RESEARCH ARTICLE



Exotic pet owners' preferences for different ectothermic taxa are based on species traits and purchase prices in the United States

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Abstract

The exotic pet trade has resulted in substantial invasion and disease risks, owing to the release of pets into new environments. Scientists have conjectured that pet owners acquire and release species with undesirable traits because they are imperfectly informed about the traits of these animals. However, few studies have used social science methods to elicit pet owners' preferences for exotic pets. In 2019 we administered a best-worst choice survey to 1,055 exotic pet owners in the United States (who own pet reptiles, amphibians, fish, or invertebrates) to examine how human preferences and incomplete information may contribute to the risks of the exotic pet trade. Respondents preferred colorful and patterned species. On average, respondents preferred medium-sized amphibians and reptiles, small fish, and large invertebrates, although they demonstrated heterogeneity in preferences with respect to the adult size of pets. Respondents also preferred amphibians and reptiles with medium life expectancies and fish and invertebrates with long life expectancies, although they again demonstrated heterogeneity in preferences with respect to pets' life span. Respondents preferred docile animals, and were more likely to purchase lower-cost pets. We found some evidence that respondents' decision to purchase exotic pets depended on whether these animals were native, rare, had unusual morphological features, and breed easily. Respondents' decision to purchase specific taxa as exotic pets also depended on their age, education, and housing. Most respondents stated that they searched for information on pets' diet, behavior, adult size, life span, costs of care such as equipment or veterinary costs, and whether the animal was captive bred before purchasing these animals. Excepting pets' diets, fewer than half of respondents had been offered information on pets' traits by sellers. On average, respondents rated the information they had been offered as average. Respondents typically obtained additional information about pets from online searches. Our results suggest that certification

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systems that provide critical information on exotic pets' behaviors, adult size, longevity, fecundity, and husbandry needs should be implemented to prevent pet owners acquiring animals that they may subsequently abandon.

Keywords

Amphibians, animal behavior, best-worst choice experiments, coloration, consumer preferences, fish, invertebrates, reptiles, size

Introduction

Although it is financially lucrative, the global exotic pet trade has resulted in the overexploitation of species, the introduction and spread of invasive species and pathogens, risks to public health and safety, and animal welfare concerns (Bush et al. 2014; Warwick et al. 2018; Gippet and Bertelsmeier 2021; Harrington et al. 2022; Toomes et al. 2022). Exotic pets are animals that are non-native and/or do not have a history of domestication, and which people purchase for companionship, ornament, or entertainment (Warwick et al. 2018). Responding to the various risks posed by the exotic pet trade is challenging because we have incomplete knowledge of the demand for exotic pets, largely derived from trade data (Lockwood et al. 2019; Sinclair et al. 2021). Better understanding of pet owners' preferences for different species and exotic pet traits (e.g., life history, aesthetic appeal, captive care requirements, monetary costs) is necessary to design interventions that appropriately target pet owners' choice of pets (Burivalova et al. 2017; Sung and Fong 2018; Lockwood et al. 2019; Hausmann et al. 2023; Street et al. 2023), in order to prevent potential risks associated with owners acquiring and subsequently releasing undesirable pets (Harrington et al. 2022; Toomes et al. 2022).

In this paper, we focus on the trade in reptiles, amphibians, fish, insects, and arachnids as exotic pets. Over 550 reptile, 170 amphibian, and 860 invertebrate species are sold as pets in the United States and United Kingdom (Warwick et al. 2018). Further estimates suggest that ~160 million ornamental fishes are kept in aquaria in the United States, encompassing ~20 million marine fish (Biondo and Burki 2020). To help elucidate how human preferences and incomplete information may contribute to the risks of the exotic pet trade, we administered a survey to exotic pet owners in the United States. We focused on 1) people's decision to acquire exotic pets, specifically which traits increase their likelihood of purchasing a pet; 2) which traits reduce the desirability of a pet; 3) how exotic pet owners acquire information about exotic pets (e.g., husbandry requirements); and 4) their assessment of the quality of the information they received when purchasing their pets.

Based on existing studies of species in the exotic pet trade, we hypothesized that pet owners would prefer species that are colorful or patterned (van Wilgen et al. 2010; Vall-llosera and Cassey 2017), and animals with distinctive or rare aesthetic or morphological features (Burghardt 2017; Sung and Fong 2018; Harrington et al. 2022; Hausmann et al. 2023). We further hypothesized that pet owners would have heterogeneous preferences with respect to the adult size and lifespan of exotic pets. People may prefer larger invertebrate species (Barua et al. 2012), but their preferences for size in pet fishes (Harrington et al. 2022) is not clear. Exotic pet owners have cited the higher longevity of certain exotic pet species relative to common domesticated animals as part of their decision to acquire exotic pets (Goins and Hanlon 2021). However, vertebrates with larger body sizes and long reproductive lifespans tend to escape captivity or be released by exotic pet owners (Toomes et al. 2022; Street et al. 2023), which suggests that these may not be preferred traits. We further posited that most pet owners would prefer species that do not require live food, and that are docile and easy to handle and maintain (van Wilgen et al. 2010; Vall-llosera and Cassey 2017). Dangerous species or species that require specialized care or housing are typically only desired by experienced pet owners and hobbyists, who are capable of caring for such species (van Wilgen et al. 2010; Vall-llosera and Cassey 2017; Hausmann et al. 2023).

We had no prior predictions on whether species rarity or captive breeding are preferred traits in exotic pets. Species that are novel, threatened or protected are traded at higher prices (Bush et al. 2014; Sung and Fong 2018; Siriwat et al. 2019). However, species that are rare in the wild may be common in the pet trade, which has been demonstrated to diminish pet owners' preferences for these species (Krishna et al. 2019). Studies are inconsistent in their findings as to whether exotic pet owners prefer wildcaught or captive-bred animals, or whether they prefer species that are common in the wild and abundant in the market (Burivalova et al. 2017; Sung and Fong 2018; Hausmann et al. 2023). What is clear from trade data is that species that are abundant in the pet trade are sold at lower prices (van Wilgen et al. 2010; Vall-llosera and Cassey 2017), which would make these pets affordable for a larger number of people. Species that are easier to breed in captivity also tend to be traded at higher volumes (van Wilgen et al. 2010). For example, amphibians and reptiles traded as exotic pets tend to have relatively high reproductive rates and long reproductive lifespans, but these species also tend to be accidentally or deliberately released by pet owners which suggests that fast life history traits are not preferred (Toomes et al. 2022; Street et al. 2023).

Although pet owners may acquire species based on a set of preferred traits (e.g., appearance, rarity), pet owners who are unable to care for pets with undesirable traits (e.g., behavior, adult size) or who are unable or unwilling to pay the veterinary expenses associated with exotic pets may abandon or release their pets into the wild (Pasmans et al. 2017). Abandonment and release of exotic pets occurs because owners are misinformed about animal behaviors (e.g., defensive behaviors such as biting and scratching) and pets' husbandry requirements and costs of care, with exotic pets often being mislabeled 'easy to keep' or 'beginner' animals (Warwick et al. 2018; Siriwat et al. 2019). However, animals' level of specialization in the wild is correlated with their temperaments and needs, which means that highly specialized species are difficult to handle and care for (Bush et al. 2014). Pet owners may lack information about pets' requirements for specific diets, habitat structure, lighting, heating, humidity, physical activity, play, stimulation, and large spaces (Bush et al. 2014; Burghardt 2017; Warwick et al. 2018). Pet owners may also be unaware that certain species exhibit aggressive or stressed behaviors when exposed to humans and multispecies assemblages (Bush et al. 2014). Poor animal husbandry is compounded by inaccurate, incomplete, and poor-quality information about exotic pet

care provided by sellers or online searches (Pasmans et al. 2017; Warwick et al. 2018). Based on the literature, we hypothesized that pet owners receive incomplete information about the traits and husbandry requirements of exotic pets, and that they rely on online searches to obtain additional information about the exotic pets they have purchased.

Methods

Survey design

We administered an online survey to exotic pet owners. We initially asked respondents to check all types of pets that they owned from an extended list that included birds and mammals. Respondents who selected reptiles, amphibians, insects, arachnids, and/or fish were directed to the questionnaire. We elicited information on both the number of exotic pets respondents owned as a child and the number of exotic pets they currently owned. We further elicited information on how respondents acquired their current pets. We asked respondents to indicate where they had purchased pets (e.g., from a breeder, commercial store, or trade show) and whether they had purchased any of their pets online.

We then asked respondents "If you were going to purchase another pet, which of these animals are you most likely to purchase?" (response options of 'snake', 'lizard/ chameleon', 'turtle', 'tortoise', 'frog/toad', 'salamander/newt', 'fish (saltwater or freshwater)', and 'insect/arachnid'). We allocated respondents questions specific to one of the taxa they had selected. We programmed the survey to ensure that (to the extent possible) an equal number of respondents were assigned questions for each taxon. We informed respondents that we were interested in their preferences for four pet traits (coloration, size, life span, and behavior) as well as the purchase price of the pet.

We presented respondents with images of different pets that varied in coloration and asked them what color and/or pattern they would prefer for their next pet ('neither colorful nor patterned', 'colorful but not patterned', 'patterned but not colorful', or 'both colorful and patterned'; Fig. 1). We also provided respondents with different examples of adult sizes for that pet type and asked them what size they would prefer the adult pet to reach ('small', 'medium', or 'large'; Table 1). We provided respondents with images of potential exotic pets when we described pet coloration and size to ensure that respondents were answering subsequent questions about whether they would purchase pets with different attributes based on identical understanding of what we meant by coloration and size. To the extent possible, we attempted to ensure that the species we presented in these images were similar in morphology (excepting coloration or size) so that respondents focused on the indicated pet trait (coloration, size). It is possible that respondents who are familiar with the species we presented took other characteristics of the species into account when answering these initial questions, but we controlled for this later in the survey (see below).

To elicit respondents' preferences for pet longevity we informed them that "The life span of potential pets can differ greatly, impacting the length of time a pet owner is responsible for their pet," and we asked them what length of time they would prefer to own their next pet. The time ranges we presented to respondents were based on the life expectancies of different species within that group of exotic pets. We defined three different levels of behavior for pets, namely: 'docile' pets that can be easily handled and/or are not aggressive towards other pets; pets with an 'intermediate' temperament that are active, can be handled, and may occasionally be aggressive; and 'aggressive' pets which are highly active, pose threats to other pets, and are difficult to handle. We modified the wording for fish to remove any reference to handling the animal. Respondents indicated which temperament they would prefer in their next pet. We also asked respondents to indicate the approximate cost of the last pet of that taxa they had acquired.

After asking respondents to consider their preferences for pet traits and what price they paid for their last pet, we presented them with six best-worst choice (BWC) questions to rigorously measure their preferences for pet traits (see below for a more detailed description of this methodology). We presented respondents with written descriptions of six different potential pets that had specific traits (coloration, adult size, longevity, and behavior) and the price at which the pet could be purchased (Fig. 2). It is important to note that we did not provide images of species in the BWC questions to ensure that respondents focused on the traits we identified, rather than other morphological or behavioral traits. We asked respondents to complete three tasks for each question: 1) to select which aspect (traits, price) of the pet they liked most; 2) to select which aspect of the pet they liked least; and 3) to indicate whether they would buy the pet exactly as described. If respondents stated that they would purchase the pet, we asked them to indicate on a 10-point scale how certain they were that they would purchase that pet (very uncertain = 1; very certain = 10). If respondents stated that they would not purchase the pet, we asked them to indicate why. The possible response options to this question were: 'I do not like the coloration of the animal'; 'I do not like the size of the animal'; 'I do not like the life span of the animal'; 'I do not like the behavior of the animal'; 'I do not like the price of the animal'; 'I do not want another pet'; or respondents could provide another reason for not purchasing the pet. We presented respondents with an example of how to complete the BWC questions before asking them to answer these questions. The different traits and prices we presented for each of the exotic pets included in the survey are presented in Table 1.

We used data provided by Stringham and Lockwood (2019) and in-person and online searches of pet retailers to identify prices at which different pets were being sold at the time of survey design to determine the prices presented in the BWC questions. We visited 4 pet retailers (2 general pet retailers that sold an array of pets and pet products; 1 retailer that specialized in pet herpetofauna, 1 retailer that specialized in aquarium fish) in person once to record prices. We searched the inventory of pets sold by 13 online pet retailers (2 general pet retailers, 2 retailers that specialized in pet turtles and tortoises, 2 retailers that specialized in pet herpetofauna, 2 retailers that specialized in pet invertebrates, 1 retailer that specialized in pet herpetofauna and invertebrates, 4 retailers that specialized in aquarium fish). Finally, we searched 3 websites that provided information about husbandry requirements and typical purchase prices for an array of exotic and traditional pets. We only visited each website once during survey development. We assumed that the prices for the regular stock of species traded by pet retailers would not vary greatly over time.

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I. Attributes
Table

	Snake	Lizard/Chameleon	Turtle	Tortoise	Frog/Toad	Salamander	Insect/Arachnid	Fish
Color (example	e species that closely mat	ched different levels of co	loration and patterning)					
Neither colorful nor patterned	Black rat snake (Pantherophis obsoletus)	Bearded dragon (<i>Pogona vitticeps</i>)	Mud turtle (<i>Kinosternon</i> subrubrum)	Burmese brown mountain tortoise (Manouria emys emys)	American bullfrog (Lithobates catesbeianus)	Jefferson salamander (Ambystoma jeffersonianum)	Dead leaf mantis (Deroplatys desiccata)	Black molly (Genus: <i>Poecilia</i>)
Colorful, not patterned	Green tree python (Morelia viridis)	Electric blue gecko (Lygodactylus williamsi)	Cumberland slider (<i>Trachemys scripta</i> <i>troostii</i>)	Cherry head red foot tortoise (<i>Geochelone</i> <i>carbonaria</i>)	Golden mantilla (Mantella aurantiaca)	Yellow-eye ensatina (Ensatina eschscholtzii xanthoptica)	Jewel beetle (Genus: Buprestidae)	Yellow tang (Zebrasoma flavescens)
Patterned, not colorful	Fire ball python (<i>Python regius</i>)	Leopard gecko (Eublepharis macularius)	Black-knobbed map turtle (<i>Graptemys</i> <i>nigrinoda</i>)	Radiated tortoise (Astrochelys radiata)	Great plains toad (Anaxyrus cognatus)	Two-lined salamander (Eurycea bislineata)	Ten-lined june bug (Polyphylla decemlineata)	Convict cichlid (Amatitlania nigrofasciata)
Both colorful and patterned	Corn snake (Pantherophis guttatus)	Panther chameleon (Furcifer pardalis)	Ornate wood turtle (Rhinoclemmys pucherrima)	High yellow pancake tortoise (<i>Malacochersus</i> <i>tornieri</i>)	Poison dart frog (Dendrobatidae spp)	Fire salamander (Salamandra salamandra)	Gooty tarantula (Poecilotheria metallica)	Mandarinfish (Synchiropus splendidus)
Adult size:								
Small	~1–3 feet long	~3–6 inches (not including tail)	~4–5 inches long, ~0.5 lbs	~8–10 inches long, 1–3 lbs	~ 1–2.5 inches long	~3–5 inches long	<0.5 inches long	~ 1–3 inches long
Medium	~4–6 feet long	~8–12 inches (not including tail)	~6–9 inches long, ~2–3 lbs	~12–18 inches long, ~15–25 lbs	~3–5 inches long	~6–14 inches long	~0.5–2 inches long	~ 5–9 inches long
Large	~8 feet or longer	~16–24 inches (not including tail)	~12–24 inches long, ~6–14 lbs	~22–36 inches long, ~70–100 lbs	~7 inches or longer	~16-45 inches long	~4 inches or longer	~12 inches or longer
Life expectancy	ż.							
Short	<10 years	<5 years	<16 years	<25 years	<5 years	<10 years	<6 months	<5 years
Average	10–25 years	5–15 years	16-40 years	25–60 years	5–12 years	10-25 years	6–12 months	5-10 years
Long	>25 years	>15 years	>40 years	>60 years	>12 years	>25 years	>12 months	>10 years
Behavior:								
Docile	An animal that is easy to handle, and not aggressive	An animal that is easy to handle, and not aggressive	An animal that is easy to handle, and not aggressive	An animal that is easy to handle, and not aggressive	An animal that is easy to handle, and not aggressive	An animal that is easy to handle, and not aggressive	An animal that is easy to handle, and not aggressive	A fish that is peaceful and does not pose a threat toward other fish
Intermediate	An animal that can be handled, and may be aggressive, but only occasionally	An animal that can be handled, and may be aggressive, but only occasionally	An animal that can be handled, and may be aggressive, but only occasionally	An animal that can be handled, and may be aggressive, but only occasionally	An animal that can be handled, and may be aggressive, but only occasionally	An animal that can be handled, and may be aggressive, but only occasionally	An animal that can be handled, and may be aggressive, but only occasionally	A fish that is active, and may be occasionally aggressive toward other fish
Aggressive	An animal that, due to its aggressive nature, is typically difficult to handle	An animal that, due to its aggressive nature, is typically difficult to handle	An animal that, due to its aggressive nature, is typically difficult to handle	An animal that, due to its aggressive nature, is typically difficult to handle	An animal that, due to its aggressive nature, is typically difficult to handle	An animal that, due to its aggressive nature, is typically difficult to handle	An animal that, due to its aggressive nature, is typically difficult to handle	A fish that is highly active, and typically poses a threat to other non-dominant fish

	Snake	Lizard/Chameleo	n Turtle	Tortoise	Frog/Toad	Salamander	r Insect/Arachnid Fish	
Price:								
Lowest	\$90	\$50	\$25	\$100	\$20	\$20	\$12 \$8	
Price 2	\$140	\$90	\$50	\$150	\$50	\$50	\$25 \$25	
Price 3	\$250	\$200	\$100	\$200	\$70	\$70	\$45 \$50	
Highest	\$450	\$450	\$250	\$400	\$110	\$100	\$90	
	 Colorful and patternik Yellow Tang Patterned and colorie Convict Cichild 	iss. For example a	Colorful and patterned: Mandarinfish Mandari	For example a	 Colorful and patternless. Fo yellow-eye Ensantina Pelterned and colorless. For two-lined salamander 	example a o	colorful and patterned. For example a fire salamander	
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Owners' preferences for exotic pet traits

We are interested in your preferences for pet snakes. In the following questions we will ask you whether you would consider buying 6 different snakes. These snakes vary in their coloration, size, life span, behavior, and cost. The snakes do not represent specific species.

In each of the following questions you will be asked to perform the same tasks. We are interested in:

- 1. Which trait of the snake you like **MOST**.
- 2. Which trait of the snake you like LEAST.
- 3. Whether you would buy a snake with ALL these 5 traits.

Snake A (select one trait you like most and one trait you like least).

Like MOST		Like LEAST
0	Colorful and patterned	0
0	Small size	0
0	Lives less than 10 years	0
0	Aggressive behavior	0
0	Price: \$90	0

Would you purchase a snake with the five traits above?

o Yes

o No

Figure 2. Best-worst choice question for a pet snake.

Once respondents had completed the BWC questions, we elicited their preferences for additional pet traits by asking them to indicate on a 5-point scale (very negative=-2, somewhat negative=-1, neither positive nor negative=0, somewhat positive=1, very positive=2) how additional traits would influence their decision to purchase an exotic pet, namely that the pet was captive-bred, wild caught, native to the area in which the respondent lives, or rare. We also asked how the pet's diet (expensive diet, diet of animal products), appearance (an unusual shape, a pre-historic appearance, an appearance that changes as the pet ages) and fecundity would influence respondents' decision to purchase a pet. We derived the term 'pre-historic' from interviews with pet trade participants, who equated 'pre-historic' with species that resembled dinosaurs, with scales, long/curved claws, wide heads, and long necks and/or tails (Episcopio-Sturgeon and Pienaar 2019). Pre-tests confirmed that survey recipients interpreted 'pre-historic appearance' as we intended.

To assess whether respondents researched the needs of pets before acquiring them, we asked respondents which information they looked up about a pet before purchasing it. We also asked which information they were offered about their current pets at the time of acquisition, how they would rate the quality of the information they received, and which information they wish they had received prior to acquiring any of their current pets. We further asked which additional information they had looked up on their pets after acquiring them and the source of that information. Finally, we collected respondents' demographic information (gender, age, education level, income level, job status, type of residence, number of household members \leq 18 years old).

Before finalizing the survey, we pre-tested the questionnaire with nine experts in the design and implementation of social sciences surveys, six invasion ecologists who study the pet trade, and 14 exotic pet owners. The final survey was approved by the University of Florida's Institutional Review Board (protocol number: IRB201802439).

Best-worst choice methodology

We used the BWC methodology (Lusk and Parker 2009), which combines best-worst scaling (BWS) with dichotomous choice experiments (DCE), to elicit pet owners' preferences for different traits of exotic pets, and whether they would purchase pets with different combinations of traits. BWS was first implemented in the field of marketing in the 1990s (Finn and Louviere 1992) to assess consumer preferences for goods and services. For the purposes of our study, respondents completed two tasks: 1) they chose which characteristics (traits and purchase price) of an exotic pet they liked most and least (the BWS task); and 2) they indicated whether they would buy the pet with the described traits at that purchase price (the DCE task).

We used optimal designs generated by SAS statistical software (JMP Version 14.1) to maximize information derived from the BWC questions while minimizing the length of the survey. The optimal design (D-efficiency = 95.02) generated 18 choice tasks (i.e., pet descriptions). We used SAS to split these 18 choice tasks into three blocks of six choice tasks to reduce respondents' cognitive burden. Accordingly, we generated three different survey versions for each exotic pet, which presented respondents with six examples of the pet that varied in traits and purchase price (see Suppl. material 1: table S1).

Analysis of the best-worst scaling data

The main advantage of BWC is that the BWS task allows researchers to directly measure the (dis)utility that pet owners derive from pet traits and the purchase price for pets (Lusk and Parker 2009). BWS allows researchers to measure both attribute 'impacts' (mean utility of an attribute across all its levels on a latent, or unobserved, utility scale) and 'level-scale values' (LSVs; utility of an attribute level, i.e., deviations from mean utility; Flynn et al. 2007; Louviere et al. 2013). The attributes (coloration, adult size, longevity, behavior, purchase price) and LSVs (e.g., docile, intermediate, or aggressive behavior) for different pets are presented in Table 1.

We used paired estimation ("maxdiff") at the respondent level to analyze the BWS data (Lusk and Briggeman 2009; Louviere et al. 2013). In completing this BWS task, respondents identified every possible pair of items available in the choice set (i.e., profile of attributes), calculated the difference in utility between each pair

of items (i.e., attribute levels), and chose the pair of items that maximized their utility difference (Flynn et al. 2007). The number of possible pairs per choice set equaled J(J-1), where J was the number of items in each choice set (J=5 for our study: type of coloration; adult size; longevity; behavior; purchase price). For a pair of items (j,k), if a respondent liked j most and liked k least then the location of $j(\lambda_j)$ on the respondent's underlying utility scale was higher than λ_k (Lusk and Briggeman 2009). The utility that individual i derived from j is given by $I_{ij} = \lambda_{ij} + \varepsilon_{ij}$ where ε_{ij} is a random error term. The probability that individual i liked j most and k least from a choice set of J items was thus equal to the probability that the difference between I_{ij} and I_{ik} exceeded the difference between all other J(J-1)-1 possible pair combinations in the choice set:

$$\Pr[(I_{ij} - I_{ik}) > (I_{il} - I_{im})]$$

where *l* and *m* were all other possible pair combinations. Assuming independently and identically distributed type I extreme value errors, the multinomial logistic estimation procedure may be used to analyze BWS data, i.e.

$$\Pr(\text{like } j \text{ most, like } k \text{ least}) = \frac{e^{\lambda_j - \lambda_k}}{\sum_l \sum_m e^{\lambda_j - \lambda_m}}$$

Thus, standard maximum likelihood techniques can be used to estimate the vector of utility parameters (λ). We estimated logistic regression models (conditioned to the J(J-1)=20 possible best-worst pair combinations per choice set) where the dependent variable took a value of 1 for the chosen pair of best and worst values and 0 for all other J(J-1)-1=19 best-worst pairs available in each choice set. The λ_j parameter estimates represented the location of item j relative to an item that was omitted to avoid the dummy variable trap and normalized to zero (i.e., we omitted the attribute impact for a pet's life expectancy from the regression). The normalized item (life expectancy attribute impact) served as the reference point for the underlying utility scale, which allowed us to directly estimate all other attribute impacts and LSVs (λ) in the same units (utility) relative to this reference point. As such, we interpreted the sign and magnitude of parameter estimates relative to the reference point.

If the coefficient value of an attribute level is twice the magnitude of another attribute level, then this implies that a respondent derives twice the utility from the preferred attribute level. We could thus identify the relative importance to pet owners of different pet traits (Flynn et al. 2007; Lusk and Briggeman 2009; Lusk and Parker 2009). For example, we could infer that pet coloration is the most preferred pet trait, but on average pet behavior (i.e., whether pets are aggressive or docile) has a higher impact on people's decision to purchase a pet. Such information provides crucial insights into why people purchase pets, and why they may choose to discard them (e.g., owners purchase a pet based on its attractive appearance but may discard the pet because they were unaware that it had an aggressive temperament). We tested for preference heterogeneity (i.e., heterogeneity across respondents in terms of their preferences for species traits) by analyzing the BWS data using a random parameters logit model (Lusk and Briggeman 2009). Accordingly, we estimated the preference parameters for each individual *i* as $\tilde{\lambda}_{ij} = \bar{\lambda}_j + \sigma_j u_{ij}$, where $\bar{\lambda}_j$ and σ_j are the mean and standard deviation of λ_j , and u_{ij} is a standardized normally distributed error term with mean zero. We assumed that preferences for the attribute levels were normally distributed (Lusk and Briggeman 2009; Louviere et al. 2013). We effects coded the attributes and LSVs to separate attribute impacts and LSVs and to map their position on respondents' underlying utility scale (Suppl. material 1: table S2).

Analysis of the dichotomous choice data

Although BWS is informative, it does not provide information on whether an individual would purchase a pet with specific traits relative to the status quo of not purchasing the pet (Flynn et al. 2008). The DCE task within the BWC methodology allowed us to determine whether respondents would purchase exotic pets and how the decision to purchase pets was influenced by pet traits, the purchase price, and respondents' sociopsychological and demographic characteristics. Incorporating the DCE task allowed us to determine if certain attribute levels would make a pet undesirable to an owner, and how owners trade off between pet traits in their decision to acquire a pet.

Respondent *i*'s utility from purchasing a pet $j(U_{ij})$ was represented by a systematic component (V_{ij}) and a random error component (ε_{ij}) :

$$U_{ij} = V_{ij} + \varepsilon_{ij} = \mathbf{X}_{ij} \boldsymbol{\beta} + \varepsilon_{ij}$$

where \mathbf{X}_{ij} is a matrix of attribute levels that describe pet *j* and the characteristics of individual *i* and $\boldsymbol{\beta}$ is the vector of estimated coefficients. We modeled the probability that individual *i* would purchase pet *j* as:

$$Pr(purchase pet j) = Pr(U_{ij} > U_{i0}) = Pr(\Delta \varepsilon_{ij} < \Delta V_{ij})$$

where $\Delta \varepsilon_{ij} \equiv \varepsilon_{i0} - \varepsilon_{ij}$ is the difference in errors and $\Delta V_{ij} = V_{ij} - V_{i0}$ is the utility difference between purchasing the pet and not purchasing pet *j*. We specified the conditional indirect utility errors (ε_{i0} and ε_{ij}) as Type I extreme value, such that the probability that individual *i* would purchase pet *j* ('yes' response to the question 'would you purchase a [pet] with the traits above?') was:

$$\Pr(\text{purchase pet } j) = \frac{e^{\Delta V_{ij}}}{1 + e^{\Delta V_{ij}}}$$

Because respondents were presented with six choice sets that varied in pet traits and purchase price, we used a random-effects logistic regression to regress respondents' decision whether to purchase a pet (yes=1, no=0) against the pet traits, purchase price and respondents' socio-psychological and demographic characteristics. In common with the BWS task, pets' coloration, adult size, life expectancy, and behavior were effects coded. Purchase price was continuously coded, and respondents' sociopsychological and demographic characteristics were a mix of binary, continuous and effects-coded variables.

We used STATA/SE v.16.1 to estimate all models. Prior to conducting our analyses, we recoded respondents' choice of whether they would purchase an exotic pet. If the respondent indicated that their certainty that they would buy the pet was ≤ 6 then we recoded their choice as choosing not to purchase the pet (Lundhede et al. 2009). We selected best-fit DCE models based on the minimum Akaike Information Criterion (AIC). We considered coefficients to be significant at the $p \leq 0.05$ level.

Survey implementation

We initially intended to administer the survey exclusively to Florida exotic pet owners because Florida has experienced considerable adverse environmental, economic, and human wellbeing consequences, owing to species invasions that are linked to the pet trade (Russello et al. 2008; Engeman et al. 2011). We paid a survey panel provider (Qualtrics Research Services) to administer the survey to Florida residents who owned one or more of the following exotic pets: snakes; lizards; chameleons; turtles; tortoises; frogs; toads; salamanders; newts; freshwater or saltwater fish; insects; and arachnids. We instructed Qualtrics to limit the number of respondents who only owned fish to 75 respondents in total (15% of the sample) to ensure that we received surveys from owners of herpetofauna, insects and arachnids.

Qualtrics administered the survey from December 6, 2018 to January 24, 2019. A total of 5,357 individuals opened the survey, and 4,229 individuals were screened out of the survey, either because the quota of responses required for that pet type had already been reached (n=2,212) or the individual did not own our targeted pets (n=2,017). An additional 454 participants were screened out because they were not Florida residents, and 31 participants failed the attention checks in the survey. The completion rate for the survey was 72.3% (465 completed surveys; 643 surveys administered to individuals who met the study criteria.)

In addition, we emailed the link to the online survey to 44 aquarium clubs, 55 herpetological societies, 31 reptile rescues, 71 aquarium shop owners and 72 pet store owners in Florida, 391 pet adopters approved by the Florida Fish and Wildlife Conservation Commission (FWC) and 3,288 Florida Class III Wildlife for Exhibition or Public Sale permit holders and Possession or Exhibition of Venomous Reptiles or Reptiles of Concern license holders. We identified the email addresses for these survey recipients (excepting FWC approved adopters and permit holders) through online searches and social media. We administered the survey in three waves (initial email and two reminder emails) from January 8 to January 29, 2019. We received 590 completed surveys from these individuals. We could not determine a response rate for this second survey effort because we could not track how many individuals were sent the survey by

hobbyist clubs, rescues, or stores. Respondents to this second survey effort were residents of the United States, and so our sample was not restricted to Florida residents. We conducted two-sample t-tests with unequal variances to test for differences in mean responses to pet ownership questions between Florida respondents and respondents from other states.

Data resources

The data underpinning the analysis reported in this paper are deposited at Zenodo, and are available at https://doi.org/10.5281/zenodo.10534609.

Results

Most respondents (n=753, 71.4%) were female (Suppl. material 1: table S3). The median age range for respondents was 35–44 years, the median education level was either an associate's or technical degree, and respondents' median gross household income was \$50,000–99,999/year. A total of 416 respondents (39.4%) had individuals \leq 18 years old living in their household. Most respondents (n=720, 68.2%) lived in a single-family home, and 617 respondents (58.5%) were Florida residents. Because the population of exotic pet owners in the United States has not been described we could not ascertain whether our sample was representative of the larger population of exotic pet owners. We oversampled Florida residents relative to exotic pet owners in other states.

Over half of respondents owned dogs (n=698, 66.2%), cats (n=550, 52.1%) and lizards/chameleons (n=544, 51.6%; Suppl. material 1: table S4). Respondents from Florida were less likely to own pet snakes (28.8% of Florida respondents, 49.8% of respondents from other states, t=6.95, p<0.001), lizards/chameleons (Florida: 42.3%, other states: 64.6%, t=7.36, p<0.001), frogs/toads (Florida: 9.1%, other states: 15.1%, t=2.90, p=0.004), salamanders (Florida: 2.8%, other states: 6.2%, t=2.57, p=0.010), and insects/arachnids (Florida: 7.9%, other states: 21.0%, t=5.85, p<0.001). Respondents from Florida were more likely to own turtles/tortoises (Florida: 43.3%, other states: 20.8%, t=-8.08, p<0.001). The largest share of respondents (n=401, 38.0%) owned 2–5 exotic pets (reptiles, amphibians, fishes, insects, arachnids) at the time that the survey was implemented. In total, 369 respondents (35.0%) owned 2–5 exotic pets when they were <18 years old, whereas 322 respondents (30.5%) owned no exotic pets when they were children. Most respondents (n=693, 65.7%) purchased their exotic pets for themselves, frequently from a commercial pet store (n=373, 35.4%), or a breeder or hobbyist (n=363, 34.4%).

