculated standard errors using the delta method (Seber 2002).

Chew-card density index calibration

We did not analyze tracking ink data because we deemed our tracking ink methods ineffective in this system and instead treated all cards as 'chew-cards' and limited our analysis to teeth impressions. We summed the cumulative number of cards with rat chews for each deployment day (1–5 days) for each grid and calculated the daily proportion of cards with rat chews. We used linear regression models and Pearson's product-moment correlations, implemented in base R, to assess the relationship between card indices and capture-mark-recapture density estimates. We conducted these analyses five times, where the predictor variable in each regression analysis was the proportion of cards that detected rats after one, two, three, four, and five deployment nights, respectively, for each grid.

Results

Capture-mark-recapture of live-trapped rats

We captured 233 individual rats a total of 444 times in 10,090 corrected trap nights over the course of our study, where one corrected trap night equaled one active trap night corrected for sprung (via target and non-target captures and false trips) and non-functioning/missing traps by considering them to represent half of a night of trapping effort and no trapping effort, respectively (Table 1; Nelson and Clark 1973). We trapped almost 11 times as many rats on Rota (n = 213 rats) as we did on Guam (n = 20rats) with approximately half the trapping effort (Table 1). We determined sex and age for 194 captured individuals. Of those, we captured more males than females and more adults than juveniles on both islands (Table 1). Collectively, rats were heavier (average Guam mass \pm SD = 193.32 \pm 62.30 g; average Rota mass \pm SD = 95.75 \pm 42.81 g; Wilcoxon rank-sum W = 2.812.5; $P = 6.09 \times 10^{-9}$ and had higher body condition indices (average Guam body condition index \pm SD = 1.32 \pm 0.36; average Rota body condition index \pm SD = 0.68 \pm 0.24; Wilcoxon rank-sum W = 2.942; *P* = 1.46 × 10⁻¹⁰) on Guam compared to Rota, but there was no difference in head-body lengths between the two islands (average Guam head-body length \pm SD = 146.05 \pm 27.25 mm; average Rota head-body length \pm SD = 135.83 \pm 29.35 mm; Wilcoxon rank-sum W = 1.798; P = 0.25; Fig. 3).

We found that rat capture probability on both islands exhibited a behavioral effect (Fig. 4; see Suppl. material 1: Tables S1–S4). There was little evidence of additional temporal variation in capture probability; a model with a two-night neophobic effect was the best-supported model for one grid on Rota (R5; $\hat{\beta} = 0.07$; \widehat{SE} [$\hat{\beta}$] = 0.24). We found no evidence of variation in capture probability among individuals (associated with body condition, age, or sex) and no evidence that capture probability varied as a function of rain (see Suppl. material 1: Tables S3, 4).

Our grids represented a range of rat density estimates (\hat{D} range = 0.00–34.73 rats/ ha) to test card indices. Rat densities on Rota (\hat{D} range = 7.09–34.73 rats/ha) were **Table 1.** Corrected trap nights[†], number of individual rats (*Rattus* spp.) captured (# indiv. rats), total number of rat captures (including recaptures; total rat caps.), sex (M = male; F = female; U = undetermined sex), age (A = adult; J = juvenile; U = undetermined age), density estimate plus/minus standard error ($\hat{D} \pm$ SE), and proportion of chew-cards with rat chews after nights 1–5 for each sampling grid in forest habitats on Guam and Rota in the Mariana Islands during June 2018–August 2019.

Grid	Live-trap dates	Corrected	# indiv.	Total rat		Sex			Age		$\widehat{D} \pm SE$		Ch	ew-ca	urd	
		trap nights†	rats	caps.									pro	porti	ons	
					Μ	F	U	Α	J	U		1	2	3	4	5
Guam																
G1	11–20 Jun 2018	1,296.5	1	1	1	0	0	1	0	0	0.26 ± 0.78	.00	.00	.00	.01	.01
G2.1	19–28 Jul 2018	1,153.0	4	5	2	2	0	4	0	0	1.37 ± 0.14	.00	.00	.02	.05	.10
G3.1	19–28 Jul 2018	879.0	3	3	2	1	0	3	0	0	0.79 ± 2.35	.00	.01	.03	.05	.11
G4	04-13 Aug 2018	1,009.5	0	0	0	0	0	0	0	0	0.00 ± 0.00	.00	.02	.04	.05	.10
G3.2	29 Nov-08 Dec 2018	1,155.5	6	11	4	2	0	5	1	0	1.01 ± 9.65	.02	.05	.06	.07	.10
G2.2	02-11 Feb 2019	1,243.5	6	7	5	1	0	5	1	0	1.93 ± 0.18	.03	.09	.14	.18	.29
	Guam total	6,737.0	20	27	14	6	0	18	2	0						
Rota																
R1	04–07 Jun 2019	286.0	20	35	9	5	6	5	9	6	7.09 ± 1.71	.28	.47	.61	.67	.73
R2	11–14 Jun 2019	334.0	12	14	4	4	4	2	6	4	20.83 ± 12.65	.02	.07	.14	.25	.39
R3	11–14 Jun 2019	311.0	17	27	3	5	9	7	1	9	9.37 ± 1.79	.27	.76	.84	.87	.87
R4.1	28 Jun–07 Jul 2019	1,285.5	92	196	48	44	0	51	41	0	34.73 ± 4.52	.12	.59	.83	.91	.94
R4.2‡											19.99 ± 4.52	.69	.89	.96	.96	.96
R5	27 Jul-05 Aug 2019	1,136.5	72	145	40	32	0	56	16	0	21.86 ± 3.21	.02	.10	.21	.36	.48
	Rota total	3,353.0	213	417	104	90	19	121	73	19						
Total		10,090.0	233	444	118	96	19	139	75	19						

[†] One corrected trap night equals one active trap night corrected for sprung (via target and non-target captures and false trips) and non-functioning/missing traps by considering them to represent half of a night of trapping effort and no trapping effort, respectively:

corrected trap nights = traps × nights -
$$\left(\frac{targets + nontargets + false trips}{2}\right)$$
 - (nonfunctioning + missing)

(Nelson and Clark 1973).

⁴ We did not live-trap in Rota grid R4 twice. Instead, we euthanized all individuals that were live-trapped on 07 July 2019, the fifth/ final day of trapping, and used the total number of rats removed—including any that died incidentally during trapping—to calculate a reduced density estimate for the second chew-card session during 09–13 July 2019 (R4.2).

higher than those on Guam (\widehat{D} range = 0.00–1.93 rats/ha). At the two grids we resampled after lethal snake treatments on Guam, G3.2 and G2.2, rat density increased by 28% and 41%, respectively, but remained comparatively low even three months after snake control was applied ($\widehat{D} = 1.01$; $\widehat{SE}[\widehat{D}] = 9.65$ and $\widehat{D} = 1.93$; $\widehat{SE}[\widehat{D}] = 0.18$, respectively; Table 1; see Suppl. material 2: Fig. S1).

Chew-cards

We deployed 1,389 chew-cards during 60 days of sampling on Guam (n = 6 deployments) and Rota (n = 6 deployments). The mean proportion of cards chewed after five days was 0.12 (SD = 0.09) on Guam and 0.73 (SD = 0.24) on Rota. On average, the proportion of cards with chews increased by 0.03 (SD = 0.03) a day on Guam and 0.10 (SD = 0.10) a day on Rota.

The proportion of cards chewed by rats was correlated with density estimates when cards were left in the field for at least three nights (Fig. 5). The correlation increased daily and was highest after five nights ($R^2 = 0.74$). When chew-cards were deployed for



Figure 3. Boxplots depicting the medians (bold lines), interquartile ranges (IQRs; 25th–75th percentiles; rectangles), minimums (first quartile-1.5*IQR) and maximums (third quartile+1.5*IQR; dashed lines), and any outliers (black dots) for **A** mass, **B** head-body length, and **C** body condition index for live-trapped rats (*Rattus* spp.) in forest habitats on Guam (n = 19 rats) and Rota (n = 163 rats) in the Mariana Islands during June 2018–August 2019. Statistics shown in the bottom-left corners are for Wilcoxon rank-sum tests ($\alpha = 0.05$). Rats were **A** heavier and had **C** higher body condition indices on Guam compared to Rota, but there was no difference in **B** head-body lengths between the two islands.



Figure 4. Capture (\hat{p}) and recapture (\hat{c}) probability estimates from closed-capture conditional likelihood models for rats (*Rattus* spp.) in forest habitats on Guam (G1–4) and Rota (R1–5) in the Mariana Islands during June 2018–August 2019.

five nights, a 10% increase in the proportion of cards chewed equated to an estimated increase in rat density of approximately 2.4 individuals per ha:

rat density = 23.51 (*cumulative proportion of cards with rat chews*)

Note that an intercept (B_{ρ}) was not included in this equation because it rounded to zero.

We deployed cameras on 60 cards and processed > 24,000 photos with animals on the cards. Twenty-eight of these cards had field recordings of rat chews, and we confirmed rat identification via photos at 27 of 28 (96%) of the card/camera nights (e.g., Fig. 6).



Figure 5. Linear regressions and Pearson's product-moment correlations to assess the relationship between the cumulative proportion of cards with rat (*Rattus* spp.) chews after one, two, three, four, and five nights (*x*-axis) and capture-mark-recapture density estimates plus/minus standard error ($\hat{D} \pm SE$; *y*-axis) in forest habitats on Guam and Rota in the Mariana Islands during June 2018–August 2019.

Discussion

Our study demonstrated a positive, monotonic relationship between chew-card counts and rat density estimates across a range of densities in Guam and Rota forests, and we thus conclude that chew-cards provided a valid index of rat densities and may be effective on similar tropical islands. Specifically, counts from chew-cards deployed for 3–5



Figure 6. Trail-camera photo of a rat (*Rattus* spp.) leaving visible chews on a chew-card. We used trail cameras to confirm or refute rat-chew identification at randomly selected cards from each grid. Emma B. Hanslowe photograph captured by an automated camera trap on 10 July 2019 in forest habitat on Rota in the Mariana Islands.

nights correlated with rat capture-mark-recapture density estimates. This relationship was retained across rat density estimates ranging from 0–35 rats/ha and after management. The correlation between the proportion of cards with rat chews and capture-mark-recapture density estimates increased daily and was highest after five nights, when nearly three quarters of the variance in capture-mark-recapture density estimates was predicted by variation in chew-card proportions ($R^2 = 0.74$). Accordingly, chew-cards should be deployed for a minimum of three nights, but five nights is optimal as this duration provided the smallest standard error around the regression line. Evaluating longer chew-card deployment periods (≥ 6 nights) may be advantageous, as additional nights might have even stronger correlations with rat density. However, the proportion of cards chewed will eventually stabilize or become 1.0 when all the cards are chewed, and this may occur more quickly at high rat densities (Burge et al 2017; Forsyth et al. 2018).

While chew-cards have been widely used to assess invasive small mammal populations (Oberg et al. 2014; Rouco et al. 2017; Gormley et al. 2018; Van Vianen et al. 2018; Nottingham et al. 2019; Robinson and Dick 2020; Ross et al. 2020; Nichols et al. 2021; Campos et al. 2022), our study is one of the few to validate this index using measured (capture-mark-recapture) density estimates (but see Caughley et al. 1998). Most studies simply compare chew-cards to other relative abundance indices (Sweetapple and Nugent 2011; Kavermann et al. 2013; Ruffell et al. 2015; Forsyth et al. 2018; Balls 2019; Nottingham et al. 2021; Brown et al. 2022). Different methodologies make it challenging to compare our results with those of previous chew-card studies. However, general conclusions across successful validation studies, including ours, as well as studies that compared multiple indices were consistent: chew-cards can represent relative differences in small mammal abundances or densities (Caughley et al. 1998; Sweetapple and Nugent 2011; Kavermann et al. 2013; Ruffell et al. 2015; Forsyth et al. 2018; Balls 2019; Nottingham et al. 2021; Brown et al. 2023).

Rat chews were easily distinguished from non-target chews (e.g., feral cats [*Felis catus*] and crab [*Coenobita brevimanus*; *Birgus latro*] pinches) and correctly identified in our study, as confirmed by our camera-trap data. Specifically, rats were photographed chewing cards at nearly all cards positive for rat chews (27 of 28 [96%] card/camera nights). The single unconfirmed chew was likely not misidentified but was more likely not captured because the camera's motion detection did not trigger. We switched camera settings from motion detection to time-lapse after this occurrence to improve rat detection on cameras, and all rat chews corresponding to a camera-trap night were photographed thereafter. Our study was the first to confirm chew-card species identification with cameras, as recommended by Forsyth et al. (2018).

We encountered significant issues with tracking ink during our study. First, the Marianas' tropical climate caused the ink to run and fade. Second, a multitude of nontarget species (e.g., geckos, skinks, crabs, snails/slugs, ants, worms) left unidentifiable tracks that made distinguishing any rat tracks difficult, time-consuming, and erroneous. Similar to other studies, we found that tracking ink provided little additional information relative to chew marks alone (P. J. Sweetapple, Manaaki Whenua Landcare Research, written comm, 08 Sep 2018), and recent studies have discontinued its use in New Zealand (Ruffell et al. 2015; Burge et al. 2017; Forsyth et al. 2018; Nottingham et al. 2021). Protecting the tracking ink and contact paper from the elements (e.g., placing them in tunnels) may reduce these issues in the tropics. A 'tracking tunnel' method was successful on tropical islands in the Caribbean with a similar non-target assemblage consisting of small lizards and invertebrates (Shiels et al. 2020). However, additional work with tracking ink may not be pressing, given our promising findings with chew-cards.

Non-targets may further hinder chew-card efficacy in Mariana Island forests via bait consumption and interspecific interference. We observed bait consumption by ants in the field, and reduced bait availability likely reduces chew-card attraction/effectiveness. In forests with abundant ants, chew-cards may be ineffective (pers. obs.). Use by non-targets may also affect rat chew-card detection (i.e., interspecific interference); for example, two studies in New Zealand found that individuals of one species were less likely to chew cards if they had already been chewed by another species (Sweetapple and Nugent 2009; Burge et al. 2017). Rats may be deterred from chew-cards if other species, especially stinging ants (e.g., little fire ants [*Wasmannia auropunctata*]) or coconut crabs, known predators of rats, are present. Further investigations of the relationships among rat chew-card detection rates, bait availability, and non-target species are warranted. Non-target exclusion methods could also be explored.

Our study results suggest that chew-cards can be appropriate for monitoring changes in rat distribution or relative density over space or time in association with invasive predator (e.g., brown treesnake) occurrence or suppression efforts in Mariana Island forests. Chew-cards have several advantages over capture-mark-recapture density estimation, at the forefront of which is cost. Extrapolating from cost analyses conducted by Wiewel et al. (2009b) and Sweetapple and Nugent (2011), we calculated that a single 11 × 11 grid with 12.5-m spacing costs roughly 10 times more to employ capture-markrecapture methods (~ U.S.\$3.000) than chew-cards (~ U.S.\$300). Like many indices, the reduced cost and simplified logistics of chew-cards make them more feasible for application at larger scales. Chew-cards also require less training and impose less risk than capture-mark-recapture sampling (e.g., no animal handling) and require minimal quantitative skills to use and interpret. However, capture-mark-recapture density estimation remains vital to scientists and managers by providing measures of precision and demographic and morphological data necessary for many studies and management decisions.

Conclusion

Controlling invasive species on islands is a global conservation priority (Doherty et al. 2016), and cost-effective monitoring tools can stretch limited resources and enhance our understanding and management of islands with invasive species. Chew-cards can provide accurate indices of differences in rat densities in Mariana Island forests and, potentially, similar habitats when deployed for 3–5 nights. Chew-cards are a cheaper alternative to capture-mark-recapture sampling when relative differences in density are of interest and measures of precision or ancillary data are unnecessary. This is likely to be the case for many situations in the Marianas because rats are, foremost, prey for a more damaging invasive predator, the brown treesnake; chew-card based indices will

likely detect the major fluctuations in prey density that we expect following effective management of invasive predators (Howald et al. 2007). Further, with reduced costs and simplified logistics, chew-cards can be deployed more often or in more areas to gather estimates of relative rat densities and precision over time and space. These data could be used to inform invasive species control efforts, assess treatment efficacy, and investigate invasive predator-prey dynamics, all of which improve success of invasive species management on islands to preserve global biodiversity.

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Supplementary material I

Appendix 1, Tables S1–S4

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- Explanation note: Model selection results. Table S1. Guam: Huggins' closed-capture conditional likelihood model selection results for combined Guam grids sampled during June 2018-February 2019. Table S2. R1-3: Model selection results for spatially explicit models fit to data collected during June 2019 from grids for which we did not collect individual covariates. Results from the temporal models only (Step 1) are provided by grid. Table S3. R4: Spatially explicit model selection for rats sampled during June–July 2019. Step 1 models include all hypothesized sources of individual variation in capture probability (sex + age + BCI + temporal structures) listed below. We retained the best-supported temporal structure (behavior) when testing all possible additive combinations of individual covariates in Step 2 (sex, BCI, age, and no individual heterogeneity). Table S4. R5: Spatially explicit model selection for rats sampled during July-August 2019. Step 1 models include all hypothesized sources of individual variation in capture probability (sex + age + BCI + temporal structures) listed below. We retained the best-supported temporal structure (behavior + neophobia2) when testing all possible additive combinations of individual covariates in Step 2 (sex, BCI, age, and no individual heterogeneity).
- 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.74.80242.suppl@1

Supplementary material 2

Figure S1

Authors: Emma B. Hanslowe, Amy A. Yackel Adams, Melia G. Nafus, Douglas A. Page, Danielle R. Bradke, Francesca T. Erickson, Larissa L. Bailey Data type: pdf file

- Explanation note: Density estimator comparison. **Fig. S1.** Comparison of three density estimation approaches for rats (*Rattus* spp.) using capture-mark-recapture data from Guam (G1–4) and Rota (R1–5) forest grids during June 2018–August 2019. Black and dark gray bars represent density estimates ($\hat{D}s$) calculated from model-averaged abundance estimates ($\hat{N}s$) divided by effective trapping areas (ETAs) calculated by adding boundary strips equaling half of the mean maximum distances moved by rats captured more than once (0.5MMDM) and the full MMDM, respectively. Light gray bars represent $\hat{D}s$ from spatially explicit capture-recapture (SECR) models for sites on Rota only.
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DISCUSSION PAPER



Native-alien populations—an apparent oxymoron that requires specific conservation attention

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Abstract

Many countries define nativity at a country-level-taxa are categorised as either alien species or native species. However, there are often substantial within-country biogeographical barriers and so a taxon can be native and alien to different parts of the same country. Here, we use the term 'native-alien populations' as a short-hand for populations that result from the human-mediated dispersal of individuals of a species beyond a biogeographical barrier to a point beyond that species' native range, but that is still within the same political entity as parts of the species' native range. Based on these criteria, we consider native-alien populations to be biological invasions. However, we argue that, in comparison to other alien populations, native-alien populations: 1) are likely to be closer geographically to their native range; 2) are likely to be phylogenetically and ecologically more similar to native species in their introduced range; and 3) options to control their introduction or manage them will likely be more limited. We argue this means nativealien populations tend to differ from other alien populations in the likelihood of invasion, the types of impacts they have, and in how they can be most effectively managed. We also argue that native-alien populations are similarly a distinct phenomenon from native populations that are increasing in abundance or range extent. And note that native-alien populations are expected to be particularly common in large, ecologically diverse countries with disjunct biomes and ecoregions. Reporting, monitoring, regulating and managing native-alien populations will, we believe, become an increasingly important component of managing global change.

Keywords

Alien species, biogeographical barriers, dispersal, human agency, native species, terminology

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Introduction

The regulation and management of biological invasions often focus on the specieslevel [e.g. the current Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services thematic assessment is on 'invasive alien species' (IPBES 2019)]; however, biological invasions are fundamentally a population-level phenomenon (Essl et al. 2020). A species might be native to a part of a country (or part of another political entity at which level management decisions are made), but individuals and/or propagules can be moved by humans to another part of the country or political entity (e.g. provinces, states etc.) where the species is not native (Spear and Chown 2009). Therefore, a species can have both native and alien populations within the same country (Fig. 1 and Table 1). For example, some plants that are native to Eastern Australia have been transported by humans and have become invasive in Western Australia (e.g. *Pittosporum undulatum*) and vice versa (e.g. *Acacia saligna*) (Head and Muir 2004). The presence of the Nullabor Plain, as a biogeographic barrier separating Eastern and

Phenomenon		Description	Examples
~Stable Native range	Country 1	Species X had three native populations. There has been no significant recent changes in the number, extent or abundance of populations.	For many species endemic to South Africa, the Cape fold mountains separate potentially suitable ranges from currently occupied native ranges (e.g., Proteaceae species, Manning 2018).
Human-mediated increase in native abundance	Country 1	Recent human-induced environmental change has led to an increase in the abundance of Species X within its original native range	The rock lobster (Jasus lalandii) has increased in abundance in its native range in the Western Cape province because an increase in nutrient levels has led to an increase in food availability (Van Zyl et al. 1998)
Natural range expansion	Country 1	Species X naturally spread forming a new native population	The Murex snail (<i>Acanthinucella spirata</i>) evolved morphologically in the Pleistocene and consequently expanded its range on the coast of California in the United States of America (Hellberg et al. 2001).
Range expansion in response to human-induced environmental change	Country 1	Human modification of the environment meant that a new range became suitable for colonisation by Species X, which naturally spread to form a new native population	Birds and butterflies in Europe have shifted their ranges due to climate change (Devictor et al. 2012)
Biological invasion (within-country)	Country 1	Species X was dispersed by humans within Country 1 forming a new alien population In this paper we refer to native species with alien populations as native-alien populations.	The guttural toad (Sclerophrys gutturalis) has been introduced from its native range in KwaZulu-Natal province in South Africa and established a native-alien population in the Western Cape province where it is not native (Measey et al. 2017)
Biological invasion (between countries)	Country 2 Country 1	Species X was dispersed by humans from Country 1 to Country 2 forming a new alien population. Both the species and the population can be regarded as alien to the whole of Country 2.	240 tree species alien to South Africa have established populations (Richardson et al. 2020)
a a realized range, RR	Alter in harve range, /// Alter in	ange, meantain, enoccupied range	

🚚 Human assisted dispersal; 🔖 Natural dispersal; 🏜 Human induced environmental change

Figure 1. How the concept of native-alien populations differs from other instances of changes in range/ abundance. These are idealised versions and are not mutually exclusive. See Table 1 for a summary of how the different phenomena differ in terms of processes and properties.

Table 1. The properties of native-alien populations and other related phenomena. The presented processes and properties are based on Essl et al. (2019) and Ogden et al. (2019). The situation for a stable native range is not shown as it forms the baseline against which the other phenomena are compared.

Phenomenon	Biogeographic barrier	Survival and reproduction	Distance from native range	Range expansion within political	
Human-mediated increase in native abundance	No barrier crossed	There is likely to have been an increase in survival or reproduction	Within native range	No range expansion	
Natural range expansion	Biogeographic barrier crossed naturally	Rates need not have changed in most of the native range, but some increase in neighbouring areas	Within natural dispersal distance of native range	Within or between political entities	
Range expansion	Human-induced	There is an increase in	Within natural dispersal	Within or between	
in response to	changes might	survival and reproduction in	distance of native range	political entities	
human-induced	have weakened	neighbouring areas			
environmental	biogeographic				
change	barriers				
Biological	Biogeographic	Individuals will not always	Further than natural	Within political	
invasion (within-	barrier crossed by	survive and reproduce in the	dispersal distance from	entity	
country)	human agency	new range, but could if the	native range		
Biological	Biogeographic	Individuals will not always	Further than natural	Between political	
invasion (between	barrier crossed by	survive and reproduce in the	dispersal distance from	entities	
countries)	human agency	new range, but could if the environment is suitable	native range		

Western Australia, means that such cases are relatively clear-cut; however, in other cases, whether populations should be considered as native or alien is uncertain. For example, following the introduction of the American bullfrog (*Lithobates catesbeianus*) from the eastern United States of America (USA) to some of the country's western states, there was confusion over the species' status—populations were classified as native by some researchers and alien by others (Guo and Ricklefs 2010). Confusion over how such introductions should be classified is partly the result of uncertainties in defining native ranges (Webber and Scott 2012; Essl et al. 2018; Pereyra 2019), partly as the phenomenon has not been clearly defined, and partly as biosecurity is implemented primarily at a country's borders and not always within a country.

As for all introductions, within-country introductions can provide socio-economic benefits (Maciejewski and Kerley 2014). Moreover, assisted migration within a country might also be essential for species' survival (Hunter 2007). However, as with all types of biological invasions, such introductions can pose significant problems. In the USA, rainbow trout (*Oncorhynchus mykiss*), which is native to eastern USA, has been introduced to western USA where it hybridises with California golden trout (*O. mykiss aguabonita*) and Paiute cutthroat trout (*O. clarki seleniris*) (Lockwood et al. 2013). When the California golden trout and Paiute cutthroat trout hybridise with rainbow trout, the offspring are fertile and can mate with either parental population. This has led to introgression which threatens the genetic integrity of these rare native taxa (Moyle 2002) and has led

to declines in their populations (Lockwood et al. 2013). In South Africa, the antelope *Damaliscus pygargus phillipsi* (blesbok) is native to much of the country, but not to the Western Cape province. Blesbok were introduced and established alien populations in the Western Cape, hybridising with the endemic bontebok (*D. pygargus pygargus*) (van Wyk et al. 2017). This hybridisation has occurred between non-admixed bontebok/non-admixed blesbok and hybrids, but no F1 individuals have been identified. Only through concerted and intensive interventions was the extinction of the bontebok prevented.

Alien species that have been introduced from one country to another receive the majority of research attention and biological invasion frameworks are often developed with such introductions in mind. In contrast, those that have established alien populations within countries to which they are native have received relatively little research attention (Vitule et al. 2019). For example, in the Global Register of Introduced and Invasive Species (GRIIS), only a few countries (including Spain and the USA) report such populations-the majority of countries (including Brazil and South Africa) do not (http://www.griis.org: Data accessed 20 July 2021). Moreover, in the USA, while the presence of alien populations of species that are native to the USA have been recognised, the severity of their potential impacts has been neglected (Guo and Ricklefs 2010). Globally, established alien populations of species that are native at the countrylevel are often ignored in analyses and, consequently, the scope of biological invasions and their impacts are underestimated and management actions could be misinformed (Vitule et al. 2019). This gap in research is partly because most of the monitoring, reporting, and management of biological invasions is performed at national or larger administrative levels [e.g. through national-level reports to the Convention on Biological Diversity (CBD)]. While national and international mechanisms seek to manage the movement of species between countries (such as CITES), instruments that control the purposeful or inadvertent human-mediated within-country movement of species are, in general, lacking, and in countries where such regulations do exist the legislation is often poorly enforced (Measey and Davies 2011).

In this perspective piece we: 1) define this phenomenon; 2) contrast it with other forms of range changes; 3) discuss expectations of how this phenomenon is likely to differ from other biological invasions; 4) identify situations where it is most likely to occur; and 5) discuss the management implications.

A proposed definition

The presence of biogeographical barriers means that some species occur in the same place at the same time (sympatric speciation), while other groups of organisms are separated by a physical or geographic barrier (allopatric speciation) (Orr and Smith 1998). Sympatric speciation is defined as evolution of intrinsic barriers to gene flow in the absence of extrinsic barriers, while allopatric speciation is the evolution of intrinsic barriers to gene flow in the presence of extrinsic barriers (Orr and Smith 1998). An **Box I.** Terms used to describe native-alien populations.

The ISI Web of Knowledge and Google Scholar were searched between May and June 2020 using the following search strings: "Intra-country established alien species", "Intracontinental exotics", "within-country aliens", "within-country movement of native species", "native-alien populations", "extralimital species", "alien natives", "domestic exotics", "native alien species". Note that 'native invaders' (sensu Simberloff 2011) are distinct from 'native-alien populations', as native invaders become 'invasive' (increase in abundance or extent) within their native range (see 'Human-mediated increase in native abundance' and 'Range expansion in response to human-induced environmental change' in Table 1). The following discrete terms were found.

Domestic exotics: Species that form invasive populations outside of their natural distribution, but within the borders of the same nation (Guo and Ricklefs 2010). [6 hits]

Extralimital species: Indigenous species translocated or intended to be translocated to a place outside its natural distribution range, but excluding an indigenous species that has extended its natural distribution range by natural means of migration or dispersal without human intervention (Spear and Chown 2009). [> 20 hits]

Home-grown exotic: Species that form invasive populations outside of their natural distribution, but within the borders of the same nation (Cox 1999). [1 hit]

Intra-country established alien species: Species that are introduced and establish amongst regions or in a novel region within the same country (Vitule et al. 2019). [1 hit]

Native-alien species: Species native to some areas of a country or territory, but introduced by humans into places outside of their natural range of distribution in that country, where they become established and disperse (Pagad et al. 2018). [1 hit]

While the term 'extralimital species' was the most common, we prefer 'native-alien' as it is explicit regarding the population's status at political and biogeographic levels and as it is currently used in the Global Register of Introduced and Invasive Species (Pagad et al. 2018). However, we adapted the term (to "native-alien population") to reflect that invasions are a population level phenomenon. The global biodiversity standard, Darwin Core, currently allows for each record to be classified as either native or introduced to that site according to the term 'establishmentMeans' as: introducedAssistedColonisation, vagrant and uncertain (https://dwc.tdwg.org/em/; Groom et al. 2019). Native-alien populations would, therefore, be classified as introduced, but this will not separate native-alien populations from other alien populations, unless linked to additional information on national status, it will be important that this is clarified in any future revisions to the term.

alien species is defined as an organism whose presence in a region is due to humanmediated dispersal (i.e. direct human agency or substantial indirect human agency) across a biogeographic barrier to a site where the species has not recently naturally occurred (Essl et al. 2018). Though here we note that the use of the term 'species' is a misnomer, as biological invasions and evolution operate at the population-level. Definitions differ as to what constitutes an invasive population, but it is generally taken to be alien organisms that survive and spread from sites of introduction to form selfsustaining populations (Blackburn et al. 2011), that, in some definitions, may cause negative impacts (e.g. CBD 2002). Regardless of the precise definition, the relevant biogeographic barriers that separate native ranges from (potential) alien ranges need not coincide with political boundaries. These biogeographical barriers include abiotic barriers, such as mountain ranges and changes in climatic conditions, and biotic barriers, such as the absence of key interacting species. As a consequence, if individuals are moved by humans within a country to which they are native and this results in the establishment of a population beyond the species' native range, a species can technically be both alien and native in the same country (Spear and Chown 2009). Hereafter,

we refer to such populations using the short-hand 'native-alien populations' (see Box 1 for other terms used). This term might seem oxymoronic, but 'native' refers to the status of the population in a political entity (e.g. a country), while 'alien' refers to biogeographical status. This means that, in the context of alien-native populations, the terms alien and native can refer to status at different spatial scales. As with alien populations introduced from other countries, the status of a native-alien population can be classified as casual, established or invasive [as per the recently adopted Darwin Core term 'dwc:degreeOfEstablishment' (see Groom et al. 2019)]. To facilitate the implementation of the term native-alien populations, we have developed a protocol, based on the definition (manuscript in preparation). This means there is a process both to circumscribe the phenomenon and to confirm instances, with a clear link through to the causes and consequences (Latombe et al. 2019).

We, therefore, define a native-alien population as a population that is: (1) within a country to which the species is native, (2) founded by individuals moved by direct human agency [or substantial indirect human agency, see Essl et al. (2018)], (3) over a biogeographical barrier and (4) to an area beyond the species' native range. We believe the use of this term is justified because, while native-alien populations are a subset of alien populations, their properties are likely to differ from other alien populations and these differences are likely to have consequences for invasion success, impacts, management and regulation (Table 1). The development of a clearly-defined term that distinguishes these populations from other range changes and alien populations will be beneficial, as it will enable the development of conceptual frameworks that can be used to classify these populations and so reduce uncertainties in invasion science (Heger et al. 2021). Various terms are currently in use for the native-alien population phenomenon (Box 1) and, therefore, we encourage one terminology be used by everyone globally.