Respondents stated that they were most likely to purchase a lizard or chameleon (n=499, 47.3%), a fish (n=415, 39.3%), or a snake (n=412, 39.1%) as their next pet (Suppl. material 1: table S5). Most of these respondents (63.3–71.5%) already owned this type of pet. Florida respondents were less likely to select a snake (Florida: 27.9%,

other states: 54.8%, t=9.01, p<0.001), lizard/chameleon (Florida: 37.9%, other states: 60.5%, t=7.41, p<0.001), tortoise (Florida: 18.8%, other states: 24.2%, t=2.09, p=0.037), frog/toad (Florida: 10.9%, other states: 21.5%, t=4.55, p<0.001), salamander (Florida: 7.3%, other states: 12.1%, t=2.56, p=0.011), or insect/arachnid (Florida: 8.1%, other states: 22.8%, t=6.43, p<0.001) as their next pet. Florida respondents were more likely to select a turtle (Florida: 24.6%, other states: 11.2%, t=-5.85, p<0.001) or a fish (Florida: 43.8%, other states: 33.3%, t=-3.46, p=0.001) as their next pet.

When asked their preferences related to the appearance of their next pet, respondents typically selected a pet that is both colorful and patterned (snake: n=93, 50.5%; lizard: n=90, 44.6%; turtle: n=56, 43.1%; frog/toad: n=59, 60.2%; salamander: n=45, 60.0%; insect/arachnid: n=67, 75.3%; fish: n=92, 56.4%; Suppl. material 1: tables S6–S13). When asked their preferences related to the adult size of their next exotic pet, respondents tended to prefer a small turtle (n=77, 59.2%), tortoise (n=44, 38.6%), frog or toad (n=43, 43.9%), or fish (n=94, 57.7%). By contrast, respondents tended to prefer a medium-size snake (n=116, 63.0%), lizard/chameleon (n=102, 50.5%) or salamander (n=60, 80.0%). Most respondents who stated they would purchase an insect/arachnid preferred a large animal (n=75, 84.3%). Respondents preferred to own a snake for 10-25 years (n=108, 58.7%), a lizard/chameleon for 5-15 years (n=127, 62.9%), a turtle for 5-15 years (n=49, 37.7%), a tortoise for 25-60 years (n=44, 38.6%), a frog/toad for 5-12 years (n=49, 50.0%), a salamander for 5-9 years (n=31, 41.3%), an insect/arachnid for > 12 months (n=84, 94.4%), and a fish for 2-4 years (n=78, 47.9%). For almost all species, most respondents preferred a docile animal (snake: n=142, 77.2%; lizard/chameleon: n=150, 74.3%; turtle: n=87, 66.9%; tortoise: n=82, 71.9%; frog/toad: n=68, 69.4%; salamander: n=48, 64.0%; fish: n=106, 65.0%). However, respondents who stated they would purchase an insect/arachnid were equally likely to select an animal that is active and may occasionally be aggressive toward other animals (n=44, 49.4%) and a docile animal (n=42, 47.2%). The median price range for the pet snake respondents had most recently acquired was \$90-140. The median price range paid by respondents for their most recent pet lizard/chameleon was \$50-90. Most respondents had paid <\$25 for their pet turtle and <\$100 for their pet tortoise. Respondents paid an average of \$20-50 for their pet toad/frog or salamander. Most respondents paid <\$45 for their pet insect/arachnid, and <\$25 for their pet fish.

When asked how other traits would influence their decision to acquire an exotic pet, respondents indicated that they view captive bred pets (median=very positive) and pets with a pre-historic appearance positively (median=somewhat positive; Suppl. material 1: table S14). Respondents tended to view pets being wild-caught or having expensive diets negatively (median=somewhat negative). Respondents were generally neutral in their assessment of a pet being native, rare, requiring a diet of animal products, having an unusual shape, changing in appearance as it ages, or breeding easily (median=neither positive nor negative).

Before acquiring an exotic pet, most respondents stated that they searched for information on the animal's diet (n=962, 91.2%), behavior (n=936, 88.7%), adult size (n=911, 86.4%), life span (n=906, 85.9%), costs of care such as equipment or veterinary costs (n=791, 75.0%), and whether the animal was wild-caught or captive-bred (n=681, 64.5%, Suppl. material 1: table S15). Excepting the pet's diet (n=668, 63.3%), fewer than half of respondents ($\leq 49.5\%$) had been offered information on the pet's traits by the seller. Fewer than a quarter of respondents (n=254, 24.1%) had been offered information about costs of care for the pet, and 233 respondents (22.1%) stated that they were offered no information on the pet. On average, respondents rated the information they had been offered as average (3.1% of respondents rated the information as 'very poor', 5.8% as 'poor', 26.3% as 'average', 20.9% as 'good', and 21.0% as 'very good', while 0.8% of respondents did not take the information). Nonetheless, 545 respondents (51.7%) stated that there was no additional information they wished they had received prior to acquiring their pets. For those respondents who were not satisfied with the information they had received, the largest share stated that they would have valued information on the potential additional costs of owning their pet (n=207, 19.6%). In total, 924 respondents (87.6%) had looked up additional information on their pet or how to care for their pet since acquiring it (Suppl. material 1: table S16). Most frequently, respondents obtained this information from an online search engine (n=753, 71.4%). Respondents were less likely to seek out additional information by contacting hobbyists or breeders (n=271, 25.7%), veterinarians (n=228, 21.6%), or pet stores (n=109, 10.3%).

Best-worst scaling task

Attribute impacts: Negative signs on coefficients in the random parameters logit (RPL) models indicate that the variables fall on the negative side of the reference case, not a negative relationship with the dependent choice variable. For all RPL models, the life expectancy attribute was omitted and used as a reference case (attribute impact or mean utility across all levels=0; Tables 2, 3). For all pets, respondents exhibited preference heterogeneity with respect to pet coloration (statistically significant standard deviation coefficients [β^{SD}]; 1.054 $\leq \beta^{\text{SD}} \leq 1.529$). However, respondents uniformly placed positive value on the color of pets (positive, significant mean coefficients $[\beta^{M}]: 1.134 \le \beta^{M} \le 1.762; |\beta^{M}| > \beta^{SD})$ relative to the reference case (pets' life expectancy), excepting for lizards/chameleons and fish $(\beta^{SD} > |\beta^{M}|)$ for the color attribute impact). Respondents placed positive value on the size of turtles (β^{M} =0.311), salamanders (β^{M} =0.294), and insects/arachnids (β^{M} =0.851) and negative value on the size of fish $(\beta^{M}$ =-0.468) relative to the reference case, although respondents exhibited preference heterogeneity for the size of salamanders ($\beta^{SD}=0.350$). Respondents placed negative value on the behavior of snakes (β^{M} =-0.442), lizards/chameleons (β^{M} =-0.591), turtles (β^{M} =-0.278), tortoises (β^{M} =-0.631), frogs/toads (β^{M} =-0.405) and fish (β^{M} =-0.789) relative to the reference case, and positive value on the behavior of insects/arachnids $(\beta^{M}=0.351)$. We found preference heterogeneity for pet behavior across respondents who selected snakes (β^{SD} =0.408), lizards/chameleons (β^{SD} =0.445), turtles (β^{SD} =0.492), fish (β^{SD} =0.406), and insects/arachnids (β^{SD} =0.724). The relative magnitude of the standard deviation coefficients suggested that a subset of respondents placed higher value on pet life expectancy than behavior for turtles ($\beta^{SD}=0.492$) and insects/arach-

	Snake		Lizard/C	hameleon	Tu	Turtle		Tortoise	
	Mean Coefficient	Standard Deviation Coefficient	Mean Coefficient	Standard Deviation Coefficient	Mean Coefficient	Standard Deviation Coefficient	Mean Coefficient	Standard Deviation Coefficient	
Attribute Impacts									
Color	1.375***	1.146***	1.155***	1.303***	1.504***	1.342***	1.134***	1.054***	
Size	-0.096	0.181	-0.044	0.254	0.311***	0.138	0.209*	0.111	
Life expectancy	0.000		0.000		0.000		0.000		
Behavior	-0.442***	0.408***	-0.591***	0.445***	-0.278**	0.492***	-0.631***	0.246	
Price	-0.779***	0.407**	-1.317***	0.388***	-1.308***	1.078***	-1.085***	0.913***	
Level Scale Values									
Color:									
Neither colorful nor patterned	-1.590		-1.301		-0.850		-0.841		
Colorful, not patterned	0.392***	0.470**	0.250**	0.129	0.380***	0.372	0.308**	0.562**	
Patterned, not colorful	-0.472***	0.193	-0.151	0.108	-0.421***	0.306	-0.140	0.155	
Both colorful and patterned	1.670***	0.961***	1.202***	0.287	0.891***	0.550*	0.672***	0.620**	
Size:									
Small	-0.043		-0.149		1.105		0.074		
Medium	0.778***	0.237	0.743***	0.723***	0.291**	0.859***	0.498***	0.614***	
Large	-0.735***	1.824***	-0.594***	1.599***	-1.395***	1.156***	-0.572***	2.244***	
Life expectancy:									
Short	-1.124		-2.188		-0.444		-0.517		
Average	0.708***	0.400^{**}	1.129***	0.002	0.402***	0.393**	0.602***	0.124	
Long	0.416***	0.686***	1.059***	1.418***	0.041	1.468***	-0.085	1.100***	
Behavior:									
Docile	3.427		3.159		2.663		2.495		
Intermediate	0.098	0.998***	0.060	0.696***	0.233*	0.746***	0.147	0.373	
Aggressive	-3.525***	1.251***	-3.219***	0.816***	-2.896***	0.254	-2.642***	1.550***	
Price:									
Lowest	1.631		1.898		1.775		1.242		
Price 2	0.466***	0.030	0.929***	0.024	0.579***	0.102	0.729***	0.087	
Price 3	-0.581***	0.275**	-0.868***	0.214	-0.570***	0.490***	-0.336*	0.239	
Highest	-1.516***	0.291*	-1.959***	0.148	-1.784***	0.227	-1.635***	0.040	
Log likelihood	-2,191.13		-2,406.05		-1,610.62		-1,423.64		
AIC	4,446.252		4876.095		3,285.243		2,911.283		
BIC	4,702.329		5135.159		3,530.204		3,152.041		

Table 2. Random parameters logit for pet herpetofauna. Estimated coefficients * significant at the 10% level; *** significant at the 5% level; *** significant at the 1% level.

* significant at the 10% level; ** significant at the 5% level; *** significant at the 1% level.

nids (β^{8D} =0.724). Respondents uniformly placed negative value on the purchase price of snakes (β^{M} =-0.779), lizards/chameleons (β^{M} =-1.317), turtles (β^{M} =-1.308), tortoises (β^{M} =-1.085), frogs/toads (β^{M} =-0.888), and fish (β^{M} =-1.864) relative to the reference case, even taking preference heterogeneity into account (0.388≤ β^{8D} ≤1.078).

Level scale values: Respondents preferred colorful lizards/chameleons (β^{M} =0.250 for colorful, not patterned animals; β^{M} =1.202 for colorful, patterned animals), turtles (β^{M} =0.380 for colorful, not patterned animals; β^{M} =0.891 for colorful, patterned animals), frogs/toads (β^{M} =0.412 for colorful, not patterned animals; β^{M} =1.420 for colorful, patterned animals), salamanders (β^{M} =0.358 for colorful, not patterned animals; β^{M} =1.852 for colorful, patterned animals), and fish (β^{M} =0.505 for colorful, not patterned animals; β^{M} =2.004 for colorful, patterned animals) over animals that were not colorful (patterned or not) – even after taking preference heterogeneity into account

	Frog/	/Toad	Salam	ander	Fi	sh	Insect/Arachnid	
	Mean Coefficient	Standard Deviation Coefficient	Mean Coefficient	Standard Deviation Coefficient	Mean Coefficient	Standard Deviation Coefficient	Mean Coefficient	Standard Deviation Coefficient
Attribute Impacts								
Color	1.226***	1.160***	1.174***	1.161***	1.286***	1.529***	1.762***	1.312***
Size	0.158	0.254	0.294**	0.350**	-0.468***	0.203	0.851***	0.016
Life expectancy	0.000		0.000		0.000		0.000	
Behavior	-0.405***	0.322*	-0.103	0.259	-0.789***	0.406***	0.351**	0.724***
Price	-0.888***	0.762***	-0.313**	0.359**	-1.864***	0.894***	0.159	0.816***
Level Scale Values								
Color:								
Neither colorful nor patterned	-1.424		-1.624		-1.730		-1.837	
Colorful, not patterned	0.412***	0.330	0.358**	0.295	0.505***	0.309	0.763***	1.200***
Patterned, not colorful	-0.408**	0.047	-0.586***	0.204	-0.780***	0.220	-0.703***	0.255
Both colorful and patterned	1.420***	0.760***	1.852***	1.156***	2.004***	1.360***	1.776***	1.145***
Size:								
Small	-0.427		-0.613		0.969		-1.355	
Medium	0.443***	0.571**	0.990***	0.365**	0.502***	0.561***	-0.141	0.348^{*}
Large	-0.016	2.143***	-0.377*	1.162***	-1.471***	0.903***	1.495***	0.646***
Life expectancy:								
Short	-1.708		-0.478		-0.693		-2.102	
Average	0.897***	0.170	0.435***	0.347	0.343***	0.224	-0.676***	0.752***
Long	0.811***	1.421***	0.043	1.400***	0.350***	0.838***	2.778***	1.776***
Behavior:								
Docile	2.184		2.595		2.169		2.600	
Intermediate	0.336**	0.046	0.018	0.298	0.317***	0.657***	-0.043	0.069
Aggressive	-2.519***	1.635***	-2.613***	1.254***	-2.486***	0.673***	-2.557***	1.612***
Price:								
Lowest	1.713		1.685		2.584		1.296	
Price 2	0.233	0.265*	0.276	0.188	0.373***	0.152	0.599***	0.079
Price 3	-0.430**	0.356*	-0.475**	0.040	-1.006***	0.271	-0.529**	0.216
Highest	-1.516***	0.148	-1.486***	1.036***	-1.951***	0.867***	-1.367***	0.527***

Table 3. Random parameters logit for pet amphibians, fish, and insects/arachnids. Estimated coefficients * significant at the 10% level; ** significant at the 5% level; *** significant at the 1% level.

2,777.546 significant at the 10% level; " significant at the 5% level; "" significant at the 1% level.

-1.238.81

2,541.627

AIC

BIC

Log likelihood

 $(\beta^{SD}=0.760$ for colorful, patterned frogs/toads; $\beta^{SD}=1.156$ for colorful, patterned salamanders; β^{SD} =1.360 for colorful, patterned fish). Respondents most preferred colorful and patterned animals for each of these pets. Respondents also preferred colorful and patterned snakes (β^{M} =1.670, β^{SD} =0.961) and insects/arachnids (β^{M} =1.776, β^{SD} =1.145) relative to animals that were not colorful (whether patterned or not). Respondents demonstrated preference heterogeneity for snakes ($\beta^{M}=0.392$, $\beta^{SD}=0.470$) and insects/arachnids ($\beta^{M}=0.763$, $\beta^{SD}=1.200$) that were colorful but not patterned, although animals with coloration were still preferred to animals that were not colorful. Respondents most preferred colorful and patterned tortoises (β^{M} =0.672). On average, respondents preferred colorful (not patterned) tortoises (β^{M} =0.308) to animals that were not colorful or patterned. However, preference heterogeneity suggested that some respondents preferred patterned, not colorful tortoises to colorful tortoises without a pattern (β^{SD} =0.562).

-978.87

2,021.740

2.249.099

-1.925.29

3914.582

4166.782

-1,050.61

2,165.218

2,398.054

Respondents preferred medium-sized snakes ($\beta^{M}=0.778$), lizards/chameleons $(\beta^{M}=0.743)$, and salamanders $(\beta^{M}=0.990)$, even after taking preference heterogeneity into account (β^{SD} =0.723 for medium-sized lizards/chameleons; β^{SD} =0.365 for medium-sized salamanders). However, a subset of respondents preferred large snakes (β^{SD} =1.824), lizards/chameleons (β^{SD} =1.599), and salamanders (β^{SD} =1.162). On average, respondents preferred medium-sized tortoises (β^{M} =0.498) and frogs/toads $(\beta^{M}=0.443)$, although preference heterogeneity indicated that respondents were not uniform in these preferences (β^{SD} =0.614 for medium-sized tortoises; β^{SD} =0.571 for medium-sized frogs/toads). Respondents appeared to prefer small turtles and fish to medium-sized animals (β^{M} =0.291 for medium-sized turtles; β^{M} =0.502 for mediumsized fish), although they were heterogeneous in these preferences (β^{SD} =0.859 for medium-sized turtles; β^{SD} =0.561 for medium-sized fish). Preference heterogeneity indicated that a subset of respondents preferred large tortoises (β^{SD} =2.244) and frogs/toads $(\beta^{SD}=2.143)$. Even after accounting for preference heterogeneity, respondents did not prefer large turtles (β^{M} =-1.395, β^{SD} =1.156) or fish (β^{M} =-1.471, β^{SD} =0.903), but did prefer large insects/arachnids (β^{M} =1.495, β^{SD} =0.646).

On average, respondents most preferred snakes (β^{M} =0.708), lizards/chameleons (β^{M} =1.129), turtles (β^{M} =0.402), tortoises (β^{M} =0.602), frogs/toads (β^{M} =0.897), and salamanders (β^{M} =0.435) with a medium life expectancy, although respondents demonstrated some preference heterogeneity with respect to medium life expectancy for snakes (β^{SD} =0.400) and turtles (β^{SD} =0.393). On average, respondents most preferred fish (β^{M} =0.350) and insects/arachnids (β^{M} =2.778) with long life expectancies. Respondents were heterogeneous in their preferences for all pet types with respect to long life expectancy (0.686≤ β^{SD} ≤1.776).

Even after accounting for preference heterogeneity ($0.673 \le \beta^{\text{SD}} \le 1.635$), respondents disliked aggressive animals ($-3.525 \le \beta^{\text{M}} \le -2.486$) relative to docile animals. We found preference heterogeneity with regards to intermediate behavior in pet snakes ($\beta^{\text{SD}}=0.998$), lizards/chameleons ($\beta^{\text{SD}}=0.696$), turtles ($\beta^{\text{SD}}=0.746$), and fish ($\beta^{\text{SD}}=0.657$). The level scale values for the price of pets followed the theoretically expected pattern of decreasing preference ($\beta^{\text{M}}<0$) for higher prices. Although there was some evidence of preference heterogeneity, lower prices were always preferred.

Decision to purchase an exotic pet

Respondents were more likely to agree that they would purchase turtles (β =0.937), tortoises (β =0.718), frogs/toads (β =0.826), salamanders (β =0.757), fish (β =0.452), and insects/arachnids (β =1.090) if they were both colorful and patterned (Table 4). Respondents were less likely to purchase species that were colorful but not patterned (lizard/ chameleon: β =-0.387, turtle: β =-0.462, fish: β =-0.371) or patterned and not colorful (tortoise: β =-0.571, salamander: β =-0.503, insect/arachnid: β =-0.537). Respondents were less likely to purchase snakes (β =-0.336), lizards/chameleons (β =-0.287), and turtles (β =-0.676) that would grow to a large adult size, but were more likely to buy insects/ arachnids (β =0.380) that would grow to a large adult size. Respondents were more likely to purchase snakes (β =0.378), lizards/chameleons (β =0.510), tortoises (β =0.495), frogs/

	Snake	Lizard/ Chameleon	Turtle	Tortoise	Frog/Toad	Salamander	Fish	Insect/ Arachnid
Constant	2.291	2.330**	2.390	0.610	4.489***	-0.506	-1.088***	-0.317
Color:								
Neither colorful nor patterned	-0.320	0.064	-0.189	-0.264	-0.553	-0.354	-0.303	-0.621
Colorful, not patterned	0.158	-0.387***	-0.462**	0.116	0.062	0.099	-0.371**	0.067
Patterned, not colorful	-0.171	0.018	-0.286	-0.571***	-0.335*	-0.503**	0.222	-0.537**
Both colorful and patterned	0.332*	0.306*	0.937***	0.718***	0.826***	0.757**	0.452**	1.090***
Size:								
Small	0.209	0.126	0.545	0.002	0.086	0.005	0.235	-0.438
Medium	0.127	0.161	0.131	0.005	0.076	0.265	-0.041	0.058
Large	-0.336**	-0.287**	-0.676***	-0.007	-0.163	-0.270	-0.195	0.380**
Life expectancy:								
Short	-0.399	-0.565	0.168	0.064	-0.337	-0.297	-0.379	-0.879
Average	0.378***	0.510***	0.299^{*}	0.495***	0.339**	0.682***	0.390***	0.160
Long	0.021	0.056	-0.467***	-0.559***	-0.002	-0.385*	-0.011	0.719***
Behavior:								
Docile	1.283	1.263	1.287	1.090	0.606	1.220	0.749	0.776
Intermediate	0.178	-0.042	0.118	0.145	-0.001	0.106	-0.043	0.006
Aggressive	-1.460***	-1.221***	-1.405***	-1.235***	-0.605***	-1.326***	-0.706***	-0.782***
Price	-0.004***	-0.005***	-0.006***	-0.005***	-0.018***	-0.014***	-0.031***	-0.029***
Influence of other traits on decision to	purchase ex	otic pets:						
Captive-bed				-0.366*				
Native to area		0.422***						
Rare					-0.491***			0.390^{*}
Expensive diet	0.339	0.428***	0.389^{*}		0.453**	0.489^{*}		
Unusual shape			0.461*					0.676**
Pre-historic appearance					0.606***	-0.594*	0.304*	
Appearance changes with age	0.389^{*}					0.598**		
Breeds easily			0.785***	0.561**		0.390^{*}		
Cost of previous pet (same taxa)	0.009***	0.004**		0.006**	0.019**	0.021**	0.029*	
Current pets:								
Fish				0.699^{*}				
Insect				1.603**		1.450***		
Reptile	-1.128				1.693***			
Rodent			1.279***			0.716		-0.751
Type of housing:								
Apartment/ condominium	0.775^{*}		1.038**				1.238**	
Trailer/mobile home							1.039**	
Demographics:								
Female				-0.893*	-0.792*			
Age		-0.024**		-0.024*	-0.036**	-0.038*		
Education	-0.208**	-0.148**	-0.256**		-0.278***			
Income					-0.007	0.007	-0.005	
Individuals < 18 years old living in household						0.902**		
Log likelihood	-520.063	-555.713	-342.564	-312.343	-285.488	-203.440	-438.222	-274.446
AIC	1076.469	1145.426	721.128	662.685	613.682	451.101	910.714	578.955
BIC	1161.583	1232.127	804.995	748.717	701.217	537.395	988.882	638.881
Ν	184	202	130	114	98	75	163	89

Table 4. Logistic regression of respondents' stated decision to purchase exotic pets. Estimated coefficients * significant at the 10% level; *** significant at the 5% level; *** significant at the 1% level.

* significant at the 10% level; ** significant at the 5% level; *** significant at the 1% level.

toads (β =0.339), salamanders (β =0.682), and fish (β =0.390) with an average life expectancy. They were less likely to purchase turtles (β =-0.467) and tortoises (β =-0.559) with long life expectancies, but more likely to purchase insects/arachnids (β =0.719) with

long life expectancies. Across all taxa, respondents were less likely to purchase aggressive animals as pets (-1.460 $\leq\beta\leq$ -0.605). The likelihood that respondents would purchase pets decreased as the price of the pet increased (-0.031 $\leq\beta\leq$ -0.004). However, the likelihood that respondents would purchase snakes (β =0.009), lizards/chameleons (β =0.004), tortoises (β =0.006), frogs/toads (β =0.019), and salamanders (β =0.021) was positively correlated with the price they had paid for their previous pet from the same taxa.

Respondents who stated that an animal being native to the area would positively influence their decision to purchase a pet were more likely to purchase lizards/chameleons (β =0.422). Respondents who stated that a pet being rare would positively influence their purchase decision were less likely to purchase frogs/toads (β =-0.491). Respondents who stated that a pet having an expensive diet would negatively influence their purchase decision were less likely to purchase lizards/chameleons (β =0.428) and frogs/ toads (β =0.453). Respondents who preferred a pet with an unusual shape were more likely to purchase insects/arachnids (β =0.676). Respondents who preferred animals with a pre-historic appearance were more likely to purchase frogs/toads (β =0.606), whereas respondents who preferred animals whose appearance changes with age were more likely to purchase salamanders (β =0.598). Respondents who preferred pets that breed easily were more likely to purchase turtles (β =0.785) and tortoises (β =0.561).

Respondents who currently own insects or arachnids were more likely to purchase tortoises (β =1.603) or salamanders (β =1.450), whereas respondents who currently own reptiles were more likely to purchase frogs/toads (β =1.693). Respondents who currently own rodents were more likely to purchase turtles (β =1.279). The number of exotic pets that respondents currently own and the number of exotic pets that they owned as children did not influence their stated decision to purchase another exotic pet.

Older respondents were less likely to purchase lizards/chameleons (β =-0.024) and frogs/toads (β =-0.036). More educated respondents were less likely to purchase snakes (β =-0.208), lizards/chameleons (β =-0.148), turtles (β =-0.256), and frogs/toads (β =-0.278). Respondents with children (<18 years old) living in the house were more likely to purchase salamanders (β =0.902). Respondents who lived in apartments or condominiums were more likely to purchase turtles (β =1.038) and fish (β =1.238). Respondents who lived in trailers or mobile homes were also more likely to purchase fish (β =1.039).

Discussion

The exotic pet trade poses substantial conservation, human safety, and animal welfare risks when people purchase exotic pets that they are unable or unwilling to care for, and owners subsequently release these animals. Using surveys of exotic pet owners, we found some evidence that demographics (gender, age, education) influence people's decision to acquire an exotic pet. However, pet traits were far more important determinants of respondents' stated decision to purchase a pet. We confirmed findings from studies of species in the exotic pet trade that pet owners prefer animals that are colorful, patterned, docile, and easy to handle (van Wilgen et al. 2010; Vall-Ilosera and Cassey 2017; Hausmann et al. 2023). We also found some evidence that exotic pet owners prefer species with distinctive morphological features (Burghardt 2017; Sung and Fong 2018; Harrington et al. 2022; Hausmann et al. 2023), specifically a prehistoric appearance or an appearance that changes with age. Our results suggest that pet owners are likely to be attracted by colorful, patterned animals with distinctive morphological features that are of medium size, especially if these animals are inexpensive to purchase. However, if pet owners subsequently discover that these species have undesirable traits (e.g., they are aggressive, have long lifespans, have expensive dietary needs) then pet owners may regret the purchase of the animal and may release pets if they cannot rehome them.

Animal behavior was a trait that clearly influenced the desirability of a pet. Pet owners preferred not to purchase animals that are aggressive or dangerous (e.g., animals that engage in defensive behaviors such as biting and scratching), which suggests that pet owners who are ill- informed about the behaviors and handling requirements of the pets they have purchased may release these animals (Warwick et al. 2018; Siriwat et al. 2019). It is thus concerning that only half of respondents (49.5%) had been given information about the behavior of exotic pets at the time of purchase. Exotic pet owners should be informed prior to purchase if pets are likely to exhibit aggressive or stressed behaviors, especially when exposed to humans and multispecies assemblages (Bush et al. 2014). For example, Tokay geckos (Gekko gecko) are prevalent in the pet trade because their coloration makes them attractive to pet owners who may be unaware that they are also aggressive, territorial, and have strong bites (O'Shea and Kaiser 2020), which makes them challenging to handle and keep. Tokay geckos are an excellent example of an animal that novice, ill-informed pet owners may regret purchasing, especially if they subsequently learn that Tokay geckos imported into the US pet trade carry antibiotic-resistant bacteria and pose a public health concern (Casey et al. 2015). Selective breeding practices that generate animals with unusual coloration or morphological traits, but also increase the risk of inbreeding depression and disease, further increase the risk that ill-informed pet owners who are attracted by animals' appearance will release their pets, especially if owners are unable or unwilling to pay necessary veterinary expenses (Pasmans et al. 2017). Exotic pet owners should thus be informed about potential genetic or disease risks associated with exotic pets, to reduce the likelihood that they will purchase these pets if they are unwilling to provide appropriate veterinary care (Moorhouse et al. 2017). This is particularly important, since only 24.1% of respondents were provided with information on additional costs of care (i.e., equipment and veterinary costs) for exotic pets.

Consistent with studies on invasive species that have been introduced through the pet trade, we found that exotic pet owners preferred species that do not reach a large adult size and that have an average life expectancy, unless they are purchasing insects or arachnids (van Wilgen et al. 2010; Toomes et al. 2022). Although most pet owners (56.1%) tended to be neutral about purchasing a pet that breeds easily, 17.4% of pet owners disliked this trait. Our findings suggest that if pet owners are uninformed or misinformed about the adult size, longevity, and fecundity of the species they have purchased they may release their pets. This is important because amphibians and reptiles traded as exotic pets tend to have relatively high reproductive rates and long reproductive lifespans (Toomes et al. 2022; Street et al. 2023). Yet, less than half of respondents had been provided with information on the adult size (47.0%) and life span (44.3%) of their current exotic pets at the time of purchase. We also found that pet owners prefer lower-priced exotic pets, which would reinforce the supply of lower-priced, abundant species. Thirty-six percent of respondents who owned the type of exotic pet they selected in our survey had obtained the pet for free or had purchased a cheap pet. Our findings support speculation by ecologists that the introduction bias in exotic pets towards highly fecund species is attributable to the lower cost and higher income from breeding these prolific species, and deliberate release by pet owners who are unable or unwilling to care for multiple offspring (Street et al. 2023).

Interestingly, the likelihood that respondents would purchase an exotic pet was positively correlated with the price they paid for a pet in the same taxa. This suggests that pet owners who have purchased more valuable pets are more likely to acquire another pet of the same taxa. Typically, rare, scarce, or illegally traded species are sold at higher prices (Morgan and Chng 2018; Altherr and Lameter 2020), which suggests that if respondents have purchased scarce species then they may be more likely to acquire additional exotic pets for their collection. However, although 45.1% of respondents valued rarity in exotic pets, respondents' interest in rarity did not increase the likelihood that they would purchase any of the exotic pets included in our survey. Respondents indicated that they were more concerned about whether pets were captive bred (69.1% of respondents viewed this trait positively) or wild-caught (71.7% of respondents viewed this trait negatively). However, we also found no correlation between respondents' preference for captive-bred species and their choice of whether to purchase a pet. Admittedly, we did not include rarity, whether pets were wild caught or captive bred, or the legality of owning species in our choice experiments because including these attributes would have greatly increased the cognitive burden of the survey. Nonetheless, our results suggest that exotic pet owners prefer that the pet trade does not impact wild populations. Importantly, only 43.4% of respondents were given information about whether their current exotic pets were captive bred. Pet owners should be made aware of how pets are sourced, even if statements about conservation do not directly influence pet owners' intention to purchase an exotic pet (Moorhouse et al. 2017).

Given that respondents were provided with incomplete or no information by sellers about pets' traits, diet, and cost of care when purchasing exotic pets, it is concerning that less than 10% of respondents had been provided with information on how to find a new home for their pet if they could no longer care for it. Incomplete information provided to respondents when they acquired their exotic pets reinforces concerns about pet owners' lack of understanding of the traits and husbandry needs of these animals. Regardless of their level of experience, exotic pet owners should be provided with baseline information on the behavior, adult size, lifespan, fecundity, diet, and expected veterinary and husbandry costs for exotic pets prior to purchase. Apart from providing clear information about pets' husbandry needs, pet owners should also be provided with clear information on how much social interaction and physical activity pets need (Bush et al. 2014; Burghardt 2017; Warwick et al. 2018). Most respondents (87.6%) looked up additional information on their pets and how to care for them after they had purchased these animals. It is notable that respondents had predominantly relied on online searches and forums, rather than contacting pet stores or pet breeders and hobbyists. This may suggest that respondents did not trust sellers to provide additional, necessary information on exotic pets, especially since respondents rated the information they had received as average. However, researchers have cautioned that poor animal husbandry is compounded by inaccurate, incomplete, and poor-quality information from online searches (Pasmans et al. 2017; Warwick et al. 2018). This reinforces our suggestion that baseline information or improved education and outreach for exotic pet owners is required to prevent them acquiring and releasing undesirable pets. Pasmans et al. (2017) suggested that pet keeper education could be implemented, which would include a system of certification before individuals may acquire exotic pets. Zoological societies could assist in the design and implementation of pet keeper education programs.

Conclusion

Our study provides insights into which species are likely to be attractive to uninformed pet owners, but which will ultimately become undesirable because of behavioral traits, size, or longevity. Our results suggest that certification systems that provide critical information on exotic pets' behaviors, adult size, longevity, fecundity, and husbandry needs should be implemented to prevent pet owners acquiring animals that they will abandon (Hausmann et al. 2023). Importantly, these certification systems must be adopted by both commercial pet sellers and private breeders (Hausmann et al. 2023). Actively engaging the exotic pet trade in the design and implementation of this certification system is important to ensure widespread adoption. The alternative is to ban trade in species that pose substantial risks. However, we caution that bans can generate illegal trade (Rivalan et al. 2007), and are unlikely to be effective if species are already in the pet trade (Patoka et al. 2018). People may release pets if they are no longer legal to own (Patoka et al. 2018). Rather, our results can be used to identify which species are likely to be purchased and released (e.g., colorful and aggressive Tokay geckos), in order to help inform approved lists of pet species that are unlikely to pose invasion and disease risks.

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Supplementary material I

Additional results for reference by readers and the reviewers

Authors: Elizabeth F. Pienaar, Diane J. E. Sturgeon Data type: docx

- Explanation note: table S1. Choice sets for each of the survey versions based on the D optimal design. table S2. Effects coding for the attribute l evels. table S3. Demographic characteristics of survey respondents (n = 1,055). table S4. Respondent's pet ownership, and how they acquired their current exotic pets (n = 1,055). table S5. Respondents' choice of which exotic pet they would purchase next, and the number of respondents who already owned this type of pet (n = 1,055). table S6. Respondent's stated preferences for the appearance, size, longevity, and behavior of pet snakes, and the price they paid for their previous pet snake (n = 184). table S7. Respondent's stated preferences for the appearance, size, longevity, and behavior of pet lizards/chameleons, and the price they paid for their previous pet lizard/chameleon (n = 202). table S8. Respondent's stated preferences for the appearance, size, longevity, and behavior of pet turtles, and the price they paid for their previous pet turtle (n = 130). table S9. Respondent's stated preferences for the appearance, size, longevity, and behavior of pet tortoises, and the price they paid for their previous pet tortoise (n = 114). table S10. Respondent's stated preferences for the appearance, size, longevity, and behavior of pet frogs/toads, and the price they paid for their previous pet frog/toad (n = 98). table S11. Respondent's stated preferences for the appearance, size, longevity, and behavior of pet salamanders, and the price they paid for their previous pet salamander (n = 75). table S12. Respondent's stated preferences for the appearance, size, longevity, and behavior of pet insects/arachnids, and the price they paid for their previous pet insect/arachnid (n = 89). table S13. Respondent's stated preferences for the appearance, size, longevity, and behavior of pet fish, and the price they paid for their previous pet fish (n = 163). table S14. Responses to the question: 'Please indicate how the following traits would influence your decision to acquire [an exotic] pet.' table S15. Information that respondents obtained prior to acquiring an exotic pet (n = 1,055). table S16. Responses to the question: 'Since acquiring your [exotic] pet(s), have you looked up any additional information on the species or how to care for the animal?' (n = 1,055).
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RESEARCH ARTICLE



Clonal alien plants in the mountains spread upward more extensively and faster than non-clonal

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Abstract

Alien species are colonizing mountain ecosystems and increasing their elevation ranges in response to ongoing climate change and anthropogenic disturbances, posing increasing threats to native species. However, how quickly alien species spread upward and what drives their invasion remains insufficiently understood. Here, using 26,952 occurrence records of 58 alien plant species collected over two centuries in the Czech Republic, we explored the elevation range and invasion speed of each alien species and the underlying factors driving these variables. We collected species traits relevant for invasion (e.g., clonality, flowering time, life span, invasion status, height, mycorrhizal type, native range, naturalized range, monoploid genome size, and Ellenberg-type indicator values for light, temperature, and nitrogen), human-associated factors (e.g., introduction pathways and the sum of economic use types), and minimum residence time. We explored the relationships between these factors and species' elevation range and invasion speed using phylogenetic regressions. Our results showed that 58 alien species have been expanding upward along mountain elevations in the Czech Republic over the past two centuries. A stronger effect of species' traits than human-associated factors has been revealed, e.g., clonality was a key trait supporting the invasion of alien species into the mountains, while human-associated factors showed no effect. Our findings highlight that the characteristics associated with rapid reproduction and spread are crucial for alien species' invasion into montane regions. Identifying key drivers of this process is important for predicting the spatiotemporal dynamics of alien species in high-altitude ecosystems and thus employing apposite measures to reduce the threat to native plant species.

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Keywords

Alien plant species, clonal trait, elevation range, human activities, invasion speed, mountain invasions

Introduction

Mountains are of crucial importance for biodiversity conservation (Rangel et al. 2018; Rahbek et al. 2019). Conventionally, these regions were considered to be relatively unharmed by alien species (McDougall et al. 2011; Alexander et al. 2016). However, with intensifying climate change and human pressure, alien species are now colonizing mountains and increasing their elevation range upward, posing a significant threat to native species (Pauchard et al. 2009; McDougall et al. 2011; Pyšek et al. 2011; Alexander et al. 2016; Dainese et al. 2017; Koide et al. 2017). Consequently, a comprehensive understanding of the increasing trends in alien species distribution and the speed at which alien species are establishing at different elevations becomes imperative for effective conservation strategies in mountain regions (Iseli et al. 2023).

In mountainous areas, the influx of alien species typically commences at lower elevations before gradually spreading upward over time (Alexander et al. 2011; Pyšek et al. 2011; Marini et al. 2013). The directional ecological filtering hypothesis, a phenomenon of a decline in alien species richness with increasing elevation due to a progressive species loss, is often invoked to elucidate the distribution of alien flora along altitudinal gradients worldwide (Alexander et al. 2011). In addition, the upward expansion of alien species is associated with minimum residence time (Pyšek et al. 2011). Recent investigations have underscored its significant role in shaping the potential elevation range of alien species (Pyšek et al. 2009a, 2011, 2015; Alexander et al. 2011), because longer residence time allows for more extensive dispersal or can result in genetic adaptation (Becker et al. 2005; Haider et al. 2010; Pyšek et al. 2011).

In addition to the minimum residence time, various characteristics of alien species, e.g., introduction pathways (Alexander et al. 2011), economic utility (Balestri et al. 2018, van Kleunen et al. 2020), proximity to road networks (Dainese et al. 2017; Skálová et al. 2017), as well as their inherent traits (e.g., dispersal abilities, climatic adaptability, and genetic adaptation) (Dietz and Edwards 2006; Alexander 2010) have been recognized as key drivers of species' range expansion. Theoretically, deliberately introduced and economically valuable plants (Pergl et al. 2017; Balestri et al. 2018; van Kleunen et al. 2018) often exhibit larger propagule pressure in terms of both propagule quantity and frequency of introduction events, consequently enhancing the likelihood of successful establishment. In addition, species with rapid growth, short generations, and strong competitive ability are also more likely to become successful invaders (van Kleunen et al. 2010). For instance, plant height was found to be positively related to species' range and probability of invasion (Pyšek et al. 2009a, 2015; Divíšek et al. 2018); optimal flowering time, functioning as a reproductive trait, ensures seed fertility (Celesti-Grapow et al. 2003; Godoy et al. 2009), and species with earlier flowering can avoid competition and expedite the life cycle, leading to prompt reproduction. On a macroecological scale, clonality was reported to play a positive role in the invasion success and distribution of alien species (Pyšek 1997; Liu et al. 2006; Wang et al. 2024) and species primarily engaging in asexual reproduction also tend to exhibit broader ranges (Cosendai et al. 2013). Moreover, studies have shed light on how the geographic expansion of alien plants benefits from the presence of mycorrhizal associations, which form specialized structures aiding vegetative dispersal (Menzel et al. 2017; Correia et al. 2018; Pyšek et al. 2019).

Karyological characteristics were recently suggested as an important trait underpinning plant invasion success, and species with small genomes proved to be at an advantage in the process of alien plants' naturalization (Kubešová et al. 2010; Suda et al. 2015; Lopes et al. 2021; Pyšek et al. 2023) as the "large genome constraints" proposed (Knight et al. 2005). However, the advantage of possessing a small genome does not translate into the more advanced stage of the invasion process characterized by the rapid spread; here, the opposite is true as species with relatively large genomes are more likely to be successful invaders (Lopes et al. 2021; Carta et al. 2022; Pyšek et al. 2023).

While the magnitude of a species' native range significantly influences its preadaptation to the introduced environment, and a broader native range generally fosters greater readiness for the establishment and thriving within the novel environment (Pyšek et al. 2015; Guo et al. 2019; Fristoe et al. 2023), the inclusion of species-specific environmental preferences, e.g., optimal values or ranges for existence, development, growth, and reproduction, can offer a more nuanced understanding of species' spatial expansion patterns, particularly along environmental gradients like elevation (Di Biase et al. 2023). Despite these insights, scant knowledge exists regarding the key driving forces behind changes in the elevation range or vertical spread of alien plants (Dainese et al. 2017; Auld et al. 2022), especially when considering the relatively low propagule pressure at high elevations (Alexander et al. 2011).

In this study, we aim to estimate the speed at which alien species increase their elevation range and identify the drivers that underlie such invasions. Specifically, we calculated the elevation range and invasion speed of 58 alien species for which such data exist in the regional dataset for the Czech Republic. We also used various characteristics of these plants, including both species' inherent traits (e.g., clonality, flowering time, monoploid genome size, and Ellenberg-type indicator values reflecting ecological demands) and human-associated factors (e.g., introduction pathways and economic use types). We then used phylogenetic regressions to identify the key drivers of elevation range and invasion speed. We hypothesized that: 1) alien species are expanding upward along mountain elevations in the Czech Republic; 2) both species' inherent traits and human-associated factors contribute to the upward of alien species, and play distinct roles in determining the elevational range (and changes) and invasion speed.

Materials and methods

Species distribution records

Species data were obtained from an existing dataset (Williamson et al. 2005; Pyšek et al. 2011), originally including 65 alien species introduced to the Czech Republic after 1500 AD. The dataset compiled the elevation of alien species in the mountains since the year 1738, with 28,288 occurrence records in total. Based on this data, we calculated each species' elevation range (Elevation $_{max}$ – Elevation $_{min}$) (m), invasion speed ((Elevation $_{max}$ – Elevation $_{max}$ – Elevati

From the complete dataset, we removed abnormal values of invasion speed, e.g., values for species with no elevation record in the year of first introduction; values for species for which there was less than 40 years since their first introduction to the maximum elevation year, due to the possibility of inadequate sampling; and negative values. We used the '*WorldFlora*' R package (Kindt 2020) to standardize all taxon names before matching species trait data further.

Species traits

We obtained data on the clonality (modular species with potential vegetative reproduction or unitary species without this potential), flowering time (as the first month of flowering), life span (annual, perennial, or both), and invasion status (casual, naturalized, invasive) of the 58 alien species from existing datasets (Williamson et al. 2005; Pyšek et al. 2022). The categories of invasion status are based on a well-defined framework (Blackburn et al. 2011): alien species that do not form self-sustaining populations in the introduced regions are casuals; alien species that form self-sustaining populations for several life cycles in the introduced region without human intervention are naturalized; and alien species that can maintain self-replacing populations at considerable distances from their parents and/or sites of introduction, producing reproductive offspring and having the potential to spread over long distances are invasive.

The height of each species was extracted from the LEDA database (https://uol. de/en/landeco/research/leda) (Kleyer et al. 2008) using an average method. We then compiled Ellenberg-type indicator values for light, temperature, and nitrogen for each species, which are expert-based rankings of plant species according to their ecological optima on main environmental gradients (recently updated and harmonized: Tichý et al. 2022). Smaller Ellenberg-type indicator values represent species that are better adapted to the lower levels of a given factor. Mycorrhizal types were obtained from the FungalRoot v.2.0 database (Soudzilovskaia et al. 2022), divided into arbuscular mycorrhizal (AM) and non-mycorrhizal (NM) plant species.

Native range data for alien species were extracted from the POWO (Plants of the World Online; https://powo.science.kew.org/) database, and naturalized range

data were obtained from the GloNAF (Global Naturalized Alien Flora) database (van Kleunen et al. 2015, 2019; Pyšek et al. 2017). These two range-related data were expressed as the number of the Taxonomic Database Working Group (TDWG) level 3 regions (level 3 corresponds to "Botanical Countries") in which species are recorded as native or naturalized, respectively (Brummitt, 2001). Monoploid genome sizes (i.e., DNA content in a single chromosome set, *sensu* Greilhuber et al. (2005)) for the species were filtered from the Plant DNA C-values (https://cvalues.science.kew.org/) (Pellicer and Leitch 2020).

Human-associated factors

Information on the introduction pathway (deliberate or accidental introduction) of each alien species to the Czech Republic was taken from Pyšek et al. (2012). Then, we collated the economic use data of each species from the WCUP (World Checklist of Useful Plant Species; Diazgranados et al. 2020). The WCUP list provides information on 10 distinct economic uses, such as medicines and materials. We calculated the overall sum of economic use types for each species and set the economic use of species not included in the list as zero.

Phylogenetic tree

To consider potential phylogenetic relatedness across the species, a phylogenetic tree was created for the 58 species using the '*V.PhyloMaker*' package based on the default setting (Jin and Qian 2019). This phylogenetic tree was used to impute the missing traits values and subsequent statistical analyses.

Imputation of missing trait values

Given the presence of missing data for certain species, we undertook gap-filling processes as follows. For the 20 species lacking monoploid genome size, imputation was carried out utilizing the full dataset of 12,273 species (Pellicer and Leitch 2020) via the R package '*Rphylopars*' (Goolsby et al. 2017). The 11 species lacking Ellenberg-type indicator values and four species without mycorrhizal types were supplemented by data from the same genus, given the strong phylogenetic conservatism for these indicators (Prinzing et al. 2001). Finally, a list of 58 alien plant species with 26,952 occurrence records and complete values was obtained, and 45 species for the invasion speed.

Statistical analyses

All data analyses and visualizations were performed in R v4.2.1 (R Core Team 2023). First, Pearson correlation analysis was performed with all the continuous variables (i.e., invasion speed, flowering time, plant height, Ellenberg indicator values for light,

temperature and nutrients, native range size, naturalized range size, the sum of economic uses, monoploid genome size, and minimum residence time), which showed no strong collinearity among the variables (Appendix 1: Fig. A1; r < 0.7). Plant height, native range size, naturalized range size, and monoploid genome size were log-transformed to approximate normal distribution. To examine whether the elevation range and invasion speed varied across the categories of clonality, invasion status, life span, mycorrhizal types, or introduction pathways, phylogenetic ANOVA models were used with function '*phylANOVA*' in R package '*phytools*' (Garland et al. 1993; Revell 2012). Phylogeny is considered in the analysis because species may not represent statistically independent data points, thus avoiding unreliable model estimates (Garland et al. 1993). *P*-values were determined by Brownian motion model simulation phylogeny, which was run 10,000 times. To explore the combined effects of continuous variables on elevation range and invasion speed of alien species, phylogenetic multiple linear regression models were employed (Si et al. 2022). Models were run separately for clonal and non-clonal species and the variables selection was conducted using the 'phylostep' function in the 'Phylolm' package (Tung Ho and Ané 2014). All numerical variables were standardized to gain standardized coefficients so that comparisons between and within models could be possible (Schielzeth 2010). Finally, according to the results of the 'phylostep', several predictors were identified as key drivers for both clonal and non-clonal alien species, which were used in regressions on data of clonal species, nonclonal species, and all species.

Results

The elevation ranges of the 58 alien species varied from 132 m to 1095 m (Fig. 1). *Heracleum mantegazzianum* had the largest elevation range (1095 m) and the highest upper elevation limit, peaking at 1294 m a.s.l. In contrast, *Panicum dichotomiflorum* had the smallest elevation range (132 m) and the lowest upper elevation limit with a peak of 271 m a.s.l. As for the elevational invasion speed of the 45 alien plants analyzed, it was fastest for *Mimulus guttatus* with 8.4 m/year, whereas for *Ambrosia artemisiifolia* it was the slowest (0.5 m/year) (Fig. 1). Generally, species with large elevation ranges also had high invasion speeds (Appendix 1: Fig. A1).

Both elevation range and invasion speed differed with regard to clonality (Fig. 2a, c; P < 0.01). Specifically, clonal species had significantly larger elevation ranges (average values of clonal vs. non-clonal species = 709 m vs. 500 m, P = 0.001) and higher invasion speeds (5.4 vs. 3.7 m/year, P = 0.004) than non-clonal species (Fig. 2a, c). Casual species had the smallest elevation ranges compared to both naturalized and invasive species (357 m, 613 m, and 688 m on average, respectively, P < 0.05), which were not significantly different from each other (Fig. 2b; P > 0.05). Likewise, no significant differences were observed in invasion speed among the three categories of invasion status (Fig. 2d). Moreover, neither elevation range nor invasion speed



Figure 1. Phylogenetic tree of the examined 58 alien plant species in the Czech Republic, with their elevation ranges (m) and invasion speeds (available for 45 species, m/year) aligned.

differed among different life spans, mycorrhizal types, and introduction pathways (Appendix 1: Fig. A2).

Phylogenetic regressions revealed different factors driving the elevation range and invasion speed for clonal and non-clonal species (Figs 3, 4). Specifically, for both clonal and non-clonal species, species' invasion speed and minimum residence time showed positive effects on elevation range, whereas Ellenberg indicator values for temperature and the time of flowering showed negative effects, with species demanding a higher temperature and those that start flowering early having small elevation ranges (Figs 3a, 4). As for elevational invasion speed, native range size was the only predictor showing a negative effect on the invasion speed of non-clonal species (Fig. 3b). For clonal species, the invasion speed was negatively associated with Ellenberg indicator values for temperature, and with minimum residence time, and positively to naturalized range size (Fig. 3b).



Figure 2. Violin plots showing the elevation ranges and invasion speeds of the examined alien species, categorized based on their clonality and invasion status. Note that the invasion speed was only available for 45 species. For the boxplots inside each violin plot, the horizontal line, red dot, and box, respectively, represent the median, the mean, and the interquartile range. *P*-values were obtained from phylogenetic ANOVAs and showed in the upper part of the violin plot if significant (P < 0.05).

Discussion

Using more than 26,000 historical occurrence records of 58 alien plant species introduced to the Czech Republic after 1738, we found a substantial upward shift along the elevations for the majority of the species analyzed (Fig. 1). The speed of spread to the higher elevation ranged from 0.5 to 8.4 m/year, with species capable of reaching the highest elevation also exhibiting the fastest invasion speed (Fig. 1). Our findings are in line with a recent study showing that alien plant species were expanding their upper elevation limits in 10 out of the 11 surveyed mountains across five continents (Iseli et al. 2023). However, no upward expansion of alien plants was observed in several regional studies, e.g., 67% of naturalized invasive plant species in California showed no mean elevation shift over the past century (Wolf et al. 2016). On the island of Hawaii, both the upper and lower elevation limit of 20 alien species are moving up, but elevation ranges did not change significantly over 40 years (Koide et al. 2017). In Europe, 10% of alien species exhibited a potential downslope shift (Dainese et al. 2017). Although these contrasting findings may be due to different climates and land-use


Figure 3. Standardized estimates and associated 95% confidence intervals obtained from phylogenetic regressions for each of the elevation range and invasion speed models. Models were run separately for clonal (orange) and non-clonal (blue) species and the variables were selected via the '*phylostep*' function in the R package '*Phylolm*'. In particular, we added invasion speed as an additional variable for the elevation range. Confidence intervals that do not cross the zero line indicate that the estimates are significant (P < 0.05).

history between the studying areas (Iseli et al. 2023), an understanding of the driving factors underlying the range expansions along elevation is emerging.

Among the various factors tested in this study, alien species with clonal reproduction exhibited not only significantly broader elevation ranges but also faster upward expansion compared to their non-clonal counterparts (Fig. 2a, c). Such a result is in line with previous studies showing that species primarily reproducing through asexual means tend to have broader distribution ranges (Cosendai et al. 2013). Besides, alien clonal species were found to have better growth performance than native clonal species, highlighting the pivotal role of clonality in promoting plant invasion (Pyšek 1997; Liu et al. 2019; Wang et al. 2019). Here, we also found that among clonal alien species, the taller ones exhibited a tendency for broader elevation ranges, and those with larger naturalized ranges had greater elevation ranges and speeds (Fig. 3a, b).

For other traits tested with regard to the elevation expansion of alien species, we observed a negative association between monoploid genome size and elevation range for clonal aliens (Fig. 3a). This can be explained by the "large genome constraints" hypothesis, stating that large genome acts as a constraint in extreme



Figure 4. Relationships between elevation ranges and important variables identified from phylogenetic regressions (See Fig. 3 for details; data were log-transformed). Blue, orange, and black lines represent regression lines with non-clonal, clonal, and all species, respectively. The dashed lines represent non-significant relations. Grey shading represents 95% confidence intervals.

and inhospitable conditions (Knight et al. 2005), which can be applied to alien species' establishment in higher elevation. The invasion speed was strongly associated with clonality, and for non-clonal alien species, only their native range size was negatively related to their upward spread (Fig. 3b). Although native range size was closely related to the fitness of alien species in the introduced areas (Pyšek et al. 2015; Guo et al. 2019; Fristoe et al. 2023), the inherent characteristics and interactions with local native species may be more important in determining the invasion speed of alien species. In essence, these results highlight the multifaceted interplay between clonality, height, genetic characteristics, and climate niche, revealing the intricate dynamics that contribute to the upward expansion and invasiveness of clonal alien plants.

In addition, our study identified the minimum residence time, flowering time, and demands for temperature as key factors driving the elevation range of alien species in the Czech Republic, regardless of their clonality status (Fig. 3a). Such results indicate that alien plants which are more likely to have a wider elevation range are those that start flowering earlier, arrive earlier and have a reduced dependence on temperature conditions. These characteristics are also commonly observed in alpine plants (Alexander et al. 2016; Inouye 2020; Wang et al. 2020) and contribute to the expansion of their ecological range.

It is gradually recognized that the position on the introduction-naturalizationinvasion continuum is a good indicator of species' minimum residence time (Pyšek et al. 2009b; Moodley et al. 2013), thus aligning with the minimum residence time results: the elevation ranges of the 58 alien species increased along the introductionnaturalization-invasion continuum (Fig. 2b). While the same trend along the invasion continuum was not obtained for the invasion speed of alien species, a negative relationship was uncovered between the minimum residence time and the invasion speed for clonal species. The suitable ecological niche of the species may be responsible for this phenomenon, i.e., alien species with longer minimum residence time may have already occupied the most suitable habitats and, therefore, have slower invasion speeds. On the other hand, the relatively limited availability of valid invasion speed data for the initial two stages, in comparison to the third stage, might have introduced a degree of compromise to the statistical analysis. This potential data limitation could lead to the nuanced results observed in this context.

For human-associated factors, we hypothesized that the economic use of alien plants will positively correlate with their elevation ranges, in keeping with the wellestablished assertions of earlier research, which underline the significance of economic use in determining the success of alien plant species within introduced ranges (Guo et al. 2019, van Kleunen et al. 2020). However, our results did not match the second hypothesis we proposed. This may be due to nearly a quarter of alien species not having economic use data. Similarly, the results for the introduction pathway had no significant effect on driving alien species expansion along elevation (Appendix 1: Fig. A2). This could be caused by other human-related factors which could have greater predictability, such as distance to roads. Proximity to roads proved to be a key driver of the observed rapid upward spread of alien species (Dainese et al. 2017).

Although we considered several factors related to species' expansion along the elevation gradient, several important variables, such as climate and soil properties, were missing from the analysis. It appears necessary to include these variables in future studies to gain a more comprehensive understanding. Interactions between alien and native species are equally important to become a subject of future studies, with the potential to provide valuable insights into the mechanisms underlying the establishment and persistence of alien species in alpine habitats.

In summary, our results showed that 58 alien species have been expanding upward along mountain elevations in the Czech Republic over the past two centuries. Alien species can reach the highest elevations and exhibit the widest range of elevations, providing further support for the hypothesis of directional ecological filtering. In particular, our study explored how species traits and human-associated factors influence the elevation range and invasion speed of alien species towards mountains. We found distinct roles of species characteristics and human-associated factors in shaping species' elevational expansion, e.g., compared with non-clonal alien species, clonal alien species had a wider elevation range and faster invasion speed, while human-associated factors had no effect. Our results emphasized that rapid reproduction and spread are crucial for alien species' expansion in mountainous regions and are further facilitated by long residence time. Identifying key drivers of the distribution and spread of alien species in mountain areas and further developing a more complete understanding of how traits, human factors, and climate interact is critical. By analyzing complex temporal patterns and trends in the distribution of alien species, we can better grasp their dynamics and potential impacts on local ecosystems given the dynamic climate change worldwide.

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	ELE_range	INV_speed	FLOW_time	EN_1	EN_T	ENN	Height	NAT_range	NATLZ_range	Ecouse	Cx_value	MRT		
900 • 600 • 300 •	.ll.ll.	0.53	-0.42	-0.51	-0.63	0.035	0.067	-0.21	-0.005	-0.000	-0.23	0.46	ELE_range	
8. 6. 4. 2.		dda.	-0.22	-0.32	-0.27	0.071	0.021	-0.31	-0.088	0.3	-0.13	-0.11	INV_speed	
10 • 8 • 6 • 4 •			սեհետ	0.24	0.29	0.087	0.23	0.28	-0.096	0.085	0.16	-0.28	FLOW_time	
8. 6. 4.					0.53	-0.028	-0.066	0.2	0.22	0.067	0.21	-0.18	ENT	
8 7 6 5 4					Infutut	0.058	0.06	0.058	0.25	0.081	-0.058	-0.22	ENT	Gamalatian
8 7 6 5 4 3						ուրի	0.3	-0.26	0.27	0.22	0.24	-0.069	EN_N	1.0 0.5
4 3 2 1	The state	A STREET						-0.074	-0.025	0.37	0.1	-0.062	Height	0.0 0.5
75 • 50 • 25 • 0 •							X	llul.	0.015	0.0087	0.063	-0.039	NAT_range	-1.0
200 • 150 • 100 • 50 • 0 •		H			H.	H				0.32	-0.09	0.15	NATLZ_range	
7.5 · 5.0 · 2.5 ·						-					0.17	-0.12	Ecouse	
4 • 2 • 0 •	the the											-0.18	CX-value	
200 • 100 •			ł	-	ł		1				. T	.ulu.	MRT	
	300 600 900 0	0 2.5 5.0 7.5	4 6 8 10	4 5 6 7 8 9	5 6 7 8	4 6 8	0 1 2 3 4	0 25 50 75	0 50 100 150 200	0.0 2.5 5.0 7.5	0 2 4	0 100 200		

Appendix I

Figure A1. Pearson's correlation for all continuous variables collected in the study. ElE_range, elevation range; INV_speed, invasion speed; FLOW_time, flowering time; EIV_L, Ellenberg indicator values of light; EIV_T, Ellenberg indicator values of temperature; EIV_N, Ellenberg indicator values of nutrients; NAT_range, native range; NATLZ_range, naturalized range; Ecouse, economic use sum; Cx_value, monoploid genome size; MRT, minimum residence time.



Figure A2. Violin plots of the elevation ranges and invasion speeds for the 58 alien species considering their life spans, mycorrhizal types, and introduction pathways. For the boxplots inside each violin plot, the horizontal line represents the median, the red dot indicates the mean, and the box represents the interquartile range. *P*-values were calculated using phylogenetic ANOVA models, and none of them are significant.

Supplementary material I

Data used for the analysis

Authors: Miao-Miao Zheng, Petr Pyšek, Kun Guo, Hasigerili, Wen-Yong Guo Data type: csv

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RESEARCH ARTICLE



Substrate complexity reduces prey consumption in functional response experiments: Implications for extrapolating to the wild

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Abstract

Understanding the density-dependent impacts of an invasive predator is integral for predicting potential consequences for prey populations. Functional response experiments are used to assess the rate of prey consumption and a predator's ability to search for and consume prey at different resource densities. However, results can be highly context-dependent, limiting their extrapolation to natural ecosystems. Here, we examined how simulated habitat complexity, through the addition of substrate in which prey can escape predation, affects the functional response of invasive European green crabs (Carcinus maenas) foraging on two different bivalve species. Green crabs feeding on varnish clams (Nuttallia obscurata) shifted from a Type II hyperbolic functional response in the absence of substrate to density-independent consumption when prey could bury. Green crabs ate few Japanese littleneck clams (Venerupis philippinarum) under all densities, such that no functional response curve of any type could be produced and their total consumption was always density independent. However, the probability of at least one Japanese littleneck clam being consumed increased significantly with initial clam density and crab claw size across all treatments. At mean crab claw size and compared to trials without substrate, the proportion of varnish clams consumed were 4.2 times smaller when substrate was present, but substrate had a negligible effect (1.2 times) on Japanese littlenecks. The proportion of varnish clams consumed increased with crab claw size and were higher across both substrate conditions than the proportion of Japanese littlenecks consumed; however, the proportion of Japanese littleneck clams consumed increased faster with claw size than that of varnish clams. Our results suggest that including environmental features and variation in prey species can

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influence the density-dependent foraging described by functional response experiments. Incorporating replicable features of the natural environment into functional response experiments is imperative to make more accurate predictions about the impact of invasive predators on prey populations.

Keywords

Decapods, density-dependent predation, environmental complexity, invasive species, marine, non-native

Introduction

Owing to new introductions and ever-expanding ranges, invasive species have significant negative impacts on the biodiversity (McNeely 2001; Molnar et al. 2008; Mollot et al. 2017), trophic structure (Nilsson et al. 2012; Papacostas and Freestone 2019) and overall integrity of ecosystems (Miehls et al. 2009; Walsh et al. 2016). Marine invasions, in particular, have significant economic and ecological consequences, with coastal marine ecosystems being amongst the most invaded due to the multiplicity of co-occurring anthropogenic activities (e.g. commercial shipping, marine aquaculture etc.) (Simberloff 2013; Geburzi and McCarthy 2018; Cuthbert et al. 2019; Pyšek et al. 2020). The ever-changing seascape of invasions makes it challenging to accurately predict the potential impacts of non-native species on ecosystems and hampers effective management (Beardsell et al. 2021).

The population density of an invader is important for estimating its potential impact and the resulting consequences for the environment (DeRoy et al. 2020a; Griffen et al. 2021). The influence of invasive species on an ecosystem is often density dependent, as it is linked to both the per-capita effect of an invader and invader abundance (Parker et al. 1999; DeRoy et al. 2020a). Functional response experiments (FREs) are often used in invasion ecology to assess the rate of resource consumption, usually in the context of a predator's ability to search for and consume prey at different resource densities (Holling 1959; Alexander et al. 2012; Beardsell et al. 2021). The resulting functional response (FR) curve can reveal whether predators could have a linear (Type I), destabilising (hyperbolic; Type II) or stabilising (sigmoidal; Type III) effect on prey populations (Holling 1959), which is important for forecasting the impact of new invaders.

Functional response experiments, like many laboratory experiments, are by nature simplified representations of complex systems. They typically remove many of the biotic and environmental variables that may influence consumption rates, increasing the comparability of findings within and between species (Holling 1959; Dick et al. 2014; Beardsell et al. 2021). However, invasion dynamics can be highly context-dependent, with every invasion into a novel environment resulting in different relationships between the invader and the surrounding system. Environmental conditions and biological interactions, including temperature, prey species and the sex of the predator (Lipcius and Hines 1986; Sponaugle and Lawton 1990; Beardsell et al. 2021; Howard et al. 2022; Kattler et al. 2023), can impact the shapes and asymptotes of FR curves.

Therefore, the factors included or excluded from the design of a FRE can be integral for the interpretation of the impact of that specific invader. The incorporation of a variable, such as habitat complexity (e.g. by adding substrate into which prey can hide), in laboratory experiments has been shown to be a determining factor in the relationship between predators and prey (Barrios-O'Neill et al. 2014; Barrios-O'Neill et al. 2016; DeRoy et al. 2020b). It can increase search effort, shift the profitability of different prey sizes and alter consumption rates (Sponaugle and Lawton 1990; Barrios-O'Neill et al. 2014; Barrios-O'Neill et al. 2016; DeRoy et al. 2014; Barrios-O'Neill et al. 2016; DeRoy et al. 2020b). The addition of such a variable to a FRE could be important for understanding the context dependency of novel invasive predator-prey relationships.