Native-alien populations differ from other forms of range change

The capability of an organism to colonise suitable, but unoccupied habitats or environments through natural dispersal depends on its dispersal traits. The dispersal of a species is facilitated by three processes: (1) natural processes (evolution and natural environmental changes); (2) human-mediated dispersal (including biological invasions); and (3) human-induced environmental change (i.e. land-use change, human-disturbance, human-mediated climate change) (Table 1). Evolutionary changes that could facilitate range expansion include shifts in host range or the development of resistance to herbicides. As an example, the murex snail (*Acanthinucella spirata*) evolved morphologically in response to climatic changes in the Pleistocene and consequently expanded its range on the coast of California in USA (Hellberg et al. 2001). Similarly, native species can shift their ranges by responding to natural environmental changes. Natural range expansion and contraction has been reported in a number of taxa in response to natural climatic variation, where species ranges expand into cooler regions

when the climate warms and then contract again during cooling periods (Parmesan and Yohe 2003). Examples of this phenomenon have been reported for marine fish, limpets, barnacles, and zooplankton in the United Kingdom (Southward et al. 1995) and butterflies in Finland and Sweden (Henriksen and Kreutzer 1982; Parmesan et al. 1999). These range expansions and contractions are infrequent and usually occur adjacent to the native range. Human-assisted dispersal can occur through the intentional or unintentional transport of propagules by humans, either within or between countries, to different biogeographical regions. For example, Sclerophrys gutturalis (Guttural toad), which is native to South Africa, has been introduced unintentionally by humans to areas outside its native range within South Africa (Measey et al. 2017), while 240 tree species, alien to South Africa, have been introduced from Australia (Richardson et al. 2020). Species can also spread into new areas by tracking human-induced environmental changes, such as climate change or the removal of predators (Essl et al. 2019). For example, birds and butterflies in Europe have shifted their ranges due to climate change (Devictor et al. 2012). Alternatively, human-modification of the environment can facilitate an increase in the abundance of species within their native ranges. For example, the rock lobster (Jasus lalandii) has increased in abundance in its native range in the Western Cape province of South Africa because an increase in nutrient levels has led to an increase in food availability (Van Zyl et al. 1998). Human assisted dispersal of organisms to new regions, whether within or between countries, is likely to result in reproductive isolation as the newly-formed native-alien or alien population could be isolated from its native range by a biogeographical barrier and would result in biotic homogenisation at the species-level. In contrast, changes to the abundance and/ or range of organisms within or adjacent to their native range, due to natural processes or human-induced environmental changes, are unlikely to result in reproductive isolation, but will often also lead to biotic homogenisation (McKinney 2005).

The three processes that facilitate dispersal (natural processes, human agency and human-induced environmental change) can act synergistically to ensure that a species reaches suitable, but unoccupied habitat (Essl et al. 2019). For example, species can be moved by humans from their native range to new areas that were previously not suitable for establishment, but are now suitable due to human-induced environmental changes. In addition, many synanthropic species (e.g. the house mouse) would be expected to show increases in abundance and extent of populations within their native ranges, i.e. as a result of human modifications to the environment, they might also have formed alien populations in countries to which they are alien and in countries to which they are native to a part of. The three processes described above result in a number of distinct phenomena that will tend to differ in key features (Fig. 1, Table 1). Here, we focus on why native-alien populations will likely differ from alien populations introduced from other countries in several important ways and, as a consequence, the likelihood of invasion and the types and magnitude of impact these phenomena have are likely to differ, noting that native-alien populations will only occur under the conditions defined above.

Native-alien populations are expected to be physically much closer to their native range than alien populations introduced from other countries, with the geographic distance roughly an order of magnitude different (Fig. 2; t = 15.6, df = 64.4, P < 0.001). Given the shorter geographical distances, it is likely that native-alien populations will differ from alien populations introduced from other countries in key properties of dispersal, including propagule pressure, genetic diversity (Vilatersana et al. 2016), potential for simultaneous movement of co-evolved species, selectivity of what is moved, and the duration of dispersal opportunities (Wilson et al. 2009). Such differences may lead to quantitative and qualitative differences in the probabilities of establishment and invasion and in the types of impact that are likely to occur. For example, the relatively short distance between these native-alien populations and their native range, means that propagule pressure [i.e. encompassing the number of individuals introduced and the number of introduction events for any particular species (Lockwood et al. 2009)] will likely be higher than for alien populations introduced from other countries. In addition, the higher the number of introduction events the greater the chance that propagules come from a wide variety of sources and the higher the potential genetic diversity. Therefore, genetic diversity is potentially higher for native-alien populations than for alien populations introduced from other countries (Vilatersana et al. 2016). These differences will have consequences for invasion potential (Bossdorf et al. 2005; Roman and Darling 2007) because, if propagule pressure is low, the entire genetic diversity of the species is unlikely to be present in the introduced individuals (Wilson et al. 2009) and this could



Figure 2. Density plot showing the distance between any two random points within a country and between two random points in different countries (t = 15.6, df = 64.4, P < 0.001). The distance between random points within a country ('within-country') represents the distance between native-alien populations and their native range, while the distance between random points in different countries ('between countries') represents the distance between alien populations introduced from other countries and their native range. See Suppl. material 1 for full methods.

result in genetic bottlenecks that reduce the chances of survival (Excoffier et al. 2009). In contrast, high propagule pressure is likely to result in a large proportion of the total genetic diversity of a species being present in the introduced population, increasing the chances of species survival and invasion success (Roman 2006).

Table 2. Number of plant species with native-alien populations and alien populations introduced from other countries that are in the same genus and family as native species in their alien range, at local and national levels. Local level is the Garden Route National Park in South Africa (Baard and Kraaij 2019), while the national level is the whole of South Africa, excluding islands (SANBI 2019). These data were analysed using Chi-square tests and Fisher's exact tests (in instances where there were expected values of less than 4, see Crawley 2007). See Suppl. material 2 for full methods.

a) Local (the Garden Route National Park)			
	Native-alien	Alien to the whole	Analysis
	populations	of South Africa	
Number of species with congeners present	14 (93%)	10 (10%)	P = < 0.001, Fisher's exact test
Number of species with confamilials present	15 (100%)	72 (72%)	P = 0.020, Fisher's exact test
Number of species	15	100	
b) National (South Africa)			
	Native-alien	Alien to the whole	Analysis
	nonulations	of South Africa	
	populations	of South Affica	
Number of species with congeners present	71 (95%)	900 (23%)	$\chi^2 = 201.25, df = 1,$
Number of species with congeners present	71 (95%)	900 (23%)	$\chi^2 = 201.25, df = 1,$ P = < 0.001
Number of species with congeners present Number of species with confamilials present	71 (95%) 75 (100%)	900 (23%) 2230 (57%)	$\chi^2 = 201.25, df = 1,$ P = < 0.001 $\chi^2 = 56.008, df = 1,$
Number of species with congeners present Number of species with confamilials present	71 (95%) 75 (100%)	900 (23%) 2230 (57%)	$\chi^{2} = 201.25, df = 1,$ P = < 0.001 $\chi^{2} = 56.008, df = 1,$ P = < 0.001

In the context of invasion science, alien organisms are expected to be ecologically novel in their introduced range (i.e. evolutionarily and ecologically different from native species) (Saul and Jeschke 2015). However, as there is a relatively short geographical distance between native-alien populations and their native range (Fig. 2), there tends to be a greater number of closely-related taxa in the introduced range of nativealien populations in comparison to alien populations introduced from other countries (see Table 2) and this means that native-alien populations are likely to be less phylogenetically and ecologically distinct from native populations in their alien range (Saul and Jeschke 2015; Essl et al. 2019). This will have consequences for the probability of invasion [cf. Darwin's Naturalisation Hypothesis (Darwin 1859; Daehler 2001)] and the types of impact. As there has been less time for differentiation or reproductive isolation, native-alien populations might be less likely to possess traits that are new to the alien range (e.g. novel weapons), but more likely to occupy similar niches to those occupied by native populations (Callaway and Ridenour 2004). Consequently, native-alien populations are, in general, more likely to experience higher levels of competition (Gilbert and Levine 2013) and natural enemies (Enders et al. 2020) in their introduced range, but are also more likely to be suited to the abiotic conditions (e.g. climate), and suitable mutualists are more likely to be present. Native-alien populations are also more likely to hybridise with closely-related native populations (Bossdorf et al. 2005; Roman and Darling 2007; and examples of rainbow trout introductions in USA and blesbok introductions in South Africa, discussed above).

Which conditions give rise to native-alien populations?

Native-alien populations can be found in any nation where biogeographic barriers prevent organisms from dispersing to suitable, but unoccupied ranges. However, large countries are, generally, more environmentally heterogeneous than smaller countries (Fig. 3 and Suppl. material 3: Fig. S1b). Large countries tend to have more biomes (Fig. 3a) and more ecoregions (Suppl. material 3: Fig. S1a) than smaller countries; and have more biomes (Fig. 3b) and ecoregions (Suppl. material 3: Fig. S1b) that are non-contiguous. Therefore, while country size is an imprecise proxy of environmental heterogeneity and the presence of biogeographical barriers, nativealien populations are likely to be more common in large countries than small countries. We note that native-alien populations are likely to be particularly prevalent in countries like Russia, the USA, and India, because they have a relatively high number of biomes and ecoregions, and a high number of non-contiguous biomes and ecoregions (Fig. 3; Suppl. material 3: Fig. S1). We tried to explore this issue using a global dataset of bird introductions, but even for this well-studied group, the data quality was not sufficiently reliable (see Suppl. material 3).

We also hypothesised that taxa that are both poor dispersers, and that are likely to be moved by humans are most likely to form native-alien populations. These are taxa for which dispersal distances are short enough that the native range can be restricted to one part of a country, and suitable alien range can only be reached with substantial assistance from humans. However, we did not find a suitable dataset to test this. Testing



Figure 3. The relationship between country size and a) the number of biomes in the country (Generalised linear model: t = 19.20, df = 106, P < 0.001); and b) the number of biomes with non-contiguous patches (Generalised linear model: t = 24.45, df = 106, P < 0.001). A similar pattern is evident for ecoregions (Suppl. material 3: Fig. S1). See Suppl. material 3 for full methods. USA: United States of America, Ind: India, Chi: China, Rus: Russia.

which species-level traits are more likely to result in native-alien populations will require some careful analyses, but will be important to better understand the phenomenon and, arguably, might reveal differences in the propensity of taxa to become invasive.

Management implications

A country's conservation or biodiversity management goals play a crucial role in determining whether a population is classified as native or alien. The focus of management goals has consequences because if too much attention is paid to preventing new introductions from other countries, then within-country invasions could be missed. For example, in USA, the impacts of native-alien populations have been realised, but the management response has been delayed (Guo and Ricklefs 2010), while the impact of alien populations introduced from other countries have been given a full management response. Therefore, native-alien populations are treated and managed differently by different countries. However, there may also be differential management across lower political levels (e.g. provinces, states) and, consequently, nativealien populations could be managed in different ways (as native or alien) in different parts of the same country. However, such differential management may make sense in some cases, for example, in cases where provinces or states vary in size or vary in their biological diversity, native-alien populations may be more prominent in some provinces or states than others. National legislation can be used to guide the management of native-alien populations. For example, in South Africa, native species, such as Sclerophrys gutturalis and Hyperolius marmoratus (both amphibians), are listed under the National Environmental Management: Biodiversity Act (NEMBA) as invasive species that require compulsory control in the Western Cape province, but are not listed as invasive species in their native ranges in the Limpopo, Mpumalanga, and KwaZulu-Natal provinces (Department of Forestry Fishery and the Environment 2013; Measey et al. 2017). As these native-alien populations can result in invasions at provincial or state levels and cause negative impacts on native populations where introduced, sub-national regulation might be preferable. For example, an analysis of native and alien plant distributions by Rouget et al. (2015) supported biome-level strategies for the control of alien plant species in South Africa. There is, thus, a need for a careful alignment of management and policy between different geographic and political scales from national to local. However, while it might make more ecological sense to regulate and manage native-alien populations, based on biogeography, this is often impractical both due to bureaucracy and biology. Funds and management are often administered according to political boundaries and which biogeographic breaks are important might be highly context-specific.

Classifying the introduction status of populations relies largely on knowing where the native range is within a country. This is expected to be easy for taxa, such as large mammals, that have been monitored and tracked over time (Skinner and Chimimba 2005) and for which data on human-mediated transportation exist. Conversely, it will be very difficult for other taxa, such as microbes, for which the native range is not well circumscribed and that have been moved unintentionally by humans using vectors and pathways that are poorly understood. For example, it is difficult to identify the location of the native range of marine species due to a lack of surveys across a number of marine environments, a lack of taxonomic expertise, the use of different terms in marine invasion science and challenges with taxonomic resolution at a global scale for a number of species (Robinson et al. 2005; Mead et al. 2011; Robinson et al. 2016). Native ranges are likely to expand and contract naturally and, in some instances, species might be introduced by human action into areas where they have historically occurred. This creates problems when identifying native-alien populations as these shifts increase uncertainty when describing the native range.

Conclusion and recommendations

We have argued here that native-alien populations will likely differ from other biological invasions and other forms of range shifts in terms of geographic, evolutionary, and ecological characteristics. Native-alien populations can cause significant and often specific negative impacts [through hybridisation in particular, for example, van Wyk et al. (2017) and Lockwood et al. (2013)]. We recommend a standardised approach to be used to compile lists of native-alien populations, for example, that taken by the Global Register of Introduced and Invasive Species (Pagad et al. 2018). Management and regulation should also ideally follow relevant biogeographic barriers or at least operate at the political level most relevant for a particular group of taxa, but this is often impractical at present. To conclude, while we recognise that the phenomenon of native-alien populations is an artefact of political boundaries, it has inherent regulatory implications and so the phenomenon must be increasingly and explicitly included in conservation predictions, planning, and management so that these populations are correctly classified, included in alien species inventories, and managed as biological invasions.

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Data resources

Data for this study are available on request from the authors.

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Supplementary material I

File S1

Authors: Takalani Nelufule, Mark P. Robertson, John R. U. Wilson, Katelyn T. Faulkner

Data type: Methods (docx. file)

- Explanation note: Methods for the simulation that illustrates that native-alien populations are likely to be closer geographically to their native range than populations introduced from other countries.
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Link: https://doi.org/10.3897/neobiota.74.81671.suppl1

Supplementary material 2

File S2

Authors: Takalani Nelufule, Mark P. Robertson, John R. U. Wilson, Katelyn T. Faulkner

Data type: Methods (docx. file)

- Explanation note: Method for determining whether native-alien populations tend to have a greater number of closely related taxa (congeneric and confamilial species) in their introduced range than alien populations introduced from other countries..
- 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.74.81671.suppl2

Supplementary material 3

File S3

Authors: Takalani Nelufule, Mark P. Robertson, John R. U. Wilson, Katelyn T. Faulkner

Data type: Methods (docx. file)

Explanation note: Method for testing whether native-alien populations are particularly prevalent in large, ecologically diverse countries.

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DASCO: A workflow to downscale alien species checklists using occurrence records and to re-allocate species distributions across realms

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Abstract

Information about occurrences of alien species is often provided in so-called checklists, which represents lists of reported alien species in a region. In many cases, available checklists cover whole countries, which is too coarse for many analyses and limits capabilities of assessing status and trends of biological invasions. Information about point-wise occurrences is available in large quantities at online facilities such as GBIF and OBIS, which, however, do not provide information about the invasion status of individual populations. To close this gap, we here provide a semi-automated workflow called DASCO to downscale regional checklists using occurrence records obtained from GBIF and OBIS. Within the workflow, coordinate-based occurrence records for species listed in the provided regional checklists are obtained from GBIF and OBIS, and the status of being an alien population is assigned using the information in the provided checklists. In this way, information in checklists is made available at the local scale, which can then be re-allocated to any other spatial categorisation as provided by the user. In addition, habitats of species are determined to distinguish between marine, brackish, terrestrial, and freshwater species, which allows splitting the provided checklists to the respective realms and ecoregions. By using checklists of global databases, we showcase the usage of the DASCO workflow and revealed > 35 million occurrence records of alien populations in terrestrial and marine regions worldwide, which were back-transformed to terrestrial and marine regions for comparison. DASCO has the potential to be used as a basis for the widely applied species distribution models or assessments of status and trends of biological invasions at large geographic scales. The workflow is implemented in R and in full compliance with the FAIR data principles of open science.

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Keywords

biological invasion, checklists, coordinates, distribution, downscaling, GBIF, marine ecoregions, neobiota, open science, workflow

Introduction

The amount of biodiversity data is increasing at an unprecedented pace (La Salle et al. 2016), with occurrence records provided by the Global Biodiversity Information Facility (GBIF) amounting to more than 2 billion records at the date of publication. Other online platforms such as the Ocean Biodiversity Information System (OBIS) are expanding likewise, although at lower levels. These platforms provide the by far largest collections of species occurrence records, which make them most useful for analysing the status and trends of biodiversity in general. The data on these platforms provided a basis for numerous analyses and biodiversity assessments but also exhibited distinct biases, gaps, and heterogeneity in quality and, therefore, should be handled with care to deal with these issues (Meyer et al. 2015; Hughes et al. 2021). Many of the recorded occurrences represent records of species outside their native range, so-called alien populations. However, these databases lack information about the status of invasion, which limits the capabilities to use the data for assessing trends in biological invasions.

As the number of biodiversity records increased, so did the number of records of alien populations collected in regional to global databases. Since 2015, at least seven new global databases of alien species records have been published: five of certain taxonomic groups such as alien plants (van Kleunen et al. 2019), birds (Dyer et al. 2017), mammals (Biancolini et al. 2021), amphibians and reptiles (Capinha et al. 2017) and macrofungi (Monteiro et al. 2020), and two major cross-taxonomic databases, one database on invasive alien species (Pagad et al. 2018) and one on years of first alien species' record (Seebens et al. 2017). Numerous collections at regional levels are available in addition. The standard format of alien species records is a checklist, which represents a list of species reported in a certain region, usually a country (Pyšek et al. 2012; Brundu and Camarda 2013). While these checklists provide a first overview of the distribution of alien species at larger geographic scales, the resolution is often too coarse to perform detailed analyses. For instance, the majority of alien species are still spreading despite their first introduction being decades or centuries ago (Seebens et al. 2021), but the availability of distribution records only at a regional scale distinctly hampers the assessment of the dynamics of spread and severely limits the possibility to predict the future spread and hot spots of alien species occurrences.

The rise of biodiversity data poses new challenges to researchers as the processing of data becomes increasingly complex and time-consuming. As the steps of data processing are often similar in different projects, researchers spent much time on developing very similar approaches multiple times, which is inefficient. In addition, the complexity of data processing requires making many minor decisions of how to handle and modify data, which are usually not reported in the method section of a scientific publication. As a consequence, studies and assessments are non-transparent and not reproducible, which reduces trust in scientific results (Franz and Sterner 2018). It is therefore of rising importance to publish all steps of data processing, the so-called workflows (Hardisty and Roberts 2013). With the rise in data volumes and complexities of data processing, it also becomes crucial to make workflows accessible to others (Guralnick et al. 2007), which provides the opportunity to document all steps of the process accurately, to make studies transparent and reproducible, to increase efficiency in science by allowing others to use the workflow, and to ultimately increase trust in study results.

In recent years, much progress has been made on developing standards, workflows, and infrastructures for biodiversity information. For example, a standard terminology for biodiversity information called Darwin Core (https://dwc.tdwg.org/) has been developed, which allows sharing data more easily (Groom et al. 2019). Workflows (i.e., technical pipelines to process data) have been proposed and developed to clean biodiversity data (Zizka et al. 2019) and to transform the massive amount of occurrence data into workable formats (Guralnick et al. 2007; Jetz et al. 2019). Standard measures of biodiversity have been proposed and accepted, such as the Essential Biodiversity Variables (Pereira et al. 2013) and a range of indicators to actually measure biodiversity change. However, most of these advancements relate to biodiversity information in general, while the specifics of biological invasions were often not taken into account, and similar developments in invasion ecology are lagging behind the general trends. Efforts have been made in some parts. For example, the Darwin Core terminology has been extended to capture aspects of the status of biological invasions (Groom et al. 2019), workflows have been published to integrate global databases (Seebens et al. 2020), and indicators have been developed to measure and visualise trends in changes of biological invasions (Wilson et al. 2018), but still, information about the status of alien species population is usually provided on national scales with all the limitation inherent in such a coarse scale, although higher resolved data are available.

Here, we provide a workflow that integrates the strengths of both the comprehensiveness of point-wise occurrence records provided by GBIF and OBIS and information on invasion status provided in checklists. While GBIF provided the by far largest amount of occurrence data, OBIS represents a platform gathering information about mostly marine species occurrences. Their combination therefore provides a comprehensive compilation of species occurrences across realms. The ultimate goal of applying the workflow is to obtain occurrence records of alien populations with associated coordinates at large extent. By combining regional checklists and occurrence records, the information provided at coarse geographic scale such as regional checklists can be transferred to a finer geographic scale of local occurrences, a process often called 'downscaling' as used in e.g. climate science. Hence, the workflow can be used to downscale alien species checklists using occurrence records, and is therefore called 'DASCO', but also to re-allocate species occurrences to different delineations of regions or realms to generate checklists at alternative spatial resolutions. For instance, a single checklist may contain species from different realms, biomes, or ecotypes. By using coordinate-based occurrence records, it is then possible to split the checklists and assign species to, for example, bordering coastal areas or ecotypes such as mountainous areas within the respective region, and to generate checklists only for those areas with a resolution, which may differ from the original checklist.

In a case study, we showcase the application of the workflow at a global scale using the largest global database of alien species occurrences based on regional checklists. This case study provides an overview of the records of alien species populations globally distinguished between terrestrial, marine, and freshwater species. The DASCO workflow is fully implemented in the open-source language R (version 4.1.3, R Core Team 2022) and is published together with this article. The workflow was designed in a way that allows other users to modify and apply the scripts to their respective needs, for example, by providing their own region delineations for aggregating the occurrence data.

The DASCO workflow

The DASCO workflow is structured in a sequence of five steps of data processing (Fig. 1): 1) preparing of input data sets and folder structure, 2) obtaining occurrence records of species from GBIF and OBIS, 3) cleaning obtained occurrence records, 4) determining the invasion status (i.e., alien) of the populations, and finally 5) preparing the final output. The steps are executed in sequence and each produces output files, which are used as input of the next step. This enables the application of individual steps in isolation without the need to run the full workflow in all cases.



Figure 1. Overview of the DASCO workflow. The workflow consists of five steps (green boxes), which are executed in sequence. It requires input from external sources (column 'Input') and exports a series of output files (blue boxes) to document the process, to provide intermediate output results, and the final output files.

The essential requirements for executing the workflow are the original database of alien taxa, which is organised as a checklist at any scale, a shapefile of the polygons of the regions, R installed on a computer, and a GBIF account. A detailed description of the workflow, requirements for running the workflow, and technical descriptions of the individual functions are available in the DASCO manual, which is available as an R Markdown file together with the code (https://doi.org/10.5281/zenodo.5841930) and as a pdf (see Suppl. material 1). An overview of the individual steps of the workflow is presented in the following:

Step I: Preparation of database

In the first step of the DASCO workflow, checklists of alien species are imported and prepared for further processing. A checklist represents a list of species, which are known to occur in a certain region. Usually, regions (also called 'location') represent a country, an island, or a nature reserve, but it could be any area of any size. Column headers of the columns containing taxon names, locations, and first record are standardised according to Darwin Core terminology following Groom et al. (2019). In addition, location names are standardised according to an associated translation table. This translation table can be modified or replaced by the user to obtain a different set of location names. A standardised spreadsheet table of location-taxon records and a list of all taxa are exported. Note that taxon names are not standardised, as this could be done using other workflows (Seebens et al. 2020; Grenié et al. 2022), which could be applied before the application of DASCO.

Step 2: Obtaining occurrence data

In the second step of the DASCO workflow, available occurrence records for each species, which are listed in the checklists provided in step 1, are obtained from GBIF and OBIS. All available occurrence records are downloaded irrespective of their location or invasion status of the respective population. Depending on the length of the species list, this may result in large amounts of data, particularly for GBIF data, which may be difficult to process in one step. Thus, the number of available records on GBIF for each species is determined beforehand. By default, the request to GBIF is automatically split into three chunks, which can be processed in parallel using a single GBIF account. If the total number of records is large, the user can provide multiple accounts, the taxa are split accordingly, and individual requests for download are sent for each chunk to obtain data sets of manageable sizes. This step requires one or multiple accounts on GBIF to allow processing multiple chunks of data simultaneously (see the DASCO manual for further details).

Once the GBIF files are ready for download, they will be downloaded to a local folder. GBIF provides digital unique identifiers (DOI) for each query, which are exported by the workflow and should be kept and provided to ensure transparency and reproducibility. The downloaded files are decompressed, and an initial cleaning is conducted by removing duplicated, empty and non-numeric entries of the columns 'speciesKey', 'decimalLatitude,' and 'decimalLongitude.' In addition, obviously wrong coordinates with values being outside the coordinate systems are removed (original records are kept for cross checking). Finally, all records indicated as 'FOSSIL_SPECI-MEN' are removed.

For OBIS, the number of available occurrence records is usually much lower compared to GBIF. Therefore, it is not necessary to perform initial checks and to split download requests. Thus, all available records for species of the provided checklists are directly imported into R. Duplicated records and records, which are indicated as 'FossilSpecimen', are removed. OBIS does not provide a DOI for individual queries. Lists of all records from GBIF and OBIS are exported and saved locally.

Step 3: Cleaning occurrence data

The third step represents the most computer- and time-intensive part of the workflow as it contains the cleaning of the obtained occurrence records. Occurrence records provided on GBIF and OBIS are prone to errors and uncertainties due to inaccurate measurements or wrong entries and therefore require cleaning. First, inaccurate coordinates with fewer than two digits after the comma are removed. This is considered to be a minimum requirement, and a higher resolution might be desired depending on the geographic resolution of the study, while for large-scale databases, such accuracy should be sufficient. Subsequently, seven tests of validation are applied to identify wrong coordinates. The tests are provided by the R package 'CoordinateCleaner,' which was specifically designed to validate occurrence records provided by platforms such as GBIF (Zizka et al. 2019). These tests involve checking whether, for example, coordinates represent centroids or capitals of countries, the location of large biodiversity institutions or the headquarter of GBIF rather than actual species populations. The most important test for our purpose represents the check for outliers, which identifies records that are located at large distances to the majority of records. These records might be a result of misspecifications or erroneous entries. Records flagged as potentially wrong entries by the tests are removed from the list, which - based on experiences - represents around 5% of records. This resulted in a more conservative estimate of the actual species occurrence. These tests are applied to records of both platforms. The user has the opportunity to check the removal of records by comparing the original downloaded occurrence files with the output file of the workflow.

Due to the sheer amount of data provided by GBIF, conducting the outlier test could be time- and memory-consuming. Many of the records represent multiple counts of the same species within a narrow geographic range, which would not add new information to our workflow. To improve the efficiency and speed of the workflow, we allowed for the thinning of records to reduce the workload. Thinning was done by rounding the coordinates to the second digit after the comma, keeping only one record (but the original, not rounded coordinates) for this occurrence, and removing others. Depending on the focus of the study, thinning could be done to finer geographic scales or disabled at all. Thinning is disabled by default for records provided by OBIS but can be turned on if required.

Step 4: Determining alien occurrences and habitats

Within the fourth step of the DASCO workflow, the cleaned occurrence records and the original checklists are used to identify alien populations. This requires having a shapefile with the same region borders as provided in the checklists. Only occurrence records were kept, which were located in the regions, where the respective species was classified as being alien. In this way, it is ensured that the information about the invasion status of being an alien taxon in a certain location has been assigned to the occurrence records. Records falling outside those regions were removed. As a default, a shapefile of country borders, large islands, and marine ecoregions is provided and used. Only those combinations of a taxon and a region are kept in the workflow if at least three occurrence records within the respective region are available for the taxon. Fewer numbers of records per taxon-region combination are considered to be too uncertain and removed. The emergence of region names of the checklists, which are not matching the names provided in the shapefile, will produce a warning and an export of mismatching region names.

Checklists often contain taxa of different habitats (e.g., terrestrial, marine, freshwater). As the region of record provided in the shapefile is often a terrestrial region, such as the land of a country or island, occurrences of recorded marine taxa often fall outside the provided polygons. The availability of coordinate-based occurrence records now provides the opportunity to specify the coastal area of the region, where the taxon actually occurs. In addition to occurrence records, this requires the determination of habitats for each taxon, a delineation of marine coastal regions, and knowledge about borders of land and marine coastal regions. We, therefore, provide a list of regions and their bordering marine ecoregions based on the classification provided by Spalding et al. (2007). Occurrence records of taxa, which have been identified as being marine and alien on a regional checklist, are considered to describe alien populations in the neighbouring marine ecoregions. Thus, occurrences of a marine taxon are assigned to a marine ecoregion only if the taxon is listed as being alien for the region (i.e., a country) and has at least three occurrence records in the respective marine ecoregion.

As records of many taxa, which are actually not marine, fall into polygons of marine ecoregions, an additional step of determining habitats of a taxon has been included. For each taxon, information about the habitat is obtained from the online databases WoRMS (WoRMS Editorial Board 2022), FishBase (Froese and Pauly 2021), and Sea LifeBase (www.sealifebase.ca) if entries for the taxon exist. Multiple entries are allowed for species capable of moving between habitats. Only records of taxa identified as being marine are assigned to a marine ecoregion. As habitat information provided a number of false entries, the following taxon groups were excluded from marine ecoregions: Vascular plants, insects, spiders, bryophytes, birds, amphibians, and mammals. In addition, only those species were considered as being marine, which were explicitly mentioned as such in the aforementioned databases or in the databases provided by the user. Marine mammals are excluded because, up to now, no introduction of a marine mammal has been reported. These restrictions may result in the removal of actual true records, but overall

will ensure avoiding large numbers of false entries in the final output, which is preferred. Other habitat types were taken as provided by the online databases or the input checklist without any test using occurrence records, because occurrence data often do not provide the accuracy to distinguish between, for example, terrestrial and freshwater habitats. Habitat information can also be provided as a separate column in the input data set.

Two data sets are exported from step 4: A list of occurrence records with coordinates for alien populations with the associated name of the region and a list of taxonregion combinations. The latter represents checklists as provided in the original input file, which is now cross-checked by records from GBIF and OBIS and may include new regions such as marine ecoregions. Providing different shapefiles would allow reassigning the occurrences to an alternative set of regions.

Step 5: Merging data sets and finalising the output

In the last step of the workflow, data sets of occurrences of alien species at a regional scale will be merged and prepared for the final output. Steps 2–4 are split into parallel strands for GBIF and OBIS, which are merged here to obtain a single output. Duplicated records are removed. If information about the year of the first record has been provided, it will be assigned at this step to the respective taxon and region. If multiple first records exist due to, e.g., the usage of a different geographic classification, the earliest first record is selected.

A case study

We showcase the application of the DASCO workflow using the SInAS database. The SInAS database represents an output from another workflow (i.e., the SInAS workflow; Seebens et al. 2020) designed to integrate databases of alien species occurrences based on checklists in a semi-automated and transparent way of standardisation and integration. Here, we use version 2.4.1 of the SInAS database (https://doi.org/10.5281/ zenodo.5562892), which results from the integration of seven global databases of alien species occurrences: Five taxonomic databases, namely for vascular plants (GloNAF; van Kleunen et al. 2019), birds (GAVIA; Dyer et al. 2017), mammals (Biancolini et al. 2021), macrofungi (Monteiro et al. 2020) and amphibians and reptiles (Capinha et al. 2017), and two cross-taxonomic databases being one about temporal information of first recording (FirstRecords; Seebens et al. 2017) and one about invasive alien species (GRIIS; Pagad et al. 2022). All seven databases are based on checklists of regional (mostly country) scale. By applying the SInAS workflow, the terminologies, taxonomies, regional delineations, and event dates of the individual databases were standardised and the standardised databases were merged into the SInAS database. This version of the SInAS database contains 175.980 records of 39.191 alien taxa occurring in 264 non-overlapping regions worldwide. As the SINAS database is organised as a collection of checklists for regions, it can be directly used as input for the DASCO workflow.