Functional response experiments have been used to evaluate the predatory behaviour and potential impacts of the European green crab (Carcinus maenas, Linnaeus, 1758) (e.g. Howard et al. 2019; Ens et al. 2021; Howard et al. 2022). Native to Europe and northern Africa, green crabs are now found on every continent but Antarctica (Yamada 2001; Klassen and Locke 2007; Young and Elliott 2019) and are one of the most studied invasive species globally (Watkins et al. 2021). They are generalist predators (Yamada 2001; Klassen and Locke 2007) and have been shown to have significant negative effects on invaded ecosystems through predation (Whitlow et al. 2003; Miron et al. 2005), competition (MacDonald et al. 2007) and habitat alteration (Malyshev and Quijón 2011; Howard et al. 2019). In particular, green crabs are known to be highly effective predators of clams and have had substantial negative consequences for bivalve fisheries across the globe (Ropes 1968; Klassen and Locke 2007; Whitlow 2010). The worldwide success of green crabs is due to their ability to tolerate a wide range of environmental conditions and their broad diet (Yamada 2001; Lohrer and Whitlatch 2002; Klassen and Locke 2007; Young and Elliott 2019). These wide physiological limits and patterns of resource use make any predictions about the impact of green crabs extremely context-specific as the species' behaviour may be similar (Howard et al. 2019) or different across novel ecosystems (Howard et al. 2018). Therefore, incorporating realistic environmental elements relevant to the invasion being studied to FREs on green crabs could provide more context-specific estimates of their potential effects on prey populations.

In this study, we aimed to examine how the FR of invasive green crab foraging on bivalve species may change when prey are provided with habitat that mimics their natural environment. More specifically, we provided substratum in which bivalve prey could bury, thereby potentially increasing green crab handling time and decreasing their attack rate and maximum prey consumption when compared to FREs conducted without substrate. We also examined the effect of prey species characteristics that can impact susceptibility to predation, i.e. morphological characteristics and burial depths, in these two substrate conditions. We expected that the different burying depths of the two clam species used might give rise to a reversal in prey profitability that foraging crabs would experience in the wild, but may not be realised in typical FREs. Increasing search time in a FRE could alter predictions of the magnitude of impact invasive predators have on prey populations.

Methods

Study species and collection

Male European green crabs were collected from Bedwell Bay (49°18.55'N, 125°48.29'W) near Tofino on the west coast of Vancouver Island, British Columbia (BC), Canada, in June 2022. Crabs without evidence of moulting, free from epibionts, with a notch-to-notch carapace width of 55 to 76 mm and with both chelipeds present, were used in our experiment. We collected only males to reduce the risk of invasion via the release of fertilised eggs at the experimental facility. Varnish clams (*Nuttallia obscurata* Reeve, 1857) were collected from Robbers Passage (48°53.77'N, 125°7.25'W) in Barkley Sound, also on the west coast of Vancouver Island, while Japanese littleneck clams (*Venerupis philip-pinarum* A. Adams & Reeve, 1850) were collected from Nanoose Bay (49°15.53'N, 124°10.99'W), on the east coast of Vancouver Island. We collected clams with undamaged shells and measuring 21–40 mm in length (i.e. anterior to posterior shell margins).

Both prey species are not native to BC, but their high abundance in soft-bottom habitats throughout the region results in a high likelihood of encounters between these invasive prey and green crabs (Quayle 1964; Gillespie et al. 2001; Dudas et al. 2005; Blackburn et al. 2011). Varnish clams are a prevalent clam species found in the Salish Sea (Gillespie et al. 2001). They possess similar physical characteristics to the native bent-nosed macoma (*Macoma nasuta*) (Dudas et al. 2005; Hiebert et al. 2015; Gordon 2018) and are found at similar depths and tidal heights, burying to around 10–20 cm (Gordon 2018). The second invasive prey species used here, Japanese littleneck clams (or Manila clams), are also abundant and similar in morphology and habitat use to the native Pacific littleneck clams (*Protothaca staminea*) (Richardson 1985), to such an extent that native crabs appear to be unable to distinguish between them (Dudas et al. 2005). Due to their short siphons, both species of littleneck clams bury to depths shallower than 10 cm (Richardson 1985). These ecological similarities between varnish clams and Japanese littleneck clams with their native counterparts, as well as their widespread occurrence and abundance in coastal BC, led to our decision to use invasive prey species for our experiment.

All animals were held at the Bamfield Marine Science Centre, on the west coast of Vancouver Island, in indoor sea tables (172 cm long × 75 cm wide × 16 cm deep) with flow-through, unfiltered seawater (10 °C \pm 0.33 °C). The animals were held under artificial lighting that mimicked natural day-night cycles. Crabs were held at low densities (~20 crabs per sea table) with ample habitat including flowerpots, rocks, PVC pipes and seaweed. Crabs were fed thawed salmon pieces every four days. Clams were fed algae and Phytofeast every three days.

Experimental set-up

Functional response experiment

Trials were conducted in opaque plastic enclosures (61 cm × 41 cm × 42 cm), which were all supplied with natural seawater flowing at equal rates. Each replicate consisted

of 12 treatment combinations: two substrate treatments (enclosures with or without substrate) at each of six clam densities (1, 2, 4, 6, 10 or 16 individuals per enclosure). We placed Quikrete[®] premium play sand on the bottom to a depth of 20 cm in each with-substrate enclosure and left the bottom of the no-substrate enclosures bare. We chose to use play sand instead of natural substrate to avoid variability introduced by grain size, the possible presence of invertebrates and/or variation in oxygen levels in natural substrate. Sand was washed thoroughly before use and the seawater used in the experimental enclosures was changed and all visible detritus removed between trials. Each trial included a single clam species; there were no mixed-species trials. All 12 treatment combinations were replicated six times each for both varnish clams and Japanese littleneck clams (for a total of 144 trials). Each replicate was run over two days between 25 June and 9 July 2022. Clam density by substrate level combinations were randomly assigned to each enclosure using a random number generator. Clams were scattered in the enclosures 12 h prior to the start of each replicate and were only used once, even if they were not consumed. Each trial commenced with the introduction of a single, randomly-assigned crab. Prior to trials, we isolated and withheld food from green crabs for 48 h to standardise hunger levels (Howard et al. 2018, 2022). Each crab was used only once. Before each trial, we recorded morphological characteristics of the green crabs and both clam species, as these could influence both the ability of crabs to open a clam and the profitability of individual clams as prey (Elner 1980; Yamada 2001; Byers 2002; Dudas et al. 2005). We measured crab carapace width (CW) from notch to notch at the widest part of the carapace and cheliped height (CH) as the maximum height of the major cheliped propodus (top to bottom). We also measured clam length with calipers. Crabs were allowed to forage for 8 h beginning at 08:00 h.

At the end of each trial, crabs were removed and the number and size of clams consumed were recorded. Finally, we ran a control replicate to test for clam survival independent of predation. Temperature and salinity were measured at the start and end of each trial using a thermometer (FisherbrandTM 76 mm immersion thermometer) and refractometer (Tropic Eden PRO-1 normal seawater refractometer), respectively.

Burial depths of prey species

We ran a burial experiment to determine the average burial depth of both clam species. We glued a graduated length of monofilament fishing line to the umbo of 15 clams of each species and allowed them to bury in identical substrate conditions as the FR experiment for 12 h. After 12 h, we measured each line from the umbo of the clam to the point where the line emerged from the sand.

Statistical analysis

For each treatment combination, we attempted to fit a functional response curve to the proportion of prey eaten in relation to prey density using the R package 'frair' (frair:frair_test) (Pritchard et al. 2017). We first used the 'frair_test' function, which compares two logistic regressions on the proportion of prey consumed: one that looks at the effect of density (i.e. representing a Type II relationship) and the other that looks at the effect of density and density squared (i.e. representing a Type III relationship) (Pritchard et al. 2017). The function uses significance testing to determine if there is sufficient evidence to fit a functional response curve. A Type III response is supported when both the density and density-squared terms are significant. When the density term is significant and negative, but density squared is not, a Type II response is supported. In the case where neither term is significant, the test offers no support for the existence of either type of response. The only treatment combination that had a significant term from this initial logistic regression was that of green crabs feeding on varnish clams in the absence of substrate (see Results). The first-order terms were negative, indicating a Type II FR. Therefore, we used the random predator equation without prey replacement (Rogers 1972):

$$N_e = N_0 (1 - \exp(a (N_e h - T)))$$

where N_e is the number of prey eaten, N_0 is the starting prey density, a is the predator's attack rate, h is the handling time and T is the length of the experiment. N_e and N_0 were determined by each individual trial, while a and h were estimated from the logistic regression model. We then used frair:frair_boot non-parametric stratified bootstrapping (n = 2000 iterations) to generate a 95% confidence interval for each parameter estimate of the model. We used a bias-corrected and accelerated bootstrap interval (upper and lower BCa) to correct for any biases or skewed distributions in the bootstrapped model. There was no evidence of a functional response for any of the other treatment combinations (i.e. none of the terms was significant for the initial logistic regression), so we therefore did not generate FR equations and the associated parameters (see Results).

As the logistic regression used by the 'frair_test' did not produce any significant density terms for three of our treatment combinations, we used an additional approach to understand the role of substrate presence in green crab foraging by considering other possible explanatory variables in addition to prey density. We first ran a separate logistic regression (generalised linear model with a binomial distribution and logit link function) to examine the probability that a crab consumed a clam in relation to cheliped height, initial clam density and substrate presence, as well as interactions between clam species and substrate presence/absence and between clam species and cheliped height. However, complete separation (i.e. one variable perfectly predicts another variable) occurred in the model for varnish clams in the absence of substrate treatment. This was caused by every crab consuming at least one varnish clam in every no-substrate trial. Therefore, we incorporated bias reduction through a maximum penalised likelihood for our observations where penalisation was done using Jeffreys invariant prior (Kosmidis 2021).

We then used a second logistic regression (generalised linear model with a binomial distribution and logit link function) to assess the proportion of clams consumed in a trial in relation to the same variables and interactions as the previous model. In both models, the interaction between clam species and substrate presence/absence was included to reveal the potential trade-off between attack rate and handling time generated

by the different burial depths of the two clam species (Richardson 1985; Gordon 2018). The interaction between clam species and cheliped height was included to reflect the potential effect of clam species differences (especially in shell thickness) on the relationship between in cheliped height and consumption (Elner 1980; Hamano and Matsuura 1986; Lee and Seed 1992). All analyses were done using R version 4.2.2 (R Core Team 2022). We analysed model fit using the 'DHARMa' package (Hartig 2022) to assess the distribution of residuals, dispersion, outliers and variances for our models. We fit our generalised linear model with bias reduction using the 'brglm' package (Kosmidis 2021).

Results

There was no mortality for either varnish clams or Japanese littleneck clams in substrate and non-substrate trials when in enclosures without green crabs. Therefore, all mortality observed in the experiment was assumed to be due to green crab predation. All clams were able to bury themselves before the start of each trial. Varnish clams buried significantly deeper (mean \pm 1 SE: 8.98 \pm 0.48 cm, range: 6.2–11.6 cm) than Japanese littleneck clams (4.15 \pm 0.29, 2.3–6.2 cm) (coefficient = 4.83, p < 0.001, Suppl. material 1: fig. S1).

The lengths of varnish clams consumed ranged from 25 to 40 mm (mean \pm 1 SE: 31.09 \pm 0.30 mm), while Japanese littleneck clams that were consumed ranged from 21 to 30 mm (mean \pm 1 SE: 26.79 \pm 0.35 mm). There was no significant difference between the sizes of varnish clams that were or were not consumed, irrespective of substrate presence (post-hoc pairwise contrasts not consumed vs. consumed, with substrate: estimate = -0.47, t₆₉₁ = -0.94, p = 0.35; without substrate: estimate = -0.19, t₆₉₁ = -0.43, p = 0.67; Suppl. material 1: fig. S2). For Japanese littleneck clams in the absence of substrate, consumed clams were on average 1.68 mm smaller than those that were not consumed (post-hoc pairwise contrast, t₄₆₁ = 2.93, p = 0.0035). However, when substrate was present, there was no significant difference in the size of clams consumed (estimate = 0.62, t₄₆₁ = 0.94, p = 0.35).

Functional responses

Green crabs feeding on varnish clams in the absence of substrate exhibited a Type II hyperbolic FR, with a corresponding significant negative first-order density term (z = -4.57, p < 0.001, Fig. 1; Table 1). Due to the non-significant logistic regressions, we did not fit functional responses to the data from the other three treatment combinations (Suppl. material 1: fig. S3). The non-significant regressions are likely due to the number of trials where a crab did not consume any clams, resulting in a sample size too small to analyse (0/36, 24/36, 26/36 and 26/36 crabs did not eat varnish clams in the absence of substrate, varnish clams with substrate, Japanese littleneck clams in the absence of substrate and Japanese littleneck clams with substrate, respectively), especially at the lower prey densities (Pritchard et al. 2017).

Table 1. Parameter estimates for green crabs feeding on varnish clams in the absence of substrate. The parameter estimates, attack rate (a) and handling time (h), were derived from a Rogers Type II functional response curve. BCa CI represents the bootstrapped accelerated bias-corrected 95% confidence intervals.

Parameter	Estimate	SE	BCa CI	z	Þ
Attack rate (a)	2.89	0.77	1.72-5.59	3.74	< 0.01
Handling time (h)	0.12	0.02	0.06-0.17	5.79	< 0.01



Figure 1. Functional response curve of green crab feeding on varnish clams in the absence of substrate. The triangles show the mean number of varnish clams consumed as a function of initial clam density (1, 2, 4, 6, 12 or 16 per trial). The Type II FR curve is represented by the dashed line with the bootstrapped 95% confidence interval represented by the shaded ribbon.

Drivers of predator consumption

The probability of an individual clam being consumed increased with cheliped height and initial clam density across all treatment combinations considered (Table 2). In the absence of substrate, the probability that green crabs would consume at least one clam was significantly higher for varnish clams than for Japanese littlenecks (coefficient = 6.48, p = 0.05). The probability that at least one Japanese littleneck clam was consumed was similar whether there was substrate or not when all other variables were constant (Table 2). However, there was a significant interaction between clam species and substrate presence. In the absence of substrate, green crabs were 10.84 times more likely to consume at least one varnish clam than at least one Japanese littleneck clam when the initial clam density was one. In contrast, in the presence of substrate, the probability of at least one varnish clam being consumed was only 1.28 times greater than that of Japanese littleneck clams when the initial clam density was one (Fig. 2, Table 2).

Table 2. Results of a generalised linear model (GLM) with bias reduction, binomial distribution and logit link function examining the effect of various factors on the probability that a green crabs would consume at least one clam during a trial. Substrate refers to the presence or absence of substrate in an enclosure, initial clam densities were 1, 2, 4, 6, 12 or 16 clams and clam species included varnish clams or Japanese littleneck clams. The baseline factor levels for the model are Japanese littleneck clams in the absence of substrate (n = 144 trials).

Factor	Estimate	SE	z	p
Intercept	-6.91	1.79	-3.86	< 0.001
Cheliped height	0.24	0.083	2.95	0.003
Initial clam density	0.19	0.049	3.88	< 0.001
Clam species	6.48	3.37	1.93	0.05
Substrate	-0.096	0.62	-0.16	0.87
Clam species × substrate	-5.93	1.73	-3.43	< 0.001
Clam species × cheliped height	-0.015	0.18	-0.085	0.99



Figure 2. Probability of a green crab consuming a clam as a function of initial clam density. Lines represent model predictions for each treatment combination (varnish clams or Japanese littleneck clam in the presence or absence of substrate) during a trial in relation to initial clam density and ribbons represent 95% confidence intervals. Data points represented individual crabs (n = 36 for each density x substrate treatment).

This trend continues at higher initial clam densities. For example, when 10 clams were initially present, varnish clams had a 2.79 times higher probability of at least one clam being consumed in the absence of substrate than Japanese littleneck clams, but only



Figure 3. Proportion of varnish clams or consumed in relation to green crab cheliped height (mm). Lines represent model predictions for each treatment combination (varnish clams or Japanese littleneck clam in the presence or absence of substrate) and ribbons represent 95% confidence intervals. Data points represented individual crabs (n = 36 for each density x substrate treatment).

1.19 times higher probability when substrate was present (Fig. 2, Table 2). There was no significant interaction between clam species and crab cheliped height (Table 2). Overall, the probabilities of clam consumption by green crabs feeding overlapped extensively across all treatment combinations, with the exception of varnish clams in the absence of substrate (Fig. 2).

Crab cheliped height, clam species and the interactions between clam species and substrate presence and between clam species and cheliped height all had a significant effect on the proportion of clams consumed during a trial (Table 3). The proportion of clams consumed increased weakly, but non-significantly, with cheliped height in both substrate conditions (Fig. 3). There was a significant negative interaction between cheliped height and clam species (Table 3). At the mean cheliped height of 18.08 mm, the proportion of Japanese littleneck clams consumed increased by 1.19 times with a one-mm increase in cheliped height in both the absence and presence of substrate (Fig. 3). The proportion of varnish clams consumed only increased by 1.008 times with a one-mm increase above the mean cheliped height in the absence of substrate, but by 1.02 times in the presence of substrate (Fig. 3). While the presence of substrate had no significant effect on the proportion of clams eaten overall, there was a significant negative interaction between clam species and the presence of substrate (Table 3).

Table 3. Results of a generalised linear model (GLM) with a binomial distribution and logit link function examining the effect of various factors on the proportion of clams consumed during a trial as a function of green crab cheliped height. Substrate refers to the presence or absence of sand in an enclosure, initial clam densities were 1, 2, 4, 6, 12 or 16 clams and clam species included varnish clams or Japanese littleneck clams. The baseline factor levels for the model are Japanese littleneck clams in the absence of substrate (n = 144 trials).

Factor	Estimate	SE	z	p
Intercept	-5.81	1.051	-5.53	< 0.001
Cheliped height	0.19	0.049	3.87	< 0.001
Initial clam density	-0.0305	0.018	-1.66	0.10
Clam species	6.11	1.26	4.84	< 0.001
Substrate	-1.15	0.36	-0.42	0.67
Clam species × substrate	-2.13	0.43	-4.92	< 0.001
Clam species × cheliped height	-0.17	0.064	-2.62	0.009

Specifically, while the proportion of Japanese littleneck clams consumed was similar in trials with and without substrate (increased by 1.13 times at the mean cheliped height in the absence of substrate), the mean proportion of varnish clams consumed was 4.19 times higher without than with substrate at the mean cheliped height (Fig. 3). Overall, the proportion of Japanese littlenecks consumed was significantly lower than the proportion of varnish clams consumed (Table 3, Fig. 3).

Discussion

European green crabs did not always forage on clams in the density-dependent manner described by functional responses. Green crabs feeding on varnish clams in the absence of substrate consumed prey in every trial and exhibited a Type II hyperbolic FR, indicating a potentially destabilising effect on this prey species at low densities. However, green crabs in the other three treatment combinations (varnish clams in substrate and Japanese littleneck clams with and without substrate) consumed too few clams to exhibit a significant density term to support a density-dependent Type II or III response. The probability of a crab consuming at least one prey increased with prey density and crab crusher claw size and prey species interacted with substrate condition. A lower proportion of varnish clams were consumed in trials with than without substrate, but no difference was detected for Japanese littleneck clams. Our findings suggest that the results of FREs and, hence, the conclusions drawn about the potential effect of predators on wild populations, are heavily influenced by their experimental simplicity.

We had originally expected that the addition of substrate in our experiments would alter the shape and/or asymptotes of the resulting FR curves. More specifically, we had predicted that search time and handling time might increase and maximum prey consumption might decrease, when substrate was present. We had also expected that adding substrate might reverse the profitability of the two clam species due to their contrasting features (i.e. differences shell thickness and burial depth) (Byers 2002; Dudas et al. 2005) and, hence, transpose their FR curves. These predictions were only partly supported. Consumption of varnish clams by green crabs was lower in the presence than in the absence of substrate, as expected. When varnish clams could bury, most green crabs failed to eat, such that we could not reliably model a FR curve and associated parameters as the resulting confidence intervals would cross zero. This was also true for Japanese littlenecks in the presence and absence of substrate. In the absence of substrate, all green crabs consumed varnish clams, while fewer than half consumed Japanese littlenecks, which indicates a likely preference for the former prey species. This preference in the absence of substrate may be linked to shell robustness (Boulding 1984), since green crabs were unselective when foraging on varnish clams, but selected Japanese littleneck clams that were smaller and, hence, easier to crush, than the ones that remained uneaten (Suppl. material 1: fig. S2). However, clam size is no longer a factor when crabs have to dig to access buried prey. Our results, therefore, suggest that density-dependent foraging, as described by functional response theory, occurs only when green crabs forage on preferred prey and search time is minimised by the absence of habitat complexity. The fact that both environment and prey species can significantly alter the consumption rate of a predator reveals the importance of experimental design choices (Lipcius and Hines 1986; Rossi et al. 2008; Howard et al. 2018).

Density-dependent foraging still occurred in the three treatment combinations where functional responses were not supported. For varnish clams in the presence of substrate and Japanese littleneck clams in both substrate conditions, the probability of a green crab consuming a clam increased significantly with both prey density and crab cheliped height. In general, the more abundant the prey, the higher the likelihood of a prey encounter, even when prey are concealed (Sponaugle and Lawton 1990; Ebersole and Kennedy 1995; Seitz et al. 2001). In addition, the consumption rates of most decapod crustaceans are typically limited by cheliped height as it can determine both the prey species and size that they can consume (Elner 1980; Hamano and Matsuura 1986; Lee and Seed 1992; Dudas et al. 2005). However, while there was no significant interaction between crab cheliped height and clam species, green crabs were 1.5 to 9.2 times more likely to consume at least one varnish clam in the absence of substrate than in the presence of substrate, with the magnitude of the effect varying inversely with initial clam densities. The same pattern can be seen with Japanese littleneck clams, though to a lesser extent (1.04 to 1.09 times more likely in the absence than presence of substrate). For varnish clams, which are thin-shelled but bury deep (Byers 2002; Dudas et al. 2005), the probability of at least one clam being consumed in a trial fell from 100% in the absence of substrate to overlap almost completely with that of Japanese littlenecks when substrate was present. In contrast, Japanese littleneck clams, which are thick-shelled and bury shallow (Byers 2002; Dudas et al. 2005), had the same probability of being eaten in both substrate treatments. Therefore, adding substrate appeared to reduce the profitability of the preferred varnish clam species to a level similar to that of the less preferred Japanese littleneck.

The drivers of the proportions of clams consumed support our interpretation of the previous results. While cheliped height did not determine the probability of a crab consuming a clam, the proportion of clams eaten increased with cheliped height, as expected (Elner 1980; Hamano and Matsuura 1986; Lee and Seed 1992; Dudas et al. 2005), across all treatment combinations. The ability of a green crab to open a thin-shelled varnish clam does not depend as strongly on cheliped height as the ability to crack open

a thicker-shelled littleneck. This appears to be also true for the co-occurring native crab species red rock (*Cancer productus*) and Dungeness crabs (*Metacarcinus magister*) which, in the absence of substrate, consume a wide range of varnish clam sizes but a more limited size range of littleneck clams (Dudas et al. 2005). These results suggest that the extra time and energy required of green crabs to forage on varnish clams in more 'natural' substrates are substantial burdens on detection and attack rates, resulting in lowered consumption. Such burdens on green crab consumption are not evident when prey are associated with long handling times, such as thick-shelled littlenecks.

We expect that the relatively low temperature of our experiments contributed to some of the low consumption rates observed, particularly if thermal effects on foraging are prey-specific (e.g. more important when more crushing force is required). It is well documented that European green crabs consume more prey as temperatures increase (Ropes 1968; Elner 1980; Howard et al. 2022) and are less active at colder temperatures (Yamada 2001; Young and Elliott 2019). However, the experimental temperatures experienced by our green crabs (10 °C \pm 0.33 °C) were a few degrees above the minimum temperature for green crab feeding (7 °C) (Yamada 2001) and green crabs did consume prey in all treatment combinations. Such temperatures are representative of intertidal environments on the coast of British Columbia, especially between the late autumn and early spring and along the northern coast, in Haida Gwaii and in southern Alaska (USA), where green crab have recently expanded (Yamada et al. 2001; Howard pers comm, 2023). Testing green crab foraging behaviour in temperatures relevant to the invasion locale of interest is another component of considering context-dependency.

Our findings suggest that the addition of complexity, in the form of substrate in which prey can conceal themselves, as well as variation in prey species characteristics, can alter the predictions stemming from FREs about the ecological impacts of an invasive marine predator. We observed a transition from a strongly density-dependent to a density-independent consumer-prey relationship with the addition of substrate, at least for a preferred prey. The switch in dependence occurred when preferred prey, varnish clams, were allowed to implement a defence mechanism (i.e. burying) that they would naturally rely on, which lowered green crab consumption considerably. A key question arising from our study is whether the foraging behaviour of all consumers is similarly altered by complexity and prey variability. The answer is important because the predictions of impacts made by FREs are sometimes used when comparing consumer species (invasive vs. invasive or invasive vs. native) to gauge their potential relative impacts (Dick et al. 2014; DeRoy et al. 2020b). Thus, variation in the effect of contextual factors, such as environmental complexity and prey traits, on consumer foraging will directly affect these comparative predictions. Incorporating replicable features of the natural environment into functional response experiments seems imperative to predict more accurately the impact of invasive predators on prey populations.

Data availability

The code and data underpinning the analyses reported in this paper are deposited on Github at https://github.com/elizabethoishi/green-crab-functional-response.

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Supplementary material I

Supplementary data

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Data type: docx

- Explanation note: figures for the burial depths of varnish clams and Japanese littleneck clams in substrate, lengths of clams that were or were not consumed by a green crab for each treatment combination, and number of varnish clams and Japanese littleneck clams consumed by green crabs with consumption rates as a function of initial clam density (1, 2, 4, 6, 12, 16) for each treatment combination.
- 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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REVIEW ARTICLE



Outcomes of control and monitoring of a widespread riparian invader (*Tamarix* spp.): a comparison of synthesis approaches

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Abstract

Effective ecological restoration requires empirical assessment to determine outcomes of projects, but conclusions regarding the effects of restoration treatments on the whole ecosystem remain rare. Control of invasive shrubs and trees in the genus Tamarix and associated riparian restoration in the American Southwest has been of interest to scientists and resource managers for decades; dozens of studies have reported highly variable outcomes of Tamarix control efforts, as measured by a range of response variables, temporal and spatial scales and monitoring strategies. We conducted a literature search and review, meta-analysis and vote count (comparison of numerical outcomes lacking reported variances and/or sample sizes) on published papers that quantitatively measured a variety of responses to control of *Tamarix*. From 96 publications obtained through a global search on terms related to Tamarix control, we found 52 publications suitable for a meta-analysis (n = 777 comparisons) and 63 publications suitable for two vote counts (n = 1,460 comparisons total; 622 comparisons reported as statistically significant) of response to Tamarix control. We estimated responses to control by treatment type (e.g. cut-stump treatment, burning, biocontrol) and ecosystem component (e.g. vegetation, fauna, fluvial processes). Finally, we compared results of the various synthesis methods to determine whether the increasingly stringent requirements for inclusion led to biased outcomes. Vegetation metrics, especially measures of Tamarix response, were the most commonly assessed. Ecosystem components other than vegetation, such as fauna, soils and hydrogeomorphic dynamics, were under-represented. The meta-analysis showed significantly positive responses by vegetation overall to biocontrol, herbicide and cut-stump treatments. This was primarily

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due to reduction of *Tamarix* cover; impacts on replacement vegetation were highly variable. We found concordance amongst our varied synthesis approaches, indicating that increased granularity from stricter quantitative techniques does not come at the cost of a biased sample. Overall, our results indicate that common control methods are generally effective for reducing *Tamarix*, but the indirect effects on other aspects of the ecosystem are variable and remain understudied. Given that this is a relatively well-studied invasive plant species, our results also illustrate the limitations of not only individual studies, but also of reviews for measuring the impact of invasive species control. We call on researchers to investigate the less commonly studied responses to *Tamarix* control and riparian restoration including the effects on fauna, soil and hydrogeomorphic characteristics.

Keywords

Invasive species management, meta-analysis, riparian restoration, Tamarix

Introduction

Assessment of outcomes is a critical aspect of ecological restoration, although evaluating the impact of a particular restoration methodology is often limited by the prevalence, scope and quality of monitoring (and of the restoration project itself). As the effectiveness of restoration methods can be highly context-dependent and the scale of restoration is often limited relative to the extent of degradation, it is important to objectively synthesise findings across a wide range of studies when a critical mass of studies have been published. Restoration actions have been heralded as a prime opportunity to understand the response to ecosystem change more generally (Egan 2001). Frameworks, such as those articulated by Suding et al. (2015) and Gann et al. (2019), have been developed to better situate ecological restoration within the context of ecological theory, including guiding principles for monitoring based on relevant indicators. However, management often remains isolated from these frameworks due to limitations in funding (and therefore data) and the capacity to effectively analyse and interpret data. Indeed, river restoration has been criticised in general for a lack of clearly defined goals, making it difficult to determine whether outcomes can be considered "successful" (Bernhardt et al. 2005; Palmer et al. 2005). Furthermore, highly variable outcomes combined with insufficient controls can lead to incorrect conclusions about the impact of restoration activities (Brudvig and Catano 2021). Thus, it is imperative to assess available studies to be able to reach generalisable conclusions to improve understanding of ecological outcomes following restoration treatment across and within ecosystem types. Examining the overall body of literature on restoration outcomes when seeking guidance for a particular system can help build a more holistic sense of ecosystem response to restoration that individual studies lack.

Rigorous syntheses can be especially important in the context of management of invasive species. As invasive species are a leading cause of ecosystem degradation and global change (Vitousek et al. 2008; Mollot et al. 2017), their control is a common and important aspect of ecological restoration. Control of non-native invaders can be ineffective, expensive and controversial and funding for such efforts can be dependent

upon public perception (Stromberg et al. 2009). However, previous research has found that, as in the broader field of ecological restoration, studies on outcomes of invasive species control are typically limited in scope spatially, temporally and with regard to measurable aspects of the ecosystem (Kettenring and Adams 2011) and, thus, may lead to misconceptions about effects of invasive species control that can influence land management decisions and policy (Bean and Dudley 2018).

Control of invasive Tamarix spp. trees in riparian systems of the American Southwest has been an important and controversial area of study (González et al. 2015, 2017c). Invasive Tamarix in North America is predominantly either T. ramosissima, T. chinensis or a hybrid thereof (Gaskin 2013). Here, we use the genus name Tamarix in reference to these two species and their hybrids. Initial introduction as a cultivated ornamental occurred in the early 19th century and, for roughly a century, Tamarix became naturalised in some riparian systems, but was not dominant (Chew 2013). In the 1930s, it was widely used by organisations like the U. S. Army Corps of Engineers to reduce streambank erosion and sediment transport into recently-constructed reservoirs (Chew 2009). Tamarix is a drought-, salt- and fire-"resilient" shrub (Busch and Smith 1995; Glenn and Nagler 2005) that is able to rapidly colonise riverbanks and floodplains and that had expanded its range to cover an estimated 500,000 hectares across the United States by the 1960s (Robinson 1965). Suspicions about its potentially high evapotranspiration rates, potential ability to deposit salts on the soil surface and trap sediment, its association with wildfires, potential negative effects on wildlife and other changes in ecosystems led to an overall concern amongst managers and ecologists about the impact of the species (Di Tomaso 1998; Stromberg et al. 2009). In particular, the tendency of *Tamarix* to form dense monocultures and ability to access groundwater led to widespread concern about its water use and hopes that control might lead to water salvage (Shafroth et al. 2005; Stromberg et al. 2009; Nagler et al. 2010). Tamarix control efforts and numbers of associated published studies both rose steadily across the latter half of the 20th century and increased dramatically in the mid-1990s, triggered by drought and extensive wildfires associated with Tamarix stands (Sher and Quigley 2013). While years of research and management have led to changes in perceptions and motivations for control, Tamarix continues to be an important focus of research on ecosystem function and dysfunction, particularly in light of biological control efforts using *Diorhabda* spp. beetles (released in the field in 2001) and the growing impact of global climate change (Bean and Dudley 2018; Mahoney et al. 2018). A Google Scholar search reveals that hundreds of papers concerning species in the genus Tamarix in their introduced range have been published since 1995 alone.

This abundance of research provides a unique opportunity to conduct meta-analyses on the effects of *Tamarix* control; here, we seek to determine the effects of active control efforts on the entire ecosystem as measured across abiotic and biotic components. Although meta-analysis has been employed to address management of invasive species as a general category (Crystal-Ornelas and Lockwood 2020; Boltovskoy et al. 2021) and of invasive trees specifically (Delmas et al. 2011), to our knowledge, meta-analysis has rarely been done to investigate outcomes of management of a single genus in its non-

native range. Furthermore, meta-analysis on restoration outcomes typically focuses on 1-3 measures of effect (e.g. soils and insect diversity; Parkhurst et al. (2022)), from the panoply of indicators that are available and recommended in the ecological restoration literature (Ruiz-Jaen and Aide 2005; Wortley et al. 2013). In fact, control of Tamarix is motivated by many different goals (e.g. restoration of native plant communities, wildlife habitat, ecosystem services or water salvage), leading to a long list of outcomes that have been measured, including not only changes in native species cover and diversity, but also soil salinity, reduction in wildfire, water availability, habitat for animals, geomorphology and others (Shafroth et al. 2005, 2008; Hultine et al. 2010; Sher and Quigley 2013). Meanwhile, critics have charged that Tamarix control is unwarranted or even detrimental to some management goals (Chew 2009). Thus, the Tamarix literature is also an opportunity to consider a wide variety of restoration outcome metrics. Dryland riparian habitat is of critical ecological importance (Smith and Finch 2016) and has been extensively affected by Tamarix invasion in the western United States (Shafroth et al. 2005; Sher and Quigley 2013), so it is of urgent importance to determine whether goals around ecosystem structure and function are being met.