Figure 2. The number of records of alien populations obtained from GBIF (top) and OBIS (bottom).

Applying the DASCO workflow to the SInAS database required processing large amounts of occurrence data, which altogether took around four days, with the longest step being the cleaning of the GBIF data. The application of the DASCO workflow resulted in a total of 35.666.064 cleaned coordinate-based occurrence records of alien populations of 17,424 taxa (Fig. 2). The vast majority of records (99%) was obtained from GBIF, and only a comparatively small fraction stemmed from OBIS. Records of both databases are heavily biased towards Europe, North America, and Australia.

While checklists often provide comprehensive lists of taxa, more detailed information about the exact occurrences of populations is limited to a distinctly lower number of taxa. Consequently, while applying the workflow, the number of taxon-region combinations likely reduces due to the lower number of taxa in GBIF and OBIS and information gaps. Indeed, information about the occurrence of alien populations was only available for 17,424 alien taxa, which is 44% of the number of species as provided in the original database.

The application of the DASCO workflow may introduce new or intensify already existing geographic and taxonomic biases due to biases of data provided by the online platforms. Although the application of the workflow resulted in a drop in available records, the proportions of reduction are fairly constant across all large-scale regions



Figure 3. The number of taxon-region combinations before (x-axes, 'Original') and after (y-axes, 'DAS-CO') applying the DASCO workflow for different regions (upper panel) taxonomic groups (lower panel).

with an average decline of 64% (Fig. 3), with the highest and lowest values reported for the Middle East & North Africa (84.1%) and Europe & Central Asia (57.6%), respectively. Overall, there is no indication that the application of the workflow increased the geographic bias, which is certainly inherent in the original databases. Comparing records of taxonomic groups revealed a stronger decline for insects, fishes, molluscs, crustaceans, and fungi, while the decline was lower for vascular plants.

Habitat information was obtained for 21.605 taxa (64% of the requested number of 33.587 taxa). The majority of habitat records were terrestrial (58%), followed by marine (13%), freshwater (9%), and brackish (2%) (Fig. 4). Years of first records were available for 42% of all taxon-region combination. Long-term trends of the number of new alien taxa per five years revealed a clear increasing trend of the rate of first records



Figure 4. Overview of obtained habitat information. Shown are the total number of taxa with obtained habitat information (left panel) and long-term trends of alien taxon numbers distinguished by habitats (right panel).



Figure 5. Map of the number of recorded alien taxa for terrestrial (freshwater + terrestrial) and marine (marine + brackish) taxa as obtained by the DASCO workflow.

until 2005, particularly for terrestrial and marine alien taxa, while rates for freshwater and brackish taxa saturated after ca. 1950 and slightly declined until today (Fig. 4).

The application of the DASCO workflow allowed the separation of checklists by habitats and the representation of alien taxon numbers for terrestrial regions (i.e., terrestrial + freshwater) and coastal marine regions (marine + brackish) (Fig. 5). For both terrestrial and marine regions, a geographic bias towards Europe, North America, and Australasia becomes apparent. The low numbers of available records, particularly for Africa, Central Asia, and many marine ecoregions, makes it difficult to identify any variation across regions and likely results from the lack of records in the used data sources.

Discussion

Checklists of alien taxa provide valuable and often comprehensive information about the invasion status of populations at regional levels, while online portals such as GBIF and OBIS provide tremendous amounts of data at higher spatial resolution. Here, we provide

a workflow to integrate the advantages of both sources by assigning the invasion status obtained from checklists to occurrence records obtained from online portals. The DASCO workflow allows downscaling regional checklists to coordinate-based occurrences, which can then be used to re-assign occurrences to any categorisation provided by the user. In this way, the information provided in checklists, which are bound to a fixed delineation, is made accessible for a range of different purposes, including the assessment of biological invasions at resolutions, deviating from the original checklists. By applying the DASCO workflow, downscaling and re-assignment is done in a standardised, reproducible, and transparent way and in full compliance with the FAIR data principles (Wilkinson et al. 2016).

Our case study of applying the DASCO workflow to the SInAS database of alien taxa checklists resulted in a comprehensive compilation of coordinate-based occurrence records of alien populations. However, the distribution of records is highly biased towards a few well-sampled regions such as Europe, North America, Australia, and New Zealand, while particularly countries in Africa except South Africa, and Central Asia are highly under-represented (Fig. 2). This bias is even more pronounced for records obtained from OBIS. Aggregating the records back to the original regional delineation revealed a global pattern of alien taxa occurrences, which is very similar to what has been published elsewhere (Dyer et al. 2017; Pyšek et al. 2017). This is not surprising as both representations are based on the same data, but show that the application of the DASCO workflow does not distort the original maps except that the total numbers of taxa are lower.

For marine ecoregions, comparable global maps of alien marine taxa do not exist. Bailey et al. (2020) published the most recent and comprehensive compilation of marine alien taxa, which, however, still covers only approximately half the world's ecoregion at a coarser resolution than provided here. But the overall patterns are similar to our results, although distinctly higher numbers of marine alien taxa can be expected for most marine ecoregions except probably for European and North American coastal waters. Our case study highlights that downscaling and re-allocating alien species occurrences using the DASCO workflow could provide a promising way to form a basis for large-scale assessments of biological invasions for regions, which are not yet well covered in global analyses.

The DASCO workflow is limited in different ways, which should be taken into account. First of all, the output of the workflow highly depends on the information provided in online sources. As this information is often geographically and taxonomically biased (Fig. 2; Meyer et al. 2016; Rocha-Ortega et al. 2021), obtained records are likely biased as well, which, however, depends on the taxon and region considered. While for well-sampled regions and taxa, a reduction might be low, the loss of information might be very high for under-sampled cases such as microorganisms or Central Africa. In addition, provided records might be of low quality, including false or imprecise coordinates (Jin and Yang 2020), and thus obtained records should be handled with care (Zizka et al. 2019). This is particularly problematic at small geographic scales, where imprecise coordinates can make a big difference when, for example, it is unclear whether a taxon is found inside or outside a nature reserve. We included a number of tests to identify imprecise and wrong entries, but these likely do not remove all faulty records. These errors became less influential at larger scales, and thus results from the application of the DASCO workflow should be treated more carefully with increasing spatial resolution of the analysis. Furthermore, as there is no single comprehensive source of habitat information for taxa, the habitat type could not be identified for many taxa, particularly aquatic ones. All of these limitations can only be solved by increasing the amount of information provided by online sources, which is an ongoing but long-lasting process. Additional software packages and workflows have been developed to identify and, to some degree, correct errors in spatial information (Mathew et al. 2014; Jin and Yang 2020), which could be applied in addition.

Another limitation of the workflow is that it currently cannot discriminate native from alien populations. Although the workflow can identify alien populations based on regional checklists, this does not automatically mean that all records not classified as being alien belong to native populations. It might be that some records refer to alien populations, which are not included in the regional checklists. It therefore remains unsafe to classify native populations using our workflow. Still, this can cause an increase in false positive records for species, which have both native and alien ranges within the same region. Such species might be considered as being alien in the regional checklist. In this case, the workflow would assign all records within the region the status of being alien, although some populations may in fact be native. This depends on the scale, at which the checklists are provided, and can only be avoided by using checklists at subnational scale for large countries to distinguish e.g. federal states and islands.

The DASCO workflow has been designed in the context of biological invasions, but its use is not limited to this area, as coordinate-based occurrences of any kind of taxon checklist can be downscaled and re-allocated across varying delineations and realms. In addition, parts of the workflow could be applied in isolation. For example, obtaining and cleaning large amounts of GBIF records in a convenient and transparent way is likely of interest for many users for various purposes. As other potential applications, obtained records of alien taxa could be used to identify native populations, and the integration of habitat information could potentially be of interest for other research studies.

By using available and open workflows, such work becomes more efficient because work does not have to be repeated as it is often done right now in parallel projects. With the increase in the amount of data, developing and sharing workflows such as DASCO becomes more and more important to make unstructured data accessible in a reproducible and transparent way, which ultimately will increase trust in scientific outcomes (Franz and Sterner 2018).

Data and code availability

All necessary files for running the DASCO workflow, such as R scripts, the shapefile, and the marine-terrestrial region file, are available for public use at Github with version control (https://github.com/hseebens/DASCOworkflow) and releases are stored on Zenodo (https://doi.org/10.5281/zenodo.5841930). The SInAS database, which represents the input data set for the case study, is available online (https://doi.org/10.5281/zenodo.5562892). The occurrence records, which are exported by the DASCO workflow for the case study, are provided online together with a list of identifiers of original GBIF downloads (https://doi.org/10.5281/zenodo.6458083).

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Supplementary material I

Manual of DASCO

Authors: Hanno Seebens, Ekin Kaplan Data type: PDF file

Explanation note: Manual of DASCO: A workflow to down-scale alien species checklists using occurrence records and to re-allocate species distributions across realms.

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Scalability of genetic biocontrols for eradicating invasive alien mammals

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Abstract

CRISPR-based gene drives offer novel solutions for controlling invasive alien species, which could ultimately extend eradication efforts to continental scales. Gene drives for suppressing invasive alien vertebrates are now under development. Using a landscape-scale individual-based model, we present the first estimates of times to eradication for long-lived alien mammals. We show that demography and life-history traits interact to determine the scalability of gene drives for vertebrate pest eradication. Notably, optimism around eradicating smaller-bodied pests (rodents and rabbits) with gene-drive technologies does not easily translate into eradication of larger-bodied alien species (cats and foxes).

Keywords

Cat, fox, gene drive, invasive mammals, mice, rabbit, rat, spatial model

Introduction

Alien vertebrates are some of the costliest invasive alien species worldwide (Diagne et al. 2021), directly causing species extinctions (Bellard et al. 2016) and driving profound environmental change (Pyšek et al. 2020). The risk of new invasive alien species continues to increase (Seebens et al. 2017, 2021), and is intimately linked to growth in globally expanding transportation networks, widespread rapid environmental change, and geopolitical forces - including intercontinental trade agreements. In Australia, mammals are the costliest invasive taxa; with feral cats (*Felis catus*), rodents (house mice *Mus musculus* and rats *Rattus spp.*), pigs (*Sus scrofa*), rabbits (*Oryctolagus cuniculus*), and red foxes (*Vulpes vulpes*) accounting for 95% of the total costs imposed by invasive mammals over the last 50 years (Bradshaw et al. 2021). Despite some notable successes in eradications remain elusive, and are greatly hampered by a lack of socio-political resourcing and will (Pluess et al. 2012). New tools are urgently needed.

CRISPR-based gene-drive approaches promise ground-breaking tools for the eradication or suppression of invasive alien species (Esvelt et al. 2014; Webber et al. 2015). By avoiding unwanted consequences to non-target organisms, genetic biocontrols offer many advantages over classical control methods such as poison baiting, trapping or hunting (Howarth 1991). There have been promising developments in laboratories using gene-drive technology in mosquitoes (Gantz et al. 2015; Kyrou et al. 2018), fruit flies (Gantz and Bier 2015; Champer et al. 2020), mice (Grunwald et al. 2019; Weitzel et al. 2021), and proof of principle for CRISPR gene editing has been demonstrated in cats (Brackett et al. 2022). Despite great interest in developing the technology for a range of vertebrate pests (Prowse et al. 2017; Moro et al. 2018; Prowse et al. 2018; Faber et al. 2021), the feasibility of achieving large-scale eradications of these species using gene drives has not been evaluated theoretically.

Here, we investigated how differences in the life-history traits of five invasive mammals (mice, rats, rabbits, feral cats, and red foxes) interact and influence the feasibility of deploying gene-drive technologies for population suppression at large spatial scales. We used an individual-based, spatially explicit, stochastic model that provides realistic estimates of eradication probabilities and expected times to eradication, due to its ability to model large population sizes at a landscape level (see Suppl. material 1: Tables S1, S2; Birand et al. 2022). We explored the effectiveness of a Y-chromosome-linked X-chromosome-shredding drive ("driving-Y"), which targets the X-chromosome for deletion during spermatogenesis with slightly imperfect efficiency (Fig. 1A). Population suppression is achieved by producing disproportionately more male offspring, and thus limiting female numbers. Theoretical models suggest that a driving-Y strategy could be effective for population eradication (Hamilton 1967; Deredec et al. 2008, 2011; Beaghton et al. 2016; Eckhoff et al. 2017; Prowse et al. 2019; Faber et al. 2021; Birand et al. 2022), and proof-of-concept for X chromosome shredding has been demonstrated in mouse zygotes (Zuo et al. 2017). We also modelled a CRISPR homing drive (see Suppl. material 1: Tables S1, S2) targeting female fertility that is predicted to be similarly effective (Prowse et

al. 2017; Birand et al. 2022), but may be challenging to generate (Grunwald et al. 2019; Pfitzner et al. 2020; Weitzel et al. 2021). Our motivation is to explore how species-specific life history and demographic traits influence eradication probabilities and times to eradication, rather than to evaluate the efficiencies of different gene-drive strategies *per se*, which is discussed extensively elsewhere (Champer et al. 2017; Unckless et al. 2017).

Based on density estimates in Australia (*d* in Table 1), we calculated the area (*A*) that each species would occupy, assuming a population size of roughly 200,000 individuals. We then modelled the required area for each species—from 40 km² for mice to 100,000 km² for cats and foxes—as a 64 × 64 grid of patches. We used historical (or experimental) invasion records to estimate the maximum distances (Δ_i) that each species could disperse per breeding cycle. These distance estimates provide reliable representation of the distance each species would cover when the population density is low at the later stages of a successful suppression (Birand et al. 2022). A dispersal function was developed for each species that was both distance and negative density dependent, mimicking the fact that individuals would move long distances to find mates when densities are low (Diffendorfer 1998; Travis and French 2000; Matthysen 2005; Birand et al. 2022).

Results and discussions

We initially simulated various spatial gene-drive release strategies and compared the simulated times to eradication for mice (Fig. 1B, C) to find an optimal release strategy that is fast, spatially expansive, but also conservative in terms of the laboratory effort required to produce gene-drive carrying individuals for release into the wild. The number of individuals released influenced the simulated time to eradication more than the spatial release strategy used. For example, releasing 4 gene-drive carrying individuals to 16 evenly-spaced patches had the same effect as releasing 1 individual to 64 evenly-spaced patches, except when the total number of individuals released was very low (less than 16, Fig. 1B), or when the dispersal distances were small (Fig. 1C). For the remainder of our study, we assumed an achievable release size of 256 individuals released into 256 evenly-spaced patches (i.e., one individual released per patch).

In order to capture the uncertainty in some of the demographic and dispersal parameters in our simulations, we generated uniform distributions based on the parameter ranges of the probabilities of survival (ω) and polyandry (p_m), and for dispersal distances (*D*) (Table 1, also see Suppl. material 1: Tables S1, S2). We used these distributions to generate 1, 000 unique parameter combinations for each species using Latin hypercube sampling (randomLHS, R package *lhs*, Carnell 2020). We ran one simulation for each parameter combination for 500 breeding cycles (Prowse et al. 2016), and calculated the times to eradication in years based on the estimates of number of breeding cycles (n_c) in a year for each species.

The probability of eradication for small-bodied species (0.97, 1.0, 1.0, respectively for mice, rats, and rabbits) was higher than for large-bodied species (0.50, and 0.89 respectively for cats and foxes, Fig. 1D). The probabilities were lower with the homing



Figure 1. Times to eradication with various release strategies in mice and other invasive mammals using Y-drive. **A** the X-chromosome shredding Y-drive is located on the Y chromosome, and cuts the X chromosome at multiple locations during spermatogenesis (with probability $p_x = 0.96$). The X-bearing sperm are destroyed and eggs are predominantly fertilized by Y-bearing sperm, causing disproportionately more male offspring **B** interquartile ranges for the time to eradication of mice with various spatial release strategies when the number of individuals released per patch, N_i , is varied and the maximum dispersal distance D = 3 patches, and **C** when $N_i = 1$ and D is varied (100 simulations for each combination) **D** violin plots showing the distributions of simulated times to eradication (1000 simulations for each species) and circles representing areas that each species with roughly 200,000 individuals would occupy. The colors of violin plots and circles represent probabilities of eradication and density estimates, respectively.

Table 1. Parameters that are related to the demography and life-history traits for each species, along with the
areas that the species are assumed to occupy with roughly 200,000 individuals, based on density estimates
obtained from literature. We note that these density estimates are used for area (A) calculation only, and
due to the stochastic nature of the simulations, densities change through time and also across simulations.

Species	Ь	n	age _m	ω	Pm	d	A	Δ_{i}	D
Mouse	6	6	2	[0.48, 0.58]	[0.41, 0.51]	5000	40	0.4	[2, 4]
Black rat	4	6	2	[0.62, 0.67]	[0.63, 0.73]	1000	200	2	[7, 9]
Rabbit	4	4	3	[0.82, 0.87]	[0.15, 0.25]	25	8000	12.5	[7, 9]
Cat	4	2	5	[0.85, 0.90]	[0.20,0.30]	2	100000	25	[3, 5]
Fox	4	2	5	[0.88, 0.93]	[0.71, 0.81]	2	100000	45	[7, 9]

b: average number of offspring per breeding cycle; p_{\perp} : number of breeding cycles in a year; $age_{\underline{m}}$: maximum age (years); ω : probability of survival to the next breeding cycle; $p_{\underline{m}}$: probability of multiple mating; *d*: density (km⁻²); *A*: area (km²); $\Delta_{\underline{r}}$: invasion distance per breeding cycle (km); *D*: corresponding distances (number of patches) in the model.

References: Brothers et al. (1985); Williams (1996); Bowen and Read (1999); Say et al. (1999); Read and Bowen (2001); Abbott (2002); Devillard et al. (2003); Baker et al. (2004); Mutze (2009); Russell (2012); Cox et al. (2013); King et al. (2014); Shiels et al. (2014); Elliott et al. (2015); Harper and Bunbury (2015); Legge et al. (2017); Barnett et al. (2018); Moro et al. (2018); Fairfax (2019); Porteus et al. (2019); Murphy and Nathan (2021).

drive than with the Y-drive (0.88, 0.90,0.91, 0.57, and 0.87; for mice, rat, rabbit, cat, and fox, respectively) due to efficient DNA repair mechanisms resulting in the evolution of functionally resistant alleles. Based on sensitivity analysis results across all species, survival (ω) had the highest influence (50.45%) on the simulated eradication probabilities with the Y-drive, followed by dispersal (46.71%) and probability of polyandry (2.83%) (Suppl. material 1: Table S1). Cats had the lowest eradication probability of eradication in cats increased to 73.10%, Suppl. material 1: Table S1). Polyandry had higher influence in simulated times to eradication (28.95%), in which the relative influence of dispersal was reduced to 26.69%, and survival remained at similar levels (44.35%). We expect that polyandry might have a higher impact on eradication probabilities if the competitive ability of sperm from gene-drive carriers is reduced further than has been assumed here (Manser et al. 2020; Birand et al. 2022).

Median eradication times of roughly 200,000 individuals with the X-chromosome shredding drive were 17.7 years for mice, 18.5 years for rats, 48.0 years for rabbits, 142.3 years for cats, and 169.0 years for foxes, with nearly 90% population suppression achieved at half that time (Suppl. material 1: Table S2) (median times to eradication with the homing drive: 13.2, 14.3, 40.8, 121.5, and 110.5 years in the same species order). Simulated eradication times were much longer and also more uncertain in cats and foxes; in comparison to the shorter-lived species tested (Fig. 1D). Shorter dispersal distances resulted in longer times to eradication (e.g. Fig. 1C) mostly due to the emergence of "chase dynamics" where successive waves of local extinction and re-colonization by wild types prolonged the eradication attempt (Champer et al. 2021; Birand et al. 2022). In fact, the effect of dispersal in simulated times to eradication can override the effect of survival, and its relative influence in the time to eradication increase in species with low dispersal (Suppl. material 1: Table S1). For example, estimated eradication times for mice were more uncertain than those for rats (Fig. 1D). This is because, although mice have faster life histories than rats, they also have lower dispersal abilities so chase dynamics are more likely to arise. Similarly, lower dispersal in cats resulted in higher uncertainty in estimated eradication times compared to foxes. The relative influence of polyandry on simulated times to eradication also increased in species with high polyandry rates (Suppl. material 1: Table S1). It is certain that reliable estimates of the time to eradication will rely on a thorough understanding of movement and mating behaviors, particularly at low densities when gene drives begin to take effect.

Conclusion

Our results are in agreement with theoretical models suggesting that gene drives could eradicate large populations of short-lived mammals successfully, within reasonable time periods, and could be an attractive alternative to current lethal control methods (Prowse et al. 2017, 2018; Prowse et al. 2019; Champer et al. 2021; Birand et al. 2022). In contrast, delayed eradication times for large and long-lived species could

render gene drives less attractive as control tools for such species. However, due to their lower densities, even if time to eradication using gene drives is high, control of longlived species is potentially possible over very large areas.

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Supplementary material I

Tables S1, S2

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Long-term changes in the prevalence of the crayfish plague pathogen and its genotyping in invasive crayfish species in Czechia

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Abstract

The widespread presence of North American alien crayfish in Europe is a major driver of native crayfish population declines, mainly because they are chronic carriers of the oomycete *Aphanomyces astaci* responsible for crayfish plague. Screening for the crayfish plague pathogen in host populations has become a common practice across Europe, but sampling usually covers spatial but not temporal variation. Our study focuses on the current situation in Czechia, where screening for *A. astaci* was first conducted in the mid-2000s. We provide data about the distribution and prevalence of this pathogen at almost 50 sites with three host crayfish: the spiny-cheek crayfish *Faxonius limosus*, signal crayfish *Pacifastacus leniusculus*, and marbled crayfish *Procambarus virginalis*. Among these sites were 20 localities that were resampled several years (usually more than a decade) after the original screening for *A. astaci*. We did not detect any *A. astaci* infection in two studied *P. virginalis* populations but documented several new hotspots of highly infected *P. leniusculus* in Czechia, and the first site with the coexistence of the latter with *F. limosus*. Our data suggest that despite some fluctuations, *A. astaci* prevalence in North American host populations generally does not tend to change significantly over time; we only observed two cases of a significant increase and one of a significant decrease. We no longer detected *A. astaci* in several originally weakly infected popula-

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tions, but our data suggest it likely still persists in these areas and threatens native crayfish populations. At the single known site in the country where *P. leniusculus* and *F. limosus* coexist, we documented the presence of the same *A. astaci* genotype group in both crayfish species, likely due to interspecific transmission of the pathogen from the former host to the latter. However, genotyping of *A. astaci* in infected host individuals still supported the link between specific pathogen genotypes and crayfish hosts, suggesting that assessment of sources of mass mortalities from the pathogen genotyping is feasible in European regions where the mutual contact of different American crayfish species is uncommon.

Keywords

Aphanomyces astaci, infection prevalence, interspecific pathogen transmission, invasive crayfish distribution, microsatellite genotyping, mitochondrial haplogroups, qPCR genotyping

Introduction

Crayfish species native to Europe face numerous threats, such as habitat loss, deteriorating water quality, overfishing or predators, with various impacts in different regions of the continent (Souty-Grosset et al. 2006). However, the key reason for declines and local extinctions of European crayfish populations is the widespread presence of invasive non-native crayfish species of North American origin (Holdich et al. 2009; Richman et al. 2015). This is partly due to their superior competitive abilities (Lindqvist and Huner 1999; van Kuijk et al. 2021), but also because these crayfish are major chronic carriers of the oomycete *Aphanomyces astaci*, a pathogen causing crayfish plague. More information on various aspects of this disease is provided in several recent reviews (Jussila et al. 2015; Rezinciuc et al. 2015; Svoboda et al. 2017; Becking et al. 2022).

Three natural host species of *A. astaci*, the spiny-cheek crayfish *Faxonius limosus*, the signal crayfish *Pacifastacus leniusculus* and the red swamp crayfish *Procambarus clarkii*, have become particularly widespread throughout Europe, but several additional alien crayfish species of the North American genera *Procambarus, Faxonius, Cambarellus* and Australasian *Cherax* have been locally introduced as well (Holdich et al. 2009; Kouba et al. 2014; Weiperth et al. 2020). *Procambarus clarkii* and *P. leniusculus* have also been introduced to other continents (*P. clarkii* being the most widespread crayfish globally; Oficialdegui et al. 2020) and along with them the crayfish plague pathogen. The introduction and spread of *A. astaci* in new regions potentially threaten local crustacean populations, including native crayfish species (Peiró et al. 2016; Mrugała et al. 2017; Martín-Torrijos et al. 2018) and those introduced for aquaculture purposes (Hsieh et al. 2016; Putra et al. 2018).

Several studies have conducted surveys on the spatial distribution and/or prevalence of chronic *A. astaci* infections in North American crayfish populations (e.g., Sandström et al. 2014; Tilmans et al. 2014; James et al. 2017b). They have shown that the prevalence of *A. astaci* may substantially differ among species and regions as well as within regions (e.g., Tilmans et al. 2014; Maguire et al. 2016; Grandjean et al. 2017). Moreover, intensive screenings in localities of coexistence with the native noble crayfish *Astacus astacus* have suggested that not all North American crayfish populations host this pathogen (e.g., Schrimpf et al. 2013). Although host populations reaching 100% prevalence are no exception (e.g., Kozubíková et al. 2011a; Filipová et al. 2013), they usually exhibit much lower prevalence values, and individual infection loads tend to be low as well (e.g., Maguire et al. 2016; James et al. 2017b; Panteleit et al. 2019).

In addition, there have been a few attempts, using various methodological approaches, to evaluate whether the prevalence of *A. astaci* differs over time. Nylund and Westman (2000) and Jussila et al. (2017) estimated the pathogen prevalence in *P. leniusculus* populations from gross symptoms, i.e., the presence of melanised lesions; however, these symptoms or their absence do not always correspond to results of *A. astaci* molecular detection (Kozubíková et al. 2009). Matasová et al. (2011) examined temporal changes in *A. astaci* prevalence in three *F. limosus* populations over three to six years using molecular diagnostics. They did not observe significant temporal variation in one highly infected and one very lowly infected population. However, the prevalence of an intermediately infected population decreased below the detection level over six years (Matasová et al. 2011). Unfortunately, the extent of that study was rather limited (a single host species, few populations, and a moderate time scale), thus its results cannot be generalised.

In Central and Western Europe, the key crayfish plague reservoirs are invasive North American crayfish populations (Holdich et al. 2009), although chronic A. astaci infections have also been documented in some native European crayfish populations (reviewed in Svoboda et al. 2017) and in the Chinese mitten crab Eriocheir sinensis (Schrimpf et al. 2014; Svoboda et al. 2014). However, unlike in Turkey, Finland, or Eastern European countries (Svoboda et al. 2017), no cases of chronic infections in native crayfish species in the territory of Czechia have been documented, despite dedicated efforts (Mojžišová et al. 2020). Currently, two of the three main crayfish plague carriers in Europe (F. limosus, P. leniusculus) are widespread in this country (Kouba et al. 2014; Mojžišová et al. 2020), and asymptomatic infections by A. astaci in Czech populations of F. limosus, and to a lesser extent of P. leniusculus, have been well documented (Kozubíková et al. 2009, 2011a; Rusch et al. 2020). The third species, P. clarkii, has not yet been documented from the wild in Czechia. However, populations of the marbled crayfish Procambarus virginalis, another proven A. astaci carrier (Keller et al. 2014; Mrugała et al. 2015), have recently been documented in the country, presumably originating from ornamental aquaria (Patoka et al. 2016, and unpubl. data).

Although all three invasive crayfish documented from Czechia (*F. limosus*, *P. leniusculus*, *P. virginalis*) have been included in the list of invasive alien species of the European Union concern according to Regulation (EU) No 1143/2014, their spread in the country continues, either unaided (due to active dispersal along watercourses), or due to unauthorised human-mediated introductions. As a result, new populations of all three species are being discovered (see map in Mojžišová et al. 2020).

Given that North American crayfish species pose the greatest risk as vectors of crayfish plague, country-wide screenings for the presence of *A. astaci* in their populations have been performed in several countries. This study follows up the screening of Czech populations carried out more than a decade ago in pioneering studies that applied molecular diagnostics to study the distribution and prevalence of A. astaci in North American asymptomatic hosts (Kozubíková et al. 2006, 2009). In samples collected between 2004 and 2006, F. limosus populations showed great variability in A. astaci prevalence, reaching up to 100%, while P. leniusculus populations seemed to be infected less intensively, with prevalence not exceeding 37% (Kozubíková et al. 2009, 2011a). Since then, both species have expanded not only in the originally invaded areas, but also with numerous populations appearing in non-adjacent places, including areas bordering Austria, Germany and Poland (Štambergová et al. 2009; Mojžišová et al. 2020). Some of the newly reported borderland P. leniusculus populations, recently screened for A. astaci along with environmental DNA samples, have shown very high prevalence values (Rusch et al. 2020), in contrast to Czech populations examined previously. We presume that these highly infected populations could have been founded from sources other than the remaining Czech populations, possibly having their origin across the country border. Despite the limited distribution of P. leniusculus in Czechia and low A. astaci prevalences reported from most populations there, its importance as a local crayfish plague reservoir is also indicated by genotyping of A. astaci from crayfish plague outbreaks. Four mass mortalities of the native noble crayfish were caused by A. astaci genotypes assumed to originate from this host species (Kozubíková-Balcarová et al. 2014; M. Mojžišová, unpubl. data).

There is an assumption that distinct A. astaci genotype groups known from Europe are linked to their original North American crayfish carrier (for more details, see Ungureanu et al. 2020). Thus, various genotyping assays applicable on either axenic A. astaci cultures (Huang et al. 1994; Rezinciuc et al. 2014) or on mixed genome samples (e.g., Grandjean et al. 2014; Makkonen et al. 2018; Minardi et al. 2019; Di Domenico et al. 2021) should allow tracking the source of infection in crayfish plague outbreaks. Although a recent study using mtDNA sequencing has shown that A. astaci haplotypes are not host speciesspecific (Martín-Torrijos et al. 2021), these haplogroups likely include multiple strains that may differ from each other in variable nuclear markers targeted by other genotyping methods (e.g., RAPD: Huang et al. 1994; microsatellites: Grandjean et al. 2014) or in their physiological properties. Despite the increasing number of genotyping methods and studies applying them, there is still only limited data about A. astaci strains genotyped directly from North American crayfish hosts in Europe that would support the link between host species and pathogen strains co-introduced with them (reviewed in Ungureanu et al. 2020). On the contrary, some evidence for the interspecific transmission of A. astaci strains between North American hosts has been provided, both from captivity (Mrugała et al. 2015) and from the wild in the invaded range (James et al. 2017a).

Our study had thus three aims: (i) to update data about the *A. astaci* distribution and prevalence in Czechia including recently discovered alien crayfish populations; (ii) to investigate potential long-term temporal changes in *A. astaci* prevalence in populations of two alien crayfish species resampled after more than a decade; and (iii)
to genotype *A. astaci* in representative host individuals from multiple populations to further test the assumption that distinct *A. astaci* genotypes causing crayfish plague outbreaks in Europe are specifically linked to their North American crayfish carriers.

Materials and methods

Crayfish sampling

A total of 448 individuals of *F. limosus* from 25 sampling sites, 487 individuals of *P. leniusculus* from 23 sampling sites, and 36 individuals of *P. virginalis* from two sampling sites collected in Czechia between 2016 and 2020 (Table 1) were analysed for *A. astaci* infections. Sampling took place from various habitats, both running waters (from small streams to larger rivers) and stagnant water bodies (fishponds, reservoirs, flooded quarries, and sandpits). The sampling sites included selected localities for which past data on *A. astaci* prevalence were available from samples collected between 2004 and 2012 (most of them published in Kozubíková et al. 2011a), as well as new sites with recently reported invasive crayfish. Some of the samples collected in 2017, indicated in Table 1, have already been analysed within a study focusing on the detection of crayfish and *A. astaci* presence from environmental DNA (Rusch et al. 2020).

Crayfish specimens were collected manually or by trapping, and then preserved in 96% ethanol or deep-frozen and stored at -80 °C until further processing. We aimed to analyse 20 individuals per population, but this number sometimes could not be obtained due to low capture success, in which case we processed all available individuals. When more material from a given site was available, we occasionally analysed additional specimens to obtain more precise prevalence estimates for some populations. The number of individuals analysed per site thus ranged from five to 44 (Table 1).

Molecular detection of A. astaci

Crayfish tissues tested for *A. astaci* presence comprised soft abdominal cuticle and uropods; the telson was also processed from individuals with body length below 5 cm. These were homogenised by crushing after immersion in liquid nitrogen, as described in Oidtmann et al. (2006) and Kozubíková et al. (2008). DNA was extracted from up to 50 mg of the homogeneous mixture with the DNeasy tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Two negative controls consisting of 50 μ l of nuclease-free water were included in each DNA extraction batch. One was kept open during manipulation with the samples to check for potential airborne laboratory contamination, another was closed to check for potential contamination of reagents. No trace of *A. astaci* DNA was detected in negative controls.

Table 1. Summary of the sampling sites and results of *A. astaci* detection in populations of alien crayfish species *F. limosus, P. leniusculus* and *P. virginalis* in Czechia from 2016 to 2020. Counts of individuals with agent levels above A0 (no traces of *A. astaci* DNA) are provided in parentheses. Genotyping of *A. astaci* was attempted for selected *A. astaci*-positive DNA isolates only, preferably exceeding 500 PFU. The pathogen was characterised by fragment analysis at microsatellite loci (Grandjean et al. 2014), sequencing of mitochondrial small (rnnS) and large (rnnL) ribosomal subunits (Makkonen et al. 2018) and by specific TaqMan qPCR genotyping assays (Di Domenico et al. 2021). German toponyms are provided in square brackets for transboundary watercourses. *A. astaci* prevalences in populations marked by asterisks have been previously reported in Rusch et al. (2020). Abbreviations: CI: confidence interval; SSR: multilocus genotype characterised by microsatellites; mtDNA: mitochondrial haplogroup; qPCR: genotype group determined by qPCR; NA: genotyping results from that method not available. More details on genotyping are provided in Suppl. material 1: Table S1.

Site	Locality	Region	River	Geographic	Month of	Infected/	Prevalence	Agent level	SSR	mtDNA	qPCR
no.			basin	coordinates	sampling	Analysed	(95% CI)				
Fax	onius limosus										
1.	quarry in Starý Klíčov	Pilsen	Berounka	49.3914°N, 12.9646°E	Jun 2020	0 / 16	0% (0–21%)	-			
2.	Hracholusky reservoir*	Pilsen	Berounka	49.7976°N, 13.1024°E	Aug 2017	2/10	20% (3–56%)	A3	E	NA	Е
3.	Lipno reservoir	South Bohemia	Vltava	48.7395°N, 14.1015°E	Aug 2017	8 / 23	35% (16–57%)	A1(4), A2(2), A3(4), A5, A6	E	e	E
4.	Barbora surface mine*	Ústí	Labe [Elbe]	50.6401°N, 13.7509°E	Aug 2017	3 / 44	7% (1–19%)	A1(3), A2, A3(2)	NA	e	NA
5.	Zlonický brook	Central Bohemia	Vltava	50.2517°N, 13.9032°E	Jul 2017	11 / 20	55% (32–77%)	A1(2), A2(2), A3(9)	Е	NA	Е
6.	Vysokopecký	Central	Berounka	49.6652°N,	Sep 2017	2/2	100% (16–100%)	A2			
	pond	Bohemia		13.9603°E	Oct 2020	0 / 20	0% (0–17%)	-			
7.	Litavka (brook below the Vysokopecký pond)	Central Bohemia	Berounka	49.6661°N, 13.9628°E	Jul 2020	0/15	0% (0–22%)	-			
8.	Ohře [Eger] river	Ústí	Labe [Elbe]	50.4510°N, 14.1623°E	Sep 2017	6 / 20	30% (12–54%)	A1, A2(3), A3(3)			
9.	Vltava river (Podbaba)	Prague	Vltava	50.1183°N, 14.3931°E	Sep 2017	5/7	71% (29–96%)	A1, A3(5)			
10.	Vltava river (Roztoky)	Central Bohemia	Vltava	50.1454°N, 14.3974°E	Sep 2018	10 / 10	100% (69–100%)	A2(2), A3(7), A4	Е	e	Е
11.	Berounka river	Central Bohemia	Berounka	49.9803°N, 14.3623°E	May 2018	9 / 20	45% (23–68%)	A2(2), A3(3), A4(4)	Е	e	Е
12.	Vltava river under the Kořensko reservoir	South Bohemia	Vltava	49.2397°N, 14.3778°E	Aug + Sep 2019	21 / 22	95% (77–100%)	A2(2), A3(9), A4(10)	E	е	E
13.	Malše river (České Budějovice)	South Bohemia	Malše [Maltsch]	48.9752°N, 14.4709°E	Jul 2020	10 / 10	100% (69–100%)	A2, A3(7), A4(2)	NA	e	E
14.	Zlatá stoka channel*	South Bohemia	[Lainsitz]	49.0655°N, 14.6809°E	Sep 2018	1 / 8	13% (0–53%)	A1(2), A3			
15.	Baraba sandpit (Cítov)	Central Bohemia	Labe [Elbe]	50.3664°N, 14.4346°E	Aug 2019	0 / 20	0% (0–17%)	-			

Site	Locality	Region	River	Geographic	Month of sampling	Infected/ Analysed	Prevalence (95% CI)	Agent level	SSR	mtDNA	qPCR
16	Labe [Elbe] river	Central	Labe	50 3109°N	Jun 2017	6/17	35% (14-62%)	A1. A2.	E	e	E
10.	(Kly)*	Bohemia	[Elbe]	14.4961°E	Juli 2017	011,	5570 (11 0270)	A3(3), A4(2)	1	c	2
17.	Kojetice quarry*	Central Bohemia	Labe [Elbe]	50.2401°N, 14.5149°E	Aug 2017	14 / 20	70% (46–88%)	A1(3), A2(14)			
18.	Konopišťský brook	Central Bohemia	Sázava	49.8401°N, 14.6795°E	Oct 2018	13 / 20	65% (41–85%)	A1(3), A2(6), A3(7)	E	NA	E
19.	Pšovka brook (Střemy)	Central Bohemia	Labe [Elbe]	50.3869°N, 14.5439°E	Jun + Jul 2020	0 / 20	0% (0–17%)	-			
20.	Pšovka brook (Harasov)*	Central Bohemia	Labe [Elbe]	50.4107°N, 14.5686°E	Aug 2017	3/15	20% (4-48%)	A2			
21.	Proboštská jezera sandpit	Central Bohemia	Labe [Elbe]	50.1994°N, 14.6573°E	Jul 2020	2/19	11% (1–33%)	A2, A3			
22.	Výmola brook (confluence with the Elbe)	Central Bohemia	Labe [Elbe]	50.1696°N, 14.7934°E	Sep 2017	0 / 20	0% (0–17%)	-			
23.	Brno reservoir	South Moravia	Dyje [Thaya]	49.2390°N, 16.5092°E	Jul 2020	8 / 20	40% (19–64%)	A1(6), A2(6), A3(2)			
24.	Prudník brook	Moravia- Silesia	Odra [Oder]	50.2982°N, 17.7437°E	Aug 2020	10 / 10	100% (69–100%)	A2, A3(7), A4(2)	E	e	Е
Site	Site with syntopic F. limosus (F) and P. leniusculus (P)										
25.	Malý Klikovský pond	South Bohemia	Lužnice [Lainsitz]	49.0971°N, 15.1433°E	Jun 2020	F: 1 / 13 P: 1 / 20	8% (0–36%) 5% (0–25%)	A1, A4 A4	B B	b b	B B
Paci	fastacus leniusculi	us									
26.	Kouba [Chamb] brook	Pilsen	Danube [Donau]	49.3120°N, 13.0075°F	Jul 2019	0 / 20	0% (0–17%)	-			
27.	Liščí brook	Pilsen	Danube [Donau]	49.3138°N, 13.0180°E	Sep 2017	0 / 20	0% (0–17%)	-			
28.	Křesanovský brook	South Bohemia	Otava	49.0605°N, 13.7582°E	Sep 2016	0 / 22	0% (0–15%)	-			
29.	Blanice river	South Bohemia	Otava	49.1550°N, 14.1710°E	Sep 2020	0 / 20	0% (0–17%)	-			
30.	Malše [Maltsch] river (country border)*	South Bohemia	Malše [Maltsch]	48.6146°N, 14.5279°E	Aug 2017	16 / 20	80% (56–94%)	A1(3), A2(8), A3(8)	В	Ь	NA
31.	Pěněnský pond	South Bohemia	Lužnice [Lainsitz]	49.0988°N, 15.0412°E	May 2018	2 / 20	10% (1–32%)	A1, A2, A3	В	NA	NA
32.	Dračice brook [Kastenitzer Bach]*	South Bohemia	Lužnice [Lainsitz]	49.0056°N, 15.0951°E	Aug 2017	20 / 20	100% (83–100%)	A3(18), A4, A5	В	b	В
33.	Kačležský pond	South Bohemia	Lužnice [Lainsitz]	49.0938°N, 15.0934°E	May 2018	1 / 20	5% (0–25%)	A2			
34.	Žďárka brook*	Vysočina	Dyje [Thaya]	49.3713°N, 15.8569°E	Aug 2017	0 / 28	0% (0–12%)	-			
35.	Staviště brook*	Vysočina	Sázava	49.5672°N, 15.9448°E	Aug 2017	0 / 42	0% (0–8%)	-			
36.	Oslava river*	Vysočina	Dyje [Thaya]	49.4201°N, 15.9864°E	Apr + Aug 2017	0 / 20	0% (0–17%)	-			
37.	Prchal pond	Vysočina	Dyje [Thaya]	49.3907°N, 15.9967°E	Mar 2017	0 / 16	0% (0–21%)	-			
38.	Šípský brook	Vysočina	Dyje [Thaya]	49.3738°N, 16.0593°E	Aug 2020	0 / 20	0% (0–17%)	-			
39.	Stržek pond	Vysočina	Dyje [Thaya]	49.3782°N, 16.0840°E	Sep 2020	0/19	0% (0–18%)	-			

Site	Locality	Region	River	Geographic	Month of	Infected/	Prevalence	Agent level	SSR	mtDNA	qPCR
no.			basin	coordinates	sampling	Analysed	(95% CI)				
40.	Dolní Tis pond	Vysočina	Dyje [Thaya]	49.4366°N, 16.0985°E	Apr 2017	0/9	0% (0-34%)	A1			
41.	Spustík pond	Vysočina	Dyje [Thaya]	49.3829°N, 16.1308°E	Sep 2020	0 / 20	0% (0–17%)	-			
42.	brook next to Ráček I pond	Pardubice	Dyje [Thaya]	49.6688°N, 16.3339°E	Jul 2020	0 / 20	0% (0–17%)	-			
43.	Besének brook	South Moravia	Dyje [Thaya]	49.4102°N, 16.4171°E	Oct 2018	0 / 20	0% (0–17%)	-			
44.	Divoká Orlice river	Pardubice	Labe [Elbe]	50.0941°N, 16.4598°E	Jul 2020	0 / 20	0% (0–17%)	-			
45.	Bobrava river	South Moravia	Dyje [Thaya]	49.1089°N, 16.6198°E	Oct 2018	16 / 20	80% (56–94%)	A2(8), A3(7), A4	NA	b	В
				49.1090°N, 16.6116°E	Aug 2020	14 / 20	70% (46–88%)	A1(2), A2(11), A3(2), A4			
46.	Morava river	Olomouc	Morava [March]	49.3531°N, 17.3204°E	Oct 2019	0/14	0% (0–23%)	-			
47.	Trňák brook	Zlín	Morava [March]	49.2131°N, 17.4020°E	Oct 2018	0 / 16	0% (0–21%)	-			
Proc	cambarus virgina	lis									
48.	Vršíček pond	Ústí	Labe [Elbe]	50.5536°N, 13.8264°E	Sep 2019	0/6	0% (0-46%)	-			
					Aug + Sep 2020	0/15	0% (0–22%)	-			
49.	Prostřední pond	Prague	Vltava	50.1495°N, 14.4401°E	Sep + Oct 2020	0 / 15	0% (0–22%)	-			

For detection of *A. astaci* DNA, TaqMan Minor Groove Binder (MGB) quantitative PCR (qPCR) was used on an iCycler iQ5 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). The assay targeting the internal transcribed spacer 1 (ITS) in the nuclear ribosomal gene cluster was performed according to Vrålstad et al. (2009) with minor adjustments to increase specificity (as in Svoboda et al. 2014). It has been recently demonstrated that this assay cross-reacts with *Aphanomyces fennicus*, an oomycete related to *A. astaci* isolated from noble crayfish in Finland (Viljamaa-Dirks and Heinikainen 2019). Nevertheless, *A. fennicus* has not yet been reported from Central Europe, and its presence was not confirmed in any of our qPCR-positive samples that were characterised by other molecular markers allowing differentiation of *A. fennicus* and *A. astaci* (i.e., mtDNA sequencing, Makkonen et al. 2018; qPCR genotyping, Di Domenico et al. 2021). We thus interpreted positive signals in the qPCR-based screening of DNA isolates from North American host crayfish as *A. astaci* infections.

The qPCR results were evaluated using iQ5 Optical System Software version 2.0 (Bio-Rad). As the results might be biased in cases of inhibition of the PCR reaction, approx. 25% of DNA isolates were randomly selected from each population, 10-fold diluted and analysed once more for the presence of *A. astaci* DNA (Vrålstad et al. 2009; Kozubíková et al. 2011a). No sign of significant PCR inhibition was observed in any samples for which dilutions were performed.

As a positive control, we used a 251-bp long synthetically assembled DNA fragment with a sequence identical to the region of *A. astaci* internal transcribed spacer contain-

ing both primer and probe binding sites. Four standards of known concentration of the target DNA (a serial four-fold dilution with the starting concentration of 5.01×10^5 PFU) were used to quantify pathogen DNA in PCR-forming units (PFU) in a reaction according to Vrålstad et al. (2009). PFU values were used as a basis for the determination of semiquantitative levels (A0–A7), where agent levels A0 (PFU = 0) and A1 (PFU ≤ 5) were not considered *A. astaci*-positive (Vrålstad et al. 2009; Kozubíková et al. 2011a).

The *A. astaci* prevalence in analysed crayfish specimens from each locality and 95% confidence intervals were calculated in R v. 4.0.2 (R Core Team, 2020) using the function "epi.conf" from the library epiR (Stevenson et al. 2021). A potential significant change in prevalence between samples analysed before 2013 and the most recently collected ones from the same locality were compared by Fisher's exact test using the function "fisher.test". To correct for the effect of multiple testing, p-values were adjusted by Holm-Bonferroni method using the "p.adjust" function (Table 2).

Aphanomyces astaci genotyping

Three molecular assays allowing to assign *A. astaci* strains to genotype groups in mixedgenome samples – microsatellite genotyping (Grandjean et al. 2014), mtDNA sequencing (Makkonen et al. 2018), and qPCR-based genotyping (Di Domenico et al. 2021) – were performed on 20 selected chronically infected crayfish individuals from 18 sampling sites (Suppl. material 1: Table S1). These included representatives of both species from the only known Czech locality where *F. limosus* coexists in syntopy with *P. leniusculus*. In addition to recently sampled sites, we also analysed DNA isolates from individuals representing three earlier-studied *F. limosus* populations: Jickovický brook sampled in 2004 (Kozubíková et al. 2006), Pšovka brook sampled in 2005, and Prudník brook sampled in 2006 (Kozubíková et al. 2009). Another *F. limosus* individual collected from the last-mentioned site in 2020 was genotyped to check whether the presence of the genotype is consistent over time.

Infected North American crayfish tend to have relatively low A. astaci agent levels (e.g., James et al. 2017a), which may reduce the success of pathogen genotyping. Amplification of the target DNA fragments for available genotyping methods is usually successful for isolates with agent levels A4 and higher (over 1000 PFU as determined in the ITS-based qPCR detection of the pathogen) and for some isolates of the A3 level (Grandjean et al. 2014; Makkonen et al. 2018; Di Domenico et al. 2021). Therefore, we primarily selected DNA isolates from highly infected hosts (agent level A4 and higher) for the genotyping. We also used A3-level isolates (PFU ranging between 225 and 887) from seven localities, and attempted to increase their genotyping success by concentrating DNA by precipitation with the GlycoBlue Coprecipitant (Thermo Fisher Scientific, Invitrogen). The initial isolate volume before precipitation differed between ca 120 and 160 µl, depending on the sample availability, but each precipitated sample was diluted to a final volume of 50 µl. To save DNA isolates for genotyping, A. astaci DNA concentration was not quantified in those samples after the precipitation step. However, qPCR quantification of A. astaci ITS in other DNA isolates used in the preliminary evaluation of the suitability of this method indicated an up to fourfold increase of target DNA concentration.

Microsatellite genotyping: Variation at nine microsatellite loci was analysed to determine *A. astaci* multilocus genotypes and assign them to genotype groups as described in Grandjean et al. (2014) and amended in Mojžišová et al. (2020). Amplification using the QIAGEN Multiplex PCR Kit (Qiagen, Hilden, Germany) followed the original protocol, but was performed separately for each locus to improve genotyping success. Fragment analysis was performed on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and allele sizes were determined in GeneMarker software version 1.95 (Soft-Genetics LLC, State College, PA, USA). The results were compared with reference *A. astaci* genotypes (Grandjean et al. 2014; Mojžišová et al. 2020), in particular those originating from studies by Huang et al. (1994), Diéguez-Uribeondo et al. (1995) and Kozubíková et al. (2011b). In case of a failure to amplify some of the microsatellite loci, the given isolate was tentatively assigned to a likely genotype group if successfully scored microsatellite markers (at least three informative loci) allowed differentiating among known *A. astaci* multilocus genotypes (Suppl. material 1: Table S1). These tentative assignments were subsequently compared with the results of the remaining genotyping methods.

Sequencing of mtDNA markers: Mitochondrial small (rnnS) and large (rnnL) ribosomal subunits of *A. astaci* were amplified and sequenced according to the protocol of Makkonen et al. (2018). The amplified fragments were sequenced in both directions on a 3500 Genetic Analyzer (Applied Biosystems), chromatograms edited in Chromas 2.6 (Technelysium, Brisbane, Australia), and the obtained sequences compared with the publicly available reference sequences of known *A. astaci* haplotypes (Makkonen et al. 2018; Martín-Torrijos et al. 2018).

qPCR-based genotyping: Genotyping by qPCR targeting five anonymous nuclear markers as described in Di Domenico et al. (2021) was performed on an iCycler iQ5 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). The qPCR assay followed the original protocol with only minor alterations. The assay for genotype groups B and E were run together in duplex reactions instead A/B and E/D, and the PCR cycling conditions were the same as for *A. astaci* detection described above, except for the annealing temperature, which was set to 60 °C. The results were evaluated using iQ5 Optical System Software version 2.0 (Bio-Rad).

Results

Distribution of A. astaci infections

A substantial difference in the proportion and spatial distribution of *A. astaci*-positive populations was observed among the tested non-native crayfish species in Czechia (Table 1, Fig. 1). Whilst no trace of the crayfish plague pathogen DNA was detected in either of the two tested *P. virginalis* populations, *A. astaci* was confirmed in 18 out of 25 sampling sites with *F. limosus* (72%), and in six out of 23 sites with *P. leniusculus* (26%).

When the crayfish plague pathogen was detected, the proportion of infected individuals among those tested ranged from 5 to 100% in populations of both host species (but note the wide confidence intervals of the prevalence estimate; Table 1). The individual pathogen load usually reached low to moderate agent levels (A2–A4; Table 1). We did not confirm the presence of *A. astaci* in four populations of *F. limosus*, 17 populations of *P. leniusculus*, or either population of *P. virginalis* (in most cases, 20 host individuals were tested per population). In all but one case, the qPCR assay revealed no trace of *A. astaci* DNA (agent level A0) in isolates from those populations. An exception was a DNA isolate from one *P. leniusculus* individual from the Dolní Tis fishpond (site no. 40), in which a potential presence of *A. astaci* DNA in trace amounts was indicated (agent level A1, conservatively interpreted as negative according to the original recommendations by Vrålstad et al. 2009).

In cases of *F. limosus*, populations with confirmed *A. astaci* infections were scattered across the whole country (Fig. 1). Those sampled from large rivers (Elbe, Vltava, and their major tributaries) were mostly infected. Some of the populations from isolated standing water bodies (quarries, sandpits) were infected and some were not, without any apparent spatial pattern. In contrast, recent unambiguous infections of *P. leniusculuss* were all restricted to the southern part of the country. In a single locality where both North American invasive species co-occurred (site no. 25), the infection was confirmed in one individual of each host, at a moderate agent level (A4). Interestingly, two of three *P. leniusculus* populations with particularly high *A. astaci* prevalence (exceeding 75%) were located in the immediate vicinity of the border with Austria (sites 30 and 32; Fig. 1).



Figure 1. Distribution of populations of invasive crayfish in Czechia screened for *Aphanomyces astaci* infection between 2017 and 2020. The shape of the symbol distinguishes host species. Populations where the pathogen was detected are marked by symbols with a full red border, those without *A. astaci* detection by a black dotted border. The fill colour indicates the pathogen genotype group (dark green: group B; yellow: group E). Site no. 25 is the only locality with a known co-occurrence of *F. limosus* and *P. leniusculus*, genotype group B was detected in both host species there.

Temporal changes in A. astaci prevalence

A slight decrease in *A. astaci* prevalence was frequently observed over time, in a total of 13 *F. limosus* and seven *P. leniusculus* populations re-examined after several years. However, these changes were usually not significant when the number of tested individuals was considered (Table 2). A significant change in *A. astaci* prevalence was observed in only three *F. limosus* populations (Table 2). Specifically, a decrease from 61% to below the detection level in the Pšovka brook near Střemy (site no. 19), already reported by Matasová et al. (2011), was confirmed by additional sampling in 2020. In contrast, a significant increase of *A. astaci* prevalence was observed in populations from the Malše river in České Budějovice (site no. 13; from 25% in 2005 to 100% in 2020) and a flooded quarry in Kojetice (site no. 17; from 15% in 2006 to 70% in 2017).

Furthermore, contrasting results of *A. astaci* detection were obtained from the *F. limosus* population in the Vysokopecký pond (site no. 6; Table 1). Two individuals were obtained from that locality in 2017, both weakly infected by *A. astaci* (agent level A2). However, when additional crayfish were collected there three years later for analysis of a larger sample (to improve the pathogen prevalence estimate), no traces of *A. astaci* DNA were detected either in 20 individuals from that pond or in 15 individuals from the Litavka brook just below the outflow from the pond.

Aphanomyces astaci genotyping

By combining available information from the three applied genotyping methods, we successfully assigned *A. astaci* to a genotype group and/or haplogroup for all 20 tested host crayfish individuals (see details in Suppl. material 1: Table S1). The success rate of the methods nevertheless varied. All three genotyping methods were successful for all six isolates exceeding 5500 PFU in the qPCR-based *A. astaci* detection (although all nine microsatellite markers were scored for three of them only). With decreasing concentrations of target DNA in the isolates, it became increasingly common that genotyping failed for some of the methods (see Suppl. material 1: Table S1). Assignment by all three genotyping methods was possible for nine cases and by two methods in seven cases. In four cases, only one of the genotyping methods was successful. When results from multiple methods were available, they were always congruent; this was the case also when a tentative assignment to a genotype group was based on a limited number of microsatellite loci. It is noteworthy that even when an insufficient number of informative microsatellite loci were scored, the observed microsatellite allele sizes never contradicted results from other genotyping methods.

Genotyping of *A. astaci* was successful for all the isolates precipitated by GlycoBlue, in which the original agent levels in the sample were low (agent level A3). For four of these, results of two methods were available; for the remaining three, only one of the genotyping methods succeeded, without any consistent pattern (Table 1; Suppl. material 1: Table S1).

Table 2. Comparison of *A. astaci* prevalence in *F. limosus* and *P. leniusculus* populations screened before 2013 and recently. If intermediate time points are shown, only the oldest with the newest are compared statistically. Significant changes in prevalence are highlighted in bold, p-values are given after Holm-Bonferroni correction for multiple testing. Site no.: Sampling site numbers as in Table 1. CI: confidence interval; NA: data not compared statistically due to a low number of individuals in old samples.

Site no.	Locality	Month, Year	Infected/ Analysed	Prevalence (95% CI)	p-value
Faxonia	is limosus				
1	Lomeček quarry (Starý Klíčov)	Mar 2006†	1 / 40	2.5% (0-13%)	1
		Jun 2020	0 / 16	0% (0–21%)	
2	Hracholusky reservoir	Jun 2006†	3 / 20	15% (3–38%)	1
		Aug 2017	2 / 10	20% (3–56%)	
4	Barbora surface mine	Oct 2005†	0 / 2	0% (0-84%)	NA
		Aug 2017	3 / 44	7% (1–19%)	
7	Litavka brook	Sep 2013§	0 / 6	0% (0-46%)	NA
		Jul 2020	0/15	0% (0-22%)	
8	Ohře river¶	Oct 2008‡	3/7	43% (10-82%)	NA
		Sep 2017	6 / 20	30% (12-54%)	
12	Vltava river near Kořensko	Apr 2004†	2/3	67% (9–99%)	NA
	reservoir#	Aug + Sep 2019	21 / 22	95% (77-100%)	
13	Malše river (České Budějovice)	Sep 2005	3 / 12	25% (6-57%)	0.009
		Jul 2020	10 / 10	100% (69–100%)	
15	Baraba sandpit (Cítov)	Oct 2005 + Jan 2007†	2 / 10	20% (3-56%)	1
		Aug 2019	0 / 20	0% (0-17%)	
17	Kojetice quarry	Aug 2006†	3 / 20	15% (3–38%)	0.02
		Aug 2017	14 / 20	70% (46-88%)	
19	Pšovka brook (Střemy)	Jun 2005†	11 / 18	61% (36-83%)	0.0005
		Jun + Jul 2020	0 / 20	0% (0-17%)	
20	Pšovka brook (Harasov)	2012 - 2013§	0/18	0% (0–19%)	1
		Aug 2017	3 / 15	20% (4-48%)	
21	Proboštská jezera sandpit	Sep 2005†	6 / 17	35% (14-62%)	1
		Oct 2019	0/7	0% (0-41%)	
		Jul 2020	2/19	10.5% (1-33%)	
24	Prudník brook	Oct 2006†	11 / 11	100% (72–100%)	1
		Aug 2020	10 / 10	100% (69–100%)	
Pacifast	acus leniusculus				
26	Kouba brook	May 2006†	1 / 11	9% (0-41%)	1
		Jul 2019	0 / 20	0% (0-17%)	
29	Blanice river	Sep – Oct 2006†	2/8	25% (3-65%)	1
		Sep 2020	0 / 20	0% (0-17%)	
35	Staviště brook	Jul 2012‡	2/6	33% (4–77%)	NA
		Aug 2017	0 / 42	0% (0-8%)	
38	Šípský brook	Jun 2010‡	0 / 10	0% (0-31%)	1
		Aug 2020	0 / 20	0% (0-17%)	
39	Stržek pond	Oct 2006†	2 / 20	10% (1-32%)	1
		Sep 2020	0/19	0% (0–18%)	
41	Spustík pond	Oct 2006†	2 / 13	15% (2-45%)	1
		Aug 2008‡	0 / 10	0% (0-31%)	
		Sep 2020	0 / 20	0% (0–17%)	
42	Ráček pond system††	Apr + Oct 2006†	2 / 23	9% (1–28%)	1
		Jul 2020	0 / 20	0% (0-17%)	

† Results included in Kozubíková et al. (2011a). ‡ Unpublished data. § Results included in Kozubíková-Balcarová et al. (2014). ¶ Old data were obtained from a fishpond connected to the river. The distance between the sampling sites is ca 400 m. # Old data were obtained from Karlovka, tributary of the Kořensko reservoir. The difference between the sampling sites is ca 2.5 km. †† Population in an interconnected pond system. Old data were obtained from the pond Ráček II, which is about 150 m from the new sampling site (stream bypass of the pond Ráček I). Out of 14 sampling sites with *F. limosus*, molecular markers corresponding to *A. astaci* genotype group E were detected in 13 cases. These represented localities across the whole invaded range of that species within Czechia (Fig. 1). In the Prudník brook (site no. 24), the same genotype group was confirmed both in 2006 and 2020 (Suppl. material 1: Table S1). However, at the site where *F. limosus* coexisted in syntopy with *P. leniusculus* (Malý Klikovský pond, site no. 25), genotype group B was confirmed in the infected *F. limosus* individual by all three molecular methods applied (Table 1, Fig. 1). Genotyping of *A. astaci* in infected individuals of *P. leniusculus* revealed in all cases the genotype group B; this also included an individual coexisting with *F. limosus* in the Malý Klikovský pond (Table 1, Fig. 1).

Discussion

Our data, extending the pilot study by Matasová et al. (2011), evaluated for the first time long-term changes in the prevalence of the crayfish plague pathogen in chronically infected invasive crayfish species on larger temporal and spatial scales. We statistically compared *A. astaci* prevalence in 14 sampling sites with *P. leniusculus* and *F. limosus* after more than ten years, and screened new sites with the documented presence of alien crayfish. Consistently with previous studies (Kozubíková et al. 2009, 2011a), the proportion of infected populations and prevalence values tended to be higher for *F. limosus* than for *P. leniusculus*. However, several hotspots of infected *P. leniusculus* were recently discovered in the country.

In our study, significant changes in *A. astaci* prevalence after a decade were observed only infrequently. Some fluctuations of *A. astaci* prevalence may reflect seasonality (Matasová et al. 2011), changes in host population density, or possibly the stress level to which the crayfish hosts are exposed. This might have caused the highly significant increases in *A. astaci* prevalence observed in two previously studied *F. limosus* populations. In several previously crayfish populations with low prevalences and infection levels (Kozubíková et al. 2011a), we no longer detected any trace of *A. astaci* DNA; however, the wide overlap of prevalence confidence intervals (see Table 2) indicates that the pathogen presence cannot be ruled out, and the decrease of prevalence was not significant in such cases.

A significant decrease to below the detection level in the *F. limosus* population from the Pšovka brook, already reported by Matasová et al. (2011), thus remains a notable exception. That study indicated a decrease in the prevalence of *A. astaci* to below the detection limit over six years (2004–10), and we did not detect *A. astaci* in the same brook stretch even a decade later (Table 2). Several kilometres upstream from that area, close to a zone where *F. limosus* was getting into contact with *A. astacus* as this latter species recolonised the stream, no *A. astaci* was detected in 2012 and 2013 (Kozubíková-Balcarová et al. 2014). However, we recently found *A. astaci* infections in three out of 15 tested *F. limosus* individuals there (Table 1; Rusch et al. 2020), indicating that the pathogen continues to persist within the host population and thus is an ongoing threat to native crayfish. In fact, a sudden disappearance of *A. astacus* from a several-km-long stretch of the brook upstream of the contact zone was observed by local conservation authorities in autumn 2021 (L. Beran, pers. comm.), presumably due to an unreported crayfish plague outbreak, as predicted by Kozubíková-Balcarová et al. (2014). Therefore, any presumed disappearance of the pathogen from a previously infected host population should be considered with caution.