The history of our understanding of Tamarix and the impact of its control reflects the evolving nature of science and public opinion (Suppl. material 1: table S1). In 1998, the first comprehensive review of Tamarix research was published by Di Tomaso (1998), which concluded that any benefits of the species were outweighed by a myriad of costs. Both scientific and media coverage of the invasive tree began growing exponentially (Sher 2013). Friedman et al. (2005) determined that Tamarix had become the second most dominant and third most frequently occurring woody riparian plant in the American Southwest. Documented impacts included that Tamarix could replace native trees under some conditions (Frasier and Johnsen 1991; Friedman et al. 2005) and increase wildfire intensity and frequency (Busch and Smith 1995), amongst other negative effects including high water use (Sala et al. 1996) and unsuitable habitat for wildlife (Hunter et al. 1988). Zavaleta (2000) estimated that Tamarix was costing 127-291 million USD annually due to loss of water for irrigation, municipal use and hydropower and also by increasing overbank flooding. Spurred by water shortages and wildfires in the early 2000s, there was a burst in funding and policy promoting Tamarix control (Carlson 2013). However, it was eventually established that early estimates of water use were flawed; Tamarix did not consume more water than other riparian tree species, such as *Populus* spp. (cottonwoods) and *Salix* spp. (willows) and its control did not predictably or sustainably result in more water for anthropogenic needs (Shafroth et al. 2005; Nagler et al. 2010; Cleverly 2013; Nagler and Glenn 2013).

In the intervening years, there has been a continued effort to reduce *Tamarix* cover along western waterways, but goals and scientific focus have shifted away from water salvage and towards general ecosystem health, ecosystem services and project-specific targets. Specifically, the focus of research on *Tamarix* ecology and management changed to quantifying its impacts on changes in plant and animal communities (Bateman et al. 2010, 2013; Sogge et al. 2013; Strudley and Dalin 2013), soil chemistry (Merritt and Shafroth 2012; Ohrtman and Lair 2013), soil ecology (Meinhardt and Gehring 2013), fire regime (Drus 2013) and river geomorphology (Auerbach et al. 2013). More recently, there has been controversy around the ecological value of *Tamarix* as habitat for the endangered south-western willow flycatcher (*Empidonax trailii extimus*; abbr. SWFL) and potentially other native birds (Sogge et al. 2013). This led to lawsuits against the biological control programme, which resulted in the termination of beetle releases and mandated SWFL habitat conservation (Bean and Dudley 2018).

As a result of these changing paradigms and the controversy surrounding the *Tamarix* system, reviews of the literature have been written regularly (Suppl. material 1: table S2). These reviews have consisted of narrative synthesis of prevailing trends and findings, but to date, a quantitative review has not been conducted on the reported outcomes of *Tamarix* control.

Here, we focus on tracing the effects of *Tamarix* control efforts in recent years (1990s-present), as this period covers much of the recent shift in attitudes and management goals away from *Tamarix* eradication and towards holistic ecosystem perspectives, while older paradigm shifts have been well-documented and are no longer as relevant to ongoing research and management. Our study thus focuses on modern objectives associated with *Tamarix* control (including improved wildlife habitat and increased native plant species cover) rather than past goals, such as streambank stabilisation or water salvage. Overall, monitoring has comprised a range of response variables, temporal and spatial scales and sampling techniques (González et al. 2015, 2017b, c, 2020a, b; Bean and Dudley 2018; Sher et al. 2018, 2020; Henry et al. 2023). Understanding these various components of the ecosystem is a step towards a holistic evaluation of restoration outcomes that will ultimately assess fundamental properties of ecosystems, such as resilience, stability or complexity (Moreno-Mateos et al. 2020).

Assessing a wide variety of properties is a well-established goal of restoration ecology, but comprehensive understanding remains rare (Gann et al. 2019). Various literature synthesis methods have relative benefits and drawbacks; in general, more quantitative methods, such as meta-analysis, allow for more precision in measured outcomes at the expense of sample size, while more qualitative methods, such as narrative reviews, allow for more comprehensive coverage of the entire body of literature at the expense of measurable outcomes (Koricheva and Gurevitch 2014; Haddaway et al. 2015). Some consider a middle ground to be a "vote counting" approach, in which numerical outcomes can be roughly quantified in the absence of reported variances and/or sample sizes.

To cover a range of approaches, we conducted three tiers of literature review with successively more restrictive rules for inclusion: qualitative success ranking, vote counting and meta-analysis. These review methods investigated metrics of response across a range of biotic and abiotic ecosystem components. With this approach, we could synthesise disparate literature sources and identify the broad outcomes of this dominant invasive species. In addition, we identified current knowledge gaps and relatively under-studied dimensions of *Tamarix* control outcomes. In addition, we sought to determine whether increasing granularity of literature review methods would result in biased outcomes.

We predict the following: (1) Tamarix control will broadly show successful biological outcomes within the studied time frame, particularly in terms of (a) reducing Tamarix and (b) promoting increased abundance of native plant species (González et al. 2017a; Sher et al. 2018); (2) Effects on animal communities will be highly varied, as habitat preferences and tolerances have been shown to be species-specific even amongst similar taxa in this system (Bateman et al. 2008, 2013; Mosher and Bateman 2016; Raynor et al. 2017); and (3) We expect that synthesis techniques with more stringent requirements for inclusion of sources (e.g. meta-analysis requiring that each outcome report variance and sample size) might bias results through exclusion of some publications, but it is difficult to predict directionality (better/worse outcomes) of the bias (Gurevitch and Hedges 1999; Koricheva and Gurevitch 2014). Understanding the outcomes of Tamarix control projects on a large scale will provide insight into the current state of the field and will allow researchers and practitioners to make more informed decisions about future projects, both in terms of desired outcomes and strategies for monitoring and reporting data. While Tamarix invasion has been most severe and long-lasting in the western United States, species in the genus have been reported as invasive across the globe, including in South Africa, Argentina, Australia, the Mediterranean Region and the Pacific Islands (Rejmánek and Richardson 2013) and biological control programmes have been proposed in Argentina (Mc Kay et al. 2018) and South Africa (ultimately rejected due to insufficient host specificity; Marlin et al. (2017, 2019)). Synthesis of empirical data on *Tamarix* control outcomes in the United States can, thus, provide a better basis for decision-making in areas where similar control attempts may occur in the future. More broadly, it is beneficial to the discipline of ecological restoration to detect whether the changing paradigms of the field are being reflected in evaluation.

Methods

Literature search and data collection

First, we separately conducted systematic reviews of the literature with the goal of finding all published primary sources on ecological outcomes of *Tamarix* control in the American Southwest. We conducted a literature search in October 2019 using the following search terms: "(*Tamarix* or tamarisk* or saltcedar) and (restor* or remov* or biocontrol or *Diorhabda*) and (river or riparian or floodplain or stream)", filtered by "Article" in Web of Science. To provide a second set of starting sources (specifically seeking non-journal sources in addition to journal sources), we then conducted a search in March 2020 using the following search terms: (tamarisk or *Tamarix* or "salt cedar" or saltcedar) and (remov* or (invasive* and (control* or manag*))), filtered by "Article" in the following databases: Aquatic Sciences and Fisheries Abstracts, ProQuest Agricultural and Environmental Science Collection, Academic Search Complete, Biological Abstracts, GreenFILE and Web of Science Core Collection. The March 2020
search yielded an initial total of 1,320 articles, the October 2019 search yielded 266 and the February 2021 search yielded 42. In addition, we manually added 15 sources not found in the literature searches, based on professional judgement of their fit with the goals of the study and finally conducted another identical database search in February 2021 to identify newly-published literature since the March 2020 search; four new sources were added as a result of this search. Peer-reviewed published articles, doctoral dissertations and government reports were ultimately included as sources.

Initial filtering (duplicates, title and abstract relevance) happened separately for each search. All filtering at this stage was based on the same criteria; sources were included if they investigated some aspect of an ecological outcome of active or biological control of *Tamarix* spp. in North America, which automatically also narrowed the papers to only those with a reported focus on T. ramosissima and/or T. chinensis and its hybrids. We first filtered out duplicate sources in each search, then filtered the resulting list based on title; papers excluded in this first round were mostly concerned with other aspects of Tamarix biology and ecology not related to control. The March 2020 search had a high number of duplicate sources (n = 758) due to searching multiple databases. Very few papers (n = 9) were excluded solely due to research taking place outside North America: other research conducted outside North America studied Tamarix in its native range or was not related to ecology. Following removal of duplicates between the two searches, this step yielded a total of 109 sources that we read in their entirety, subsequently filtered to 81, based on full text content. Papers were excluded at this stage if they did not address intentional anthropogenic treatment of *Tamarix* or only involved greenhouse studies without in situ field data. Papers from the final filtering stage were combined with the sources we manually selected, for a final sample size of 96 sources (Fig. 1A). We then conducted the three tiers of analysis, with increasing restrictions, based on what quantitative measures were included (Fig. 1B). This was done also to investigate the hypothesis that a traditional meta-analysis could bias the findings, a common criticism of a strict quantitative approach (Gurevitch and Hedges 1999; Koricheva and Gurevitch 2014; Haddaway et al. 2015; Westgate and Lindenmayer 2017; Lilian et al. 2021).

For inclusion in any of the three tiers of analysis, these papers needed to explicitly address active control of *Tamarix* and/or biocontrol and include some measure of the effect of treatment on an ecosystem component (either measurement before and after treatment ["BA"] or a control group compared to an impact group ["CI"]); of these, 96 papers were ultimately selected for use in tier 1: tracing *Tamarix* control evaluation, 63 for tier 2: qualitative vote count and 52 for tier 3: meta-analysis. Criteria for including or excluding papers for tiers 2 and 3 are described in detail below. While searches included multiple databases, all papers ultimately selected (including dissertations and agency reports) were catalogued in Web of Science. Refer to Suppl. material 2 for a complete list of publications.

For each paper, we recorded sampling location data (river basin; Upper Colorado River Basin, Lower Colorado River Basin, Rio Grande River Basin and Humboldt River Basin), study design, control and/or restoration actions (using definitions outlined



Figure I. A summary of searches and source filtering B summary of the three tiers of analysis.

in González et al. (2017b); Table 1), response variables (Table 2) and study duration (first year of restoration, last year of restoration, first year of monitoring and last year of monitoring; Morandi et al. (2014); González et al. (2015)). Each measure of an ecosystem component (e.g. native plant species richness following biocontrol) was recorded separately as a row of data. Within a paper, we designated separate "case IDs", based on whether multiple rows of data were independent replicates of each other; for example, if a source reported multiple results of the same test in two separate sites, they would be considered distinct cases, whereas multiple replicates at one site would be designated as the same case.

Tier I: Tracing Tamarix evaluation through qualitative success ranking

Tracing trends in the literature is important to understand how priorities and approaches have changed over time and to identify knowledge gaps (Dufour et al. 2019), particularly since restoration ecology is a relatively young discipline and its protocols are still subject to further development (Hobbs 2018). It also has fewer constraints than quantitative analysis, allowing us to include all publications that investigated response to restoration in areas with *Tamarix* invasion (n 96; Fig. 1). We summarised basic trends in study foci over time and conducted a qualitative success ranking based on publication abstracts to assess messages in the literature at coarser resolution.

For each selected source, restoration treatments and measured ecosystem component responses to restoration were categorised (e.g. plants, water, invertebrates) to

Category	Restoration action
Primary control methods	Mechanical treatment with heavy machinery
	Cut-stump with herbicide
	Cutting, no herbicide
	Herbicide only
	Biological control
	Burning
Secondary methods/follow-up treatments	Environmental water introduction (deliberate flooding)
	Dead biomass removal
	Dead biomass chipping/mulching
	Dead biomass burning
	Regrading channels and floodplains
	Follow-up herbicide application
	Active revegetation

Table 1. Summary of restoration actions for control of invasive Tamarix spp. considered in the dataset.

Table 2. Hierarchical ecosystem component categories as considered by both the vote count and metaanalysis in published papers that measured a variety of responses to the control of invasive *Tamarix* spp. Categories are broadly patterned after Gann et al. (2019), but adapted based on what was reported in the literature. Vegetation is sub-categorised by growth habit (either overstorey or understorey). Growth habit was often not explicitly stated in the publications; determinations were made, based on classification by USDA PLANTS (USDA, NRCS 2024). In some cases, studies reported data on tree seedlings; these were coded as understorey species. "Understorey + overstorey" denotes cases where a specific indicator could feasibly consist of either ground or canopy vegetation or both (for instance, *Tamarix* cover often extends continuously to ground level). In analyses, the "understorey + overstorey" category consists of all applicable vegetation indicators. Where Category 3 is "NA", there was insufficient replication within Category 2 to further divide groups of indicators.

Category 1	Category 2	Category 3
Biotic	Vegetation	Understorey
		Overstorey
		Understorey + overstorey
	Fauna	Avifauna
		Herpetofauna
		Invertebrates
	Soil organisms	NA
Abiotic	Soil physio-chemical properties	NA
	Water (e.g. evapotranspiration, river flow rate)	NA
	Climate (e.g. site temperature)	NA
	Fire	NA
	Geomorphic	NA

determine relative numbers of different abiotic and biotic characteristics addressed in restoration studies (Table 2). For the purpose of this study, we considered "restoration treatments" to include all types of intentional control of invasive *Tamarix* (e.g. chemical, mechanical, biological control) as well as follow-up actions, such as revegetation of desirable species or follow-up herbicide application. Categories were adapted from

those described in Gann et al. (2019), Ruiz-Jaen and Aide (2005), Morandi et al. (2014) and González et al. (2015) and combined where necessary.

We then conducted a qualitative review of "success" of Tamarix control reported in the abstracts of each of the 96 papers, using a scale of 1 to 5, plus a category for those that appeared inconclusive (Table 3). These scores were intended to reflect the degree of positive result from restoration, as indicated by the papers themselves, such as whether outcomes were consistent with goals (e.g. reduction in invasive species abundance or increase in desirable species abundance). In other words, the published opinion of "success" could have been supported with numerical data or simply reported as a subjective observation for this tier of our literature review. Each paper was assigned a numerical value of "success" by averaging scores assigned to it by four of the authors (AG, EG, AS, AH) in a blind review (i.e. scorers did not view each other's scores until afterwards). Most papers were already familiar to us; due to time constraints, we made our success categorisations based primarily on the abstracts, but reviewed the discussion/conclusions and introduction if needed for additional context. If scorers disagreed on whether a paper was inconclusive or not, the majority opinion was accepted and, in the case of a tie, we revisited the abstract and made a decision. To help detect any bias in paper selection, based on the three tiers of analysis, we also compared the breakdown of success rankings amongst the three pools of papers (i.e. all 96 papers evaluated in tracing, the subset of 63 papers evaluated in the vote count and the subset of 52 papers evaluated in the meta-analysis). We averaged success rankings by scorer for each paper and counted the number of papers whose average ranking fell within each range.

Tier 2:Vote counting

For identifying quantitative directional trends with the largest possible sample size, we then conducted vote counts of general outcomes for the 63 sources that included quantitative measures of impact of *Tamarix* control (Fig. 1). All sources included in

Table 3. Success rankings. Success was considered in relation to the stated goals of the study (if present); for instance, a bird-focused study which found bird populations to decline following *Tamarix* control (primary goal) was considered a partial failure even if there was a large reduction in invasive cover (failure to increase bird abundance mitigated by success in reducing invasive plant cover) (secondary goal). If a goal was not explicitly stated, we assumed success, based on established goals of *Tamarix* control projects; higher native plant and animal abundance, as well as overall higher species diversity, is desired, while higher invasive plant abundance is not desired (Shafroth et al. 2008).

Score	Success ranking	Description
5	Clear success	Positive message
4	Partial success	Positive message overall with some qualifiers
3	Neutral	No effect or equal negative and positive effects
2	Partial failure	Negative message overall, but some positive, including predicted positive outcomes
1	Clear failure	Negative message
NA	Inconclusive	Paper focuses on methods instead of ecological outcomes or does not have a clear
		message (as opposed to a neutral message)

the vote counts were also included in the success ranking section. Response metrics differed in terms of desirability (e.g. sources may report changes in *Tamarix* abundance as well as abundance of native species). As our goal was to make direct comparisons amongst all methods, it was necessary to standardise the directionality to always refer to desired outcomes. Therefore, each row of data was designated either desirable, undesirable or neutral for the purpose of calculating effect sizes with consistent directionality (i.e. the dependent variable in all cases is "improvement," which could consist of either reduction in undesirable environmental characteristics or increase in desirable characteristics). Desirability was categorised on the basis of stated goals of each project and the general assumption that higher native plant and animal abundance, as well as overall higher species diversity, is desired, while higher invasive plant abundance is not desired (Shafroth et al. 2008). Thus, we counted non-noxious exotic species (as defined by USDA PLANTS; USDA, NRCS (2024)) as desirable since they contribute to biodiversity. Data reported in graph form were digitised manually to the highest possible accuracy using GraphGrabber v.2.02 (Quintessa Inc.). All records were checked by someone other than the coder at least once.

The first vote count tallied all outcomes of *Tamarix* control that were reported as statistically significant (n = 622 outcomes). As not all cases within a paper reported results of an associated statistical test (for example, if results simply showed a list of before and after values for cover of multiple species), we then conducted a separate vote count across the entire dataset, regardless of significance (n = 1,460 outcomes). All ecosystem components were assigned a vote count value, based on whether the response variable significantly increased, decreased or did not significantly change over time (for before-after comparisons; abbr. BA) or between the control and impact groups (abbr. CI). When a case was reported as a BACI design (Before-After-Control-Impact; reporting before-after data for both the control group and the treatment group), we split it into separate BA and CI cases (or rows in the database). We also recorded effect size regardless of statistical significance. We then calculated relative percentages of increased/decreased/no change metrics for each possible combination of restoration treatment and ecosystem component.

Tier 3: Meta-analysis

Finally, we conducted a meta-analysis to statistically test the hypothesis that *Tamarix* control activities resulted in positive outcomes (Shafroth et al. 2008), as measured in terms of various biotic and abiotic factors (n = 52, Tables 1, 2). Mean, sample size and variance were required for a measurement to be included in the meta-analysis, making it the synthesis method with the most stringent requirements. We used the metafor package in R (Viechtbauer 2010) to calculate effect sizes of each case, represented as standardised mean differences (Viechtbauer 2010). To standardise directionality of metrics, based on desirability (e.g. increases in native species cover were considered desirable, but so were decreases in invasive species cover), we multiplied the effect size by -1 when a response metric was considered undesirable and response metrics desig-

nated as neutral were excluded from analysis. We added a small constant (0.000001) to all standard deviations in order to allow for calculation of effect sizes in cases where the variance was zero. We then constructed separate multi-level error models for each possible combination of restoration actions and ecosystem components (e.g. all vegetation responses to biocontrol). A restoration action was included in each model as a moderator and the effect size of each action on each ecosystem component was used as a dependent variable. Case ID, nested within paper ID (unique identifier for each paper), was included as a random effect in all models. In many cases, there was insufficient replication (fewer than three replicates) to subset the data by a given combination of response metric and restoration action; we did not report an effect size for these subsets. In addition, some categories (e.g. fauna subcategories) were combined to improve replication, as sample sizes were lower in the meta-analysis than in the vote counts.

In addition to calculating effect sizes by treatment and response variable, we conducted a sub-study using all restoration actions and vegetation (the most well-represented ecosystem component) divided by desirability category (desirable, *Tamarix* and undesirable other than *Tamarix*) and growth habit (understorey, overstorey, both). We also examined the impact of temporal scale on vegetation outcomes, using the following metrics for elapsed time: (1) number of years between end of treatment and start of monitoring; (2) number of years between end of treatment and end of monitoring.

We tested effects of various characteristics of restoration projects (duration and geographic location, by river basin) to determine whether they affect the effect sizes. In some cases, there were few to no between-paper replicates of a specific restoration action/response metric combination; we report both number of papers addressing each metric and number of discrete measurements of each restoration action/ecosystem component combination. We also tested for funnel plot asymmetry using Egger's test (Egger et al. 1997) and calculated fail-safe N values using the Rosenthal method (Rosenthal 1979; Orwin 1983) for each model to determine whether significant results were being influenced by insufficient sample sizes (Viechtbauer 2010). Three data points were excluded from analysis due to extremely high variance.

All analyses were conducted in R version 4.1.1 with RStudio version 2022.02.03 using the following functions from the metafor package: "escalc" (calculates effect sizes from means, SDs, Ns), "rma.mv" (mixed model calculation), "fsn" (calculates fail-safe N value) and "funnel" (creates funnel plots to visualise asymmetry; Viechtbauer (2010)).

Results

Tier I: Tracing monitoring and evaluation

Publication trends by year

The bulk of papers on the effect(s) of *Tamarix* control were published between 2011 and 2020 and the largest number (10) were published in 2017. However, there were no clear directional trends over time. Most of the papers included in

our analysis focused on vegetation metrics (78% of reported outcomes were on vegetation; Suppl. material 1: table S3), with particular years featuring more measurements of fauna (2015) or abiotic responses (2017). There were transitions over time with regard to treatment methods, with the cut stump method appearing less often, as heavy machinery and biocontrol became more common around 2011 and, ultimately, were the two best-represented treatment methods investigated (Suppl. material 1: table S4). However, it is notable that, like other measures, publications reporting response to biocontrol did not increase over time, instead having peaks in 2011 and 2020.

Success rankings

Our success rankings showed that outcomes reported in paper abstracts were, on average, slightly positive, i.e. between "neutral" (no effects or some positive effects on some components compensate negative effects on others) and "partial success" (the message is positive, but there is a "however"); mean = 3.61; median = 3.75; SD = 0.99. Similarly, the counts of averaged success rankings show a high proportion of abstracts reporting partially successful outcomes (Fig. 2). Agreement between scorers (AG, AS, EG, AH) was high; we had perfect agreement on 29 of 96 papers and near-perfect agreement (three out of four scorers agreed and the fourth ranking was an adjacent value) on 45 of 96 papers. We did not find a difference amongst the distributions of success rankings across the full 96 papers, the 63-paper vote count subset and the 52-paper meta-analysis subset (ANOVA: F = 1.23, p = 0.30, df = 2).

Tier 2:Vote count

The vote counts found that most vegetation responses to *Tamarix* control efforts showed more positive than negative outcomes (Fig. 3; blue predominated in stacked bar charts of first row), but "no change" predominated when only examining reported statistically significant changes (Fig. 4; grey predominated in stacked bar charts of first row). Sample size of fauna outcomes in response to *Tamarix* control efforts was very low (two or fewer publications per combination of treatment method and response metric), but showed relatively high numbers of negative outcomes; birds showed the most negative outcomes (negatively affected by biocontrol and cut-stump with herbicide; Figs 3, 4, second row) and herpetofauna were negatively affected by biocontrol in all cases (statistically significantly in half of cases), but showed generally positive or neutral outcomes from other treatment methods. Abiotic results were mixed; there were more positive "water" outcomes (primarily reductions in evapotranspiration) than negative (Fig. 3, rows 7–12), but more negative hydrological outcomes, and geomorphic outcomes were almost entirely value-neutral (Figs 3, 4, final two rows).

The vegetation-only vote count on differences regardless of statistical significance showed broadly that *Tamarix* cover was reduced in most cases and non-*Tamarix* undesirable vegetation was heavily reduced in the overstorey, but not the understorey



Figure 2. Distribution showing counts of averages of scorers' rankings for "success" of projects as inferred from language in each publication abstract. "Success" was considered in relation to the stated goals of the study (if present). Outcomes described in papers were considered "inconclusive" if the majority of scorers reported that the authors of the paper discussed *Tamarix* control, but focused on methods rather than outcomes. n = 96. See Table 3 for definitions of success categories.



Figure 3. Summary of vote counting by treatment method used to control *Tamarix*, based on whether any change at all was reported in the publication. Each cell represents a combination of the listed treatment method and response variable. Bars represent the numbers of desirable outcomes (shown in blue), undesirable outcomes (red), neutral outcomes (dark grey) and no-change (light grey). Width reflects sample size, with number of observations (number of papers in parentheses) reported in each cell.



Figure 4. Summary of vote counting by treatment method used to control *Tamarix*, only if change was reported as statistically significant by the published source. Each cell represents a combination of the listed treatment method and response variable. Bars represent the numbers of desirable outcomes (shown in blue), undesirable outcomes (red), neutral outcomes (dark grey) and no-change (light grey). Width reflects sample size, with number of observations (number of papers in parentheses) reported in each cell.

(Fig. 5). Response of desirable vegetation was mixed, with slightly better outcomes in the overstorey than the understorey (Fig. 5). The vegetation-specific vote count on significant differences found that "no change" was very common, but reduction in overall *Tamarix* metrics and non-*Tamarix* invasive overstorey species were seen in most cases (Fig. 6). Changes in desirable vegetation were mixed, but there were more positive than negative outcomes of total desirable vegetation cases.

Tier 3: Meta-analysis

Total sample size for the meta-analysis was 777 outcomes within 52 publications. The overall model without considering any moderator was heterogeneous (Q(df = 771) = 9,238 p < 0.0001)) and there was a significant, but small positive effect of *Tamarix* control (estimated effect size = 0.5465, SE = 0.2732, Z = 2.0002, p = 0.045). The fail-safe N calculation on effect sizes via the Rosenthal method was significant (p < 0.0001), with a fail-safe N of 409,193.



Figure 5. Summary of vote counting by vegetation types, change in vegetation in response to *Tamarix* control efforts regardless of reported statistical significance in the published paper. Each cell represents a combination of invasive classification (desirable/undesirable/total) and growth habit (overstorey/understorey/both). Bars represent the numbers of desirable outcomes (shown in blue), undesirable outcomes (red), neutral outcomes (dark grey) and no-change (light grey). Width reflects sample size, with number of observations (number of papers in parentheses) reported in each cell. Note that "overstorey" and "understorey" sample sizes do not add up to "overstorey + understorey" sample sizes, as response variables were not always reported as specific overstorey/understorey metrics.

By treatment method

Most of the significant effects of treatments on ecosystem components were positive (treatments were associated with more desirable outcomes). Restoration treatments were broadly seen to either decrease cover of undesirable plant species, increase cover of desirable plant species or have no effect; herbicide had the highest significant positive effect on desirable outcomes. Amongst the 19 response variables, we found the effect sizes of six to be significantly different from zero, including the following combinations with treatments: biocontrol, cut-stump with herbicide, herbicide and cutting were associated with positive vegetation outcomes (biocontrol: est. = 0.3985, Z = 1.98, p < 0.05; cut-stump: est. = 0.26, Z = 2.12, p < 0.05, herbicide: est. = 1.30, Z = 3.70, p < 0.001; cutting: est. = 0.20, Z = 4.72, p < 0.0001), cut-stump treatment was associated with negative fire outcomes (est. = -0.333, Z = -3.44, p < 0.001; Fig. 7). Some response variable categories are condensed in the meta-analysis relative to the vote counts due to lower sample sizes (e.g. while the vote counts differentiate between birds, fish, mammals and herpetofauna, the meta-analysis combines all fauna).



Figure 6. Summary of vote counting by vegetation types, only if change in vegetation type in response to *Tamarix* control effort was reported as statistically significant by the published source. Each cell represents a combination of invasive classification (desirable/undesirable/total) and growth habit (overstorey/understorey/both). Bars represent the numbers of desirable outcomes (shown in blue), undesirable outcomes (red), neutral outcomes (dark grey) and no-change (light grey). Widths reflect sample size, with number of observations (number of papers in parentheses) reported in each cell. Note that "overstorey" and "understorey" sample sizes do not add up to "overstorey + understorey" sample sizes, as response variables were not always reported as specific overstorey/understorey metrics.

Vegetation by growth habit and category

Total vegetation (including desirable, *Tamarix* and other undesirable; Fig. 8) showed statistically significant positive responses to treatment across growth habits (overstorey+understorey: Z = 2.40, p < 0.05, k = 607; overstorey: Z = 2.19, p < 0.05, k = 97; understorey: Z = 2.35, $p \le 0.05$, k = 191). However, this result appears to be primarily driven by the significant response of *Tamarix* reduction in the overstorey+understorey (Z = 3.02, p < 0.01, k = 275), as cover of desirable and non-*Tamarix* undesirable vegetation was not observed to change significantly as a result of *Tamarix* control efforts.

River basin

We did not find a significant difference amongst river basins when looking at all metrics, all vegetation metrics or desirable vegetation; however, we found that the Upper Colorado River Basin had significantly greater *Tamarix* reduction than any other Basin (intercept = 2.1231, Z = 2.68, k = 275, p < 0.01).



Figure 7. Summary of quantitative meta-analysis examining responses of multiple ecosystem components to control of *Tamarix* by multiple methods as reported in published papers. Dots represent the effect size estimate, calculated as the standardised mean difference. Horizontal lines represent 95% confidence intervals and vertical dotted lines denote zero. Asterisks next to dots indicate statistical significance; sample sizes are shown next to dots with number of studies reported in parentheses. Blue dots represent significantly positive effect sizes and red dots represent significantly negative effect sizes.



Figure 8. Summary of quantitative meta-analysis of vegetation-only data from published sources (for all treatment methods used to control *Tamarix*) by vegetation types (understorey/overstorey/both, desirable/undesirable/all). Dots represent the effect size estimate, calculated as the standardised mean difference. Horizontal lines represent 95% confidence intervals and vertical dotted lines denote zero. Asterisks next to dots indicate statistical significance; sample sizes are shown next to dots with number of studies reported in parentheses. Blue dots represent significantly positive effect sizes.

Vegetation by time elapsed

The average elapsed time was small across cases; the mean of end of treatment – start of monitoring was -1.66 years (due to the prevalence of Before-After study design, monitoring often started concurrently with treatment or before treatment) and the mean of

end of treatment – end of monitoring was 0.76 years. We found a statistically significant negative relationship between overall vegetation cover and time elapsed between the end of treatment and the start of monitoring (estimate = -0.3213, Z = -11.4755, k = 552, p < 0.0001). We found a significantly positive relationship between understorey vegetation cover and time elapsed between the end of treatment and the end of monitoring (estimate = 0.15, Z = 2.6667, k = 179, p < 0.01). Elapsed time did not significantly explain outcomes in any other subset of vegetation metrics.

Discussion

Effects of Tamarix control on vegetation

Our results indicate that while *Tamarix* is successfully reduced by control efforts, other ecosystem components are less clearly affected and, in some cases, are negatively impacted. When examining all vegetation metrics, we see a generally positive effect that is mostly being driven by reduction of *Tamarix* in both the understorey and overstorey. While most effect sizes for native vegetation metrics were non-significant (and all were small in magnitude, with high variance), all were positive. This is consistent with previous research showing that increases in native cover following *Tamarix* control and related restoration actions are often very slow and small (e.g. González et al. (2017b); Goetz et al. (2022); but see Sher et al. (2018)). In addition, previous meta-analyses in other systems have similarly found that control of a dominant invader does not necessarily improve the condition of the native plant community (Thomas and Reid 2007; Kettenring and Adams 2011).

The negative effect of time on total combined vegetation metrics was likely a function of the short time elapsed between the end of treatment and the start of monitoring. First, due to the disturbance inherent in restoration treatments, indirect outcomes associated with native species are likely to worsen before they can improve (González et al. 2017b). Second, the effect of time is driven, in part, by short-term decreases in undesirable species cover directly following treatment; later, more subtle changes in community composition are, thus, "worse" than the initial major improvement. In addition, many of the studies used a Before-After comparison, meaning treatment may have started at the same time as monitoring; this may obscure underlying mechanisms. Likewise, understorey metrics had a positive relationship with time, likely due to a peak in undesirable pioneer species directly after disturbance followed by the longer-term establishment of more functionally diverse native vegetation (González et al. 2017a). However, it is important to note that the time frame was still relatively short, with an average of 0.8 years between the end of treatment and the end of monitoring. Many changes in the plant community were missed in the absence of long-term monitoring and many of the reported end states of restoration projects may not be indicative of the ecosystem's broader trajectory; this is a major limitation somewhat inherent to the field, despite frequent recommendations to engage in longer-term monitoring (e.g. Gann et al. (2019)). Indeed, it is possible that, at this timescale, we are only able to observe initial response to treatment itself rather than long-term ecological change following removal of an invader. More recent published studies in this system have incorporated longer-term monitoring (e.g. González et al. (2020a, b); Henry et al. (2023)); we suggest that future work continue to examine long-term ecosystem trajectories following *Tamarix* control and follow-up on past projects to determine whether there has been sufficient time to see the anticipated effects of restoration efforts.

We did not observe a significant effect of geographical location (in terms of river basin) on any vegetation outcomes other than *Tamarix* reduction itself. This suggests that, despite differences in environmental conditions (Sher et al. 2020) and dominant treatment strategies (González et al. 2017b) amongst river basins, variation in outcomes is driven by other factors. The greater reduction of *Tamarix* in the Upper Colorado River Basin may have been caused, in part by greater incidence of the biocontrol beetle, particularly during earlier study years (Bean and Dudley 2018). Given that degree of defoliation (especially over time) and indirect responses to biocontrol have been mixed (González et al. 2017c, 2020b; Sher et al. 2018; Henry et al. 2021), this result is perhaps unsurprising. A lack of large-scale geographic effects on indirect outcomes (e.g. native vegetation, wildlife) may also highlight the need for careful project strategy at a local scale (Shafroth and Briggs 2008); it is likely that small-scale variation within river basins and, crucially, the human decisions around restoration planning (Sher et al. 2020) play a large role in determining whether common restoration goals are met.

Effects of Tamarix control on fauna

We found some evidence to suggest that wildlife may be negatively impacted by *Tamarix* control in the aggregate, but it was difficult to elucidate trends due to low replication and lack of a comprehensive body of literature across taxa. Though the metaanalysis did not show any significant relationships between fauna and *Tamarix* control, the vote count found that birds were negatively affected by biocontrol and cut-stump treatments in most reported cases, while herpetofauna were negatively affected by biocontrol in all reported cases. but positively impacted by other treatment methods in most cases. However, this was likely influenced by the low sample sizes, both in terms of outcomes and publications. This synthesis of the literature does show some support for concerns surrounding the effects of *Tamarix* control on wildlife (e.g. Bateman et al. (2014); Raynor et al. (2017)), but mainly indicates that more research is required before a clear consensus can be reached.

Methodological effects on study outcomes

Our results found meta-analysis to be an effective technique for synthesising the literature on control of a well-studied plant invader. In conducting meta-analysis, restrictions on which types of studies can be included have the risk of biasing the results relative to more comprehensive strategies like narrative reviews. In our experience, the specific and stringent requirements for inclusion in meta-analysis (reporting of effect sizes, variance and sample sizes) tend to exclude "grey" literature, older publications and publications that use multivariate modelling techniques for data analysis. Conversely, qualitative tracing and vote counting may offer a greater sample size in terms of publications (nearly half of the publications used for tracing were excluded from meta-analysis), but with a lower strength of evidence due to more assumptions and less granularity. Success rankings involved human interpretation of each publication's overall "message," which applied to each publication as a whole rather than in terms of individual comparisons. By this metric, the most common outcomes of papers were "partial success" and "clear success," respectively. Likewise, as synthesis techniques became more granular, the precision of our findings increased, but it became more difficult to make generalised claims about outcomes; the transition from vote counting "any" effect to statistically significant effects only greatly increased the number of "no change" outcomes and we found few statistically significant effect sizes in the meta-analysis.

Our results do not show evidence for inherent publication bias in meta-analysis; if publications were excluded, based on the meta-analysis requirements in a truly biased manner, we would expect more discrepancy between meta-analysis and other review techniques than was observed. Success rankings, based on language used in publication abstracts, did show some bias in favour of positive outcomes, but this is more likely a result of authors "putting a positive face" on their work than publications with negative outcomes being left out of other analyses; there was not a significant difference in success rankings amongst papers included only in tracing, tracing + vote count or tracing + vote count + meta-analysis. The tendency towards relatively optimistic language in publication abstracts may also be related to issues surrounding a common lack of clearly stated a priori goals and objectives in restoration projects (Bernhardt et al. 2005; Palmer et al. 2005; Brudvig and Catano 2021). In addition, some projects had stated or implicit goals relating solely or largely to reduction in *Tamarix* abundance, so it was more likely that these would show "clear success" than a project with more varied goals surrounding indirect responses of other ecosystem components. This is consistent with a previous finding that river restoration outcomes are often reported more optimistically than is accurate, partially due to vague goals and objectives (Jähnig et al. 2011). The relative lack of observed publication bias may be due, in part, to the fact that we focused on one study system rather than attempting to synthesise responses to a conceptual hypothesis across systems (see Gurevitch and Hedges (1999); Koricheva and Gurevitch (2014)). We also did not find evidence that the "file drawer effect" (tendency for negative results to remain unpublished) affected our conclusions, based on our calculated fail-safe number.

Many combinations of treatment methods and ecosystem components, including all combinations showing significant negative relationships, had very low replication in terms of both cases and publications. As a result, for many ecosystem component/treatment combinations, our conclusions are essentially the same as the primary sources themselves. A particular artefact of limitations in paper selection for the metaanalysis is that, while several sources have stated that *Tamarix* control reduces fire risk and severity assuming dead *Tamarix* is not left in the system (Drus 2013), the only fire metrics used in our analysis came from a single paper that found the opposite (Drus et al. 2013). In the vote count, we saw very different results between metrics reported to have significant change versus any change at all; with the requirement of reported significance, "no change" was often the most common outcome. This was consistent with the high variance we saw in the meta-analysis and speaks to the importance of reporting statistical significance of results. Of the total 1,461 cases used in the vote count, 837 did not have statistical significance reported. In addition, several newer papers conduct multivariate modelling and do not report group means and variances; these also had to be excluded from our meta-analysis. Further, many sources do not report numerical data in a way that allows for meta-analysis; for this reason, our quantitative results do not necessarily reflect the entire body of literature on Tamarix control (especially given that, despite our efforts to include non-journal sources, we were unable to access many government reports through our searches). Many sources did not report sample sizes or applicable measures of variance. Our conclusions regarding issues with monitoring and reporting are consistent with many prior review papers on this topic (e.g. Kettenring and Adams (2011); Wortley et al. (2013); Morandi et al. (2014); Ruiz-Jaen and Aide (2005); González et al. (2015); Dufour et al. (2019)), all of which found that the scope of monitoring is often limited in scale, time and breadth of ecosystem components. The limited scope of monitoring is also linked with underlying issues regarding a lack of explicitly stated goals and objectives of restoration projects. Despite consistent recommendations for clearer goal-setting in riparian restoration (Landers 1997; Bernhardt et al. 2005; Palmer et al. 2005; Shafroth et al. 2008; Mcdonald et al. 2016; Gann et al. 2019), unclear goals remain a common criticism of recent projects (Kroll et al. 2019; Brudvig and Catano 2021).

The issue of lingering uncertainty is by no means limited to our study system; the field of restoration ecology remains young and norms continue to be established regarding monitoring and evaluation, with implementation limited by logistical constraints (Kettenring and Adams 2011; Gann et al. 2019). Regardless, we urge practitioners and scientists working in this field to consider under-studied aspects of the ecosystem, to report data that meet the standards for meta-analysis and to better enable the science of monitoring by defining clear baselines, goals and expectations for projects. Prior work has found that there is successful information exchange between science and practice regarding best approaches to *Tamarix* control (Clark et al. 2019), indicating a positive trajectory for better understanding of broad-scale outcomes. The philosophical shift away from a single-species approach and towards one that encompasses the entire ecosystem has been an important development in this field and is indicative of overall directions in restoration ecology, but monitoring of restored systems has typically fallen short of addressing whether control measures have contributed to whole-ecosystem success.

Conclusions

Tamarix control has been a priority for managers and an object of debate for regional scientists for many years, but uncertainty remains regarding broad-scale conclusions of its impact on the entire ecosystem. Due to changing paradigms in how *Tamarix* is considered in the context of the ecosystem, several important shifts in focus took place over time and current research remains situated in the context of controversy. Previ-

ous reviews of the *Tamarix* literature (e.g. DiTomaso (1998); Sher and Quigley (2013)) showed a focus on assessing effects of *Tamarix* on the ecosystem, with post-control trajectories remaining somewhat uncertain. Our results indicate that, despite additional research in the intervening years, we are still unable to make broad declarations regarding post-control trajectories and non-target effects. Importantly, this shows that, while the general attitude around *Tamarix* has shifted away from reduction as a main goal and towards a more holistic view of conservation and ecosystem resilience (Sher 2013), our understanding of the study system remains focused primarily on control with relatively little knowledge of indirect impacts on desirable species and ecosystem processes. From a global perspective, our findings indicate that significant reduction in *Tamarix* abundance is certainly possible in invaded areas, but it is unlikely to be a reliable means of promoting overall ecosystem recovery and all planning must be considered within the context of specific, local-scale objectives. Additionally, our findings support the use of meta-analysis as a method for literature synthesis; we did not find evidence of significant bias caused by exclusion of data that did not fit the stricter criteria for inclusion in analysis.

Many aspects of *Tamarix*-invaded riparian ecosystems remain under-researched despite a large body of literature on the topic. Published data on ecosystem components other than vegetation was rare; abiotic conditions were especially under-represented, as were animals other than birds and herpetofauna. We, thus, suggest that future studies consider aspects of the environment beyond the commonly-studied ecosystem components, as it is difficult to draw any conclusions about the effects of *Tamarix* control on anything other than vegetation. Even within the category of vegetation, much of the data collected only focuses on the target species itself.

Future directions

Additional coverage of multiple ecosystem components would allow for better informed land management decisions. For instance, given that the biotic components of riparian ecosystems are highly linked with hydrogeomorphic factors, further knowledge of the impacts of *Tamarix* control on hydrogeomorphic processes could provide information for decisions in areas where increased erosion is likely to occur due to vegetation reduction. In addition, the role of invasive *Tamarix* as both a factor of anthropologic ecosystem change and an ecosystem engineer in its own right (Johnson 2013) provides opportunities to explore fundamental ecological questions around biotic/abiotic feedbacks and interactions, many of which remain unexplored.

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Supplementary material I

Additional summaries of published literature

Authors: Alexander R. B. Goetz, Eduardo González-Sargas, Mayra C. Vidal, Patrick B. Shafroth, Annie L. Henry, Anna A. Sher

Data type: docx

- Explanation note: table S1. Timeline of important events (highlighted in grey) and publications on control, monitoring, and evaluation of *Tamarix* spp. in the American Southwest. Highly cited papers (>200 citations as of November 2023) are listed, as are those that were the first to put forth a new framework for assessing Tamarix ecology or its management. table S2. Summary of prior reviews of the literature on Tamarix spp. control in the American Southwest. "Important findings" are stated answers to research questions or our takeaways regarding major steps or paradigm shifts shown in each review. table S3. Number of measured ecosystem responses to control of invasive Tamarix spp. in the American Southwest as reported in the literature, by publication year and ecosystem response category. N = 1,460 reported outcomes within 63 publications. table S4. Number of measured ecosystem responses to control of invasive Tamarix spp. in the American Southwest as reported in the literature, by publication year and primary treatment method. In this case, "biocontrol" denotes that biological control via Diorhabda spp. was the treatment method evaluated in the study, i.e. only present in the experimental treatments. N = 1,460 reported outcomes within 63 publications.
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- Link: https://doi.org/10.3897/neobiota.91.111628.suppl1

Supplementary material 2

Reviewed publications

Authors: Alexander R. B. Goetz, Eduardo González-Sargas, Mayra C. Vidal, Patrick B. Shafroth, Annie L. Henry, Anna A. Sher

Data type: csv

- Explanation note: List of publications used in of outcomes of control and monitoring of a widespread riparian invader (*Tamarix* spp.), with digital object identifier (DOI) or other identifier listed for each source. Columns 4–6 identify whether a source was used in each tier of analysis (see Methods).
- 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



Effect of residence time on trait evolution in invasive plants: review and meta-analysis

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Abstract

The success of invasive species is often attributed to rapid post-introduction evolution, due to novel selection pressures at the introduced range. However, evolutionary shifts in invasion-promoting traits can also take place within the introduced range over time. Here, we first present a review of the proposed hypotheses regarding the selection pressures and trait divergence along gradients of invasion history and the studies that examined them. In addition, we present the results of a meta-analysis aimed to provide a more general overview of current knowledge on trait evolution with time since introduction. Invasionpromoting traits, including growth, competitive ability and dispersal ability, were proposed to decline in more established populations with a long invasion history due to the attenuation of selection pressures, such as enemy release or interspecific competition, while herbivore defence was suggested to increase. Our meta-analysis results reveal a general indication for the evolution of invasive plants with residence time for most of the studied traits. However, this divergence did not have a consistent direction in most traits, except for growth, which, in contrast with our prediction, increased with residence time. The lack of empirical support for the predicted change in most of the studied traits over time suggests trait evolution might be affected by other context-dependent factors such as climatic gradients along invasion routes. Similarly, the increased allocation to size in older and more established populations may be driven by increased conspecific competition pressure experienced in these populations. The general temporal effect found in our meta-analysis stresses the need to consider population age when comparing attributes of invasive plants between native and invasive ranges. Moreover, the increased size of invasive plants in older populations, suggests that the dominance of these plants might not attenuate with time since introduction, thus highlighting the need to further explore the long-term dynamics between invasive plants and their recipient native communities.

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Keywords

Chronosequence, enemy release hypothesis, evolution of competitive ability hypothesis, invasive plants, novel weapons hypothesis

Introduction

A main interest in the study of plant invasion is the characterisation of traits associated with invasive success, such as high growth rate, competitive ability and phenotypic plasticity and the processes governing the prevalence of these traits in introduced compared to native ranges (Baker 1965; Thébaud and Simberloff 2001; Vila and Weiner 2004; Blumenthal and Hufbauer 2007; Pyšek and Richardson 2008; Van Kleunen et al. 2010; Hodgins et al. 2018). While traits that determine invasiveness could be preselected for invasion (Schlaepfer et al. 2010), the success of invasive species has often been attributed to rapid post-introduction evolution, due to novel selection pressures at the introduced range, such as release from native enemies and co-evolved competitors (Blossey and Notzold 1995; Sakai et al. 2001; Keane and Crawley 2002; Callaway and Ridenour 2004; Bossdorf et al. 2005; Prentis et al. 2008). Such trait evolution in invasive plants has been examined in ample studies that provided support for evolutionary shifts between native and introduced ranges in traits such as defence, growth, fecundity, competitive ability, allelopathy and phenotypic plasticity (see meta-analyses: Thébaud and Simberloff (2001); Davidson et al. (2011); Felker-Quinn et al. (2013); Rotter and Holeski (2018); Zhang et al. (2018)).

In addition to trait divergence between the native and introduced ranges, evolutionary shifts in invasion-promoting traits can take place within the introduced range over time, due to varying selection pressures that might come into play at different invasion stages (Siemann et al. 2006; Strayer et al. 2006; Lankau 2011; Dostál et al. 2013; Gruntman et al. 2017). For example, while populations at the invasion front can experience release from enemies or co-evolved competitors, this selection pressure is likely to attenuate over time in fully-established populations, where herbivores and pathogens could become increasingly accustomed to the invasive plant (Hawkes 2007; Brändle et al. 2008; Diez et al. 2010; Mitchell et al. 2010; Dostál et al. 2013; Flory and Clay 2013) and intraspecific competition could intensify (Inderjit et al. 2011). Such changes in selection pressures might, in turn, lead to divergence in invasion-promoting traits along gradients of invasion history, with attributes that facilitate greater invasion potential in populations at the invasion front, compared to core populations.

Compared to the evolution of invasive plants between their native and introduced ranges, fewer studies have looked at potential evolutionary shifts within the introduced range. However, studying the idea that invasive plants might undergo rapid selection with time since their introduction might provide a unique model system to explore fundamental questions related to adaptive divergence in plant traits. Moreover, if the adaptive advantage of invasion-promoting traits might decline in more established populations with a long invasion history, this might lead to changes in the effect of such invasive plants on local communities (Strayer et al. 2006; Dostál et al. 2013; Pyšek et al. 2015; Crystal-Ornelas and Lockwood 2020). Hence, knowledge on the potential evolution of invasive populations at different invasion stages might also be valuable for predicting the long-term effects of invasive plants and evaluating alternative management practices (Strayer et al. 2006; Wilson et al. 2007; Bucharova and Van Kleunen 2009).

Studying the evolution of invasion-promoting traits with time since introduction presents a challenge, as knowledge on population ages and residence times might not be readily available. However, despite these difficulties, an increasing number of studies have recently focused on exploring changes in invasion-promoting traits of invasive plants over time (Suppl. material 1: fig. S1). Here, we first present a review of the proposed hypotheses regarding the selection pressures and evolution of trait divergence along gradients of invasion history and the studies that examined them. In addition, we present the results of a meta-analysis aimed at providing a more general overview of current knowledge on trait evolution with time since introduction. In both the review and meta-analysis, the chosen species were defined as "invasive" according to the definition of the CBD, i.e. alien species that threaten ecosystems, habitats or species (CBD 2000).

Review of trait evolution with invasion history

Approaches for studying trait divergence in invasive species over time

Two main approaches have been employed to study changes in invasion-promoting traits over invasion-history gradients, with advantages and drawbacks to both. In the first approach, plant traits are compared amongst different invasive species with varying residence time within a region (e.g. Hawkes (2007); Iacarella et al. (2015); Sheppard and Schurr (2019); Sheppard and Brendel (2021)). Such an interspecific approach can provide a generalised overview across multiple species and requires only general knowledge on residence time of species within a region, such as the earliest report of introduction time (e.g. at the country level). However, results of multi-species experiments might be confounded due to historic biases in the types of introductions (Sheppard and Brendel 2021) or due to discrepancies between the time of introduction to certain regions and the age of the sampled populations (but see Iacarella et al. (2015)). In the second approach, plant traits are compared within a species across different populations along gradients of invasion history (e.g. Lankau et al. (2009); Gruntman et al. (2017); Tabassum and Leishman (2018)). This intraspecific approach requires knowledge on population age (a chronosequence approach) or the identity of source populations and invasion trajectories (i.e. distance to source populations), as well as information on the possibility of multiple introductions. Regardless of the approach used, the study of divergence in invasion-promoting traits is best examined in controlled common garden experiments, which expose plants with varying invasion

histories to the same environmental conditions. Such studies can reveal inherent variations in trait levels and exclude variations due to plastic responses to field conditions at different sites.

Two additional approaches should be noted due to the alternative advantages they offer to the study of trait evolution along invasion-history gradients. The first approach is the use of herbaria collections, which can provide historical samples of invasive plants (reviewed in: Meineke et al. (2018); Lang et al. (2019)). Such collections can be compared at the intraspecific level to identify divergence over time since introduction in physiological and morphological traits, such as herbivore defence compounds or plant size (Zangerl and Berenbaum 2005; Buswell et al. 2011). Herbaria records can, thus, be an important tool in the study of invasion history. The second approach is the use of selection gradients (Lande and Arnold 1983) on invasion-promoting traits, which are measured in the field in introduced populations with different invasion histories. Such field measurements of selection can provide important knowledge on the adaptive relevance of a focal trait in different populations along invasion gradients, but, to date, it has mainly been applied to study variations between native and introduced populations (e.g. Franks et al. (2008); O'Donnell and Pigliucci (2010); Colautti and Lau (2016)). However, as for other studies using field-collected measurements or samples, both approaches cannot exclude plastic responses to field conditions as an explanation for trait variation rather than evolutionary change (excluding herbaria studies that examine the genetic makeup of plants: Vandepitte et al. (2014)).

The following sections provide a review of the hypotheses suggested to explain the effect of time on the evolution of different invasion-promoting traits, including defence, growth, competitive ability and dispersal ability, focusing on studies that examined them under common garden conditions.

Divergence in traits associated with defence

One of the most well-studied hypotheses to explain the success of invasive plants is the evolution of increased competitive ability hypothesis (EICA) (Blossey and Notzold 1995). This hypothesis proposes that plants in their introduced range experience reduced damage by natural enemies, such as pathogens and specialist herbivores, which selects for reduced allocation to defence traits and a consequent increase in resources available for growth and competitive ability (Blossey and Notzold 1995; Bossdorf et al. 2005). Alternatively, the shifting defence hypothesis (SDH) proposes that the loss of specialist enemies at the introduced range can result in an evolutionary shift from investment in defence against specialists (digestibility reducers such as trichomes and tannins) to defence against generalists (toxins such as glucosinolates and alkaloids) (Müller-Schärer et al. 2004; Joshi and Vrieling 2005).

Both the EICA and the SDH assume that invasive plants experience release from their specialist enemies at the introduced range. This attenuating selection pressure is not likely to change with time since introduction, except due to the unintentional introduction of specialist herbivores or pathogens (e.g. Zangerl and Berenbaum (2005); Wan et al. (2019)) or their use as biocontrol agents (Stastny and Sargent 2017). Nevertheless, several studies have shown that enemy pressure on invasive plants increases over time. In particular, more established populations have been shown to experience greater colonisation rates and attacks from local herbivores (Hawkes 2007; Brändle et al. 2008; Dostál et al. 2013; Harvey et al. 2013; Schultheis et al. 2015; Gruntman et al. 2017; but see Carpenter and Cappuccino (2005)), increased pathogen accumulation (Siemann et al. 2006; Hawkes 2007; Mitchell et al. 2010; Flory and Clay 2013) and increased negative soil feedback (Diez et al. 2010). These results imply that compared to range-edge populations, older populations might incur greater attacks even from generalist enemies. Such increased enemy load could be attributed to increases in the density and abundance of invasive plants in older populations, which could increase their attraction as hosts (Agrawal et al. 2006).

Increased enemy pressure with time since introduction might re-select for increased allocation to defence traits in plants and particularly against generalist herbivores (Fig. 1). However, we are aware of only three studies to date that examined divergence in defence-related traits along invasion history gradients under common garden conditions. Gruntman et al. (2017) found that Impatiens glandulifera plants from younger populations within the invasive range have reduced herbivore resistance against a generalist herbivore, coupled with reduced production of secondary defence compounds, to an extent similar to those of native populations. In contrast, Siemann et al. (2006) found no effect of population age on Sapium sebiferum survival in common gardens exposed to local herbivores, even though herbivory levels increased in older populations. Similarly, Harms and Walter (2021) found no effect of population age on herbivore defence against a generalist herbivore in the invasive plant Butomus umbellatus. Additional studies are, therefore, required to provide a general understanding of the effect of invasion history on the evolution of enemy defence in invasive plants. Moreover, such future studies should compare their relative allocation to defence against specialist vs. generalist enemies across populations with different invasion histories.

Divergence in growth traits

Invasive plants are commonly associated with increases in growth rate and size at their introduced compared to native range (Pyšek and Richardson 2008; Van Kleunen et al. 2010). While such increases in growth-related traits can be a result of plastic responses to the environment, they might also be attributed to evolutionary change. The latter idea was proposed in the EICA hypothesis, which predicts that, due to an allocation trade-off, the decrease in defence experienced by plants at the introduced range is likely to result in an evolutionary increased allocation to traits related to growth (Blossey and Notzold 1995; Bossdorf et al. 2005; Joshi and Vrieling 2005). The same defence-growth trade-off might govern the evolution of growth traits within the introduced range of invasive plants. Specifically, if as suggested above, older and more established populations incur more enemy pressure that selects for increased investment in defence, these populations are also predicted to evolve decreased allocation to growth (Fig. 1).



Figure 1. Schematic representation of the predicted effect of population age on divergence in different invasion-promoting traits, including positive effects (light green), negative effects (dark green) and no effect (grey).

We found seven common garden studies that explicitly investigated the effect of invasion history on the evolution of plant growth and their results provide contrasting patterns. Kilkenny and Galloway (2013) and Evans et al. (2013) provide support for the hypothesised negative effect of invasion history on plant size in studies on the invasive plant *Lonicera japonica*, where plants from core populations within the introduced range were smaller than their conspecifics from edge populations. In contrast, Wan et al. (2018) found that *Plantago lanceolata* plants from populations with a long invasion history were larger and more fecund compared to populations at the invasion front and similar results were shown by Fenesi and Botta-Dukát (2012) in *Ambrosia artemisiifolia* and VanWallendael et al. (2018) in *Reynoutria japonica*. Finally, Monty and Mahy (2009) found no effect of population age on biomass production of *Senecio inaequidens* and Tabassum and Leishman (2018) similarly found no effect of distance to source population on the height of *Gladiolus gueinzii*.

A lack of consistent results regarding the effect of invasion history on plant size might reflect the variety of selection pressures that likely act on such a fundamental life-history trait. For example, local climate across elevational and latitudinal gradients, as well as levels of primary productivity, might also change along invasion routes and exert strong selection on plant size and growth rate (Colautti et al. 2009; Monty and Mahy 2009). Thus, although the defence-growth trade-off might be key to the invasive success of many plant species, other factors can contribute to divergence in growth traits within the introduced range.

Divergence in traits associated with competitive ability

Two hypotheses were suggested to account for the evolution of competitive ability in invasive plants and can be similarly applied for divergence in competitive ability within the introduced range. First, as suggested above and following the premise of the EICA

hypothesis, older populations are predicted to undergo selection for increased defence associated with decreased allocation to growth and plant size. This decrease in size is, therefore, likely to be manifested in reduced competitive ability via resource competition in older and more established populations. In contrast, populations at the invasion front are predicted to undergo selection for decreased defence and increased size and competitive ability.

The second hypothesis suggested to explain the evolution of increased competitive ability in invasive plants is the novel weapons hypothesis (NWH: Callaway and Aschehoug (2000); Callaway and Ridenour (2004)). The NWH suggests that the production of toxic allelochemicals should be selected for in introduced populations due to its enhanced negative effects on naïve native competitors compared to co-evolved ones at the native range (Callaway and Ridenour 2004; Prati and Bossdorf 2004; Abhilasha et al. 2008). Hence, this hypothesis proposes increased selection for competitive ability via interference competition in introduced populations. As for plant size and its derived competitive ability, the adaptive advantage of allelopathy might also decline with time since introduction, due to three selection pressures. First, the above-suggested need for increased production of defence compounds in older populations, might favour decreased production of allelochemicals due to a trade-off between these secondary compounds (Inderjit et al. 2011; Gruntman et al. 2017). Secondly, in older and more established populations, plants might experience a shift from competition with heterospecific to conspecific neighbours, which are usually unaffected by conspecific allelochemicals (Inderjit et al. 2011). Thirdly, with time since introduction, co-occurring native species or their mutualist soil biota might also evolve resistance to the novel allelochemicals of the invasive plants (Callaway et al. 2005; Lankau 2011; Dostál et al. 2013).

Competitive ability can be attributed to two components that were suggested to be associated with different traits (Goldberg 1990, 1996). Competitive effect, which is the ability to suppress neighbours, can be attributed to rapid resource acquisition and growth or to allelopathy; while competitive response, which is the ability to withstand competition, can be attributed to tolerance of low resource levels or to neighbour avoidance (Goldberg and Landa 1991; Cahill et al. 2005). Thus, the two aforementioned hypotheses regarding the evolution of decreased competitive ability with time since introduction via allocation to plant size or allelopathy, are mostly related to competitive effect (Fig. 1). In contrast, competitive response might be either not affected by these processes or even increase, if lower growth rate and smaller size correlate with stress tolerance (Fig. 1).

Changes in competitive ability at the introduced range over time were examined in several studies. Some of these studies used an interspecific approach and examined the competitive effect of multiple invasive species with different residence times, using either common garden experiments (Sheppard and Schurr 2019; Sheppard and Brendel 2021) or a meta-analysis approach (Iacarella et al. 2015) and their results reveal different patterns. For example, Sheppard and Brendel (2021) used a common garden experiment to study the competitive effect of 47 non-native Asteraceae species on native plants and found that species with longer residence time had stronger competitive effects. However,

this study referred to plants of varying non-native status rather than strictly invasive species and the variation in their competitive effect was better explained by this status (casual vs. established neophytes, archaeophytes or native species). In contrast, Iacarella et al. (2015) performed a meta-analysis of studies that examined the competitive effect of 36 invasive species with a known residence time at the collection sites and found that competitive effect of the plants decreases with time since introduction. The results of this study provide compelling evidence for temporal shifts in the evolution of competitive ability, because they are based on introduction time of the studied population rather than the entire invaded region. However, such an interspecific approach might be confounded due to variations in competitive ability amongst species, which can be avoided by comparing competitive ability of conspecifics with different residence time.

A few common garden studies used an intraspecific approach and explored divergence in competitive ability amongst populations of the same species across invasion gradients. While some of these studies have attributed competitive ability to growth traits, such as plant height and biomass (see "Divergence in growth traits" above), we found only six studies that have explicitly examined divergence in competitive ability and all compared competitive effects on the performance of neighbours or the production of allelochemicals. Lankau et al. (2009) and Lankau (2012) provide support to the predicted decrease in competitive effect with invasion age, showing that the production of allelochemicals in invasive Alliaria petiolata declines in older populations. Similarly, Oduor et al. (2022) found that invasive Solidago canadensis plants from older populations had a lower competitive effect on native species and a greater competitive response to them, although these interactions depended on plant-soil feedbacks, suggesting that soil biota has an important role in these interactions. Evans et al. (2013) also found a reduced competitive effect in older Lonicera japonica populations under competition with conspecifics. In contrast, Huang and Peng (2016) found that the competitive effect of the invasive vine Mikania micrantha in intraspecific competition is higher in more established core populations, while Gruntman et al. (2017) found no effect of population age on allelopathic ability of invasive Impatiens glandulifera.

The lack of consistent results for the effect of invasion history on the competitive ability of the studied plants might be attributed to variations in competitive environments experienced by these plants. For example, as suggested above, invasive plants often experience a shift from inter- to intraspecific competition with time since introduction, which could select for different competitive strategies. Indeed, in this review, the three studies whose results support the predicted decrease in competitive effect used heterospecific neighbours, while a study that employed conspecific competitors found the opposite trend (Huang and Peng 2016). Further studies are, therefore, needed to differentiate between the effect of invasion history on inter- vs. intraspecific competitive ability.

Divergence in dispersal ability

As for other invasion-promoting traits, evolution of traits related to dispersal ability might also take place between different invasion stages within the introduced range. The most common hypothesis in this regard proposes that, during range expansion, higher dispersal ability is likely to be selected for in individuals arriving at the invasion front compared to core populations (Hargreaves and Eckert 2014; Hodgins et al. 2018) (Fig. 1).

The notion that dispersal ability should be selected for at range edges of invasive species has been suggested in several theoretical models (Travis and Dytham 2002; Phillips et al. 2008; Travis et al. 2009) and supported by several studies on invasive animal species (e.g. Hughes et al. (2003); Phillips et al. (2006); Lombaert et al. (2014); Ochocki and Miller (2017)). However, evolutionary shifts in dispersal ability at range edges of invasive plants have been relatively less studied, of which we are aware of only two studies that were conducted with seeds collected from plants grown under common garden conditions. These studies, by Monty and Mahy (2010) and Huang et al. (2015), provide support for increased dispersal ability (i.e. increased investment in seed pappus) at the invasion front of the invasive plants *Senecio inaequidens* and *Mikania micrantha*, respectively. A few additional studies examined dispersal ability in seeds that were collected directly from field populations and not from plants growing under common garden conditions, showing similar results (Tabassum and Leishman 2018; Robinson et al. 2023; but see Bartle et al. (2013)).

In summary, accumulating evidence provides support for different ways in which invasion-promoting traits such as defence, growth, competitive ability and dispersal might evolve in the introduced range over time. However, our review of the studies did not reveal consistent directions in divergence for most of the studied traits, which could be attributed to other selection pressures that might vary along invasion gradients. Moreover, existing studies that have explicitly explored trait divergence along gradients of invasion history are still very few, ranging from two to seven studies per trait, thus precluding our ability to reach generalised conclusions and highlighting the need for further studies on the subject. The aim of the following meta-analysis is to provide a more general overview on the subject.

Meta-analysis of trait divergence with invasion history

Studies that examine divergence in plant characteristics with residence time often vary in the specific traits and the methodology used to measure them, as well as the way residence time is evaluated and compared across populations. For example, different studies used either time of introduction or distance from core population(s) to estimate chronosequence effects. Therefore, to provide a more general overview of current knowledge on trait divergence with time, we employed a meta-analysis approach that synthesises published literature on the subject. However, as apparent from the literature review above, only very few studies compared trait variations of invasive populations across invasion gradients and even fewer compared these traits under common garden conditions, rendering the data insufficient from which to draw conclusions. To tackle this issue, we employed an additional approach in our meta-analysis, whereby we analysed data from common garden experiments that measured invasion-promoting traits across several populations and used information on the age of these populations from additional sources. Using the two approaches, we asked whether invasion-promoting traits, including herbivore defence (in general or against generalist or specialist species if known), plant growth, competitive ability (effect and response) and dispersal ability, change with residence time across populations of the same invasive plant species. In addition, we asked whether such an overall change has a similar direction within or across traits, corresponding to the predictions outlined above, including an increase in defence, particularly against generalist herbivores; and a decrease in growth, competitive ability, particularly competitive effect, and dispersal ability (Fig. 1).