To obtain more reliable data about the occurrence of *A. astaci* in populations where the pathogen prevalence may be low, very high numbers of individuals per population need to be examined (see Schrimpf et al. 2013). Negatively tested individuals in the low dozens per site, as in our present study, cannot prove the absence of the pathogen. One example of a likely underestimation of *A. astaci* occurrence may be the Vysočina region (sites no. 34 to 41 in Fig. 1, Table 1), the area of the first successful introduction of *P. leniusculus* to the Czech territory (Filipová et al. 2006). We did not reliably confirm the pathogen in recently collected samples from anywhere in this region, including populations with a previously reported *A. astaci* presence (Table 2; Kozubíková et al. 2011a). Although this may possibly represent a long-term regional decrease of *A. astaci* prevalence, disappearance of the pathogen from the entire region is highly unlikely. In this context, it should be noted that in one out of nine crayfish individuals from the newly screened population in the Dolní Tis fishpond (site no. 40), a trace amount of *A. astaci* DNA was consistently detected (Table 1).

An extreme case where the absence of *A. astaci* detection likely represents a false negative result at the whole-population level might be the Vysokopecký pond (site no. 6). There, we confirmed the infection in two *F. limosus* individuals in 2017, but three years later no trace of *A. astaci* DNA was detected either in 20 individuals from the pond or in 15 individuals from the adjacent Litavka brook (Table 1). The long-term presence of the pathogen in the brook may be nevertheless assumed, as a crayfish plague outbreak caused by *A. astaci* genotype group E was confirmed in the section just below the Vysokopecký pond in 2011 (Kozubíková-Balcarová et al. 2014)

A contrasting difference between older and more recent samples, but in the opposite direction, was also observed in the Bobrava river in the south-eastern part of the country (site no. 45). Consistently high *A. astaci* prevalence (\geq 70%) in *P. leniusculus* was detected there in 2018 and 2020 (Table 1), but the pathogen was not detected by qPCR in 10 individuals collected approx. 12 km upstream in 2010 (E. Kozubíková-Balcarová, unpubl. data). Genotyping of the pathogen in *P. leniusculus* from this river confirmed the genotype group B, which is generally associated with this host species. Corresponding strains have been repeatedly isolated and/or genotyped from *P. leniusculus* originating in the USA (Huang et al. 1994; Makkonen et al. 2019) as well from individuals collected across its invaded range in Europe (reviewed in Ungureanu et al. 2020). Considering that the nearest locality to the Bobrava river known to host crayfish infected by *A. astaci* is inhabited by *F. limosus* (site no. 23, located within the same river basin), we presume that the long-term persistence of *A. astaci* in its original *P. leniusculus* host is a likely explanation for its recent confirmation in the Bobrava.

Genotype group B was also confirmed in all other genotyped individuals of *P. leniusculus* from Czech localities (Fig. 1). Populations of that species with sufficient infection levels to allow genotyping were located only in the southern part of the country. Except for the Bobrava river mentioned above, all those localities are close to the state border with Austria (in two cases, in the immediate vicinity). According to the species occurrence database of the Nature Conservation Agency of the Czech Republic (accessed 12/2021), they were all discovered after 2010. Nevertheless, *A. astaci* had likely been spreading from *P. leniusculus* to native crayfish earlier: four mass mortalities of native *A. astacus* caused by genotype group B have been confirmed in various regions of Czechia (Kozubíková-Balcarová et al. 2014; M. Mojžišová, unpubl.). The first was reported in 2007 from the tributary of Pěnenský pond (site no. 31), in which we confirmed the presence of infected *P. leniusculus* and genotyped the pathogen only a decade later.

In all but one case, we identified *A. astaci* genotypes that were expected to be found in European populations of their respective North American crayfish carriers (Fig. 1, Table 1). This indicates that original sources of the pathogen in crayfish mass mortalities can be reasonably assumed from genotyping results in regions where the coexistence of different North American invasive hosts is uncommon or absent. However, in a single locality in Czechia where the syntopic presence of *P. leniusculus* and *F. limosus* was discovered in 2020 (Malý Klikovský pond; site no. 25), we unambiguously identified *A. astaci* genotype group B in both host species. This indicates a likely interspecific transmission of *A. astaci* from *P. leniusculus* to *F. limosus*, as in a previously reported case from the UK where the recipient taxon was a member of the virile crayfish species complex, *Faxonius* cf. *virilis* (James et al. 2017a). Regions where multiple invasive *A. astaci* carriers coexist or may come into contact, such as the Netherlands (Tilmans et al. 2014) and Hungary (Weiperth et al. 2020), may thus yield hardly predictable host taxon – pathogen genotype combinations, making it difficult to track the origin of possible crayfish plague outbreaks.

Our experience with the inconsistent success of the applied genotyping methods confirms that characterising *A. astaci* genotypes chronically infecting their original carriers is challenging, and various methodological approaches may complement each other. In the relatively rare cases when a heavy infection of an American host is observed, all genotyping methods are likely to succeed. In already preserved material, increasing the pathogen DNA concentration in the isolate, such as with the use of the GlycoBlue Coprecipitant in our study, may increase the chance for successful genotyping. Alternatively, when live crayfish are available, the growth of the pathogen may be enhanced by their exposure to stress (as in Kozubíková et al. 2011b) or by analysis of host moults, which seems particularly promising (Martín-Torrijos et al. 2021).

Our data suggest that long-term significant changes in *A. astaci* prevalence in its North American hosts were not common within the studied populations. In several originally weakly infected populations (in particular of *P. leniusculus*) we no longer detected the pathogen, but it is likely that it persists in the area. The re-appearance of infected *F. limosus* individuals in the Pšovka brook (moreover, associated with the recent disappearance of susceptible *A. astacus* from an adjacent section of the brook) confirms that *A. astaci* prevalence at low levels (<5%) still poses a threat to local native crayfish.

The preventive rescue transfer of *A. astacus* from the Pškovka to another local watershed without alien crayfish (Kozubíková-Balcarová et al. 2014) was apparently a timely measure that contributed to the species conservation in the region. However, highly infected populations of invasive crayfish (and nearby populations of the susceptible native species) should be prioritised in any management strategies. In this context, the apparent cross-border invasion by strongly infected *P. leniusculus*, likely of different origin from the long-established weakly infected populations of that species in Czechia, is of particular concern.

Despite evidence of the apparent interspecific transmission of *A. astaci* from *P. leniusculus* to *F. limosus* at one site, our results generally support the link between specific pathogen genotypes and particular North American crayfish hosts invading European waters. This suggests that *A. astaci* genotyping is a relevant approach to tracking of sources of the pathogen in crayfish plague outbreaks in Central and Western European countries. Overall, our study highlights the importance of routine country-wide screening for relevant aquatic wildlife pathogens as an integral part of relevant conservation strategies. In the case of *A. astaci*, the screening accuracy might be improved by combining the analyses of host tissues and environmental DNA (e.g., Rusch et al. 2020; Troth et al. 2020; Sieber et al. 2022).

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Supplementary material I

Table S1

Authors: Michaela Mojžišová, Jitka Svobodová, Eva Kozubíková-Balcarová, Eva Štruncová, Robin Stift, Michal Bílý, Antonín Kouba, Adam Petrusek

Data type: genotyping

- Explanation note: Detailed results of *Aphanomyces astaci* genotyping in individual DNA isolates. Allele sizes for each microsatellite locus are provided for all analysed samples and for relevant pathogen reference genotypes. Strains representing genotype groups B and E and highlighted in bold.
- 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.

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RESEARCH ARTICLE



Scanning the horizon for invasive plant threats using a data-driven approach

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Abstract

Early detection and eradication of invasive plants are more cost-effective than managing well-established invasive plant populations and their impacts. However, there is high uncertainty around which taxa are likely to become invasive in a given area. Horizon scanning that combines a data-driven approach with rapid risk assessment and consensus building among experts can help identify invasion threats. We performed a horizon scan of potential invasive plant threats to Florida, USA—a state with a high influx of introduced species, conditions that are generally favorable for plant establishment, and a history of negative impacts from invasive plants. We began with an initial list of 2128 non-native plant taxa that are known invaders or crop pests. We built on previous invasive species horizon scans by developing databased criteria to prioritize 100 taxa for rapid risk assessment. The semi-automated prioritization process

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included selecting taxa "on the horizon" (i.e., not yet in the target location and not on a noxious weed list) with climate matching, naturalization history, "weediness" record, and global commonness. We derived overall invasion risk scores with rapid risk assessment by evaluating the likelihood of each of the taxa arriving, establishing, and having an impact in Florida. Then, following a consensus-building discussion, we identified six plant taxa as high risk, with overall risk scores ranging from 75 to 100 out of a possible 125. The six taxa are globally distributed, easily transported to new areas, found in regions with climates similar to Florida's, and can impact native plant communities, human health, or agriculture. Finally, we evaluated our initial and final lists for potential biases. Assessors tended to assign higher risk scores to taxa that had more available information. In addition, we identified biases towards four plant families and certain geographical regions of origin. Our horizon scan approach identified taxa conforming to metrics of high invasion risk and used a methodology refined for plants that can be applied to other locations.

Keywords

certainty, consensus building, Florida, horizon scan, invasion, prevention, rapid risk assessment

Introduction

Invasive species can negatively impact ecosystems, economies, and human health (CBD 2009). Managing potential impacts of invasive species, and invasive plants in particular, is daunting given the many species introduced to novel areas each year, with rates predicted to increase in the future (Seebens et al. 2017). When governments and private landowners take action, they often manage invasive plants and mitigate negative impacts after establishment. However, preventing the introduction and initial spread of invasive plants is generally more effective and avoids potential ecological and economic losses (Keller et al. 2007; Sheley et al. 2015). Unfortunately, the benefits of prevention are difficult to quantify and involve high uncertainty, making post-invasion control the more common approach (Finnoff et al. 2007; Early et al. 2016). Thus, programs that help identify which non-native plant taxa have a high probability of becoming problematic invaders are essential for providing the first line of defense against plant invasions.

Horizon scanning is the systematic search to identify potential threats, emerging issues, and opportunities that can inform research and action (Sutherland and Woodroof 2009; Amanatidou et al. 2012). The goal of horizon scanning in conservation science is to preemptively identify threats so researchers can provide timely and informed input on policy and decision-making (Sutherland and Woodroof 2009). In Europe, horizon scanning of emerging invaders has involved acquiring lists of potentially invasive species for a specific region, assessing the likelihood of arrival, establishment, and impact for each species, and, in some cases, building consensus among experts around a list of species ranked by risk (Parrott et al. 2009; Matthews et al. 2014; Roy et al. 2014; Gallardo et al. 2016; Lucy et al. 2020). These horizon scans have informed policy and guided resource allocation towards research and prevention efforts.

Florida is one of the most important states for regulating invasive plants in the United States because nearly 85% of all non-native plants imported to the contiguous

United States enter through one of Florida's shipping ports or airports (Gordon and Thomas 1997). As international trade continues to grow, so too does the frequency of intentional and accidental introductions (Early et al. 2016). In addition to being an entry point for invasive species to the rest of the country, Florida is particularly vulnerable to the establishment of invasive plants due to its tropical/subtropical climate and diverse ecosystems (Simberloff 1997; Pyšek et al. 2017). Management of invasive plants in Florida's conservation areas costs nearly \$45 million annually (Hiatt et al. 2019) and invasive species (including plants, insects, and pathogens) cost Florida's agriculture industry at least \$179 million annually (Coffman et al. 2001). Identifying potential invaders before or soon after they enter Florida can reduce ecological and economic losses to the state as well as prevent the spread of invasive plants nationally.

Here, we developed a horizon scan approach to create a ranked list of non-native plants that are likely to arrive and establish in Florida and have impacts on native biodiversity, the economy, or human health in the near future. We started with a large initial list of plant taxa that were associated with invasion. We then developed criteria and used publicly available datasets to prioritize taxa for risk assessment. This step builds on previous horizon scans, which were able to assess all taxa on initial lists. We present a ranked list of potential invasive plant threats to Florida, which can be used to inform research, management, and policy aimed at reducing invasive plant impacts.

Methods

This horizon scan was part of the Florida Invasion Threats Horizon Scan, which assessed invasion threats of freshwater and terrestrial plants (reported here), marine taxa, freshwater invertebrates, terrestrial invertebrates, and non-marine vertebrates (Lieurance et al. in review). We adapted and revised the horizon scanning method outlined by Roy et al. (2014, 2015) to develop a ranked list of invasive plant threats and their potential pathways for arrival to the target location (Florida) in the near future (e.g., 5–15 years). We chose this time frame to prioritize upcoming threats, to establish a minimum frequency for updating the horizon scan with new information (once every 5–15 years), and to evaluate risk within current climate conditions (i.e., omitting future climate change scenarios). We kept this time frame in mind by considering current arrival pathways and environmental conditions in the target location.

Expert panel and workshop

We (the authors) formed the expert panel for freshwater and terrestrial plants, providing knowledge of Florida's natural systems, existing invasive plants, relevant policy, and data analysis. Along with experts of other taxonomic groups described above, we convened a workshop for the Florida Invasion Threats Horizon Scan in December 2019. During the workshop, we designed criteria for prioritizing taxa to assess (see Assembling a list) and discuss the rapid risk assessment tool (see Assessing and scoring the taxa).

Assembling a list

Using the horizon scan tool developed by the Centre for Agriculture and Biosciences International (CABI; an inter-governmental not-for-profit organization that provides information and expertise on agriculture and the environment), we generated an initial list of invasive taxa and crop pests (Suppl. material 1). The tool consolidates information from the CABI Invasive Species Compendium and Crop Protection Compendium, which are science-based encyclopedic databases (CABI 2018). Based on these databases, the tool generated a list of 2128 plants and algae that were not known to be present in Florida.

We corrected the list for synonyms and accepted names using (in the order of our assigned authority): the Atlas of Florida Plants (Wunderlin et al. 2019), the Integrated Taxonomic Information System (Integrated Taxonomic Information System 2000), and the Taxonomic Name Resolution Service (Boyle et al. 2015; see Suppl. material 1 for more details). We then identified taxa that were growing in at least one location with similar climate to the target location (Kottek et al. 2006; CABI 2018), not already naturalized in the target location (Wunderlin et al. 2019), not on a local (i.e., Florida) or national noxious weed list, naturalized outside of their native ranges (van Kleunen et al. 2019), and historically weedy (Randall 2017; Fig. 1, Suppl. materials 1, 2). We next used expert opinion to remove two taxa: one taxon that had already been assessed by a panel member and one that was only specified to genus level (Suppl. material 1). Finally, we selected the top 100 most globally common taxa for further assessment (GBIF.org 2022, Suppl. material 1), which was the largest number of taxa that nine assessors could evaluate given 20 hours of assessment time each (and 40 hours for one assessor). Global commonness serves as a proxy for propagule pressure and establishment success (Shah et al. 2012; Blackburn et al. 2015).

Assessing and scoring the taxa

Nine assessors evaluated taxa using a rapid risk assessment tool modified from Roy et al. (2014). First, we used a species not included in the assessment list to evaluate the tool for clarity, timing, and assessment consistency. Then, we completed risk assessments with a standardized set of resources (Suppl. material 3). Because the risk assessments are designed to be completed rapidly, we aimed to spend less than two hours on each taxon.

We identified one or more potential pathways for taxa to arrive in Florida based on an established framework (Hulme et al. 2008; CBD 2014; Harrower et al. 2018). Briefly, the pathways included "release in nature" (intentional release, such as for erosion control), "escape from confinement" (intentional commodity that escapes, such as a horticultural taxon), "transport contaminant" (associated with the transport of a specific commodity, such as a seed contaminant), "transport stowaway" (other forms of unintentional transport, such as through soil on equipment), "corridor" (through human infrastructure linking previously unconnected areas, such as a waterway), and unaided (natural dispersal).









Figure 1. Methods for selecting and evaluating taxa as invasive plant threats for a target location (Florida, United States). Data-based list processing led to the prioritization of 100 taxa for risk assessment. Rapid risk assessments performed by an expert panel included pathways for arrival and likelihood scores and certainty aged to get an overall certainty. Each risk assessment was evaluated with two rounds of review and a consensus-building discussion before the expert panel confirmed ratings for arrival, establishment, and impact. The three component likelihood scores were multiplied to get an overall score and certainty ratings were roughly avertaxa rankings We scored the likelihoods of arrival, establishment, and negative impacts (environmental, socioeconomic, and human health) on a scale of 1 (very low) to 5 (very high; Fig. 1). To estimate the likelihood of arrival, we considered the current distribution of the taxon, the availability of the taxon for purchase, history of invasion by the taxon in other regions, and the presence of a plausible arrival pathway (Table 1).

To estimate the likelihood of establishment (i.e., developing a self-sustaining population), we considered the distribution and number of records of the taxon within regions with Köppen-Geiger climate zones matching Florida (Table 1). This evaluation expands on the use of Köppen-Geiger climate zones to select taxa for our assessment list, in which records in only one matching location were needed to pass the criterion (Fig. 1). We also considered ecological properties of both the taxon and target location habitats, including time to reproductive maturity, reproduction rate, dispersal mechanism, propagule pressure, tolerance of a broad range of environmental conditions, resource availability, natural enemies, and amount of nurturing required (e.g., weeding, irrigation, fertilization, pest control; Petri et al. 2021). Geographic thresholds for arrival and establishment likelihood scores (Table 1) were chosen based on distance, ease of movement through ground transportation, and low barriers to introduction by travel or mail (USDA APHIS 2017a, b).

To estimate the likelihood of negative impacts, we used a scoring rubric modified from the Invasive Species Environmental Impact Assessment protocol (Branquart 2009), the Environmental Impact Classification of Alien Taxa (Blackburn et al. 2014; Hawkins et al. 2015), and the Socio-Economic Impact Classification of Alien Taxa (Bacher et al. 2018; Table 1). The overall risk score was the product of arrival, establishment, and impact likelihood scores (Fig. 1; Roy et al. 2015). We provided brief justifications for our scores and assigned certainty ratings that ranged from very low (i.e., all scores were equally likely) to high (i.e., could confidently eliminate all other scores). The overall certainty rating was the rating most consistent with three component certainty ratings (Suppl. material 3).

Review and consensus building

Assessments were peer-reviewed by the panel (Suppl. material 1). During the virtual consensus-building meeting, we discussed taxa in descending order of scores and removed one taxon because of ambiguity about whether it was already naturalized in Florida (Suppl. material 1). Because reviewers used a range of criteria for arrival and establishment justifications that were inconsistent across taxa, we created rubrics (Table 1) and reviewed scores again. After confirming overall scores with the panel, we categorized taxa as follows: taxa scoring ≥ 64 (i.e., an average score of 4 for each category of arrival, establishment, and impact) as high risk, taxa scoring ≥ 27 (i.e., an average score of 3 for each category) and < 64 as medium risk, and taxa scoring < 27 as low risk. This process resulted in a final list of 99 taxa that moved through the assessment, review, and analysis steps (Fig. 1).

Category	Criteria	Score				
Arrival [†] Closest observation to target location [‡] and closest online seller to target location are outside of re						
	Closest observation to target location is within region, but not nearby ⁸ , and closest online seller to	2				
	target location is outside of region.					
	Closest observation to Florida and closest online seller to target location are within region, but not	3				
	nearby or closest observation to target location is nearby, but not in target location, and closest online					
	seller to target location is outside region.					
	Closest observation to target location is nearby, but not in target location, and closest online seller is	4				
	within region or nearby, but not in target location.					
	The taxon has been observed or sold within target location.	5				
Establishment [†]	No observations in areas with matching Köppen-Geiger (KG) zones to target location.	1				
	Few observations in one area with matching KG zones to target location.	2				
	Many observations in one area or few observations in multiple areas with matching KG zones to target	3				
	location.					
	Many observations in multiple areas with matching KG zones to target location.	4				
	Criteria for score 4 plus evidence of a biological strategy that aids establishment or evidence of estab-	5				
	lishment in target location.					
Impact	Unlikely to cause negative impacts on the native biota or abiotic environment, human well-being, or	1				
	economic systems.					
	Likely to cause (a) declines in the performance (e.g., biomass, body size) of native biota, but no de-	2				
	cline in native population sizes or (b) income loss, minor health problems, higher effort or expense to					
	participate in activities, increased difficulty in accessing goods, or minor disruption of social activities,					
	but no significant impact on participation in normal activities.					
	Likely to cause (a) declines in the population size(s) of native species, but no changes to the structure	3				
	of communities or to the abiotic or biotic composition of ecosystems or (b) changes in the size of					
	social activities, with fewer people participating, but the activity is still carried out. These changes to					
	social activities could be linked to accessibility to the activity area or mild effects to human health					
	(e.g., allergies).					
	Likely to cause (a) the local or population extinction of at least one native species, leading to reversible	4				
	changes in the structure of communities, the abiotic or biotic composition of ecosystems or (b) the					
	local disappearance of a social or economic activity from all or part of the area invaded by the alien					
	taxon, collapse of the specific activity, switch to other activities, abandonment of activity without					
	replacement, emigration from region, or moderate effects to human health.					
	Likely to cause (a) the replacement and local extinction of native species and will produce irreversible	5				
	changes in the structure of communities and the abiotic or biotic composition of ecosystems or (b)					
	local disappearance of a social or economic activity from all or part of the area invaded by the alien					
	taxon or major effects to human health.					

Table I. Rubrics for scoring likelihood of arrival, establishment, and impacts of potential invasive plants.

[†]Arrival and Establishment rubrics were applied during the second review phase. Scores were adjusted by up to one point based on additional information in the assessments.

[‡]target location = Florida, United States; observations based on GBIF.org (2020) or information provided by the assessor or reviewer.

[§]For our purposes, "region" is contiguous United States and "nearby" are the states of Georgia, Alabama, South Carolina, North Carolina, Tennessee, and Mississippi.

Observations based on GBIF.org (2020) or information provided by the assessor or reviewer. Florida's Köppen-Geiger zones include Af, Am, Aw, and Cfa (Kottek et al. 2006).

Analysis of risk scores

We evaluated whether peer-review and consensus building significantly affected overall risk scores with a paired two-sample *t*-test (before vs. after). We also evaluated how assessors and characteristics of the taxa affected overall risk scores. We fit a generalized linear regression with a negative binomial error structure to the overall risk scores with the expert who completed the assessment (N = 9), expert certainty about the overall score (very low, low, medium, or high), whether the typical habitat is terrestrial or

aquatic, the number of records in the United States, and the year of the earliest occurrence record in the United States (cultivated, naturalized, and otherwise) as independent variables. We assumed the number of records and earliest record were proxies for propagule pressure (the former metric), residence time (the latter metric; Pyšek et al. 2009), and existing information in the literature, internet, and held by experts (both metrics). We used the package 'rgbif' (Chamberlain et al. 2021) to extract all Global Biodiversity Information Facility (GBIF) records in the United States for each taxon, selecting records that had coordinates and no geospatial issues (GBIF.org 2021). Number of records and earliest record from this dataset were centered and scaled and were not significantly correlated with each other (r = 0.04, P = 0.68). We fit the model using the 'MASS' package (Venables and Ripley 2002), evaluated the fit using the 'DHAR-Ma' package (Hartig and Lohse 2020), tested the significance of each independent variable using likelihood ratio tests, and compared estimated marginal means of factor levels with the Tukey method using the 'emmeans' package (Lenth et al. 2021). All analyses were conducted in R version 4.0.2 (R Core Team 2020).

Plant families and geographic ranges

We evaluated whether plant taxonomic families were under- or overrepresented in the CABI plant list and in the final list using a resampling procedure (Daehler 1998). We first extracted all accepted species names and their family names from The Plant List using the 'taxize' package (Chamberlain and Szoecs 2013, TPL 2013), resulting in a dataset of 373,847 taxa. The CABI list contained 158 families (with 2091 taxa) in The Plant List (vascular plants and bryophytes). We re-sampled 2091 taxa without replacement from The Plant List dataset 10,000 times. Taxa were replaced between iterations and we counted the number of taxa per family at each iteration. We set the threshold for statistical significance to P < 0.0003 (0.05 divided by the number of families, consistent with a Bonferroni correction; Daehler 1998). Therefore, if the number of taxa sampled from a family was greater (less) than or equal to the number of taxa from that family in the CABI list in fewer than three iterations, we considered the family overrepresented (underrepresented) in the CABI list. We repeated this procedure with different values for the final list: 34 families with 98 taxa, 1,000 iterations, P < 0.0015, and families with one or fewer iterations.

To evaluate the native and introduced ranges of taxa in the final list, we researched their distributions using the Plants of the World database (for 95 of the 99 taxa; POWO 2021), the CABI Invasive Species Compendium (CABI 2021), the Global Compendium of Weeds (Randall 2017), and GBIF (GBIF.org 2020). We summarized and mapped distributions using the World Bank Development Indicator regions in the 'countrycode' package (Arel-Bundock et al. 2018). One species, *Aegagropila linnaei*, was omitted from the map because we were unable to clearly define its native range.

Data availability

Data and code are available at https://doi.org/10.5281/zenodo.6211243.

Results

Analysis of risk scores

We found no significant difference in the means of overall risk scores before and after peer-review and consensus building (t = -1.41, 95% CI = -4.43-1.61, df = 97, P = 0.357) with an average score (\pm SE) of 21.3 ± 2.1 before and 22.7 ± 2.1 after. However, the overall risk scores of 14 taxa increased enough to move them into a higher risk category, with one taxon (*Avena fatua*) moving two categories higher. Additionally, the overall risk scores of ten taxa decreased enough post-review and consensus building to move them into a lower risk category, with one taxon (*Campylopus introflexus*) moving two categories lower. These larger changes in overall risk scores resulted from assessors reconsidering how to interpret available information following consensus building and rubric review (Table 1, Suppl. material 3).

There was strong evidence that the assessor and certainty level affected the overall risk score (Table 2). Four out of 36 pairwise comparisons of assessors were significantly different with P < 0.05. Taxa with higher overall certainty ratings also had higher overall risk scores (Fig. 2C). Taxa with earlier first records in the United States received higher overall risk scores than taxa with later first records (Fig. 3A, Table 2). Taxa with more records in the United States did not receive significantly higher overall risk scores (Table 2), although there was a positive trend (Fig. 3B).

Plant families and geographic ranges

Four families were significantly overrepresented in the final list of 99 taxa compared to the number of accepted species in the family (Suppl. material 4): Juncaceae (3 taxa out of 581 accepted species), Poaceae (21 taxa/11883 accepted species), Polygonaceae (4 taxa/1584 accepted species), and Rosaceae (7 taxa/5325 accepted species). These four families were also significantly overrepresented in the CABI list (Suppl. material 5): 21 taxa (1% of the CABI list) were in Juncaceae, 226 taxa (11%) were in Poaceae, 37 taxa (2%) were in Polygonaceae, and 80 taxa (4%) were in Rosaceae. None of the families present on the final list were significantly underrepresented.

The majority (93%) of taxa on the final list had native ranges that included Europe and Central Asia, 75% included the Middle East and North Africa, and 67% included East Asia and the Pacific (Fig. 4A). Other regions were included in 43% or fewer of the taxa's native ranges. The United States was included in the native ranges of

Variable	χ²	df	Р
Assessor	27.02	8	< 0.001
Certainty	21.40	3	< 0.001
Earliest U.S. record	3.85	1	0.05
Records in United States	1.67	1	0.20
Habitat (terrestrial vs. aquatic)	0.07	1	0.79

Table 2. Model summary of overall risk scores, evaluated with likelihood ratio tests of nested models.

Α

Plant species



Figure 2. Overall likelihood scores from the horizon scan of potential invasive plant threats to Florida **A** the overall risk scores for 99 taxa, divided into groups of high risk (score ≥ 64), medium risk (27 \leq score < 64), and low risk (score < 27) and shaded by overall certainty rating **B** the number of taxa associated with each of the pathways of arrival. Multiple pathways could be assigned to a single taxon. **C** the relationship between certainty and the overall risk score, averaged across all taxa. Letters above bars indicate significant differences in overall risk score among certainty ratings with *P* < 0.05.



Figure 3. Earliest record and number of records. The overall risk score and **A** the year of the earliest record in the United States and **B** the number of records (displayed on a \log_{10} scale for clarity) in the United States for the 99 taxa on the final list. Points represent data while line and shading represent model-estimated mean \pm SE.



Figure 4. Ranges of taxa **A** native and **B** introduced ranges of the final list of taxa generalized at the country level. Countries with darker shades indicate a greater number of taxa native or introduced to the area. The target location (Florida) is in red.

11 taxa: Bolboschoenus maritimus, Carex nigra, Deschampsia cespitosa, Elodea nuttallii, Fragaria vesca, Geranium robertianum, Juncus articulatus, Lupinus polyphyllus, Phalaris arundinacea, Potamogeton natans, and Sanguisorba officinalis. Although some native populations of *P. arundinacea* exist in North America, most populations are Eurasian genotypes (Jakubowski et al. 2014). The remaining ten taxa are native to some U.S. states, but are not in the target location (Florida; USDA 2019). The majority (89%) of the taxa on the final list have been introduced to North America (Fig. 4B). This region was followed closely by East Asia and the Pacific (79%), Europe and Central Asia (71%), and Latin America and the Caribbean (69%). Other regions were included in 40% or fewer of the taxa's introduced ranges.

High risk taxa

Six plant taxa received risk scores of at least 64 (Figs 2, 5), indicating that they are likely to invade Florida in the near future. We had high certainty about the risk scores for four taxa: *Ligustrum vulgare*, *Cytisus scoparius*, *Phalaris arundinacea*, and *Avena fatua*. We had medium certainty for the other two taxa: *Agrostis capillaris* and *Persicaria hydropiper*. Three were considered very likely to arrive in Florida (arrival score = 5 out of 5): *L. vulgare*, *A. fatua*, and *P. hydropiper*. This conclusion was based on herbarium specimens indicating historic, but not current, presence in Florida; observations of presence without naturalization within the last 20 years; and records of seeds sold within the United States at the time of the assessment (Suppl. material 3). All six taxa were considered very likely to establish in Florida (establishment score



Figure 5. The six taxa that were designated as high risk for invasion in Florida. Overall risk scores are in black circles (maximum possible score is 125). (Photos: Meneerke bloem, Isidre blanc, Andreas Eichler, Stefan.lefnaer, CC BY-SA 4.0; Robert Flogaus-Faust, CC BY 4.0; Rasbak, CC BY-SA 3.0; Willow, CC-BY 2.5; Mary Joyce, Katrice Baur, scottq1, rae117, CC BY-NC 4.0; Christian Grenier, CC0 1.0).

= 5 out of 5) because they occur in other regions of the world with climates similar to Florida's and in some cases, they are known to have high reproductive capacity (Suppl. material 3). Four taxa were considered likely to cause loss of native species, loss of social or economic activity, or moderate human health effects (impact score = 4 out of 5): *L. vulgare, C. scoparius, P. arundinacea,* and *A. capillaris.* Impacts of the high risk taxa included suppressing native vegetation through competition, producing pollen that can be a human allergen, and reducing crop yields (Table 3, Suppl. material 3). Information about the six taxa from a handful of sources can help inform potential future policy actions (Table 3): the taxa have global distributions; they have cultural and economic uses that have facilitated their introduction to new regions; they are managed through various, often integrated, approaches; and they are included in non-Florida U.S. state noxious weed lists or laws.