Methods

Literature search

To test for directional changes in the different traits along the invasion-history gradient, we used two literature review procedures. In the first procedure, we searched for studies explicitly investigating divergence in plant characteristics along invasion gradients at the introduced range, which included information on population ages. The literature was searched using two databases, Web of Science Core Collection (WOS) and Google Scholar. We first screened the literature in WOS (last accessed on 17 January 2023), using the search terms (chronosequence OR time-since-introduction OR invasion-history OR residence-time OR range-expansion OR colonization-history OR introduction-history) AND (plant*) AND (invasi*). We then complemented our search and screened the literature in Google Scholar (last accessed on 6 July 2022), using similar search terms.

Papers selected for the analysis had to meet the following criteria: (1) the study aimed to test the relationship between residence time of an invasive plant and at least one of the following traits: defence against herbivores (measured as, for example, the inverse of leaf damage or herbivore mass following feeding or the production of defence metabolites), plant growth (e.g. plant biomass or height), competitive effect (e.g. effects on the performance of native species or allelopathy), competitive response (e.g. performance of the invasive species under competition with native species) and dispersal ability (e.g. the ratio between the size of dispersal structures such as wing or pappus and seed mass); (2) a gradient of invasion history was explicitly reported in the paper, either as differences in time (generally in years, although papers that reported residence times at large geographical scales, such as country were not included) or as a distance from source to expanding populations; (3) the study reported the results of controlled experiments under common garden conditions, thus ensuring that variations amongst populations in the studied traits are the result of genetic differentiations rather than plastic responses to environmental conditions at the site. A total of 24 cases from 19 papers were included after meeting these criteria.

We carefully checked whether species were defined as invasive (rather than, for instance, alien or naturalised), based on the terminology given in the specific studies as well as in the CABI compendium digital library, invasive species section
(https://www.cabidigitallibrary.org/product/qi). Moreover, in several cases, in which naturalised vs. invasive ranges of introduced species were compared, only the invasive range was used in our analysis.

In the second literature review procedure, we searched for studies investigating variation in characteristics of invasive plants across populations under common garden conditions, but that did not explicitly include data on invasion history. Instead, these data were extracted from additional sources. The literature was searched using the Web of Science Core Collection (WOS) (last accessed on 18 January 2023), using the search terms (common garden OR greenhouse) AND (population OR accession) AND (plant*) AND (invasi*). Data on the location of the collection sites used in the different studies were extracted when possible. Invasion history of the populations was obtained when possible from additional papers that studied the same populations. For other cases, this information was extracted from additional sources such as the Global Biodiversity Information Facility database (https://www.gbif.org/) and CABI compendium digital library- invasive species section. Such information was extracted only for the same locations or for nearby locations at the scale of kilometres. In cases where information on the age of certain populations was missing, such populations were excluded from the analyses. Moreover, when information was given in the literature on the location of the first introduction of the invasive plant (given mostly at the local scale, for example, city), this location was used to estimate the distance from the source population with Google Earth Pro. In such cases, distance to source population was used instead of population age in the analysis. Papers selected for the analysis had to meet similar criteria as in the first literature review procedure, with the exception that information on population ages was not provided, but could be extracted from external sources, following which a gradient of invasion history was used to compare across sites.

Using the two literature review procedures, a total of 79 cases from 62 papers were included after meeting our inclusion criteria in the final dataset of the meta-analysis (see Suppl. material 1: fig. S2 for more details on paper screening and selection process).

Data analysis

Data on the relationship between invasion history (population age or distance from source population) and the studied traits were extracted for each of the selected study cases. When source data were not available, the data were extracted from figures using the software GetData Graph Digitizer ver. 2.26 (http://getdata-graph-digitizer.com). In studies where several treatments were applied (e.g. water or nutrients addition or different disturbance levels), only a subset of the data, representing standardised controlled conditions, was used, such as high water availability (see Suppl. material 1: table S1 for details on specific studies).

As considerable variation could be found amongst studies in the ranges of ages or distances across the studies populations, both the invasion history and measured trait data were first transformed using z-standardisation (standardised by subtracting the mean from each value and dividing by the standard deviation). A linear regression was then performed between the measured trait values and population age/distance. The standardised slope of the regression (β) was taken as the estimated effect size and the variance of the estimate of the standardised slope (SE squared) was taken as the estimated sampling variance (see Suppl. material 1: table S1). When other geographic variables were provided for the populations, such as elevation or mean annual temperature, the estimated standardised slope and variance were generated using linear model or linear mixed model analyses, with these variables as covariates (see Suppl. material 1: table S1).

The effect of invasion history on overall trait divergence (regardless of its direction) was examined with the absolute value of the estimated standardised slope ($|\beta|$), while the effect on directional changes in traits was examined with the standardised slope (β) as an effect size. For both meta-analyses, a random-effects model was used in order to combine the estimated effect sizes from the different studies. Such random-effects models allow for both variation of effect sizes amongst studies and sampling variation within studies (Koricheva and Hayes 2018). In order to estimate model parameters, a Restricted Maximum Likelihood (REML) approach was used. In order to determine means and confidence intervals for the different trait categories, trait category was used as a moderator in the models. As some studies used the same invasive plant species or different traits were sometimes measured in the same study (see Suppl. material 1: table S1), study as well as species identity were also included as random factors in the models. Effect sizes were considered significant if the 95% confidence intervals did not overlap zero. Meta-analyses were performed using the METAFOR package v.4.4 (Viechtbauer 2010) in R-Studio v.2023.9.1.494 (Posit team 2023) and R (R Core Team 2023).

Effects of type of study and origin of information on effect sizes

Study cases selected for the meta-analyses included information on invasion history data extracted from different sources, i.e. reported in papers (n = 36 cases) or estimated from external databases (n = 43). In addition, invasion history was measured in two ways, i.e. residence time (n = 65) or distance from source populations (n = 14) (Suppl. material 1: table S1). A meta-regression model was, therefore, conducted to assess the effect of these moderators on the magnitude of effect sizes. Here, a random-effects meta-regression was performed, in which data origin and type of study were served as moderators, study as well as species identity were included as random factors and an REML approach was employed to estimate model parameters. To interpret the significance of the chosen moderators, Q_m statistics was used to test the extent of heterogeneity explained by the moderators. The meta-regression was performed using the METAFOR package v.4.4 (Viechtbauer 2010) in R-Studio v.2023.09.1.494 (Posit team 2023) and R (R Core Team 2023).

Publication bias analysis

The magnitude and significance of effect sizes may affect the publication and/or visibility rates of studies (e.g. based on the impact factor of journals) (Koricheva et al. 2013). To test for a possible publication bias in cases where the overall effect sizes in our meta-analyses were found to be significant, several approaches were used. First, we tested for temporal publication bias, examining a potential correlation between effect sizes and publication year. Additionally, we checked for a potential correlation between effect sizes and the journal's impact factor at the year of publication. We also tested for the possibility that differences in age and distance ranges amongst studies could influence effect sizes. To that end, an LMM analysis was used, in which publication year, journal's impact factor and age/distance ranges per study served as the independent variables and study identity and the invasive plant species served as random factors. Finally, we estimated the fail-safe numbers using the Rosenberg method (Rosenberg 2005), which indicates the number of additional studies with effect size of 0 needed to reduce the significance level of the observed average effect size to $\alpha = 0.05$. This analysis was conducted using the Fail-Safe Number Calculator software (https://www.rosenberglab.net/Rosenberg2005FailSafe.html). Statistical analyses, unless indicated otherwise, were performed using JMP Pro 17.1 (SAS Inst. Inc.).

Meta-analysis results

A total of 79 observations from 62 studies were included in our meta-analysis after meeting our criteria (Suppl. material 1: table S1). Population age in these studies ranges between 0 and 230 years and distance from source to peripheral populations ranges between 0 and 1000 km (Suppl. material 1: table S1). Overall, invasive species exhibited divergence in the studied traits over time, when averaged across traits (p < 0.001, Fig. 2A). This overall change is also shown for traits related to defence (p < 0.001) and defence against generalists (p < 0.007), growth (p < 0.001), dispersal ability (p = 0.023), overall competitive ability (p < 0.001) and competitive effect (p < 0.001), but not for defence against specialists (p = 0.146) or competitive response (p = 0.363) (Fig. 2A).

In contrast to the overall trait divergence, the direction of change was not affected by residence time for most trait categories, including overall defence (p = 0.135), competitive ability (p = 0.95) or dispersal ability (p = 0.105) (Fig. 2B). However, plant growth increased with residence time (estimated slope \pm SE = 0.187 \pm 0.057; p = 0.001; Fig. 2B).

Effects of type of study and origin of information on effect sizes

The meta-regression results indicate no significant effect of the two moderators on absolute effect sizes ($Q_M = 3.193$, p = 0.203, residual heterogeneity $Q_E = 162.94$, p < 0.001). Specifically, there were no significant differences in absolute effect size between the two types of study (residence time: mean \pm SE = 0.272 ± 0.041 ; distance: mean \pm SE = 0.334 ± 0.084 , p = 0.48; Suppl. material 1: fig. S3). Yet, a non-significant trend was found for the origin of data, according to which absolute effect size values of data reported in papers was slightly higher than data estimated using external databases (reported in papers: mean \pm SE = 0.369 ± 0.085 ; estimated from external sources: mean \pm SE = 0.247 ± 0.097 , p = 0.075; Suppl. material 1: fig. S3).



Figure 2. Meta-analysis results showing **A** mean absolute effect sizes ($|\beta| \pm 95\%$ confidence intervals; $0 \le |\beta| \le 1$) of differences along invasion history gradients for the grand mean for all categories (blue) and each trait category separately and **B** mean effect sizes ($\beta \pm 95\%$ confidence intervals; $-1 \le \beta \le 1$) of differences along invasion history gradients for each trait category. Mean effect sizes are significantly different from zeroes if the confidence intervals do not include zero values, indicating significant trait changes. Negative effect sizes in **B** indicate a negative slope of decreased trait values away from core populations, while positive values indicate an increase towards core populations. Trait categories in light green, dark green and grey, indicate predicted postive, negative or no effect, respectively (see Fig. 1). Sample sizes (number of cases) are indicated in parentheses.

Publication bias

The chosen studies were published between the years 1994–2022. When testing for temporal bias, publication year had no effect on absolute effect size (LMM results: slope \pm SE = 0.0038 \pm 0.0058, *p* = 0.51). Nevertheless, a significant effect of the journal's impact factor was found on absolute effect size, according to which studies with *lower* absolute effect sizes were published in higher impact journals (LMM results: slope \pm SE = -0.039 \pm 0.017, *p* = 0.039; Suppl. material 1: fig. S4A). Moreover, a positive correlation was found between the journal's impact factor and the study's total sample size (Pearson's correlation (log-log scale): r = 0.539, *p* < 0.001; Suppl. material 1: fig. S4B). In addition, when time/distance ranges per study was considered, no significant effect of differences in ranges on absolute effect size was observed (LMM results (log range): slope (\pm SE) = 0.036 \pm 0.076, *p* = 0.63). Finally, the estimated Rosenberg's fail-safe number (i.e. additional number of studies with an average effect size of 0 needed to reduce the significance level of the observed average effect size to $\alpha = 0.05$) was 4767, suggesting our results of overall absolute effect size are robust against possible publication bias.

Discussion

Our meta-analysis results reveal overall divergence in invasion-promoting traits with residence time. This divergence was exhibited for all traits, except for competitive response and defence against specialists, which could be attributed to the lower sample sizes of studies that examined these traits in the meta-analysis. However, both our review and meta-analysis results show that, for most studied traits, their divergence lacks a consistent direction.

The only trait for which our meta-analysis revealed a directional shift was plant growth. However, in contrast with our prediction, growth-related traits, such as height and vegetative biomass, increased over time. Invasive plants in older and more established populations were predicted to undergo selection for decreased allocation to growth and competitive ability compared to populations at the invasion front, due an increase in herbivore and pathogen pressure and an allocation trade-off with defence traits. However, of the seven studies explored in our review, only two studies, conducted with the same species (Lonicera japonica), provide support for this prediction (Evans et al. 2013; Kilkenny and Galloway 2013). An increased allocation to size in older and more established populations might be driven by increased competition pressure. Specifically, plants in these populations might experience shifts from interspecific competition with diverse neighbours to intraspecific competition with neighbours of similar resource requirements (Lankau et al. 2009; Inderjit et al. 2011), which might be stronger and require greater allocation to growth (Huang et al. 2021). However, the increase in growth with residence time did not translate to increased competitive ability in the meta-analysis results, which might be attributed to the fact that most invasion history studies tested for inter- rather than intraspecific competition (but see Huang

and Peng (2016); Harms and Walter (2021)). Studies that compare competitive ability with heterospecific vs. conspecific neighbours across invasion history gradients are, thus, needed to examine this hypothesis.

The lack of significant consistent directional divergence in most of the traits tested in our meta-analysis could be attributed to varying selection pressures that might be context-dependent and vary with habitat type and resilience of the native communities. Moreover, some gradients of invasion history might take place along geographical gradients, where variation in invasion history could be confounded with other factors, such as changes in ambient temperatures, season length and primary productivity across sites, which could affect the observed patterns (Colautti et al. 2010; Colautti and Barrett 2013; Liu et al. 2017; Hulme and Bernard-Verdier 2018; Irimia et al. 2019; Hierro et al. 2020; Kühn et al. 2021). Several studies have shown that local climates can play an important role in the rapid adaptation of invasive plants and their range expansion (Colautti and Barrett 2013; Vandepitte et al. 2014; Colomer-Ventura et al. 2015; van Boheemen et al. 2019; Haider et al. 2022). For example, Colautti et al. (2009) analysed data from experiments that examined trait divergence of invasive plant species from native and introduced populations and showed that such divergence is highly affected by geographic clines. While we incorporated climatic factors in the analyses whenever available, this was not the case for most of the chosen studies. If such climatic variables can result in divergence in invasion-promoting traits, they may confound divergence due to invasion history. In addition, the evolution of invasive populations might be affected by additional factors whose strength might change with time. For example, as suggested above, while selection pressures that promote greater plant size and competitive ability, such as enemy release, could attenuate with time since introduction, strong competitive ability might still be selected for in older populations if the effect of intraspecific competition is much stronger than that of native heterospecifics.

In addition to different context-dependent selection pressures, the lack of consistent directional change with time since introduction can be attributed to neutral non-adaptive evolutionary processes that might have taken place within the introduced range of some invasive species, such as founder effects and genetic drift. For example, multiple introductions could involve different samplings from the native range, resulting in repeated founder effects of populations with different invasion histories (Parker et al. 2003; Kliber and Eckert 2005; Dlugosch and Parker 2008; Keller et al. 2009). Similarly, successive founder events along invasion routes can result in non-adaptive (or even mal-adaptive) differentiation (Amsellem et al. 2000; Colautti and Lau 2016). In order to discern between evolution due to selection vs. neutral processes along gradients of invasion history, further studies should, therefore, use genetic information of these populations and/or employ reciprocal transplant experiments that test for local adaptations along such gradients (Colautti and Barrett 2013; Moran and Alexander 2014; VanWallendael et al. 2018).

Another explanation for a lack of directional effects of residence time found in this meta-analysis is that, unlike our predictions, trait evolution might follow a non-linear trajectory. For example, recently established populations at the invasion front might

exhibit initial lags in their responses to selection pressures if they are derived from different source populations of different ages or due to factors such as small population sizes. Moreover, evolution of core populations might decelerate if the intensity of selective pressures they experience, such as herbivore load, attenuate with time (Hawkes 2007; Gruntman et al. 2017).

Finally, the lack of a clear directional change might result from the small number of studies on the subject in some of the categories. This is particularly true for traits related to dispersal ability for which we were able to find only three studies that compared dispersal ability across different populations in the introduced range that used common garden experiments. Several other studies have examined the effect of residence time on dispersal ability under field conditions, providing support for such divergence (Tabassum and Leishman 2018; Robinson et al. 2023). However, additional studies that incorporate the effect of residence time on this trait under common garden conditions could provide valuable information and expand the empirical basis and knowledge on its impact on the evolution of invasive plants.

In this review and meta-analysis, we looked at four main categories of invasion-promoting traits for which temporal changes are predicted within the introduced range, including defence, growth, competitive ability and dispersal ability. Yet, additional traits that could contribute to the invasive success of plants might be affected by time since introduction. For example, phenotypic plasticity has been suggested to evolve at the introduced range and facilitate plant invasion in varying habitats and climates (Richards et al. 2006; Davidson et al. 2011). Similarly, selection pressures that might favour phenotypic plasticity are likely to be stronger at the invasion front, where invasive plants encounter novel environmental conditions (Richards et al. 2006; Matesanz et al. 2010). However, while several studies have examined changes in plasticity in response to range shifts (Matesanz et al. 2010), only very few looked at such changes in plasticity along gradients of invasion history (VanWallendael et al. 2018; Wan et al. 2018).

Conclusions

Adding a temporal dimension to studies on traits of invasive plants is challenging because it entails knowledge on the timing of population establishment or distance to known core populations. However, the potential for rapid evolution of invasive plants within their introduced range across different invasion stages provides a unique opportunity to study fundamental questions related to adaptive divergence in plant traits. Here, we reviewed several hypotheses regarding divergence in invasion-promoting traits, which propose that the effect of varying selection pressures might attenuate with time since introduction. However, while our meta-analysis results reveal a general indication for the evolution of invasive plants with residence time, they do not provide support for a consistent directional divergence, except for growth. Here and in contrast with our prediction, growth parameters were found to increase with invasion history, which might reflect greater competition pressure in these populations. The general temporal effect found in this study highlights the need to take into account the potential confounding effect of population age when sampling populations to explore attributes of invasive plants (e.g. comparing trait evolution between native and invasive ranges) and particularly when evaluating the long-term effects of invasive plants on native communities and ecosystems. Moreover, the increased size of invasive plants in older populations found in this study, suggests that, although some selection pressures that drive the evolution of invasiveness, such as enemy release, can decrease with time, their dominance and effects on the native communities and ecosystems in the introduced range might not attenuate. Studies that further explore both trait divergence and community effects across invasion routes in the introduced range will be crucial for understanding the long-term dynamics between invasive plants and their recipient native communities.

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Supplementary material I

Supplementary information

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Data type: docx

- Explanation note: fig. S1. Temporal trends (between 1977–2022) in studies focusing on exploring variation in invasion-promoting traits of invasive plants over time. Data was extracted after searching the Web of Science Core Collection database using the search terms (chronosequence OR time-since-introduction OR invasionhistory OR residence-time OR range-expansion OR colonization-history OR introduction-history) and plant* and invasi* and (biomass OR defense OR competiti* OR dispersal OR allelopathy OR herbivor* OR plant-height). The smooth curve (indicated in blue) was added for visual interpretation. fig. S2. Schematic representation of the two literature searches used in the meta-analysis, using both Web of Science and Google Scholar databases. fig. S3. Meta regression results for the effects of the different moderators on mean absolute effect sizes ($|\beta| \pm 95\%$ confidence intervals; $0 \le |\beta| \le 1$). Moderators include the origin of information (invasion history data reported in papers vs. estimated using external databases) and type of invasion history measurement (residence time vs. distance). Sample sizes (number of cases) are indicated in parentheses. fig. S4. Correlations between (A) absolute effect size of the different studies and the impact factor of the journal at time of publication or between (B) journal's impact factor and total sample size per study. Dashed lines indicate significant negative correlations (r = -0.357, p = 0.0017, n = 75 and r = 0.539, p < 0.001, n = 75, respectively). table S1. Information on the invasive species, variables and factors used for the meta-analysis, as well as the respective effect sizes and variances.
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RESEARCH ARTICLE



Can gardeners identify 'future invaders'?

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Abstract

It is estimated that there are 30 million gardeners in Britain, who could play a crucial role in being the 'first contact' for reporting ornamental plants in gardens with invasive potential. Invasive species are one of the five drivers of the global nature crisis, many of which were originally introduced through ornamental horticulture. Ornamentals confined to gardens and those which have already naturalised, but are not yet shown to be invasive, represent a 'pool' of species with invasive potential - 'future invaders'. An online survey asking gardeners to report ornamentals they had noticed invading or taking over their garden resulted in 251 different taxa being reported (including cultivars). The future invaders were prioritised with a simple yet structured scheme, looking at the domestic and global naturalised and invasive status of each taxon, including in the Global Register of Introduced and Invasive Species (GRIIS) and the Global Naturalized Alien Flora (GloNAF) databases. The structured scheme identified a shortlist of nine ornamentals of concern which should be prioritised for further analysis, such as a formal risk assessment. Identifying and preventing future invaders before they escape gardens is critical, to prevent future threats to nature. There is also a gap in the identification of potentially invasive ornamentals, which are not currently invasive, yet are beyond the scope of formal horizon scanning because they are naturalised. Here we explore whether surveying gardeners can be a suitable approach to prioritising future invaders while also being an opportunity to increase awareness of invasive species. This positive feedback loop between gardeners and invasion scientists could help reduce the risk of future invaders.

Keywords

Invasive plant species, invasive potential, online survey, ornamental horticulture, public engagement

Introduction

In Britain and Ireland, non-native (sensu Macpherson et al. 1996) plants now constitute over half of the wild flora (Stroh et al. 2023). Similarly, at least 75% of the naturalised flora globally is thought to have escaped domestic gardens (van Kleunen et al. 2018). There is also a continued increase in plant introductions (first record rate) globally (Roy et al. 2012, Seebens et al. 2017). This is projected to result in an increase in the number of naturalised species particularly in Europe (Seebens et al. 2021). Although the main introduction pathway or source of invasive species globally is ornamental horticulture (Drew et al. 2010; Dehnen-Schmutz 2011; Hulme et al. 2017; van Kleunen et al. 2018; Arianoutsou et al. 2021), only a relatively small number have so far become invasive (Stace and Crawley 2015). Invasive species threaten native biodiversity (IUCN 2000) and/or have economic, human health, or quality of life impacts (IPBES 2019) and have cost the UK economy between £5.4 and £13.7 billion since 1976 (Cuthbert et al. 2021). Garden ornamentals (Cubey et al. 2022) confined to gardens and those which have already naturalised, but not yet shown to be invasive, represent a 'pool' of species with invasive potential – 'future invaders' sensu Mayer et al. (2017) – or an invasion debt (Essl et al. 2011; Haeuser et al. 2018).

Despite the risks of invasive species and future invaders, ornamental horticulture brings with it many benefits such as to human health (e.g. Hoyle 2021) and by providing ecosystem services (e.g. Salisbury et al. 2015, 2017). The ornamental horticulture and landscaping industry also contributed (e.g. through retail and production of ornamentals) £28.8 billion to UK GDP in 2019 with a potential increase to £41.8 billion by 2030 (Ornamental Horticulture Roundtable Group 2021).

Hence, identifying and preventing future invaders before they escape gardens is critical, both ecologically and economically, and gardeners may have a key role in this. Here we explore whether surveying gardeners can be a suitable approach to prioritising future invaders.

How do ornamentals escape, and become invasive?

Numerous frameworks have been developed to better understand why certain species become invasive and to improve links between invasion science, policy and management (Wilson et al. 2020). One example is the Unified Framework for Biological Invasions (Blackburn et al. 2011), referred to here as the 'unified framework'. This combines the concepts of stages (Williamson 1996; Williamson and Fitter 1996) and barriers (Richardson et al. 2000) in invasion science (Wilson et al. 2020). Barriers can be described as limiting factors that restrict a species from 'succeeding' to the next stage. The unified framework is arguably the most applicable framework for ornamental horticulture because it recognises human-imposed cultivation barriers (e.g. garden fences) between introduction and naturalisation. Blackburn et al. (2011) recognise that it is possible for species to 'skip' this barrier if introduced directly into the wild unintentionally. However, there is no recognition that this barrier can also be 'skipped'

due to intentional introduction into the wild *sensu* Roy et al. (2014). Note that the unified framework of Blackburn et al. (2011) does not incorporate the impact of an invasive species. The stages of the invasion process are not independent of each other. For example, Milbau and Stout (2008) found that an early first record in the wild was one of the factors increasing the likelihood of a non-native plant transitioning from being a casual to being naturalised. One factor important in this specific context of ornamental horticulture is hybridisation which can increase the invasive potential of ornamentals and is linked with climate change (Kohn et al. 2009; Klonner et al. 2017).

Can gardeners identify potentially invasive ornamentals?

Gardeners have a crucial role in reducing the risks associated with invasive species, including at a practical level, for example through their choice of ornamentals to grow and steps to adopt while gardening to limit the spread of invasive species into the wild (Jones et al. 2024). Gardeners also have a role in identifying ornamentals with invasive potential because they often have expert knowledge (Dehnen-Schmutz and Conroy 2018) of how different ornamentals are performing in their garden including those showing 'invasive behaviour'. This expert knowledge can help identify a potentially invasive species early (e.g. before escaping gardens) which is both ecologically and economically advantageous (Hulme 2006) as it allows for prevention as a management approach. It is also important to prioritise species for control (Shackelford et al. 2013; Head et al. 2015). For ornamentals, this means identifying which of the around 70,000 plants available for gardeners (Cubey et al. 2022) have invasive potential, before escaping from gardens. There are also many non-native (sensu Macpherson et al. 1996) ornamentals which are not currently invasive (Stace and Crawley 2015) but are beyond the scope of horizon scanning because they are already present in the wild (Roy et al. 2019) either as: i) casuals: plants surviving in the wild (i.e. outside of cultivation) due to repeated introductions; ii) survivors: plants that are persistent in an area simply due to longevity but do not reproduce; or iii) having naturalised (syn. established): a plant which is self-reproducing or increasing year-to-year by sexual or vegetative means (Stace and Crawley 2015). This leaves a gap – as identified by Dehnen-Schmutz (2011) - in the identification of potentially invasive ornamentals. This gap can be addressed by looking at 'non-invasiveness' to determine green lists (Dehnen-Schmutz 2011) or engaging with gardeners to identify potentially invasive ornamentals. The latter is the focus of this study.

Dehnen-Schmutz and Conroy (2018) tested a citizen science approach using an online survey to identify potentially invasive ornamentals (Johnson et al. 2020) and reported the naturalisation status of 121 species (including 17 native species). Eight species were not known to have escaped gardens, i.e., with no naturalised records in Britain at the time of their study. Since the Dehnen-Schmutz and Conroy (2018) study, a long-term citizen science project called Plant Alert has been launched – led by the Botanical Society of Britain and Ireland (BSBI) and Coventry University – to monitor potentially invasive ornamentals, asking gardeners to record invasive plants

in their garden (Plant Alert 2023). Such data can be used, for example, in risk assessments and for advising gardeners (Webb 2020). A survey can also act as an educational mechanism for gardeners (Reichard and White 2001; Hulme et al. 2017).

The challenge is identifying which ornamentals could become invasive in the future, not just naturalisation status. An important aspect is therefore to also look at invasive status elsewhere in the world. In this study, we identify gardeners as the target audience for engagement to identifying future invaders, i.e., species invasive potential. This has great potential for achieving Target 6 of the Kunming-Montreal Global Biodiversity Framework (CBD 2022). A structured scheme for prioritising future invaders reported by gardeners is shown below (Fig. 1).



Figure 1. A structured scheme for prioritising future invaders.

This structured scheme for prioritising can be adapted depending on data and geographic scale. See Methods section for list of data sources.

We engaged with gardeners to address the research question: can gardeners identify future invaders? By doing so, we aimed to explore whether surveying gardeners can be a suitable approach to prioritising future invaders in Britain and Ireland.

Methods

Two complementary surveys were designed and conducted, which differed in their method of participation, but had the same target audience (Tweddle et al. 2012; Varner 2014) of gardeners (amateur or professionals) in Britain and Ireland. Both surveys were hosted by Jisc Online Surveys (www.onlinesurveys.ac.uk) and passed ethical review prior to implementation. Neither survey offered a comprehensive explanation of the term 'invasive' to participants because: 1) doing so might not match how gardeners use

the term in their gardens, see Jones et al. (2024); and 2) the purpose of the study was not to ask gardeners what ornamentals are having an impact in the wild (as invasive species *sensu stricto*) but rather those showing 'invasive behaviour' in gardens.

Scoping survey

A scoping survey asked gardeners to: '*list up to three ornamental plants you've noticed invading/taking over your garden*'. Participants could report up to three plants (the first being the most invasive) and the first part of their postcode (UK) or Eircode in Ireland meaning no personal data was collected. See Suppl. material 1. The survey was launched in August 2018 using the RHS's social media Twitter account (@The_RHS with 159,000 followers at the time). It was also publicised elsewhere including the November issue of The Garden (RHS 2018a), with a circulation of over 510,000 (RHS 2018b), and The Hardy Plant Society's Newsletter and the RHS's December email circulation, reaching 183,306 RHS members. Targeting existing gardening groups such as this can be effective (Tweddle et al. 2012) in recruiting participants through non-probability convenience sampling (Callegaro et al. 2015; Vehovar and Manfreda 2017). The scoping survey closed on May 19th 2019.

Chelsea survey

The scoping survey informed a follow-up survey (henceforth the Chelsea survey) which was launched at the RHS Chelsea Flower Show (RHS Chelsea) in London May 20th– 25th 2019. The Chelsea survey was tested with potential participants beforehand, using regular gardening volunteers from the Friends of the Harris Garden, at the University of Reading. Minor improvements were made to the survey as a result. The Chelsea survey was approved by the Ethics Committee of the School of Biological Sciences at the University (reference number SBS18-19 36).

Relevant to this study is the question: '*what is the main ornamental plant you have noticed invading or taking over your garden?*'. This was a drop-down question consisting of the ten most reported ornamentals (based on preliminary analysis) in the scoping survey. Four of the drop-down options were for genera only which then prompted an additional question asking the participant if they could specify which species and/ or cultivar. Participants could also select 'other' to report a different ornamental. See Suppl. material 1. The question could be repeated up to two times. Visitors at Chelsea who had indicated that they wished to participate at a later date were emailed with a direct link. The Chelsea survey closed in December 2019.

Data cleaning

To ensure participants of both surveys were from Britain or Ireland, the postcodes or Eircodes were geolocated using www.geocode.xyz. Responses which could not be geocoded were discarded. The plants reported in both surveys were then taxonomically standardised in three steps: 1) manually correcting spelling errors and giving scientific names to vernacular names. This was done through expert judgement and checking RHS references (RHS 2008, 2020; Cubey et al. 2018, 2020). 'Japanese anemone' sensu lato is treated here as Anemone × hybrida Paxton. Any reports with a vernacular name which could not confidently be assigned a scientific name were discarded; 2) the scientific names were checked using the Global Names Resolver (gnr resolve) function as part of the taxize package (Chamberlain and Szöcs 2013) in R version 4.0.4 (R Core Team 2021). The data source was the International Plant Names Index (IPNI 2020). The nomenclature thus follows IPNI, except for infra-specific (including cultivars) and inter-specific taxa (hybrids); and 3) infra- and inter-specific examples checked against the aforementioned RHS references, but the nomenclature to species level still follows IPNI. The standardised list was checked for duplicates and reports which were only at genus level were removed, i.e. species, subspecies and varieties as well as hybrid taxa were retained and analysed as such (except for cultivars). Native taxa (Morais and Reichard 2018; Pagad et al. 2018, 2022) as listed by Stace (2010), were also removed. Data cleaning resulted in 318 responses being discarded - including responses from the Republic of Ireland or Northern Ireland which could not be geolocated - out of the total 876 responses (562 in the scoping survey and 314 in the Chelsea survey).

Data analysis

Global and domestic invasive status (i.e. evidence of impact) was taken from the Country Compendium version 1.0 of the Global Register of Introduced and Invasive Species (GRIIS) (Pagad et al. 2018, 2022). Stace and Crawley (2015) and The Global Naturalized Alien Flora (GloNAF) database (van Kleunen et al. 2015; Pyšek et al. 2017) were used for determining domestic status, including naturalisation. The point of the study is not to make a direct comparison with Plant Alert (2023) because the questions are different but both share the principle of identifying potentially invasive ornamentals. We have therefore added the respective number of reports via Plant Alert (BSBI 2023; Plant Alert 2023) into Table 1 for context with what has been done since the data of this study was collected. Species richness of reported species (Pergl et al. 2016) and the completeness of the sampling strategy ("sample coverage", Chao and Jost 2012) were investigated using iNEXT (Chao et al. 2014, 2022; Hsieh et al. 2016). Briefly, the sampling-unit-based incidence data approach was used for interpolation and extrapolation, treating each gardener as the sampling unit, based on the gardener's expert knowledge, and the identity of their reported species as incidence data. Here we assume that while every garden has a different size and overall combination of species, each gardener accurately assessed the identity of plants that were 'invasive' within their own garden. In this approach "sample coverage" is the proportion of overall species occurrences that can be attributed to identified taxa. For the purposes of these analyses: i) cultivars were not included; and ii) observations of varieties and subspecies for which the species was already present in the dataset were combined with the observations of their respective species.