Species	Native range [†]	Introduced countries [‡]	Common uses [§]	Potential impacts [§]	Management	States
					approaches [§]	listed
Ligustrum vulgare	Europe, western Asia, northern Africa	Argentina, Australia, Brazil, Canada, New Zealand, South Africa, United States	landscape (planted as a hedge or bor- der), medicinal	host crop pests, compete with na- tive plants, pollen allergens, poison- ous berries	mechanical (pull- ing, digging, cut- ting), herbicides	11
Phalaris arundinacea	Asia, Europe, Central America, North America ⁵ , southern/eastern/ northern Africa	Ethiopia, Kenya, Tanzania, Uganda	erosion control, fodder crop, fiber, ornamental, biofuel	obstruct waterways, compete with na- tive plants, reduce wildlife habitat quality	integrated control, burning, discing, mowing, herbicides	10
Cytisus scoparius	Europe	Argentina, Australia, Bolivia, Brazil, Canada, Chile, China, India, Iran, Japan, New Zealand, South Africa, United States	ornamental, medici- nal, nurse plant	compete with native plants, facilitate other invasive species, alter nutrient and water availability	integrated control, burning, grazing, mulch- ing, pulling, cut- ting, herbicides, biological control	14
Agrostis capillaris	central/western/ southwestern Asia, Europe, North Africa,	Argentina, Australia, Bhutan, Brazil, Canada, Chile, Green- land, India, New Zealand, Saint Helena, Saint Pierre and Miquelon, South Georgia and the South Sandwich Islands, United States	turf grass (lawns and golf), fodder, pasture, erosion control, landscape rehabilitation	competes with native plants, indirectly reduce moth population sizes through loss of native plants, pollen allergens	crop rotations, pulling, herbi- cides	5
Avena fatua	Central Asia	Canada, United States (pre- sent in 74 other countries, but "introduced" status not provided)	fodder, forage, gene source for disease resistance, medicinal	reduce crop yields	straw burning, crop rotation, herbicides, soil cultivation, soil solarization	4
Persicaria hydropiper	Europe	"introduced" status not provided, but present in 48 countries	culinary, medicinal	crop and pasture weed	herbicides	1

Table 3. Summary of the six high risk species using three of the main references used in rapid risk assessment.

[†]Geographic regions where the taxon is native (CABI 2021, Native Plant Trust 2021).

[‡]Countries where the taxon has been introduced (CABI 2021).

See Plant families and geographic ranges section for more details.

[§]Uses, impacts, and management approaches in CABI database (2021). This information was used, along with other sources, in rapid risk assessments.

U.S. states in which the taxon is included in a prohibited list or law (EDDMapS 2021).

Medium risk taxa

Twenty-three taxa received medium risk scores ($27 \le \text{score} < 64$; Fig. 2). Two taxa, *Matricaria chamomilla* and *Symphytum officinale*, were considered very likely to arrive in Florida (arrival score = 5) because they had records in Florida, including two for *S. officinale* that suggested escape (Suppl. material 3). Both taxa were also considered very likely to establish in Florida (establishment score = 5) and cause declines in native species' performances or minor human social, economic, or health problems, but not more negative impacts (impact score = 2). Three taxa, *Hypericum perforatum, Malva sylvestris*, and *Mentha aquatica*, were considered likely to arrive in Florida (arrival score = 4), very likely to establish in Florida (establishment score = 5), and likely to cause declines in native species' population sizes or human participation in social activities (impact score = 3). All five taxa are sold as ornamental plants within the United States and have been reported in the southeastern United States in the past 20 years (Suppl. material 3). We had high certainty about the scores of two taxa (including *H. perforatum*), medium certainty about the scores for 18 taxa, and low certainty about the scores for three taxa (including *S. officinale*).

Low risk taxa

Seventy taxa received low risk scores (< 27; Fig. 2). *Poa trivalis* was considered very likely to arrive in Florida (arrival score = 5) because it is in the southeastern United States, has been used in at least one research experiment in Florida, and is planted in golf courses in the southeast both intentionally and unintentionally (seed contaminant). *Poa trivalis*, however, is unlikely to establish in Florida (establishment score = 2) and have severe impacts (impact score = 2). *Sambucus nigra* ssp. *nigra* was considered very likely to establish in Florida (establishment score = 5) because the species *Sambucus nigra* occurs in multiple locations with climate similar to Florida's (Suppl. material 3). However, the subspecies has few recorded occurrences globally, which led to very low certainty about the establishment score = 1) and likely to cause declines in native species' performances or minor human social, economic, or health problems, but not more negative impacts (impact score = 2). We had high certainty about the scores of 16 taxa, and very low certainty about the scores of three taxa.

Pathways of arrival

The most likely pathway of arrival for the taxa on the final list was escape from confinement (Fig. 2B). Taxa are also likely to arrive in Florida as transport contaminants, transport stowaways, or with unaided dispersal. It is less likely that plants will arrive through intentional release into nature or through a constructed corridor.

Discussion

Our horizon scan of invasive plant threats to Florida identified six taxa that have a high risk of becoming invasive in the state in the near future (5-15 years). The horizon scanning process helped us identify taxa that should undergo more thorough risk assessments and potentially receive policy restrictions or research priority. Our reliance on existing databases allowed us to quickly evaluate many taxa in a manner than can be applied to future horizon scans. Further, we used this case study to assess biases in the horizon scan process that should be taken into consideration in future horizon scans of invasive plants.

Although we used databases to reduce the number of taxa on our list, it was necessary to use expertise to perform rapid risk assessments, review, and consensus building. These expert-based processes are therefore not repeatable, but we aimed to increase transparency by providing the assessments and reviews (Suppl. material 3). The identity of the assessor significantly affected the overall risk scores. Two assessors who had 3–8 years of risk assessment experience scored taxa higher on average than two assessors who had less than one year of risk assessment experience. Because our sample size of assessors is small, we are unsure whether this outcome is coincidental (due to the taxa assessed by these individuals) or due to assessor experience. To address differences in experience, future horizon scans could calibrate scores among assessors with a set of test taxa, a more rigorous approach than our calibration with a single taxon, or derive composite scores from multiple assessors, for example through structured expert judgement (Wittmann et al. 2015). Discrepancies in experience highlight the importance of rubrics, peer review, and consensus building; although experience may have influenced assessors during the risk assessment phase, all assessors agreed on the final ranking of taxa.

Overall risk scores were positively related to overall certainty ratings. We hypothesize that this occurred because more available data can contribute to higher certainty and provide more evidence that a taxon may arrive, establish, or have impacts. Similarly, risk scores were negatively related to the year of the earliest U.S. record. We hypothesize that taxa with earlier and more records of occurrence in the United States are likely to be better represented in English-language texts than less common or more recently detected taxa, leading to more evidence for arrival, establishment, and impacts. Efforts to synthesize and standardize information about invasive species (Simpson et al. 2019; CABI 2021) could reduce these potential sources of bias. The relationships between risk scores and earliest record (negative) and number of records (positive) may also indicate that taxa with longer residence time and larger population sizes, respectively, have greater risk of arrival, establishment, and impact (Pyšek et al. 2009).

We evaluated taxonomic and geographic biases in the final horizon scan list and taxonomic biases in the initial CABI list. These biases may indicate shared characteristics of invasive plants or cultural biases in the CABI databases. While we cannot distinguish between these two causes, we look to previous studies for insights. The families Juncaceae (rushes), Poaceae (grasses), Polygonaceae (knotweeds), and Rosaceae (roses) were significantly overrepresented in both the final horizon scan list and the initial CABI list compared to the number of taxa in these families. These families are similarly overrepresented in global lists of naturalized plants (Daehler 1998; Pyšek et al. 2017). The overrepresented families may indicate shared characteristics of invasive plants. Taxa in these families are characterized by traits that can aid invasion, including high reproduction, broad environmental tolerance, and high human use frequency (Hummer and Janick 2009; Canavan et al. 2019; Ashby et al. 2020). In addition, mis-identified invasive rushes and grasses may go undiscovered for long periods, allowing them to establish self-sustaining populations before being controlled (Scott and Hallam 2003). Such general trends can help identify families on which to concentrate risk assessment resources.

Most of the taxa that made our final list were native to Europe, Asia, and North Africa. This result is likely a combination of shared characteristics of invasive plants and cultural biases in the initial CABI list. Europe is the native range for a disproportionately high number of naturalized plant species relative to the number of native plant species (van Kleunen et al. 2015), which may be influenced by plant adaptations to European pastoralism and cultivation-practices that have been widely adopted (Mac-Dougall et al. 2018)—and historical exchange between Europe and other geographic regions (Pyšek et al. 2015). Temperate Asia is also a major source of global naturalized plant species (van Kleunen et al. 2015). Because Florida's Köppen-Geiger climate zones most consistently overlap with Central and South America, central Africa, and southern and eastern Asia (Kottek et al. 2006), our final list likely omits key high risk taxa. Further, the scoring systems for arrival and establishment likelihoods may better estimate risk by including key locations outside of the contiguous U.S., such as Hawaii, Puerto Rico, and the U.S. Virgin Islands. Future horizon scans could focus more on taxa from geographic regions with a similar climate to the target location and strong trade and tourism ties. Although we did not evaluate the geographic ranges of taxa on the initial CABI list, our results from the final list indicate that this analysis could be an important initial step of the horizon scan process to identify whether invasive or naturalized species lists from underrepresented geographic regions need to be obtained.

Overall scores were calculated by multiplying likelihoods of arrival, establishment, and impact (Roy et al. 2015). By equally weighting these three processes, we assumed that each was crucial to a taxon becoming invasive (Blackburn et al. 2011, 2014; Bacher et al. 2018). Four taxa (*Ligustrum vulgare, Cytisus scoparius, Phalaris arundinacea*, and *Avena fatua*) had high overall risk scores with high certainty. Although we did not independently validate these results, staff at the University of Florida (including and trained by one of the authors) assessed these taxa with a more rigorous 49-question predictive tool and found them all to be high invasion risks (University of Florida, Institute of Food and Agricultural Sciences 2018). In our horizon scan, two taxa (*Agrostis capillaris* and *Persicaria hydropiper*) had high overall risk scores, but medium certainty. Because we were unsure how *A. capillaris* would fare in competition with native Florida grasses, competition studies could increase certainty. Similarly, agricultural impact studies of *P. hydropiper*, which interferes with crops and grazing in other regions, could increase certainty about the risk of this taxon. Taxa with high overall risk scores are included in noxious weed lists or laws for 1–14 states (EDDMapS 2021), raising
the question of whether they have already arrived in Florida, but failed to establish. While we considered many environmental factors and plant traits in our assessment of establishment likelihood, establishment experiments may be valuable in informing how much resources should be allocated to preventing invasion of these taxa. On the other hand, the arrival and establishment of these taxa in Florida may be in a lag phase (Taylor and Hastings 2005; Aiko et al. 2010).

We identified "escape from confinement" as the most likely pathway for taxa on our final list to arrive in Florida's natural areas, which is consistent with a global analysis of invasive plants (Hulme et al. 2008). This pathway includes escape from agriculture, botanical gardens, forestry, research facilities, horticulture, and ornamental purposes other than horticulture (CBD 2014). Domestication can select for traits that increase invasion risk, including fast growth rates, high fecundity, and the ability to hybridize (Petri et al. 2021). However, selection for traits that reduce invasion risk and do not interfere with the commercial purposes of plants could help prevent escape from confinement (Petri et al. 2021).

Taxa on our final list were also likely to arrive in Florida's natural areas as transport contaminants or transport stowaways. Florida's seaports are some of the most active in the country (U.S. Army Corps of Engineers 2018), hosting international and domestic trade, as well as millions of cruise passengers (Florida Department of Transportation 2017). Florida is also a top tourist destination, attracting well over 100 million visitors each year (VISIT FLORIDA 2020). These high movement rates provide ample opportunities for plant propagules to enter the state. The risk of introducing taxa through trade routes, however, can be mitigated by identifying steps in the process of importing, processing, and storing goods that can be modified to reduce plant survival (Hulme 2009).

This horizon scan of invasive plant threats to Florida provides a first step in reducing the impacts of invasive species on Florida's natural systems. Like other horizon scans of potential invasive species, the generated list informs future research efforts and policy (e.g., Matthews et al. 2014; Roy et al. 2014; Gallardo et al. 2016; Lucy et al. 2020). Our horizon scan builds on previous invasive species horizon scans, however, in important ways. First, we began with a list of 2128 potential invasive taxa, which was too large a list to perform rapid risk assessments (approximately 2 hours each) in a reasonable timeline (approximately one year between initial workshop and all taxa consensus building; Lieurance et al. in review). We therefore developed data-based criteria to filter the list to 100 taxa. The databases and code we used are publicly available (Suppl. material 1, Kendig et al. 2022) and could be used for other horizon scans of potential invasive plants. Second, the rapid risk assessments and peer reviews led to enough consensus among experts that our final rankings relied entirely on scores from that process (e.g., in contrast to Roy et al. 2014; Lucy et al. 2020). Consensus building led to important methodological changes (i.e., removing a taxon with too much uncertainty, revisiting assessments with arrival and establishment rubrics), but did not directly alter the rankings. A major advantage of this approach is that the rapid risk assessment tool and rubric can increase transparency of the horizon scan process, especially as they become more refined with future horizon scans.

Conclusion

Here we presented a horizon scan of 2128 plant taxa, identifying six with a high invasion risk for Florida in the near future and 93 with medium or low invasion risk. The horizon scan process therefore can potentially reduce the number of taxa requiring thorough risk assessments by three orders of magnitude. The results provide researchers, regulators, and private and public land managers with a practicable list of high risk taxa to focus on. Given the substantial impacts and costs of invaders in Florida, the ability to differentiate and focus efforts on high probability threats is critical.

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Supplementary material I

Methods S1

Authors: Amy E. Kendig, Susan Canavan, Patti J. Anderson, S. Luke Flory, Lyn A. Gettys, Doria R. Gordon, Basil V. Iannone III, John M. Kunzer, Tabitha Petri, Ian A. Pfingsten, Deah Lieurance

Data type: Supplementary methods

- Explanation note: Methods for trimming the list of potential invasive species based on several criteria.
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Link: https://doi.org/10.3897/neobiota.74.83312.suppl1

Supplementary material 2

Table S1

Authors: Amy E. Kendig, Susan Canavan, Patti J. Anderson, S. Luke Flory, Lyn A. Gettys, Doria R. Gordon, Basil V. Iannone III, John M. Kunzer, Tabitha Petri, Ian A. Pfingsten, Deah Lieurance

Data type: Horizon scan criteria.

Explanation note: Potential invasive plant species provided by the CABI Horizon Scan Tool, their synonyms, and their values for criteria described in Suppl. material 1.

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Link: https://doi.org/10.3897/neobiota.74.83312.suppl2

Supplementary material 3

Table S2

Authors: Amy E. Kendig, Susan Canavan, Patti J. Anderson, S. Luke Flory, Lyn A. Gettys, Doria R. Gordon, Basil V. Iannone III, John M. Kunzer, Tabitha Petri, Ian A. Pfingsten, Deah Lieurance

Data type: Rapid risk assessments.

Explanation note: Reviewed rapid risk assessments of the 99 plant species in the final list, ordered by overall score.

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Link: https://doi.org/10.3897/neobiota.74.83312.suppl3

Supplementary material 4

Table S3

Authors: Amy E. Kendig, Susan Canavan, Patti J. Anderson, S. Luke Flory, Lyn A. Gettys, Doria R. Gordon, Basil V. Iannone III, John M. Kunzer, Tabitha Petri, Ian A. Pfingsten, Deah Lieurance

Data type: Statistical results.

Explanation note: Test of under- or overrepresentation of plant families in the final horizon scan list based on resampling of accepted species from The Plant List database.

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Link: https://doi.org/10.3897/neobiota.74.83312.suppl4

Supplementary material 5

Table S4

Authors: Amy E. Kendig, Susan Canavan, Patti J. Anderson, S. Luke Flory, Lyn A. Gettys, Doria R. Gordon, Basil V. Iannone III, John M. Kunzer, Tabitha Petri, Ian A. Pfingsten, Deah Lieurance

Data type: Statistical results.

Explanation note: Test of under- or overrepresentation of plant families in the initial CABI list based on resampling of accepted species from The Plant List database.

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RESEARCH ARTICLE



Presence of an alien turtle accelerates hatching of common frog (Rana temporaria) tadpoles

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Abstract

The presence of a predator affects prey populations either by direct predation or by modifying various parts of their life history. We investigated whether the hatching time, developmental stage, and body size at hatching of common frog (*Rana temporaria*) embryos would alter in the presence of a red-eared slider (*Trachemys scripta elegans*) as a predator. The presence of a predator affected all factors examined. We found that in the absence of the slider, the embryos hatched in 12 days, while hatching was accelerated by two days in slider treatment. At the same time, the embryos hatched smaller and at a lower stage of development with the slider than without it. Our study extends the range of predators studied, including the effect on different phases of development of potential amphibian prey.

Keywords

Antipredator defence, embryonic development, invasive predator, predator-cued hatching, predation risk, *Rana temporaria, Trachemys scripta elegans*

Introduction

The impacts of invasive species on native communities are still difficult to generalise due to the limited number of species and environments researched (Rolim et al. 2015; Tricarico et al. 2016; Griesemer et al. 2018; Ramírez-Albores et al. 2019). However, inappropriate responses of individuals to invasive predators can strongly affect native populations (Mooney and Cleland 2001). In amphibians, predation can account for a significant proportion of the total mortality of all their developmental stages (Nyström

et al. 1997; Chivers et al. 2001; Laurila et al. 2002; Gunzburger and Travis 2005). The ability to detect, recognise, and respond to potential predators is, therefore, an important part of antipredatory behaviour (Bennett et al. 2013; Polo-Cavia and Gomez-Mestre 2014), and native populations can have especially serious problems facing the presence of new alien predators (Polo-Cavia et al. 2010; Gomez-Mestre and Díaz-Paniagua 2011; Nunes et al. 2019). In general, embryonic and early larval stages are the most vulnerable to predation (Laurila et al. 2002; Wells 2007), and the ability to respond to the presence of a predator can therefore significantly increase the fitness of an individual and thus the viability of the entire population (Warkentin 1995; Vonesh and Bolker 2005).

Whether intentionally or unintentionally introduced, the recent wide occurrence of the red-eared slider (Trachemys scripta elegans) in Europe (GISD 2021) presents a new opportunity to investigate the responses of naive native amphibian populations to a new predator. Although the red-eared slider (hereafter referred to as slider) is not reproductively successful throughout Europe (Cadi et al. 2004; Ficetola et al. 2009; Mikátová and Šandera 2015; Standfuss et al. 2016), even the mere presence of adults may pose a certain risk to native species. In previous studies, we found that the presence of the sliders affects several life history parameters of common frog (Rana temporaria) tadpoles, such as movement activity, trajectory of movement (Berec et al. 2016), time to metamorphosis, or size at metamorphosis (Vodrážková et al. 2020). Although sliders are usually still hibernating at the time of common frog breeding (Gibbons et al. 1990; Speybroeck et al. 2016), which eliminates the risk of direct predation, chemical cues (kairomones) released by sliders into the aquatic environment provide amphibians with information about their presence. Since the slider is an opportunistic predator and can consume frog eggs (Ernst and Lovich 2009), some response of common frog embryos is to be expected.

For frog embryos, there are two basic strategies for avoiding predation or significantly reducing its effects: the development of egg unpalatability and hatching plasticity (Wells 2007). The unpalatability of eggs is a passive strategy in which the embryo relies on the predator's inability or unwillingness to consume eggs, which imposes costs on its host even if the host never comes in contact with the predator; environmentally cued hatching is characterised by an embryo's active capability to alter the time of hatching according to the conditions it encounters during embryonic development. Hatching plasticity has been documented many times in amphibian embryos, and predator presence has been shown to trigger early hatching from eggs incubated in both air and water (Chivers et al. 2001; Warkentin 2011). In terrestrially laid eggs, hatching can be stimulated by vibrational cues during the direct physical attacks of predators, such as snakes (Warkentin 1995; Jung et al. 2019), frogs (Vonesh and Bolker 2005), katydids (Poo and Bickford 2014), wasps (Warkentin 2000), or egg-eating fly larvae (Vonesh and Bolker 2005). In aquatic environments, these responses are induced mainly by chemical cues from predators (kairomones) or by chemical cues that are released from injured prey during predation events (Petranka et al. 1987; Dodson 1988; Tollrian 1994; Nicieza 1999, 2000; Laurila et al. 2002; Smith and Fortune 2009).

This study aimed to shift our previous focus (Berec et al. 2016; Vodrážková et al. 2020, 2022) to a different developmental stage, namely, embryos in eggs. We investigated whether the presence of a slider can alter the hatching time of common frog embryos. We hypothesised that the presence of a slider would accelerate the hatching time, so the ontogenetic stage and body size at hatching were also measured. The uniqueness of this study lies in the use of a stage-nonspecific predator, which is virtually absent in the literature. At the same time, it is an alien predator from a taxonomic group to which the prey has no common history.

Materials and methods

Five freshly laid clutches of common frogs were collected in a pool between Holubov and Vrábče, South Bohemia, the Czech Republic (48.9078633°N, 14.3485608°E), on 2 April 2021. Collection locality was monitored daily to collect egg clutches laid during the night before. Neither the slider nor any other species of turtle occurs at the collection locality, so the eggs and their parents are naive prey relative to the turtles. The experiment was performed in six glass tanks – three replications with the sliders and three replications of control without the sliders. Glass tanks (size: $100 \times 55 \times 50$ cm) filled with 20 cm of aged tap water were equipped with a Claro 300 filter pump (300 L.h⁻¹) and rinsed three times a week. The room temperature was set at 15 °C and the datalogger (Dostman LOG200 PDF) recorded a mean air temperature of 14.8 ± 0.4 °C (\pm S.D.; measured at hourly intervals) during the experiment. Fluorescent tubes (2×36 W) with a light regime of 12 h/12 h were used. During the dark phase of the day, the glass tanks were illuminated with red light to allow permanent monitoring of egg hatching.

Three adult sliders (carapace length: 18 cm, 20 cm, and 21 cm) were used as predators. A slider was placed in each of three glass tanks over the course of three days to release kairomones into the water before the experiment was initiated and fed three times a week with ReptoMin Tetra turtle gammarus. To prevent physical but not chemical contact between the slider and frog eggs, a glass barrier was placed inside each glass tank with a 6 cm gap at both ends so that water could flow freely throughout the tank. On the other side of this barrier, five perforated opaque boxes (20×14 cm) with holes 1 mm in diameter were glued to the bottom of the glass tanks to contain the eggs (Fig. 1).

Six fragments of approximately 150 eggs each were taken from the collected clutch and randomly placed in five boxes, one in each glass tank. This procedure was repeated for all five clutches, so that there were five boxes in each tank with a fragment from each clutch. Each glass tank was continuously monitored using a camera (Niceboy Stream Pro) to distinguish hatched tadpoles occasionally returned to the inside of the egg capsules from tadpoles just before hatching. Hatched tadpoles were counted every 24 h. Hatching was defined as the moment at which the whole hatchling had left the protective jelly of the eggs. To maintain a good processing of the camera recordings



Figure 1. Diagram of the glass tank showing the position of the slider (if present) and the boxes for clutch fragments. These were placed randomly in the boxes in each glass tank (see Materials and methods). Three replications with the sliders and three replications without them (control) were used. Slider drawing by Jakub Berec.

(the large number of hatched tadpoles in a small box makes it difficult to count them), hatched tadpoles were transferred every six hours to a depot tank. At the time when half of the eggs in each box had hatched, two tadpoles were taken from the group of tadpoles hatched in the last six hours. These tadpoles were photographed under a stereomicroscope (Olympus SZX 7) and measured (to the nearest 0.01 mm) using QuickPHOTO MICRO 3.2 software. Their developmental phase was determined according to Gosner (1960).

Three different dependent variables connected with different aspects of the hatching of common frog tadpoles with/without the presence of a slider were measured as described above: hatching time, developmental stage, and size at hatching. The potential impact of four independent variables – (1) slider: presence/absence, (2) glass tank: three tanks with slider and three controls without slider, (3) box: five boxes in each glass tank at the same position within the glass tank, and (4) clutch: six fragments of each clutch – on each of the dependent variables was then analysed. Thus, three separate analyses were performed to fulfil the aim of the present study. According to the experimental design, a linear main effect ANOVA model from the general linear model family (GLM) was used for analysis of experimental data (Quinn and Keough 2002). The factor 'slider' was set in all three analyses as a fixed factor, as both levels of this factor (presence/absence) were of direct interest to our study and are not interdependent. The other three factors (glass tank, clutch, and box) were set as random factors (Allen 2017). The overall fit of all parameters in the GLM was tested using a test of the sum of squares of whole model versus sum of squares of residuals. An overparameterized model based on the indicator variable approach (Midway 2019) was used in type III sums of squares test (TIBCO 2017) to represent the effects of all four independent variables (factors) on the dependent variable. Adjusted R-squared was used in the overall fit of all parameters as a measure of the variability explained by the GLM because more than one independent variable was used in the model. The effect sizes of all four partial factors were evaluated using partial eta-squared (Richardson 2011). Given the number of eggs, the statistical significance was assessed at the 99.9% level (Steel et al. 2013). All calculations were performed in Tibco Statistica (TIBCO 2017).

Results

The GLM for hatching time with the effects of all factors analysed (overall fit of all parameters) was statistically significant (all effect: $F_{11,2988} = 809.2$, p<<0.001; adjusted $R^2 = 0.75$). In the partial effects analysis, we found a significant difference in hatching time between the presence and absence of the slider ($F_{1,2988} = 8672.4$; p<<0.001). The effect of this factor on hatching time (partial eta-squared 0.74) prevailed over the effect of the other factors. In the absence of the slider accelerated hatching by two days (10 ± 0.6 days) (Fig. 2A). The partial effects of the random factors were also significant: glass tank ($F_{2,2988} = 11.7$; p < 0.001), box ($F_{4,2988} = 7.6$; p < 0.001), and clutch ($F_{4,2988} = 44.1$; p<<0.001). However, compared to the effect of slider presence, the effect sizes of these factors were negligible (partial eta-squared for clutch: 0.06, glass tank: 0.01, and box: 0.01) (Suppl. material 3: Table S1).

The GLM for developmental stage of all the factors analysed (overall fit of all parameters) was statistically significant (all effect: $F_{11,48} = 33.2$, p<<0.001; adjusted $R^2 = 0.86$). In the analyses of partial effects, the presence of the slider was the only significant factor in the model ($F_{1,48} = 358.0$; p<<0.01). In the presence of a slider, embryos hatched at developmental stage 20 ± 1.5 (mean ± S.D.), while in control, freshly hatched embryos had developed to stage 23 ± 1.0 (Fig. 2B). The developmental stage was not significantly influenced by glass tank, clutch, and box (Suppl. material 3: Table S2).

Similarly to the previous life history parameters, the GLM for size at hatching of all factors analysed (overall fit of all parameters) was statistically significant (all effect: $F_{11,48} = 23.8$, p<<0.001; adjusted R² = 0.81). In the analyses of partial effects, the significant difference was found between the size of freshly hatched embryos in the presence of the slider and without it ($F_{1,48} = 245.3$; p<<0.001). In the presence of a slider, the embryos hatched with an average size of 5.92 ± 1.460 mm (mean ± S.D.), while in the control, the average size of the freshly hatched embryos was 10.77 ± 1.042 mm (Fig. 2C). As for developmental stage, the presence of the slider was the only significant factor in the model (Suppl. material 3: Table S3).



Figure 2. Histogram of **A** hatching time **B** developmental (Gosner) stage, and **C** size at hatching of the embryos of common frogs in the presence of red-eared slider and control.

Discussion

Developmental plasticity is an adaptive response of anuran embryos and larvae to the risk of predation (Altig and McDiarmid 1999; Benard 2004; Warkentin 2011). Here, we present evidence for the developmental plasticity of common frog embryos in the presence of a red-eared slider and, in addition to a previous study (Vodrážková et al. 2020), provide a comprehensive insight into the influence of this alien predator on the early phases of the common frog life cycle. We have previously shown (Vodrážková et al. 2020) that, in the slider presence, tadpoles of common frogs are capable to modify the duration of larval development. In the present study, we confirmed a similar response in common frog embryos, which hatched earlier in the presence of a slider. At the same time, the embryos were smaller and less developed when exposed to the chemical signals of a predator.

In the presence of stage-specific predators, amphibians can modify the duration of the relevant developmental stage (Chivers et al. 2001; Ireland et al. 2007; Mitchell et al. 2017). In anuran embryos, specifically, the presence of egg predators has mostly been shown to induce early hatching of embryos (Warkentin 1995, 2000; Chivers et al. 2001; Laurila et al. 2001; Johnson et al. 2003; Segev et al. 2015), while tadpole predators induce delayed hatching (Sih and Moore 1993; Laurila et al. 2002; Schalk et al. 2002; Mitchell et al. 2017), thus increasing their chance of survival by escaping possible attacks. However, the slider is not a stage-specific predator, as it is capable of consuming both amphibian eggs and larvae (Brown et al. 1995; Chen 2006; Ernst and Lovich 2009); thus, the allocation of risk between developmental stages of the frog may be more complex in this case (Warkentin 2011). Studies that examine predator effects on the developmental rates of both eggs and larvae are rare because few predators consume both eggs and larvae simultaneously. Muraro et al. (2021) used a stage-nonspecific predator (*Procambarus clarkii*) and found, in concordance with our results, a reduction in hatching time in *Rana latastei* embryos. However, they did not study larval development. Ireland et al. (2007) solved the problem of predator stage specificity by simultaneously exposing frog eggs to stage-specific predators of eggs (leech: *Nephelopsis obscura*) and larvae (dragonfly: *Aeshna canadensis* nymphs), which resulted in no change in hatching time, while tests with separately acting predators produced the expected response of a reduction in hatching time in the egg predator treatment and an increase in hatching time in the larval predator treatment. This study on embryos and a previous study on tadpoles (Vodrážková et al. 2020) jointly clarify that the embryos/tadpoles of the common frog responded to the presence of a predator by shortening the stage of development during which the embryo/tadpole would be exposed to the predator. It would be interesting to analyse how common frog tadpoles react to the presence of a slider if the entire development from eggs to metamorphosis was taking place with this predator present.

However, some studies have shown that frog embryos, including the common frog, do not always respond specifically to stage-specific predators by shortening hatching time (Laurila et al. 2001, 2002; Schalk et al. 2002; Saglio and Mandrillon 2006; Touchon et al. 2006; Capellán and Nicieza 2010; Touchon and Wojdak 2014). The published differences in embryo responses may correspond to different signal intensities of the presence of a specific predator, and thus the responses to indirect waterborne cues might be weaker than those to the direct, mechanical cues of a predator attack (Warkentin 2011). An evident response to water-borne cues of sliders may be related to a markedly stronger signal of a much larger-sized predator in our experiment compared to commonly tested invertebrate predators. The ability to scale predator danger and adjust hatching time accordingly has been found, for example, in embryos of southern leopard frogs (Lithobates sphenocephalus) (Johnson et al. 2003). Moreover, a possible absence of a change in hatching time does not necessarily imply a complete lack of response to the presence of a predator. It may be manifested by other types of response, such as changes in the body shape of tadpoles (Laurila et al. 2001; Saglio and Mandrillon 2006; Mandrillon and Saglio 2007; Touchon and Wojdak 2014) or their behaviour (Saglio and Mandrillon 2006; Touchon and Wojdak 2014).

Native and naive prey can fail to detect the novel predator adequately as a dangerous threat, resulting in no (Cox and Lima 2006; Sih et al. 2010) or inefficient antipredator responses to counter the predator's attack strategies (Strauss et al. 2006; Sih et al. 2010). However, when responses in hatching time in naive prey are detected, they are often explained by the presence of syntopic, taxonomically related predators (Sih et al. 2010; Melotto et al. 2021; Muraro et al. 2021), although the time since invasion may also play an important role (Gomez-Mestre and Díaz-Paniagua 2011; Nunes et al. 2013). Our results suggested that a common evolutionary history is not necessary for a detectable response. Such a result has already been published for tadpole development time (Stav et al. 2007; Vodrážková et al. 2020), but as far as we know, it has not yet been published for the hatching time in frog embryos. An explanation for embryo response to an alien slider may be in the ability of embryos to detect a kind of general "smell of fear" that is elicited by most predators, regardless of taxonomic classification (Sih et al. 2010).

Finding a general tendency in the phenotypic plasticity responses of prey across a broad range of animal predators (different taxa and feeding spectra), environmental and experimental conditions is a challenge even in anurans themselves (Relyea et al. 2018). However, in frogs, the earlier hatching time was generally associated with smaller size at hatching (Chivers et al. 2001; Laurila et al. 2002; Capellán and Nicieza 2007; Ireland et al. 2007) and lower developmental stage (Chivers et al. 2001; Laurila et al. 2002; Capellán and Nicieza 2007; Ireland et al. 2007; Muraro et al. 2021), and our results confirm this relationship. In some cases, earlier hatched tadpoles performed higher growth rate and reached the size of later hatched tadpoles at metamorphosis (Capellán and Nicieza 2007). However, if tadpoles are unable to compensate for their smaller size at hatching, this can impose significant costs in later developmental phases. These costs have been demonstrated through increased mortality during the larval stage (Smith 1987; Warkentin 1995; but see Vonesh and Bolker (2005) where early hatchlings survived better), reduced size at metamorphosis (Vonesh and Bolker 2005; Vodrážková et al. 2020), lower post-metamorphic survival (Berven 1990; Altwegg and Rever 2003), change of behaviour (Buckley et al. 2005; Capellán and Nicieza 2007), delayed maturity (Smith 1987) and lower reproductive success (Smith 1987).

The hatching time was also influenced by the clutch, glass tank, and box. The clutch effect can be explained by a possible difference in the age of the collected clutches. Although freshly laid clutches were always collected in the morning after the actual reproductive event, differences of several hours in the age of the clutches cannot be excluded. The box effect could be attributed to the different distances of each box from the pump filter and/or the slider compartment. We can rule out a temperature gradient in the experimental room as the most likely cause of the glass tank effect, as regular temperature measurements during the experiment did not detect one. Nevertheless, all partial eta-squared of clutch, glass tank, and box are an order of magnitude lower than partial eta-squared for slider presence/absence. This confirms the importance of the slider presence/absence on the hatching time. Moreover, the statistical significance of the above-mentioned random factors need not be functionally relevant.

Although the results are fairly straightforward, we are aware of certain limitations of our experiment. First, the five clutches used originated from a single pool. For this reason, the general validity of our results cannot be confirmed, as some studies also point to a genetic component of variability in some features of ontogenetic development (Lind et al. 2008; to our knowledge, the genetic component of hatching time variability in frogs has not yet been investigated). An additional potential statistical complication could be the placement of multiple boxes in a glass tank and multiple eggs (a fragment of clutch) in a box. Having individual eggs in individual tanks with their own maintenance system and with a separate water supply from the glass tank with or without a slider would prevent this issue. At the same time, it solves the problem of different box distances from the pump filter and/or the slider compartment. However, although this solution is technically feasible, division of the clutches into individual eggs remains problematic in terms of embryo survival.

Our work added a slider as an additional predator inducing changes in the embryonic developmental rate in Ranidae. Since the impact of earlier embryo hatching (lower body

size and lower stage of development) on fitness has been confirmed in several frog species (Warkentin 1995; Laurila et al. 2002; Vonesh and Bolker 2005; Touchon et al. 2013), the same impact can be expected for the common frog. The existence of defensive responses in slider-exposed embryos may reduce the threat that the spreading of this invasive species poses in Europe. On the other hand, the reduced size at hatching and developmental stage of common frog hatchlings represents additional risks of negative fitness impacts, and at the very least, the presence of sliders in non-native areas should receive increased attention.

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All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Czech Ministry of Agriculture, Department of Animal Welfare according to article No. 15, section 2 of the act registered under number 9103/2009-17210.

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Supplementary material I

Hatching time

Authors: Magda Vodrážková, Irena Šetlíková, Josef Navrátil, Michal Berec Data type: excel file.

- Explanation note: Data of hatching time (days) with four independent variables slider presence (0/1), glass tank (1–6), box (1–5), and clutch (1–5) (n = 3000).
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Supplementary material 2

Developmental stage, size

Authors: Magda Vodrážková, Irena Šetlíková, Josef Navrátil, Michal Berec Data type: excel file.

- Explanation note: Data of developmental (Gosner) stage and size at hatching (mm) with four independent variables slider presence (0/1), glass tank (1-6), box (1-5), and clutch (1-5) (n = 30).
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Supplementary material 3

Tables 1–3

Authors: Magda Vodrážková, Irena Šetlíková, Josef Navrátil, Michal Berec Data type: Docx file.

- Explanation note: Results of linear main effect ANOVA models for hatching time (Table S1), developmental stage (Table S2), and size at hatching (Table S3). All three tables include the overall fit of all parameters including adjusted R2 and then univariate results for all factors tested including partial eta-squared. Abbreviations: SS = Sum of Squares, d.f. = Degrees of Freedom, and MS = Mean Square.
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RESEARCH ARTICLE



Comparing field-based management approaches for invasive Winter Heliotrope (Petasites pyrenaicus, Asteraceae)

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Abstract

Winter Heliotrope (Petasites pyrenaicus, previously P. fragrans), is a persistent, rhizome-forming species found throughout the Mediterranean region and North Africa and is an Invasive Alien Plant (IAP) in the UK and Ireland. P. pyrenaicus excludes native flora by forming a dense, compact canopy that persists for much of the growing season, and is often found growing in rough ground, riparian areas and along communication routes, incurring significant management costs at sites of conservation interest. Our study describes the first field-based assessment of P. pyrenaicus control treatments, testing 12 physical and/or chemical treatments in replicated 1 m² plots over four years and one chemical treatment over three years. Treatments focused on understanding phenology and resource allocation to exploit rhizome sourcesink relationships in *P. pyrenaicus*. Multiple-stage glyphosate- and picloram-based treatments reduced leaf canopy cover to zero (%) over time, though no treatment completely eradicated P. pyrenaicus. When designing management strategies, effective P. pyrenaicus control may be achieved by a single annual soil and/or foliar application of picloram at 1.34 kg AE ha⁻¹ in spring, or by a single annual foliar application of glyphosate in spring at 2.16 kg AE ha⁻¹. Control is not improved by the addition of other herbicides or physical treatment methods, underlining the importance of these herbicides for perennial invasive plant management. This work confirms the importance of considering plant phenology, resource allocation and rhizome source-sink relationships, to increase treatment efficacy and reduce the environmental impacts associated with the management of *P. pyrenaicus* and other invasive, rhizome forming species.

Keywords

field trial, herbicide, Integrated Weed Management (IWM) system, invasive alien plants (IAPs), invasive non-native species (INNS), *Petasites fragrans, Petasites pyrenaicus*, rhizome source-sink, Winter Heliotrope

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Introduction

Winter Heliotrope (Petasites pyrenaicus (L.) G. López, previously known as P. fragrans (Vill.) C. Presl): Asteraceae) is a persistent dioecious, rhizomatous, herbaceous perennial native to the Mediterranean region and North Africa (Desjardins et al. 2016; Stace 2019). The non-native range of *P. pyrenaicus* includes Europe, New Zealand, Australia and the northwest coast of the United States; in the British Isles (United Kingdom (UK) and Ireland) it is one of several Petasites spp. considered as invasive alien plants (IAPs) (National Roads Authority 2010; GB Non-Native Species Secretariat 2011a, b, c; Global Biodiversity Information Facility 2020). It was introduced as an ornamental plant to the UK in 1806, first recorded as naturalised by 1835 (Clement and Foster 1994; GB Non-Native Species Secretariat 2011a; Stace 2019), and its range continues to expand throughout the UK (except in northern England and Scotland) and Ireland, typically associated with rough ground, riparian areas and communication routes (Clement and Foster 1994; National Roads Authority 2010; GB Non-Native Species Secretariat 2011a; Desjardins et al. 2016; Stace 2019). P. pyrenaicus primary mode of spread in its non-native range is clonally through asexual dispersal, i.e. rhizome expansion and fragmentation. Anthropogenic and natural disturbance has been reported to increase dispersal (GB Non-Native Species Secretariat 2011a; Cornwall LNR's 2013). Desjardins et al. (2016) reported hybridisation of P. japonicus (Giant butterbur) with *P. pyrenaicus* in southern England (UK), the hybrid offspring of which (*P. japonicus* × *P. pyrenaicus*) were highly fertile.

P. pyrenaicus excludes native flora by light exclusion from a low growing, compact leaf canopy (Fig 1.). Beneath the canopy, a persistent mulch of dead leaves suppresses native plant species germination (GB Non-Native Species Secretariat 2011a; Booy et al. 2015). Belowground *P. pyrenaicus* rhizome growth is largely within the first 50 cm of the soil profile (Fig. 1) but varies depending upon establishment of the patch and local ground conditions (Jones 2015). Rhizomes grow laterally at 0.5–1.0 m yr⁻¹ (Hoare 2014), with new ramets spreading aboveground growth and adventitious roots (Jones 2015) leading to growth of dense monospecific patches. In riparian habitats, the relatively low soil binding capabilities of *P. pyrenaicus* rhizomes and adventitious roots leads to increased bank erosion (Fig. 1; Jones 2015).

Long-term, field relevant research to underpin the management of many IAPs is lacking (Kettenring and Adams 2011). Specifically, in the UK there is limited guidance available for the control of *P. pyrenaicus* and other introduced *Petasites* spp. including *P. japonicus* (Giant butterbur) and *P. albus* (White butterbur), which incur significant management costs at sites of conservation importance and along roadsides (Parrott 2008; National Roads Authority 2010; Stace 2019; GB Non-Native Species Secretariat 2011a, b, c). Management practices for rhizome-forming species must account for the linkage between above and belowground tissues to inform the correct timing, concentration and intensity of control treatment application (Jones et al. 2018). Extensive above and belowground biomass may hamper efforts to deplete rhizome reserves and strong seasonal changes in *P. pyrenaicus* rhizome source-sink strength affects herbicide translocation to belowground tissues (Jones 2015).



Figure 1. *P. pyrenaicus* - Winter Heliotrope. Where **A** adaxial and **B** abaxial leaf surfaces (immature leaf and leaf bud is also shown). Leaves are suborbicular and not lobed; up to 20 cm across, petioles to 30 cm **C** inflorescence (November-February). Erect flowering stems (to 30 cm) bear few medium-broad bracts and a panicle of capitula; flowers are white tinged purple and strongly almond-scented **D** and **E** low growing, compact, closed canopy of leaves growing adjacent to a road (**D**) and stream (**E**) **F** *P. pyrenaicus* growing on the bank of Roath Brook (Cardiff, UK). Note depth of rhizome system (bank is ~2 m above the river channel), that the majority of rhizome is concentrated in the top 50 cm of the soil profile and erosion of the riverbank due to ineffective binding of soil by *P. pyrenaicus* rhizomes and roots. (Images courtesy of D. Jones)

To our knowledge, only one source of information for the control and management of *P. pyrenaicus* exists, which is not based on empirical data (National Roads Authority 2010). The use of glyphosate, an aromatic amino acid (AAA) synthesis inhibitor, and metsulfu-

ron-methyl, an acetolactate synthase (ALS) inhibitor is advised for roadside management. Alternatively, complete physical excavation of above and belowground (rhizome) biomass and integration of physical with chemical treatments is also recommended (National Roads Authority 2010). Methods involving cutting roadside vegetation will increase the dispersal of vegetative *P. pyrenaicus* propagules, similar to other rhizome-forming species, e.g. Japanese Knotweed (*Reynoutria japonica*; Bashtanova et al. 2009; Jones et al. 2020).

The primary objective of this study was to employ an evidence-based experimental approach to provide a robust, appropriately scaled field evaluation of *P. pyrenai*cus management strategies. The Integrated Weed Management system approach tested three treatment response categories: physical (e.g. covering), chemical (e.g. application of herbicide) and integrated (e.g. digging before herbicide spraying). Our study linked *P. pyrenaicus* physiology (i.e. resource allocation and rhizome source-sink strength) with physical or chemical control method target (i.e. resource depletion, uptake, movement and metabolism) within a four-stage mechanistic model (Fig. 2). This approach to treatment efficacy evaluation was similar to that successfully employed in Japanese Knotweed (R. japonica) control (Jones et al. 2018). Briefly, Stage 1; summer disruption of new P. pyrenaicus aboveground growth and depletion of rhizome reserves (note that this stage was not tested specifically in the current experiment). Stage 2, autumn treatment against metabolism and growth, reducing resource acquisition. Stage 3, winter treatment at maximum leaf expansion, targeting the transition point where the rhizome becomes a reserve. Stage 4, spring coupling of aboveground resource translocation to the rhizome with herbicide application, maximising translocation to belowground tissues.



Figure 2. Conceptual four stage mechanistic model of phenological changes in *P. pyrenaicus* growth, resource allocation and rhizome source-sink strength during the temperate northern hemisphere growing season (adapted from Jones et al. 2018). LAI = leaf area index. Note linkage of above and belowground growth processes with changes in source-sink strength and that rhizome tissue sink strength increases through the winter from November, reaching a peak in April-June during senescence.

Here we report on the first, multi-year evaluation of 13 control strategies for *P. pyrenaicus*, following an Integrated Weed Management system approach. In particular, we considered whether targeting the rhizome source-sink switch can provide more effective and sustainable *P. pyrenaicus* control, by reducing pesticide application to minimise ecological impact.

Methods

Field trial site selection

The four-year experiment was conducted at a single site in south Wales (UK; Fig. 3) and the geological and hydrological conditions of the site are provided in Suppl. material 1. *P. pyrenaicus* was extensive and well established at the site, being present *in-situ* for more than 20 years. For the present study, control methods were applied from 2013 to 2017.

Experimental design

Thirty 1 m² treatment and control plots were established (Suppl. material 2), with each plot surrounded by a 10 cm buffer zone. Physical, chemical and/or integrated treat-



Figure 3. Map of the study area. Inset shows location of Invasives Research Centre (IRC) in south Wales, UK (WGS 84: 51.534124, -3.259120).

ments were applied to the whole of each treatment plot. Each treatment group was replicated twice with the exceptions of the untreated control plot and covering treatment (Covering, N/A, Win.; Table 1) which were replicated once, and one glyphosate-based herbicide treatment replicated four times (Gly., 2.16, Fol., Spr.; Table 1). No dummy treatments were applied to the untreated plots as the application of dilute quantities of herbicide from the spraying equipment may have influenced untreated plot responses. Treatment assignment was randomised, with the exception of the picloram treatment group (Pic., 1.34, Soil+Fol., Spr.; Table 1) which could not be legally sited near watercourses (Suppl. material 3).

In the first year of treatment (2013), plot assessment was undertaken on 01 May prior to treatment application, and again on 21 August following treatment application. In subsequent years, assessment was undertaken while the plant was in full growth (between 16 April and 01 July), with the final assessment made following application of all treatments on the 01 September 2017, while the plant was in full growth and prior to senescence. Aboveground *P. pyrenaicus* leaf canopy percentage cover (%) was recorded from each plot as the response variable.

Table 1. Physiochemical Winter Heliotrope treatments, showing treatment group abbreviation, concentration of herbicide active ingredient (a.i.) within each product tested (g L⁻¹), application rate measured in kilogrammes acid equivalent per hectare (kg AE ha⁻¹), application method (e.g. foliar spray) and seasonal timing. Underlined herbicide active ingredients indicate product mix; italicised processes represent physical components of integrated physiochemical control treatments; Roman numerals represent multi-seasonal application of physiochemical control treatments. Specific timing of seasonal application: autumn (stage 2) = September-November; winter (stage 3) = December-March; spring (stage 4) = April-June. Treatment group abbreviations are provided in the format: treatment, application rate, application method, season of application. Abbreviations used in the treatment groups are as follows: 2,4-D = 2,4-D amine; Ami. = aminopyralid; Clo. = clopyralid; Flu. = fluroxypyr; Gly. = glyphosate; Pic. = picloram; Tri. = triclopyr; Fol. = foliar application; Exc. = excavation; Spr. = spring; Aut. = autumn.

Treatment group abbreviation	a.i. (g L ⁻¹)	Application rate (kg AE ha ⁻¹)	Application	Application timing
Gly., 3.60, Fol., Spr.	Glyphosate (360)	3.60	Foliar spray	Spring
Gly., 2.16, Fol., Spr.	Glyphosate (360)	2.16	Foliar spray	Spring
Gly., 3.60, Fol., Aut.	Glyphosate (360)	3.60	Foliar spray	Autumn
Gly., 2.16, Fol., Spr.+Aut.	Glyphosate (360)	2.16	Foliar spray	i) Spring ii) Autumn
2,4-D, 4.50, Fol., Spr.	2,4-D amine (500)	4.50	Foliar spray	Spring
2,4-D, 4.50, Fol., Aut.	2,4-D amine (500)	4.50	Foliar spray	Autumn
Ami.+Flu., 0.06+0.20, Fol., Spr.	Aminopyralid (30)	0.06 & 0.20	Foliar spray	Spring
	& Fluroxypyr (100)			
2,4-D+Dic., 1.20+0.42, Fol., Spr.	2,4-D amine (344)	1.20 & 0.42	Foliar spray	Spring
	& Dicamba (120)			
Tri.+Clo., 0.05+0.48, Fol., Spr.	Triclopyr (240) &	0.29 & 0.05	Foliar spray	Spring
	Clopyralid (60)			
Pic., 1.34, Soil+Fol., Spr.	Picloram (240)	1.34	Soil and foliar spray	Spring
Ami.+Tri., 0.05+0.48, Fol., Spr.	Aminopyralid (12)	0.05 & 0.48	Foliar spray	Spring
	& Triclopyr (100)			
Exc.+Gly., N/A+3.60, Fol.,	Excavation	3.60	Foliar spray	i) Winter ii) Spring
Win.+Spr.	Glyphosate (360)			
Covering, N/A, Win.	Covering	N/A	Cardboard	Winter

Herbicide product selection and control treatment timing

Herbicide product selection and application timing of the 13 treatments (Table 1) was informed by the consideration of established *P. pyrenaicus* source-sink relationships, and methods used against other rhizome-forming species (Jones et al. 2018; Fig. 2). Full herbicide and spray adjuvant information, including physical properties, areas of use, legal designations, UK inclusion date and manufacturers, is supplied in Suppl. material 3.

Details of treatment groups

Herbicide control treatments - Soil and foliar spray application

Herbicide product(s) were applied at a fixed rate of active ingredient(s) per unit area (L or kg AE ha-1) using a Cooper Pegler CP3 (20 L) Classic knapsack sprayer, fitted with a Cooper Pegler blue flat fan nozzle (AN 1.8). All herbicide products were applied with dye, adjuvant (Topfilm; 1.2 L ha⁻¹) and water conditioner (EasiMix; 1.2 L ha⁻¹) to ensure even coverage and maximise herbicide active ingredient absorption. Herbicide products containing aminopyralid (synthetic auxin) were applied with antifoaming agent (Foam Fighter). All herbicides were foliar applied, except for picloram, which was also applied to any bare ground within the field trial plot due to the persistent soil activity of this herbicide. Following application of all herbicides at the specified application rate (kg AE ha⁻¹; Table 1) the knapsack sprayer was cleaned with 10 L clean water. Following application of herbicide products containing 2,4-D, this was supplemented with 50 ml ammonia-based cleaning fluid (Extra Clear). Weather forecast information (UK Met Office weather app) was consulted prior to treatment application to ensure that no rain was forecast for a minimum of 8 hours post-application. Note that spring aminopyralid and triclopyr foliar spray (TG g1) treatment was tested for 3 years only as this product combination of herbicide active ingredients was newly introduced to market one year after field trial establishment.

Integrated physiochemical control treatment - Excavation

Excavation of the full 1 m² field trial plot, to a depth of 0.5 m, was undertaken with a hand shovel in winter (stage 3), breaking up the rhizome system; excavated soil containing rhizome was left *in-situ*. The following spring (stage 4), glyphosate was applied as a foliar spray, at full rate (FR, 3.6 kg AE ha⁻¹), following regrowth of the *P. pyrenaicus* canopy. Excavation and glyphosate foliar spray were repeated in each subsequent winter and spring, respectively.

Physical control treatment - Covering combined with hand pulling

Prior to covering in spring (stage 4), the full 1 m^2 field trial plot was excavated using a hand shovel in winter (stage 3) to a depth of 0.5 m, breaking up the rhizome system;

excavated soil containing rhizome was left in-situ. The treatment area was fully covered for the duration of the experiment, by laying five layers of thick (4.0 mm) cardboard annually over the treatment area and weighted to remain in position (new layers of cardboard being laid over the top of old layers). Visible *P. pyrenaicus* growth emerging around the covering was then hand pulled and left *in-situ* underneath the covering and/or additional covering added to prevent further growth. Covering was the only physical control treatment trialled, as other physical control treatments (pulling, digging and burning) were considered too costly, labour intensive and increased the risk of *P. pyrenaicus* spread.

Data analysis

Following the recommendation of Warton and Hui (2011) for dealing with % data, we applied a logit transformation to the *P. pyrenaicus* leaf canopy percentage cover (%; 1 m²) data by first converting the % coverage in each field trial plot to proportion coverage (*PC*), with the addition of the smallest recorded coverage value (0.5%) to both numerator and denominator, to avoid problems with log transformation of the 0% coverage values. This gives an untransformed response variable *PC* = (% cover + 0.5)/100.5, to which the logit transformation is then applied: $y = \log_e(PC/[1-PC])$. The logit transformed data was analysed using a linear model (ANCOVA) considering the interaction between days after treatment (DAT) and treatment group (TG).

We focussed on the change in logit transformed *P. pyrenaicus* cover over time within each individual treatment group, rather than directly comparing slopes across treatments or the untreated control group. This is appropriate to maintain statistical power, given the independence of plots in the sampling design and the relatively low levels of replication within treatment groups. Model residuals were checked and did not violate the assumption of normality (Shapiro test, W = 0.99, p = 0.31).

All data were analysed using R v3.6.3 (The R Development Core Team 2020). The 'emmeans' package (Lenth 2020) was used to determine 95% confidence intervals for each Treatment Group's slope estimates and the 'ggplot2' package (Wickham 2016) was used to generate plots.

Results

Three treatments provided greatest control of aboveground *P. pyrenaicus* growth, defined by reduced leaf canopy cover (Table 2; Fig. 4): spring glyphosate full rate (FR) foliar spray (Gly., 3.60, Fol., Spr.; Table 1), spring glyphosate half rate (HR) foliar spray (Gly., 2.16, Fol., Spr.; Table 1) and spring picloram FR soil and foliar spray (Pic., 1.34, Soil+Fol., Spr.; Table 1). Neither the untreated control group, nor any of the other treatment groups, showed any significant change in *P. pyrenaicus* cover over time (Table 2; Fig. 4).

Application of the synthetic auxins 2,4-D amine (2,4-D, 4.50, Fol., Spr.; 2,4-D, 4.50, Fol., Aut.; Table 1), aminopyralid and fluroxypyr (Ami.+Flu., 0.06+0.20, Fol.,

Table 2. Linear model parameter estimates for changes in Logit transformed Winter Heliotrope canopy cover (%, m²) as a function of time (days) after treatment started (Fig. 4). Full model statistics: $F_{27,134} = 5.5$, p < 0.001, R² = 0.53. Slope estimates in bold differ significantly from 0 at the a = 0.05 level. Treatment group abbreviations are provided in the format: treatment, application rate, application method, season of application. Abbreviations used in the treatment groups are as follows: 2,4-D = 2,4-D amine; Ami. = aminopyralid; Clo. = clopyralid; Flu. = fluroxypyr; Gly. = glyphosate; Pic. = picloram; Tri. = triclopyr; Fol. = foliar application; Exc. = excavation; Spr. = spring; Aut. = autumn.

Treatment group abbreviation	Intercept \pm S.E.	Slope \pm S.E.	Slope 95% CI
Untreated control	1.92 ± 1.16	-0.0003 ± 0.0013	-0.0029, 0.0023
Gly., 3.60, Fol., Spr.	-2.39 ± 0.83	$\textbf{-0.0021} \pm \textbf{0.0010}$	-0.0040, -0.0002
Gly., 2.16, Fol., Spr.	-2.71 ± 0.58	$\textbf{-0.0013} \pm \textbf{0.0007}$	-0.0027, -0.000003
Gly., 3.60, Fol., Aut.	-1.43 ± 0.77	-0.0008 ± 0.0009	-0.0027, 0.0012
Gly., 2.16, Fol., Spr.+Aut.	$\textbf{-0.90} \pm 0.77$	0.0003 ± 0.0010	-0.0016, 0.0022
2,4-D, 4.50, Fol., Spr.	-1.90 ± 0.83	0.0013 ± 0.0010	-0.0006, 0.0032
2,4-D, 4.50, Fol., Aut.	$\textbf{-0.91} \pm 0.77$	0.0006 ± 0.0009	-0.0012, 0.0025
Ami.+Flu., 0.06+0.20, Fol., Spr.	-1.18 ± 0.83	0.0001 ± 0.0010	-0.0018, 0.0020
2,4-D+Dic., 1.20+0.42, Fol., Spr.	-1.75 ± 0.83	0.0016 ± 0.0010	-0.0003, 0.0035
Tri.+Clo., 0.05+0.48, Fol., Spr.	-1.30 ± 0.82	0.0003 ± 0.0009	-0.0016, 0.0025
Pic., 1.34, Soil+Fol., Spr.	-3.07 ± 0.83	-0.0020 \pm 0.0010	-0.0039, -0.00002
Ami.+Tri., 0.05+0.48, Fol., Spr.	1.96 ± 1.05	-0.0003 ± 0.0015	-0.0033, 0.0027
Exc.+Gly., N/A+3.60, Fol., Win.+Spr.	-0.63 ± 0.82	-0.0003 ± 0.0009	-0.0022, 0.0015
Covering, N/A, Win.	1.28 ± 1.17	-0.0013 ± 0.0014	-0.0040, 0.0015

Spr.), 2,4-D amine and dicamba (2,4-D+Dic., 1.20+0.42, Fol., Spr.), triclopyr and clopyralid (Tri.+Clo., 0.05+0.48, Fol., Spr.), aminopyralid and triclopyr (Ami.+Tri., 0.05+0.48, Fol., Spr.) did not reduce *P. pyrenaicus* canopy cover in the long-term, regardless of application timing (stages 2 and 4, Fig. 4). In contrast, picloram (Pic., 1.34, Soil+Fol., Spr.) significantly reduced *P. pyrenaicus* canopy cover throughout this four-year study, despite picloram only being applied for two years between 2013 and 2015 (picloram was withdrawn from European Union (EU) use 30 June 2015). This treatment rapidly led to 0% cover in both replicates by autumn 2013, with the only brief reappearance being 1% cover in one replicate in spring 2014, which then returned to 0% cover for the remainder of the trial following subsequent treatment application.

Discussion

This study forms the first assessment of *P. pyrenaicus* control treatments, specifically targeting the rhizome source-sink switch (Fig. 2) and utilising an Integrated Weed Management system experimental design. Field-relevant experimental designs are fundamental to inform the control of long-lived perennial, rhizome-forming invasive species. Our approach was designed to account for long-term control response in 12 treatment groups across 28 treatment plots (1 m²) over four years; spring aminopyralid and triclopyr foliar spray (Ami.+Tri., 0.05+0.48, Fol., Spr.; Table 1) treatment was assessed in two treatment plots over three years following initial treatment application.




Physical, chemical and integrated control treatment application was combined with our biological understanding of *P. pyrenaicus*. Autumn (stage 2, Fig. 2) treatments were targeted at metabolism and growth to limit belowground resource acquisition. Winter (stage 3, Fig. 2) treatments tested were intended either to increase efficacy of subsequent herbicide treatment (stage 4, Fig. 2) through disruption (excavation; Exc+Gly., N/A+3.60, Fol., Win.+Spr.; Table 1) of belowground tissues, or deplete rhizome resources through resource restriction (light, covering; Covering, N/A, Win.; Table 1) throughout the growing season. Herbicide-based control methods applied in spring (stage 4, Fig. 2) were either coupled to mass flow of photosynthates through the phloem to rhizome sink tissues (glyphosate, synthetic auxin herbicides other than picloram), or targeted to the foliage and bare soil to directly disrupt and suppress growth above and belowground (picloram, synthetic auxin). Although no treatments were applied in summer (stage 1, Fig. 2), these would be directed toward emergent aboveground growth, depleting rhizome reserves.

The only treatments that showed significant reductions in *P. pyrenaicus* cover over the study period included annual spring (stage 4, Fig. 2) foliar application of glyphosate at FR (3.60 kg AE ha⁻¹) or HR (2.16 kg AE ha⁻¹), or soil and/or foliar application of picloram (1.34 kg AE ha⁻¹). We note that due to the residual activity of picloram in soil (at least one year; USDA Forest Service 2000), it can be applied throughout the calendar year (stages 1 to 4). Glyphosate was most effective where application timing was coupled to photosynthate flow to the rhizome (stage 4, Fig. 2). No significant control effect of foliar applied glyphosate at FR (3.60 kg AE ha⁻¹) was observed when resources are being mobilised to aboveground tissues in autumn (stage 2, Fig. 2). This highlights the importance of integrating species ecophysiology with perennial IAP management.

Prior to annual senescence in rhizome-forming plants (stage 4, Fig. 2), glyphosate is transported to metabolically active sink tissues during mass transit of photosynthate to the rhizome (Jones et al. 2018). Glyphosate accumulation within sink tissues (i.e., P. pyrenaicus leaf clump and rhizome buds (meristems) prevents regrowth in subsequent growing seasons by blocking indole-3-acetic acid (IAA) biosynthesis resulting in extensive localised cell and tissue death (Jiang et al. 2013; Gomes et al. 2014; Jones et al. 2018). The control effect of glyphosate is largely independent of dose, beyond a threshold application rate, because distribution across different tissues (i.e., leaf, petiole and rhizome) is determined by sink strength (Jones et al. 2018). Effective control can therefore be achieved at lower application rates i.e., glyphosate HR application rate (Gly., 2.16, Fol., Spr.; Table 1). Effective management using lower doses of glyphosate-based herbicide also optimises material and labour inputs. Based on our biological understanding of Japanese Knotweed (R. japonica; Jones et al. 2018), we propose that the *P. pyrenaicus* glyphosate application window may be extended to include the transitional phenological source-sink stage in winter (stage 3), increasing the potential application timeframe. Winter management of P. pyrenaicus could further enhance economic and environmental sustainability and minimise non-target effects of herbicide application because the plant is one of few species in leaf (and flower) in winter and would, therefore, be readily located.

We tested a range of synthetic auxin herbicides drawn from three chemical families: phenoxy-carboxylic acids (2,4-D amine), benzoic acids (dicamba) and pyridinecarboxylic acids (aminopyralid, clopyralid, fluroxypyr, picloram, triclopyr; Grossman 2009; Busi et al. 2017). The synthetic auxin herbicides tested did not significantly reduce *P. pyrenaicus* cover through depletion of rhizome reserves (stage 2, Fig. 2) and only picloram significantly reduced *P. pyrenaicus* cover via poisoning of rhizome buds/ meristems (stage 4, Fig. 2). These results suggest that *P. pyrenaicus* synthetic auxin herbicide sensitivity is not based on chemical family, but rather it is the dose of herbicide active ingredient accumulated within rhizome buds/meristems which determines herbicide control efficacy.

Synthetic auxin herbicides mimic the main endogenous auxin (indol-3-acetic acid, IAA) and cause plant death by the overinduction of the auxin response leading to the deregulation of natural auxin regulatory mechanisms (Kelley and Riechers 2007; Grossman 2009). Tissue concentration of synthetic auxin herbicides is not determined by sink strength to the same degree as glyphosate-based herbicides and global accumulation in planta is proportional to herbicide dose (i.e., a classical dose-response relationship is observed; Streibig 2013). Meristematic tissues are most sensitive to synthetic auxin herbicides, and consequently these herbicides are highly effective at low application rates for the control of immature dicotyledonous weeds. In contrast, established, rhizome-forming plants possess a greater number of larger and more structurally robust meristems that allow rapid regeneration following disturbance (Ott et al. 2019). Consequently, a greater dose of herbicide must accumulate globally to poison these structures effectively. Picloram is effective for P. pyrenaicus control because it is persistent in the soil and remains in contact with rhizome meristems at sufficiently high concentration (and duration) to cause tissue accumulation and poisoning. Conversely, at the doses tested, foliar application of the other synthetic auxin herbicides was ineffective presumably due to insufficient accumulation within meristematic tissues (Krzyszowska et al. 1994; USDA Forest Service 2000).

Integration of winter excavation with spring glyphosate application (Exc+Gly., N/ A+3.60, Fol., Win.+Spr.; Table 1) did not reduce *P. pyrenaicus* canopy cover in the long-term, despite greater labour and equipment requirements and cost, compared with the application of glyphosate alone. We suggest that this is due to disruption/ damage of emerging aboveground tissues, reducing source tissue (leaf) strength and subsequent glyphosate translocation to active rhizome buds/meristems. Moreover, as clonality is a common adaptation to physical disturbance (Harper 1977; Ott et al. 2019) this management approach may be counter intuitive for invasive, rhizomeforming species. Physical covering (Covering, N/A, Win.; Table 1) was ineffective at controlling aboveground *P. pyrenaicus* canopy cover, indicating that long-term depletion of rhizome resources to achieve successful control is unfeasible. Physical covering is the only practical physical control treatment that can be applied at scale; other treatments such as pulling and cutting are too costly, labour intensive and likely to increase the risk of *P. pyrenaicus* spread. Due to difficulties in obtaining accessible field sites of sufficient size, we acknowledge the relatively limited replication within our experimental design. However, we suggest that our long-term field-scale evaluation approach, incorporating multiple herbicide products and active ingredients, provides more realistic management data than short-term (less than 2 growing seasons) pot- and/or field-based experiments. This is because short-term experimental designs may overextrapolate the efficacy of treatments which disrupt aboveground growth (e.g. cutting, certain synthetic auxin herbicides) and conversely, do not detect the long-term efficacy of treatments that display limited aboveground control effects (symptomology), but are effectively poisoning belowground tissues (i.e., glyphosate-based herbicides; Child 1999; Skibo 2007; Jones et al. 2018). Where insufficient empirical data is available to underpin control of invasive plant populations, resulting ineffective management strategies are frequently characterised by excessive herbicide and labour inputs, and herbicide resistance may develop (Hutchinson et al. 2007; Kettenring and Adams 2011).

While we welcome trends toward less toxic and persistent active ingredient(s) contained within plant protection products (PPPs), continued reduction of the number of PPPs in Europe presents challenges for the effective management of rhizome-forming IAPs such as *P. pyrenaicus*, particularly in non-agricultural settings (Myers et al. 2016; Kudsk and Mathiassen 2020). Rhizome-forming IAPs have few weak points that can be exploited for management and, as the limited range of effective tools for their management continues to decline, so too does the likelihood of effective management at the landscape scale. Consequently, withdrawal of glyphosate for the control of invasive plants such as *P. pyrenaicus* could impact negatively upon native biodiversity (particularly in areas of nature conservation) and result in the application of ineffective and unsustainable (CO₂ intensive) management practices, to the detriment of wider ecosystem services (Pergl et al. 2020). Therefore, it is timely to encourage the development of new herbicide products targeting source-sink dynamics to increase the range of effective management tools for rhizome-forming invasive plants.

Conclusions

Management of rhizome-forming IAPs such as *P. pyrenaicus* is increasingly being undertaken across a range of sectors to minimise their long-term environmental and economic impacts. However, there is often limited scale-appropriate empirical evidence to support the selection of appropriate control methods, hampering effective management. Knowledge of treatment application timing and appropriate herbicide mode of action are the most important factors for the successful control of *P. pyrenaicus*. Multiple-stage glyphosate- and picloram-based treatments applied at the appropriate phenological stage (Fig. 2) were found to be most effective, completely controlling aboveground *P. pyrenaicus* growth (leaf canopy cover reduced to 0%). However, no control treatment completely eradicated *P. pyrenaicus* within four years of the first treatment application. Picloram was withdrawn from the European market in 2015, leaving glyphosate as the only effective control treatment for the management of *P. pyrenaicus* in much of the introduced range. We recommend that ineffective synthetic auxin herbicides and physical control methods (covering, cutting), that add equipment and labour costs and increase environmental impacts (CO_2 emissions) without improving control compared to spraying alone, are discontinued. While reduced herbicide application to control *P. pyrenaicus* can be achieved by targeted application, alternative control methods currently do not provide viable mitigation against the long-term deterioration of persistently invaded habitats.

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Desk-based site geological, hydrological and historical surveys

Authors: Daniel Jones, Mike S. Fowler, Sophie Hocking, Daniel Eastwood Data type: Docx file.

- Explanation note: Geographical, geological, hydrological, current and historic landuse data for the Invasives Research Centre (IRC), Taffs Well.
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Link: https://doi.org/10.3897/neobiota.74.82673.suppl1

Supplementary material 2

Field trial site treatment group assignment

Authors: Daniel Jones, Mike S. Fowler, Sophie Hocking, Daniel Eastwood

Data type: Docx file.

Explanation note: Schematic of field trial at the Invasives Research Centre (IRC), Taffs Well.

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Supplementary material 3

Petasites pyrenaicus field trial herbicide properties, manufacturers and suppliers

Authors: Daniel Jones, Mike S. Fowler, Sophie Hocking, Daniel Eastwood Data type: Docx file.

Explanation note: Field trial herbicide properties, manufacturers and suppliers.

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RESEARCH ARTICLE



An impact assessment of alien invasive plants in South Africa generally dispersed by native avian species

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Abstract

Invasive alien plant species have been identified as a major threat to biodiversity and the relationship with native avian dispersers may increase their invasion potential. The impact of invasive plant species needs to be quantified using comparable assessment tools across different habitats and species to allocate limited resources to high-priority species. Here, we used the Generic Impact Scoring System (GISS) to assess the impacts of 16 fleshy-fruited alien invasive plant species in South Africa generally dispersed by native avian species. The results showed that fleshy-fruited invasive species have both environmental and socioeconomic impacts. The cumulated impact scores for lantana (Lantana camara) and the tree of heaven (Ailanthus altissima) were the highest, with scores of 42 and 32, respectively. Some species, such as white mulberry (Morus alba), camphor tree (Cinnamomum camphora), American bramble (Rubus cuneifolius) and Brazilian pepper tree (Schinus terebinthifolius), had low overall impact scores of 8, 18, 14 and 16, respectively, but scored the maximum impact of 5 for certain mechanisms. Environmental impacts of fleshy-fruited invasive plant species had a high impact magnitude through effects on the ecosystem and vegetation. Socio-economic impacts were mainly through effects on forest production, agriculture and human health. Species with large crop sizes, small seeds and fruit sizes had higher environmental and socio-economic impact magnitude. The information generated in this study is important for guiding resource allocation and preventing the uncontrolled introduction of invasive species in South Africa. The impact of the fleshy-fruited invasive species transcended sectors and, therefore, effective management of invasive species will require the collaboration of multiple and inter-sectoral stakeholders in South Africa.

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Keywords

environmental impacts, GISS, impact score, management strategy, NEMBA, socio-economic impacts

Introduction

Invasive alien plants have been identified as a major threat to biodiversity (Gosper and Vivian-Smith 2009; Liu et al. 2017). Depending on the species, invasive alien plants generally reduce species richness (Gaertner et al. 2009), disrupt pollination and dispersal networks (Pyšek et al. 2012), change ecosystem functioning (Andersen et al. 2004; Charles and Dukes 2008), cause economic losses (Novoa et al. 2016; Zengeya et al. 2017) and impact human well-being (Vilà et al. 2011). Invasive alien plant species are introduced either accidentally or intentionally for forestry, agriculture, horticulture (Arriaga et al. 2004), recreation (van Wilgen et al. 2008), restoration (Kumschick et al. 2012) and as ornamentals (Hulme et al. 2018). New introductions or movements of invasive alien plant species within a country are promoted by increased domestic and global travel and trade, making their management a challenge in many countries (Leung et al. 2012; Seebens 2019). Once introduced, invasive alien plants that attract and rely on generalist frugivores for seed dispersal thrive because animal-plant interactions allow for fast recruitment (Jordaan et al. 2011a, b, 2012; Molefe et al. 2020; Traveset and Richardson 2020). The spread of invasive alien plants is further exacerbated by global climate change (Ahmad et al. 2019a, b; Mofu et al. 2019). For frugivore dispersed plant species in South Africa, altered habitats trigger and sustain invasions (Bitani et al. 2020).

Like other parts of the world, South Africa is severely affected by alien plant invasion (Nel et al. 2004; McLean et al. 2018). Alien plant species are the country's most widespread and damaging group of invasives and have been reported to cover approximately 7% of the country (van Wilgen 2018). Amongst invasive alien plants, those with fleshy fruits have high-risk invasiveness (Jordaan et al. 2011b). Species traits have been shown to influence invasiveness (the likelihood of a species being introduced and spreading). Generalist birds have been identified as the most important seed dispersers of fleshyfruited invasive shrubs and trees (Richardson and Rejmanek 2011). In new habitats, fleshy-fruited invasive alien plants overcome barriers of spread through seed dispersal mutualisms (Aslan and Rejmanek 2011; Jordaan et al. 2011a, b). Bird-plant interactions are equally important to avian dispersers as they gain a nutritious supplementary fruit source (Thabethe et al. 2015; Blendinger et al. 2016). The invasion process and success of avian-dispersed invasive alien plants are influenced by plant morphological (Gosper and Vivian-Smith 2009), chemical (Jordaan and Downs 2012; Blendinger et al. 2016) and phenological traits (Marciniak et al. 2020; Nogueira et al. 2020). Certain traits favour bird-fruit interaction and allow plants to integrate into native seed-dispersal networks (Rojas et al. 2019; Marciniak et al. 2020). For example, plants that produce large fruit crop sizes have a high potential to be consumed by birds (Blendinger and Villegas 2011).

Impacts associated with invasives vary across habitats and taxa (Hawkins et al. 2015; Bacher et al. 2018), but are mainly related to changes to natural environments, society and economy (Jeschke et al. 2014; Measey et al. 2016; Kumschick et al. 2017). Consequently, impacts associated with biological invasions have led to the development of impact assessment tools intending to quantify the impacts posed by alien invasive species (Nentwig et al. 2016; Rumlerová et al. 2016; Bartz and Kowarik 2019). The impact assessment tools are based on scientific evidence (Kumschick et al. 2015; Moshobane et al. 2019), comparable across different regions and taxa (Nentwig et al. 2016) and allow for the synthesis of impact data (Vilà et al. 2019). Several tools have been developed. The two widely used ones are the Environmental Impact Classification for Alien Taxa (EICAT), developed by Blackburn et al. (2014) to quantify environmental impacts and the Generic Impact Scoring System (GISS), developed to assess environmental and economic impacts (Turbé et al. 2017). The GISS has been used for various taxa, including birds (Turbé et al. 2017; Shivambu et al. 2020), mammals (Hagen and Kumschick 2018), amphibians (Measey et al. 2016), fish (Orfinger and Goodding 2018), arthropods (Laverty et al. 2015) and selected plants (Novoa et al. 2016; Yazlik et al. 2018). Using impact quantifying approaches like the GISS gives insights into which species are detrimental so that management prioritises those species with major impacts (Rumlerová et al. 2016) and provides information for decisions relating to the introduction of species (Bartz and Kowarik 2019).

As part of the global biodiversity goals, most countries worldwide are committed to preventing the introduction of high-priority species or minimising their impacts (Moshobane et al. 2019; Verbrugge et al. 2019). The Department of Environmental Affairs (DEA, now Department of Environment, Forestry & Fisheries, DEFF), through the South African National Biodiversity Institute (SANBI), aims to eventually conduct an impact assessment for all listed species as invasive under the National Environmental Management Biodiversity Act (NEMBA). Of the 379 listed terrestrial invasive plant species, only 75 plant species have been assessed (DEA 2016). Assessing the impacts posed by listed species is important to ensure that the listing can be challenged (SANBI 2017). In response to policy-makers' information needs, we aimed to assess the ecological and socio-economic impacts posed by selected fleshy-fruited invasive plant species dispersed by native avian species in South Africa. Additionally, we explored how morphological traits of fleshy-fruited invasive plants relate to their impacts. The results from the present study will assist in providing information for decision-making, allocating resources to control alien invasive plant species and identifying less-studied plants and impacts. In addition, where the study species have not yet been introduced, it will help guide decisions around permitting or prohibiting activities.

Methods

Species selection and literature search

Sixteen fleshy-fruited alien trees or shrubs dispersed by native avian species that occur in the coastal forests of KwaZulu-Natal, South Africa, were selected for this study. The selected plants are listed as invasive under the South African NEMBA. A literature survey, based on published scientific literature and e-literature from Google Scholar (https://scholar.google.com) and Web of Science – ISI Web of Knowledge (hhtps:// apps.webofknowledge.com) and the global invasive species database, such as the Global Invasive Species Database (GISD: www.iucngisd.org/gisd) and the Invasive Species Specialist Group (ISSG: www.iucngisd.org/gisd), was conducted before assessing the risk posed by the species. For each species, species' common names, scientific names and synonyms were used to search for the literature and filter the search by the information provided in the abstracts and titles. In addition, we used terms like "invasive alien plants", "fleshy-fruited", "IAS", "introduced plant species", "non-indigenous plants", "ecological impacts", "economic impacts" and "negative impacts" to search for papers. All the references of the selected publication were screened and included as grey literature.

Impact assessments

Different impact assessment tools have been developed to quantify the impacts of invasive species (Nentwig et al. 2016; Nkuna et al. 2018). For this study, we used the Generic Impact Scoring System (GISS) as it integrates both ecological and socioeconomic impacts (Nentwig et al. 2016) and has proven to be useful in assessing the impacts of invasive plants globally, including in South Africa (e.g. Novoa et al. 2016; Nkuna et al. 2018; Shivambu et al. 2020). The GISS is divided into two main categories, environmental and socio-economic impacts, each with six different mechanisms. The environmental impacts consist of impacts (1.1) on plants or vegetation, (1.2) on animals, (1.3) through competition, (1.4) through disease transmission, (1.5)through hybridisation and (1.6) on the ecosystem. The socio-economic include impacts on (2.1) agricultural production, (2.2) animal production, (2.3) forestry production, (2.4) human infrastructure (2.5) human health and (2.6) human social life. For each category, the impact level ranges from 0 (no known impacts or data deficiency) -5 (highest impact) and the scenarios are described to ensure consistency (details on Nentwig et al. 2016). The overall impact scores (environmental and socio-economic) per species were used for analyses.

Traits of plants

Plant and fruit morphological traits influencing the invasion success of fleshy-fruited invasive alien plants are well documented. For each of the plant species, we compiled data that included mean fruit size, seed size, number of fruits and crop size (Suppl. material 1).

Data analyses

The differences between the overall and mean impact scores for each species' socioeconomic and environmental impacts were tested using a paired t-test. We tested the differences between the mechanisms for environmental and socio-economic impact for each plant species using ANOVA. We used Kendall's rank correlation to test the correlation between the overall impact scores per plant and the number of papers used for each species. To explore the effects of plant species' functional traits with the environmental and socio-economic impact (sum of the six mechanisms), we fitted linear mixed-effects models. The functional trait data were log-transformed because of the non-normal distribution. We used the package lme4, libray nlme and function lme in R with the plant species traits as explanatory variables and the impacts as the response variable. To account for the phylogenetic relatedness, the species family was specified as a random effect (random ~ 1 | a). All the data were analysed using R statistical anlysis v.3.4.4 (R Core Team 2018).

Results

A total of 103 publications were used to score the impacts of 16 fleshy-fruited invasive plant species. There was no significant difference between the overall environmental and socio-economic impacts (Welch's t.test: P = 0.42). Amongst the 16 invasive plant species, lantana (L. camara) (impact magnitude = 42) and the tree of heaven (A. altissima) (impact magnitude = 32) had the highest cumulated impact scores (Table 1). Environmental impacts scores were higher for lantana and the camphor tree (Cinnamomum camphora) than the other species (Table 1). The highest socio-economic impact scores were recorded for lantana and tree of heaven (Fig. 1). Four plant species that had relatively little environmental impact presently included guava (Psidium guajava), inkberry (Cestrum laevigatum), the forget-me-not-tree (Duranta erecta) and the wax tree (Rhus succedanea). Two species that had no socio-economic impacts were coral bush (Ardisia crenata) and white mulberry (Morus alba). The tree of heaven scored the maximum impact on the socio-economic category through human social life (i.e. loss of recreational activities and tourist attractions, see Nentwig et al. 2016; Table 2). Some species showed low overall impact scores, but scored higher (the maximum impact score of five) in some mechanisms, for example, M. alba (impacts through hybridisation), C. camphora (impacts on plants or vegetation), R. cuneifolius (impacts on ecosystems) and S. terebinthifolius (impacts on plant or vegetation) (Fig. 1; Table 2). Most of the impacts recorded for the socio-economic category were through animal production, agricultural production and human health and the least impact was on human infrastructure (Fig. 2a; Table 2). There was a non-significant negative relationship between the environmental impact score and mean seed size and a significant relationship with mean fruit size (Fig. 3; Table 3). There was a non-

			GISS score			
Scientific names	Common names	NEMBA category	Environmental	Socio- economic	Total	Region of origin
Ailanthus altissima	Tree of heaven	1b	13	19	32	Asia (China)
Ardisia crenata	Coral bush	1b	3	0	3	Asia
Cestrum laevigatum	Inkberry	1b	0	3	3	South America (Brazil)
Cinnamomum camphor	Camphor tree	1b	16	2	18	East Asia
Duranta erecta	Forget-me-not-tree	3	0	1	1	America
Eugina uniflora	Surinam cherry	1a	2	2	4	South America (Brazil)
Lantana camara	Lantana	1b	23	19	42	Central and South America
Melia azedarach	Syringa	1b	3	2	5	Asia, Australia
Morus alba	White mulberry	2	8	0	8	Asia
Psidium guajava	Guava	2	0	6	6	America
Toxicodendron succedanea	Wax tree	1	0	3	3	Asia
Ricinus communis	Castor-oil plant	1b	4	2	6	Africa
Rubus cuneifolious	American bramble	1b	10	4	14	North America
Schinus terebinthifolius	Brazilian pepper tree	1b	11	5	16	South America (Brazil)
Solanum mauritianum	Bugweed	1b	12	7	19	South America
Syzgium jambos	Rose apple	3	5	6	11	South - East Asia

Table 1. The sum of environmental and socio-economic impacts scored for 16 fleshy-fruited invasive plant species using the Generic Impact Scoring System (GISS). Species that scored a maximum impact score of 5 in any of the mechanisms are highlighted in bold.



Figure 1. Impact scores for the socio-economic and environmental impact category for all the sixteen fleshy-fruited invasive plant species in South Africa in the present study.

species assessed usi	ng the Generic Impa	ict Sco	re System (GISS)		0103 0	1 1103	11y 1	runc		1 v 431	ve p	14110
Species	Common names	Envi	ronmental mechanis	ms	Socio	econo	omic	mech	anisr	ns		
		tion	noission	total	iction ion	tion	cture	-4	ife	total	res	rature

Table 2. Environmental and socio-economic mechanism impact scores of fleshy-fruited invasive plant

		Plants or vegetation	Animals	Competition	Diseases transmissio	Hybridization	Ecosystems	Environmental tot:	Agricultural producti	Animal production	Forestry production	Human Infrastructu	Human health	Human social life	Socio-economic tot	Overall scores	Number of literat
Ailanthus altissima	Tree of heaven	4	3	2	0	0	4	13	3	0	4	4	3	5	19	32	17
Ardisia crenata	Coral bush	3	0	0	0	0	0	3	0	0	0	0	0	0	0	3	1
Cestrum laevigatum	Inkberry	0	0	0	0	0	0	0	3	0	0	0	0	0	3	3	5
Cinnamomum camphor	Camphor tree	5	3	2	3	0	3	16	0	0	0	0	2	0	2	18	1
Duranta erecta	Forget-me-not-tree	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	7
Eugina uniflora	Surinam cherry	0	0	0	2	0	0	2	2	0	0	0	0	0	2	4	1
Lantana camara	Lantana	4	4	3	4	4	4	23	4	4	4	0	4	3	19	42	25
Melia azedarach	Syringa	0	0	0	0	0	3	3	0	0	0	0	2	0	2	5	3
Morus alba	White mulberry	0	0	0	3	5	0	8	0	0	0	0	0	0	0	8	2
Psidium guajava	Guava	0	0	0	0	0	0	0	3	0	3	0	0	0	6	6	4
Rhus succedanea	Wax tree	0	0	0	0	0	0	0	0	0	0	0	3	0	3	3	2
Ricinus communis	Castor-oil plant	3	1	0	0	0	0	4	0	0	0	0	0	2	2	6	4
Rubus cuneifolious	American bramble	3	0	2	0	0	5	10	0	2	2	0	0	0	4	14	6
Schinus terebinthifolius	Brazilian pepper tree	5	3	0	0	0	3	11	0	0	2	0	3	0	5	16	10
Solanum mauritianum	Bugweed	3	3	0	2	0	4	12	3	0	3	0	1	0	7	19	8
Syzgium jambos	Rose apple	0	0	2	3	0	0	5	3	0	3	0	0	0	6	11	6



Figure 2. The mean impact scores for a the socio-economic mechanisms and b the environmental mechanisms in South Africa in the present study. (The boxes represent the mean impacts score in quantiles and the circles represent outliers).



Figure 3. Relationship between socio-economic impacts with log-transformed morphological traits **a** mean fruit crop size **b** mean fruit size **c** mean seed size and environmental impacts with log-transformed morphological traits **d** mean seed size **e** mean fruit size and **f** mean fruit crop size. (Each dot represents a species).

significant positive relationship between socio-economic impact and crop size and a positive non-significant for mean seed size and mean fruit size (Fig. 3; Table 3).

Most environmental impacts were through impacts on plants or vegetation, ecosystem and animals and the least impacts were through hybridisation (Fig. 2b; Table 2). There were no significant differences in the impact magnitude of different mechanisms

Response	Model	Df	AICc	Log likelihood	P-value
Socio-economic impa	ct				
	Log fruit size	12	93.17	-41.59	0.40
	Log seed size	12	92.91	-41.45	0.60
	Log fruit crop size	12	95.83	-42.92	0.59
Environmental impac	t				
	Log fruit size	12	91.72	-40.86	0.04^{*}
	Log seed size	12	94.10	-42.05	0.34
	Log fruit crop size	12	96.71	-43.36	0.27

Table 3. Linear mixed-effects model estimating the effect of fruit size, seed size and fruit crop size on the socio-economic and environmental impacts of avian dispersed alien invasive plant species in the current study. AIC is the Akaike Information Criterion.

in both categories: socio-economic (ANOVA: df = 5, P > 0.05) and environmental (ANOVA: df = 5, P > 0.05, Fig. 2). We found that 14 (86%) of the 16 plant species had no records of causing socio-economic impacts through impacting human life and environmental impacts through hybridisation. Most records of alien invasive plant species were mainly for environmental rather than socio-economic mechanisms. The total number of papers used for the impact assessment was 103 (see Suppl. material 1 for a list of the data sources used) and there were significant differences between the number of papers and the scored impacts per plant (Kendall's Tau: $\tau = -0.15$; p < 0.05).

Discussion

In the present study, global impacts assessment of 16 fleshy-fruited invasive species indicated that 12 species had environmental impacts and 14 had socio-economic impacts. A total of six species in the present study showed either no environmental or socio-economic impacts. Similarly, a previous study in Europe that assessed the impacts of alien invasive plant species using the GISS showed no environmental or socioeconomic impacts (Rumlerová et al. 2016). This is a consequence of studies focusing on certain impacts or the selection of species with already known impacts (Pyšek et al. 2012; Rumlerová et al. 2016; Schirmel et al. 2016; White et al. 2019). Previous studies have noted the influence of undocumented or lack of peer-reviewed information in quantitative impact assessment studies (McGeoch et al. 2012; Moshobane et al. 2019; Verbrugge et al. 2019). For example, *P. guajava* has major ecological impacts in Zululand, KwaZulu-Natal, South Africa, where this species has displaced native vegetation (C.T. Downs, unpublished data). Consequently, the impacts on the ecosystem or vegetation posed by this species are misrepresented in the present study. This highlights the importance of re-assessing the impacts of species once data are available or published in the case of using assessment tools that use peer-reviewed literature.

In the environmental category, we found impacts associated with fleshy-fruited invasive plant species were through the ecosystems, plants or vegetation impact mechanism and some species had the highest impact scores on these mechanisms, for example, R. cuneifolious, S. terebinthifolius and C. camphora. These results correspond with previous studies showing similar findings on environmental impact mechanisms associated with invasive plant species (Vilà et al. 2011; Yazlik et al. 2018). For species with high scores, impacts on ecosystem functioning manifest in different ways, including integrating into ecosystem networks and changing seed dispersal and pollination networks which are important ecological processes. Through seed mutualism interaction, fleshy-fruited invasive plants alter the dispersal of other plant species and outcompete indigenous plants for dispersal agents (Mokotjomela et al. 2016). Consequently, changes in seed dispersal networks reduce overall biodiversity (Fuster et al. 2019) through the loss of ecological processes like pollination and seed dispersal. For example, in South Africa, R. cuneifolius alters pollination networks of native communities (Hansen et al. 2018) and disrupts bird-mediated ecological processes (Reynolds and Symes 2013). Some of the species with major impacts (i.e. A. altissima, L. camara and S. terebinthifolius) had impacts on vegetation and plants through allelopathy, negatively affecting native threatened plant species and overall biodiversity (Morgan and Overholt 2005; Sharma et al. 2005; Kowarik and Samuel 2007).

Impacts on human health, forestry and agricultural production were the main socio-economic impact mechanisms associated with fleshy-fruited invasive species in the present study, with L. camara and A. altissima having the highest impacts. Similarly, a study in Turkey showed that socio-economic impact mechanisms are through agriculture and human health (Yazlik et al. 2018). The major impact on forestry production may be because forests are identified as an important introduction pathway for many invasive tree and shrub species (Rejmánek 2014; Sitzia et al. 2016). Although some of these species are forest-edge species, they must be included in forest management (Sitzia et al. 2016). Impacts on agriculture and human health were indirect through hosting pests that damage agricultural crops or threaten human health. For example, L. camara harbours pests (e.g. tsetse fly Glossina spp.), resulting in major health issues in sub-Saharan Africa (Goulson and Derwent 2004). Additionally, alien fleshy-fruited plants form thick stands that generally reduce agricultural land's productivity and viability, resulting in reduced crop production of economically-important plants and increased management costs (Shackleton et al. 2017). It is important that the management of invasive plants is not only targeting protected areas and should be implemented in agricultural areas, as impacts associated with invasive plants are both environmental and socio-economic (Yazlik et al. 2018). This is particularly important for sub-Saharan African countries with agriculture-dominated economies, where livestock and crop farming constitute the largest agricultural sector (Pratt et al. 2017). Fleshy-fruited invasive species had relatively few or generally lower impacts on human infrastructure, except for A. altissima, which scored the maximum impact. This is mainly because the impacts of alien plant species on human infrastructure (e.g. roads, and traffic infrastructure, see Nentwig et al. 2016) remain poorly explored. Some species in the present study had low overall impact scores, but had the highest magnitude score for some mechanisms, for example, *M. alba, C. camphora* and *R. cuneifolius.* In the United States of America (USA), *M. alba* has been reported to hybridise with an endangered native species *M. rubra* (Burgess et al. 2005), *C. camphora* replaces an endangered shrub *Ziziphus celata* in Florida, USA (Kaufman and Kaufman 2013) and *R. cuneifolius* threatens a grassland specialist plant in South Africa (Hansen et al. 2018). Similarly, a study that assessed the impacts of grasses using the GISS showed similar results where two grass species with low overall impact had high magnitude scores for certain mechanisms (Nkuna et al. 2018). This is particularly interesting as it raises an important question should species with high overall impact scores, but high magnitude scores for certain mechanisms, be of concern (Nkuna et al. 2018)? The overall impact scores can be useful in broad recommendations, but may negate the importance of specific species with specific impacts.

In the present study, there were significant differences between the scored impacts and the number of papers used; well-studied plant species scored significantly higher impacts than species with few or no impact studies. In general, the negative impacts of some species, especially those with economic value (i.e. P. guajava, R. communis and R. cuneifolius), are often overlooked because of their beneficial uses. The research efforts of assessing the impacts of economically-important invasive plants are potentially complicated by the trade-off between economic importance and their damage, resulting in misrepresentation of impacts. Indeed, Zengeva et al. (2017) assessed the impacts and benefits of invasive species and showed that the management of *P. guajava* has resulted in stakeholder conflict in South Africa because of the economic and intrinsic value of the plant. In addition, it has been reported that species with major economic impacts attract scientific attention, improving understanding of their ecological impacts (Pyšek and Richardson 2010). It was not the aim of this study to assess the limitations of this tool. Therefore, both scientists and decisionmakers who aim to manage alien invasive species should consider both the benefits and costs of preventing the introduction of species with high impact scores or their management after introduction and establishment. This problem highlights the need for further studies to evaluate the socio-economic and ecological impacts posed by fleshy-fruited invasive plant species. Evaluating invasive species' social impacts will increase stakeholder engagement and scientific citizenship (Estévez et al. 2014; Crowley et al. 2017; Potgieter et al. 2019).

Species traits are important in the invasion success of alien plants (Pyšek and Richardson 2008). Our results of the impact relationship with morphological traits showed that species that produce large fruit crops of small fruit with small seed sizes have relatively higher environmental and socio-economic impacts. In cases where dispersal is limited to frugivores, fleshy-fruited plant species with large crop sizes are competitive, attract most species and are successful invaders (Ramaswami et al. 2017). For example, *S. mauritianum* has higher visitation rates than native and other plants alien to South Africa with relatively small crop sizes (Mokotjomela et al. 2013). Therefore, plant traits that influence seed dispersal interaction and invasion success are important and should

be incorporated into the screening process of fleshy-fruited alien plants (Jordaan and Downs 2012; Bitani et al. 2020). Species trait data of fleshy-fruited invasive species are comparable across different regions; therefore, the data can be transferable across regions (Jordaan et al. 2012).

Conclusions

Assessing socio-economic and environmental impacts of fleshy-fruited invasive plant species in South Africa showed that these species pose both ecological and socio-economic impacts. This study also highlighted that the impacts of many fleshy-fruited invasive species are not documented. We recommend management prioritise species with high overall impact scores (L. camara, A. altissima and C. camphora), including species with low overall impact scores, but high impact magnitude for certain mechanisms (M. alba, R. cu*neifoliu*, and *S. terebinthifolius*) as the impacts are inevitable. The introduction pathways of these fleshy-fruited invasive plant species need to be identified and managed to prevent their future spread. The present study results showed that different sectors are affected by invasive plant species, emphasising the need for the collaboration of stakeholders in biological invasion management. In South Africa, not all local municipalities have the capacity to effectively implement management strategies to manage invasive species (McLean et al. 2018). Therefore, despite the different mandates for different departments or sectors in South Africa, effective management of invasive plant species requires collaboration at a national and regional level, including and adding a socio-economic dimension to the management strategies to ensure inclusivity and transparency. This study is an important contribution in guiding managing invasive plant species and allocating limited resources in South Africa. We recommend that more research be done to evaluate the impacts, especially socio-economic impacts associated with fleshy-fruited invasive plant species.

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Supplementary material I

Table S1

Authors: Nasiphi Bitani, Tinyiko C. Shivambu, Ndivhuwo Shivambu, Colleen T. Downs

Data type: Docx file.

Explanation note: The plant species' functional traits that influence seed dispersal by bird species as identified in Bitani et al. (2020).

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Link: https://doi.org/10.3897/neobiota.74.83342.suppl1

Supplementary material 2

Table S2

Authors: Nasiphi Bitani, Tinyiko C. Shivambu, Ndivhuwo Shivambu, Colleen T. Downs

Data type: Docx file.

Explanation note: References used for the data summarised in Table 2.

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