Results

The cleaned results for both surveys are presented here together with 847 reports from 558 gardeners (Fig. 2). The 847 reports included: 203 species, 8 infra-specific (4 subspecies and 4 varieties), and 13 hybrids, totalling 224 taxa. There were also 27 named cultivars resulting in 251 different taxa being reported.

Based on the 221 unique species and hybrids (see Methods section) included in the overall dataset, interpolated species accumulation of reported taxa for the survey data did not approach an asymptote (Fig. 3). For observed data the estimated 95% CI of species richness was 203.83–238.17 species (Fig. 3a, c) and sample coverage (SC) was



Figure 2. Location of the gardeners [n = 558] who participated in the scoping and Chelsea surveys.

estimated at 0.849 (95% CI: 0.825–0.873) (Fig. 3b, c). This level of SC suggests that for every 6–8 additional gardeners, an additional taxon would be added (each additional gardener surveyed (beyond 558) would add an additional 0.127–0.175 species to the total). Extrapolation to twice the number of survey responses (Fig. 3) gives an estimated 95% CI for species richness of 291.04–353.72 and SC = 0.907 (95% CI: 0.883–0.931).



Figure 3. Rarefaction (solid lines) and extrapolation (dashed lines) curves for species richness of potentially invasive plants based on incidence data from gardens. Panel **a** species accumulation curve (species richness with increasing sample number). Panel **b** sample completeness curve (sample coverage with increasing sample number). Panel **c** coverage-based sampling curve (species richness with increasing sample coverage). Shaded areas = 95% confidence intervals (based on 100 bootstrap replications). Number of sampling units = number of gardens. Sample coverage = proportion of the predicted total number of (invasive) species. Solid dot = end of observed data from surveys.

Table 1. The most commonly reported plants (by \geq 5 gardeners) with N showing number of reports (cultivars are not separated). Statuses (matching Fig. 1): a cell with beige shading (NN) = **non-native**; a cell with light orange shading (S) = **survivor**; a cell with orange shading (N) = **naturalised**; a cell with red shading (I) = **invasive**; with – meaning no record. Statuses from Stace and Crawley (2015) shown as APs for "*Alien Plants*". GB = Great Britain (England, Scotland and Wales), IE = Ireland and Northern Ireland, and BI = British Isles (i.e., GB, IE, the Channel Islands and the Isle of Man). Global invasive status lists countries (excluding GB and IE) where the taxa is listed as invasive in the GRIIS Country Compendium (using alpha-2 codes of the according to the ISO 3166 standard). The number of reports (as of November 18th 2023) via Plant Alert (BSBI 2023) are also shown.

		N		Don	nestic s	status		
			BI GB		IE			
Scientific name	This study	Plant Alert	APs	GloNAF	GRIIS	GloNAF	GRIIS	Global invasive status (GRISS)
<i>Arum italicum</i> Mill. subsp. <i>italicum</i> ¹	5	-	N	N	NN	N	NN	AR, NZ, US
Euphorbia cyparissias L.	5	2	Ν	N	NN	N	NN	EE, LT, NO, US
<i>Fallopia baldschuanica</i> (Regel) Holub	5	6	S	N	Ι	N	NN	BG, CZ, NL, PT
Geranium nodosum L.	5	3	Ν	N	NN	N	-	-
<i>Symphoricarpos albus</i> (L.) S.F.Blake ²	5	9	Ι	N	Ι	N	NN	CZ, DK, NL, NO, RU, SE
Parthenocissus quinquefolia (L.) Planch.	6	2	N	N	Ι	N	NN	BA, HR, CU, CZ, NO, RO, RU, SI, SE
<i>Rosa rugosa</i> Thunb.	6	2	Ι	N	Ι	N	Ι	DK, EE, FI, DE, LV, LT, NL, NO, RU, SE, US
Vinca minor L.	6	1	Ι	N	Ι	N	NN	EE, LT, NO, RU, SE, US
Leycesteria formosa Wall.	7	23	Ν	N	NN	N	NN	NZ
Vinca major L.	7	4	Ν	N	NN	N	NN	AR, CA, JP, KE, NZ, ZA, US
<i>Reynoutria japonica</i> Houtt. syn. <i>Fallopia japonica</i> (Houtt.) Ronse Decr.	8	31	Ι	N	Ι	N	Ι	BY, BA, CA, HR, CZ, DK, EE, FI, FR, IT, LI, LU, ME, NL, NZ, NO, PL, PT, RO, RU, SK, SE, CH, US
Impatiens glandulifera Royle	9	34	Ι	N	Ι	N	Ι	AT, BY, BA, CA, HR, CZ, DK, EE, FI, FR, IT, LV, LI, LT, LU, NL, NZ, NO, RU, SK, SI, SE, CH, US
Allium triquetrum L.	10	16	Ι	Ν	NN	N	NN	NZ, ZA
Erigeron karvinskianus DC.	10	8	N	N	NN	N	NN	CL, IN, IT, JP, MU, NP, NZ, TZ, ZM, ZW
<i>Euphorbia amygdaloides</i> Lam. subsp. <i>robbiae</i> (Turrill) Stace	10	3	N	N	NN	N	NN	_
Lysimachia ciliata L.	10	17	Ν	N	NN	-	NN	-
<i>Pilosella aurantiaca</i> (L.) F.W.Schultz & Sch.Bip. ³	10	15	N	N	NN	N	NN	CA, JP, KG, NZ, NO
Centranthus ruber (L.) DC. ⁴	11	9	Ι	Ν	NN	N	NN	ZA, US
Symphyotrichum novi-belgii (L.) G.L.Nesom syn. Aster novi-belgii L.	11	-	N	N	NN	N	NN	AT, BY, BG, CZ, DE, JP, LT, ME, SK, SE
Aegopodium podagraria L.	12	5	Ι	N	NN	N	NN	US

		N		Don	nestic s	status		
			BIC		B	IE		
Scientific name	This study	Plant Alert	APs	GloNAF	GRIIS	GloNAF	GRIIS	Global invasive status (GRISS)
Houttuynia cordata Thunb.	12	15	Ν	N	NN	-	-	NL, NZ, US
<i>Soleirolia soleirolii</i> (Req.) Dandy syn. <i>Helxine soleirolii</i> Req.	14	8	N	N	NN	N	NN	-
Pentaglottis sempervirens (L.) Tausch ex L.H.Bailey	16	25	Ι	N	Ι	-	NN	US
<i>Buddleja davidii</i> Franch.	21	78	Ι	N	Ι	N	NN	AR, BA, BG, CA, CZ, DK, FR, IN, IT, JP, LI, NL, NZ, CH, US
<i>Lamium galeobdolon</i> (L.) Crantz subsp. <i>argentatum</i> (Smejkal) J.Duvign.	21	119	N	N	-	N	_	CZ
Verbena bonariensis L.	26	8	Ν	-	NN	_	-	ET, FJ, JP, KE, RW, ZA, TZ, US
<i>Hyacinthoides hispanica</i> (Mill.) Rothm. ⁵	29	110	N	N	NN	N	Ι	US
Alchemilla mollis (Buser) Rothm.	79	6	N	N	Ι	N	NN	NL, NO, SE, US
Crocosmia × crocosmiiflora (Lemoine) N.E.Br. ⁶	82	711	Ι	N	-	N	NN	BR, JP, NZ, PG, US
Anemone × hybrida Paxton "Iapanese anemone" s /	86	1712	S	-	-	_	NN	_

Included as: ¹*A. italicum* Mill. in GloNAF; ²*Symphoricarpos albus* (L.) C.Koch in GRISS; ³*Pilosella aurantiaca* subsp. *aurantiaca* syn. *Hieracium aurantiacum* L. in GRISS for CA and NO; ⁴*C. ruber* (All.) Lam. & DC. in GRISS for GB and as *C. ruber* DC. for US; ⁵gardeners often mistakenly refer to bluebells grown in gardens as *Hyacinthoides hispanica* or using the vernacular name 'Spanish bluebell' (see Discussion also); ⁶included as *Crocosmia crocosmiiflora* (Nicholson) N.E.Br. in GRISS for BR, JP, NZ, PG and the US. ⁷Specifically *Lysimachia ciliata* 'Firecracker'; ⁸including *B. davidii* 'Black Knight'; ⁹included as *Lamiastrum galeobdolon* subsp. *argentatum* (Smejkal) Stace; ¹⁰with an additional three treated as *Hyacinthoides hispanica* agg.; ¹¹included as records of *Crocosmia* Planch.;¹²including one record of *Anemone* × *hybrida* 'September Charm'.

The most commonly reported taxa (by \geq 5 gardeners) are shown in Table 1 along with their domestic status, and invasive status globally. Table 1 also includes Plant Alert results (BSBI 2023) as of November 18th 2023 for the respective data.

All taxa in Table 1 are neophytes except for *Aegopodium podagraria* and *Vinca minor* which are archaeophytes (Stace and Crawley 2015). The 251 reported taxa included 5 casuals and 13 survivors (two of which are listed in Table 1) as listed in Stace and Crawley (2015). See Suppl. material 2, for full list.

Discussion

Citizen science has great potential to improve our understanding of invasive species (Johnson et al. 2020) especially in identifying invasive potential (e.g. Dehnen-Schmutz and Conroy 2018). It also has the added benefit of being an opportunity for public

engagement and science communication, informing participants about issues (Miller-Rushing et al. 2012; Tweddle et al. 2012) such as invasive species (Hulme et al. 2017). The focus here is on a citizen science approach to identify future invaders.

Shortlist of future invaders

Ornamentals reported by \geq 5 gardeners (Table 1) are prioritised here to generate a shortlist. None of the taxa in Table 1 were included in the green list of Dehnen-Schmutz (2011). Although Table 1 does not include any species not known to have escaped gardens in GB or Ireland (Stace and Crawley 2015), it does include two survivors. One of which, *Anemone* × *hybrida*, was the most reported but it has no invasive status globally (Pagad et al. 2022). It was also the joint most frequently reported in Dehnen-Schmutz and Conroy (2018) with six reports, although reported in their study as *Anemone scabiosa* H.Lév. & Vaniot.

Of the reported taxa which have already escaped gardens in GB and/or Ireland but are not yet invasive (Table 1) it is important to focus on those with an invasive status globally (Pagad et al. 2022) as shown in Fig. 1. This gives a shortlist of nine ornamentals: Arum italicum subsp. italicum, Erigeron karvinskianus, Euphorbia cyparissias, Houttuynia cordata, Lamium galeobdolon subsp. argentatum (see note below on data sources), Leycesteria formosa, Pilosella aurantiaca. Symphyotrichum novi-belgii, and Verbena bonariensis. It is also worth noting, that of the reported plants (Table 1) considered invasive, Crocosmia × crocosmiiflora, Hyacinthoides hispanica were the most frequently reported in Dehnen-Schmutz and Conroy (2018) with six reports each (jointly with Anemone scabiosa and L. galeobdolon subsp. argentatum). Of the shortlisted ornamentals, the following are also listed as the most frequently reported via Plant Alert (2023) as of November 18th 2023: E. karvinskianus, H. cordata, L. galeobdolon subsp. argentatum, L. formosa, P. aurantiaca and V. bonariensis.

One problem with prioritising is the differences in status between data sources. For example, as is the case with *Fallopia baldschuanica* (Table 1) and *Lamium galeobdolon* subsp. *argentatum* is arguably already invasive and is listed in Schedule 9 of the Wildlife and Countryside Act which applies in Great Britain. Critically, none of the shortlisted taxa or those listed in Table 1 – except for *Akebia quinata* – were identified during the most recent horizon scanning process for GB because they were beyond the scope of the exercise; e.g. they were already present in the wild (Roy et al. 2019). The approach here is therefore effective in addressing the gap in the identification of potentially invasive ornamentals.

Based on the trajectory of the species accumulation in the survey data (Fig. 3) we suggest that there may be a significant number of additional future invaders that were not reported. This trend, shown in Fig. 3, is similar to that found by Thompson et al. (2003) (albeit of quadrant data not from a survey). Extrapolation from our data suggests that the number of reported species could increase by approximately 1 for every additional 5–8 gardeners surveyed. Very tentatively, the species accumulation curve appears to approach an asymptote at 350–400 species. However, this estimation requires caution as we note it may be a consequence of limiting the total number of

species recorded by each gardener to a maximum of three. This necessarily increases the number of observations required to reach sampling saturation (SC = 1). For more accurate estimation in future surveys, we recommend allowing the observer to enter as many species as they wish (as is now the case with Plant Alert).

Native ornamentals

Native species were removed because they cannot be considered invasive sensu stricto. However, there were 169 reports of native species with the most reported species being: Hedera helix L. [n = 24], Carex pendula Huds. [n = 22], and Convallaria majalis L. [n = 10]. This is also a factor with Plant Alert, e.g. with nine reports of C. pendula as of November 18th 2019 (BSBI 2023). This raises two important points: 1) the idea of 'cryptic invasions'; and 2) how gardeners understand the term 'invasive'. Firstly, cryptic invasions (Novak 2011) have an impact on native genetic diversity (see Morais and Reichard 2018) with an increasing number of hybrids recorded in Britain and Ireland (Stace et al. 2015) and due to the introduction of non-native genotypes. For example, six different cultivars of native species were reported including Hedera helix 'Ivalace' [n = 1]. Secondly, the reports of native species suggest gardeners conflate the term 'invasive' with garden weeds and 'thugs' (Jones et al. 2024). C. pendula is a good example of being native but widely considered a weed or 'thug' in gardens (e.g. RHS 2021a), and is even described in Stace and Crawley (2015, 468) as an "invasive native". Conversely, Geranium nodosum (Table 1) has no invasive status globally (Pagad et al. 2022) but could be considered by many gardeners to be a weed. This possible conflation of terms has implications for this approach (see below).

Limitations and improving the approach

Dehnen-Schmutz and Conroy (2018) found two limitations to their approach: 1) difficulty with identification skills (see also Johnson et al. (2020)); and 2) motivation of participants. Our study differs from that of Dehnen-Schmutz and Conroy (2018) by targeting gardeners rather than botanists. Their argument was that botanists (mainly members of the Botanical Society for Britain and Ireland) would have better identification skills, but gardeners are arguably more familiar with ornamentals grown in gardens. Furthermore, it is estimated that there are 30 million gardeners in Britain (RHS 2021b), compared to around 3,400 members of the Botanical Society of Britain and Ireland (BSBI 2022), which is a much better opportunity for recruiting participants. This study suggests gardeners are very interested in the issue of invasive species and their role in identifying future invaders. The main limitation in surveys appears to be in the distinction between an invasive - or potentially invasive - species and garden weeds or 'thugs'; specifically in how gardeners understand the term 'invasive' (Jones et al. 2024). This should therefore be explicitly explained before asking gardeners to report future invaders such as by providing a definition or multiple-choice question. An additional consideration is that, thanks to the efforts of gardeners, garden ornamentals

often grow outside the core niche of their native distribution (Yesson and Culham 2006). Hence, species that are thriving inside gardens, and potentially taking over these highly managed spaces, may not be as successful in the wild. Also, there is a degree of uncertainty when asking gardeners to report through a survey due to challenges in identification. For example, bluebells grown in gardens are likely to be *Hyacinthoides* × *massartiana* Geerinck rather than *H. hispanica* (Ruhsam et al. 2023) with the latter being uncommon in gardens and rarely escape into the wild (Rumsey 2023). However, gardeners often refer to them as *H. hispanica* or by using the vernacular name 'Spanish bluebell'. There can also be nomenclature problems such as with the vernacular name 'Japanese anemone' which is applied to several scientific names including *Anemone hup-ehensis* (Lemoine) Lemoine and *A.* × *hybrida* (Cubey et al. 2022), and under '*A. scabiosa'* (Dehnen-Schmutz and Conroy 2018). Using a drop-down question which searches for a taxon (as is the case now with Plant Alert) does not necessarily address these challenges because it still relies on gardeners identifying the correct taxon in their report. It does, however, reduce the number of reports being discarded through data cleaning.

This approach, if improved as suggested above and by allowing gardeners to report as many species as they wish, could be adopted as a form of horizon scanning for identifying future invaders even if it is not looking at 'door knocker' species (Seebens et al. 2018). This gives the opportunity to prioritise the approximately 70,000 ornamentals (Cubey et al. 2022) available for sale in the UK. This could also be extended beyond individual gardeners as the target audience (as in this study) to better engage with a wider range of gardeners and landscapers, such as those working in public or botanic gardens and in residential areas. This would build on work already done in the Czech Republic (Kutlvašr et al. 2019, 2020), which could be replicated in Britain and Ireland, to ensure selection of ornamentals do not include future invaders. Central to the approach is the structured scheme for prioritising future invaders (Fig. 1). This is also important for deciding which taxa need the invasive potential to be measured; for example, by adopting a species distribution modelling and/or trait-based approach (e.g. Fournier et al. 2019). This would result in both identifying future invaders and measuring their invasive potential.

Conclusion

Identifying future invaders before they can become invasive in the wild is an important yet challenging issue for invasion science. Gardeners have a crucial role here in being the 'first contact' for reporting ornamentals with invasive potential because ornamental horticulture is a main introduction pathway or source of invasive species globally. By addressing the research question of this study we have shown that data collected by gardeners can be used in a simple yet structured approach with the scheme for prioritising future invaders. This structured scheme is applied here to prioritise species in need of further analysis, such as a risk assessment, and has resulted in a shortlist of nine ornamentals of concern. Importantly, the shortlisted taxa were not identified as potentially invasive through horizon scanning. Furthermore, the approach has considerable potential for increasing awareness of invasive and potentially invasive ornamentals through engagement with gardeners by notifying them of the ornamentals of concern. This positive feedback loop between gardeners and invasion scientists could help reduce the risk of more ornamentals becoming invasive in the future.

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Supplementary material I

Scoping survey

Authors: Tomos Siôn Jones, Alastair Culham, Brian John Pickles, John David Data type: docx

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Link: https://doi.org/10.3897/neobiota.91.110560.suppl1

Supplementary material 2

Full list of reported taxa (cultivars not included) along with a unique ID for each gardener (survey participants)

Authors: Tomos Siôn Jones, Alastair Culham, Brian John Pickles, John David Data type: csv

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Rapid risk assessment of plant pathogenic bacteria and protists likely to threaten agriculture, biodiversity and forestry in Zambia

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Abstract

A prioritisation study was conducted to address the lack of adequate information about potential pests likely to be introduced in Zambia and become invasive. The study was conducted by subject matter experts from relevant institutions in and outside Zambia. Although this study focused on major pest categories, this paper only addresses bacteria and Protista. A list of 306 bacterial and 10 Protista species adjudged to affect plants was generated using CABI's Horizon Scanning Tool. The 316 (total) pest species were refined to focus on pests that affect value chains important to Zambia's economy. This resulted in a final list of 133 bacteria and eight Protista. Four additional bacteria species considered of phytosanitary interest were added and all 137 bacteria and eight Protista species were subjected to a rapid risk assessment

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using agreed guidelines. Vectors reported to transmit any of the pathogenic organisms were also subjected to a risk assessment. A proportion of 53% (n = 77 of 145) comprising 73 bacteria and four Protista species were reported as present in Africa. Of these, 42 (57%, n = 73) bacterial species and two (n=4) Protista species were reported in neighbouring countries. Considering a cut-off of 54, the highest scoring pests were 40 bacteria (highest score of 140) and three Protista (highest score of 125). Three actions were suggested for high-scoring pests, a detection surveillance, a pest-initiated pest risk analysis (PRA) or a detection surveillance followed by pest-initiated PRA. A "no action" was suggested where the risk was very low although, for some pathogenic organisms, a "no action" was followed by periodic monitoring. This information will contribute towards proactive prevention and management of biological invasions.

Keywords

Horizon scanning, invasive alien species, pest prioritisation, pest risk, risk assessment

Introduction

A number of alien species¹ have been introduced in sub-Saharan Africa (SSA) in the last couple of years through intentional or unintentional human-mediated activities (Faulkner et al. 2020; Uyi et al. 2021; Mulema et al. 2022). The majority of these aliens have become invasive² (here referred to as invasive alien species or IAS) as evidenced by their effects on agricultural productivity, human health, livelihoods and biological diversity (Early et al. 2016; Paini et al. 2016; Pratt et al. 2017). In phytosanitary terms, such organisms are considered pests³ and classified as quarantine⁴ pests if not yet widespread within a target region. The primary objective of National Plant Protection Organisations (NPPOs) is to prevent the introduction and spread of quarantine pests through regulation. The effect of IAS on agricultural productivity is characterised with loss of income due to reduced crop yields, compromised quality of harvested produce and increased management costs (Eschen et al. 2021).

For instance, Eschen et al. (2021) estimated losses associated with the invasive lepidopteran insect, *Spodoptera frugiperda* in SSA at USD 9.4 Bn annually. It has also been estimated that the invasive plant pathogenic bacterium, *Xylella fastidiosa*, will cause losses ranging from USD 1.9 to USD 5.2 Bn if no corrective measures, such as de-

¹ A species introduced outside its natural past or present distribution.

² A species whose introduction and/or spread by the human agency directly or indirectly threatens biological diversity.

³ The term "pest" is used within the context of the International Plant Protection Convention (IPPC) and refers to any species, strain, or biotype of plant, animal, or pathogenic agent injurious to plants or plant products (International Standards for Phytosanitary Measures (ISPM) Number 5). Pathogenic agents include bacteria, fungi, oomycetes, phytoplasma, viroid and virus while animals may include arthropods, molluscs and nematodes (IPPC Secretariat 2021).

⁴ A pest of potential economic importance to the area endangered thereby and not yet present there or present, but not widely distributed and being officially controlled (ISPM Number 5), (IPPC Secretariat 2021).

ploying resistant cultivars and application of appropriate phytosanitary measures⁵, are implemented (Schneider et al. 2020). Such phytosanitary measures include control of vectors that transmit the bacterium, suppression of inoculum and removal of infected host plants (Almeida et al. 2005; Liccardo et al. 2020; Castro et al. 2021; Quetglas et al. 2022). In SSA, management of IAS is associated with extensive indiscriminate application of mostly hazardous inorganic pesticides due to limited cost-effective and efficient pest control options (Siddiqui et al. 2023). This has resulted in the production of unsafe food and feed for human and animal consumption and reduced biodiversity due to the adverse effects of hazardous agro-chemicals on non-target species (Martinez et al. 2020).

The most cost-effective, efficient, sustainable and practical management option for IAS is through restricting entry or enabling early detection in case of entry, followed by prompt mitigation of pest spread and associated adverse effects of the IAS. However, this requires availability of adequate and up-to-date information about potential invasions (Mulema et al. 2022). Horizon scanning is one approach through which such information can be generated and availed to risk managers, policy and decision-makers (Sutherland et al. 2010, 2020; Matthews et al. 2017). It is the systematic search for potential biological invasions and an assessment of their potential impacts on the economy, society and environment considering possible opportunities for mitigating the impacts (Sutherland et al. 2008, 2010, 2020; Roy et al. 2014). Information generated from horizon scanning can be used to support planning on management of IAS at country and regional level and provide information for policy and practice (Caffrey et al. 2014).

At country level, horizon scanning has been used to prioritise IAS in countries, such as Cyprus (Peyton et al. 2019), Spain (Gassó et al. 2009; Bayón and Vilà 2019), United Kingdom (Sutherland et al. 2008), see also Great Britain (Roy et al. 2014) and recently in Ghana and Kenya (Kenis et al. 2022; Mulema et al. 2022). At the regional level, horizon scanning has been utilised in the European Union (Roy et al. 2019), Central Europe (Weber and Gut 2004) and Western Europe (Gallardo et al. 2016). CABI is also considering assessing at regional level, the risk of new IAS to the Regional Economic Blocks of the East African Community (EAC), Economic Community of West African States (ECOWAS) and Southern African Development Community (SADC). There is a paucity of information on potential biological invasions in most SSA countries resulting in reduced capacity for timely detection, mitigation and management of pertinent pest threats in the region. Therefore, the current study applies the horizon-scanning approach to generate useful pest-related information for Zambia that will enhance timely action on IAS. The study was conducted with the ultimate objective of prioritising pests that are not currently recorded as present in Zambia, but could be introduced and become invasive in future, thereby threatening the economy by negatively impacting on agriculture, biodiversity and forestry.

⁵ Any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests or to limit the economic impact of regulated non-quarantine pests (ISPM Number 5), (IPPC Secretariat 2021).

The full horizon-scanning assessment covered plant pests in the categories, Arthropoda, Bacteria, Chromista, Fungi, Mollusca, Nematoda, Protista, Viruses and Viroids. Previously, lists of candidate IAS for risk assessment were generated by experts through extensive literature searches (Weber and Gut 2004; Sutherland et al. 2008; Gassó et al. 2009; Roy et al. 2014; Gallardo et al. 2016; Bayón and Vilà 2019); howev-er, CABI has developed a Horizon Scanning Tool to support identification of pests for risk assessment. The Horizon Scanning Tool was previously applied in studies conducted in Kenya in 2018 (Mulema et al. 2022) and Ghana in 2020 (Kenis et al. 2022). The tool can be accessed directly from https://www.cabi.org/HorizonScanningTool and via the CABI Compendium (https://www.cabidigitallibrary.org/cabicompendium).

Materials and methods

Selection of pests from horizon scanning

A preliminary selection of pests that had not been reported as present in Zambia was conducted using the premium version of the Horizon Scanning Tool. In this tool, information from datasheets available in the CABI Compendium was used to generate a list of pest species that are not yet reported in the selected 'area at risk' (Zambia), but reported in specified "source areas" (such as trading partner countries). However, due to gaps in pest reporting mechanisms by some countries, non-availability of a presence record for a given pest in the area at risk is not necessarily a confirmation of a pest's absence. In the Horizon Scanning Tool, the following parameters were used.

The area at risk was identified as Zambia. This was followed by selecting areas from which likely invasive pests could be introduced (source areas). These areas included all geographical areas within all continents (Africa, Asia, Europe, North America, Oceania and South America), except Antarctica. The search under source areas could be further refined by emphasising countries with matching climatic conditions, based on the Köppen-Geiger climate classification (Rubel and Kottek 2010); however, this option was not considered because all geographical areas within all continents were selected. The search could be refined by selecting likely pathways of introduction, affected plant hosts, affected plant parts that may be used in trade, habitats, impact outcomes and type of organism. However, all these parameters were left open, except for the type of pest organism.

The type of pest organism considered for this study were bacteria, viruses (included viroids) protists, fungi and chromista (oomycetes) and invertebrates (included arthropods, molluscs and nematodes). Other pest categories although not considered for this study, were plants, vertebrates and diseases of unknown aetiology. Plants were not considered due to lack of the appropriate guidelines for risk assessment. In addition, the resulting pest list may be refined to retain only pests with enhanced (full) datasheets, only those that affect plants and those that have been established to be invasive. For this analysis, only pests known to affect plants were retained. The enhanced datasheet and invasive options were left open. The list generated from the tool was downloaded as an excel (.xlsx) file for downstream analysis.

The list was manually assessed to remove pests that do not affect value chains of interest to Zambia and pests represented by their genera instead of species names. The final list was subjected to risk assessment by 24 Subject Matter Experts (SMEs) convened from national and international agricultural research institutions, academia and extension institutions. The SMEs had experience in the fields of bacteriology, entomology, mycology, nematology and virology acquired from diverse backgrounds including policy, regulation, industrial and academic research. The SMEs were allocated to three thematic groups, based on their expertise: Entomology, Nematology and Plant Pathology. Plant pathology included the field of Bacteriology (bacteria and phytoplasmas), Mycology (included Chromista (oomycetes and fungi) and Virology (viruses and virolog).

Description of the scoring system

The risk scoring system used was based on that described by Roy et al. (2019). This scoring system (guidelines) had been modified in previous studies by Mulema et al. (2022) and Kenis et al. (2022). Roy et al. (2019) assessed the likelihood of arrival, establishment, spread and magnitude of potential negative impact on biodiversity and ecosystem services, whereas in this assessment, the likelihood of entry (arrival), establishment and potential magnitude of socio-economic impact and potential magnitude of impact on biodiversity were assessed. The likelihood of spread was considered under establishment; however, once an alien species arrives on the African continent, exponential spread within and between countries in SSA has been observed (Guimapi et al. 2016; De Groote et al. 2020). This is majorly assisted by human-mediated activities especially if the criteria for entry and establishment are met and the key pathways⁶ are available (Mahuku et al. 2015; De Groote et al. 2020). A 5-score system for the four parameters (entry, establishment, socio-economic and biodiversity impact) was used, where a score of 1 suggested unlikely to enter or establish or minimal impact and a score of 5 suggested very likely to enter or establish or major impact. The full guidelines and a description of the 5-score system for the four parameters are presented in Suppl. material 1, but briefly outlined below.

To assess the likelihood of entry, a score of 1 suggested absent from Africa and unlikely to be in the imported commodity; 2, absent from Africa, but likely to be infrequently imported on a commodity; 3, present in Africa (not in neighbouring countries) and spreads slowly; or absent from Africa, but recently spreads very fast on several continents or often associated with a commodity commonly imported or frequently intercepted in Zambia; 4, present in Africa (not in neighbouring countries) and spreads fast or in a neighbouring country and spreads slowly; and 5, present in a neighbouring country (Angola, Botswana, The Democratic Republic of the Congo (DR Congo), Malawi, Tanzania, Mozambique, Namibia and Zimbabwe) and spreads fast. To assess the likely pathways of arrival, three likely pathways as defined by Hulme et al. (2008) were considered.

⁶ The term "pathway" is used within the context of the IPPC and refers to any means that allows entry and spread of a pest (ISPM Number 5) (IPPC Secretariat 2021).

Hulme et al. (2008) defined three mechanisms through which alien species may enter a new geographical or political region. They included importation of a commodity, arrival of a transport vector and natural spread from a neighbouring region. The three mechanisms comprised six pathways namely, contaminant, escape and release under the importation of a commodity mechanism; stowaway under the arrival of a transport vector mechanism; corridor and unaided under the natural spread from a neighbouring region mechanism. Only three pathways were considered, contaminant, stowaway also referred to as hitchhiker and unaided, abbreviated in the tables as CO, ST and UN, respectively. Pathogenic organisms especially bacteria, viruses and viroids which could be carried by vectors, the stowaway pathway was considered although the contaminant pathway was also considered if the pathogenic organism is seed-borne⁷ and seed-transmitted⁸. The stowaway pathway was also considered for soil- and refuse-borne pathogenic organisms which could unintentionally be introduced with soil or plant debris.

To assess the likelihood of establishment, a score of 1 suggested Zambia is climatically unsuitable or host plants are not present; 2, only few areas in Zambia climatically suitable; or host plants rare; 3, large areas in Zambia climatically suitable and host plant rare; or only few areas in Zambia climatically suitable, but host plants at least moderately abundant; 4, large areas in Zambia climatically suitable and host plants moderately abundant; and 5, large areas in Zambia climatically suitable and host plants very abundant. For the potential magnitude of socio-economic impact, a score of 1 suggested the species does not attack plants that are cultivated or utilised; 2, the species damages plants that are only occasionally cultivated or utilised; 3, the species damages plants that are regularly cultivated or utilised, but without threatening the cultivation, utilisation or trade of this crop; 4, the species has the potential to threaten, at least locally, the cultivation of a plant that is regularly cultivated or utilised; or to regularly attack a crop that is key for the Zambian economy without threatening this latter; and 5, the species has the potential to threaten, at least locally, a crop that is key for the Zambian economy. For potential magnitude of impact on biodiversity, a score of 1 suggested the species will not affect any native species; 2, the species will affect individuals of a native species without affecting its population level; 3, the species has the potential to lower the population levels of a native species; 4, the species has the potential to locally eradicate a native species or to affect populations of a protected or keystone species; and 5, the species has the potential to eradicate a native species or to locally eradicate a keystone species.

Scoring of species

After a group training of SMEs at the initial workshop conducted in July 2022, the scoring of species was done independently by all SMEs. In September 2022, a consensus follow-up workshop was held to review the risk assessments for each attribute one by one and any discrepancies between the scores were discussed amongst the assessors. The assessors had the opportunity to modify their scores according to the opinions

⁷ A seed-borne organism is any organism or pathogen that is carried in or on or with seed.

⁸ Seed-transmission refers to the transfer and re-establishment of a seed-borne pathogen from seed to plant.

of the other SMEs. The risk score was validated through consensus and, in cases of disagreement, the individual scores and the evidence on which they were based were re-discussed. Confidence was estimated for each score recorded for species for the like-lihood of entry; establishment; potential magnitude of socio-economic impact; and potential impact on biodiversity; likely pathway of arrival; and for the overall score following Blackburn et al. (2014). The rating proposed by Blackburn et al. (2014) was originally modified from the European and Mediterranean Plant Protection Organisation (EPPO) pest risk assessment decision support scheme (OEPP/EPPO 2012). The information to support the scores and confidences and the likely pathways was obtained from CABI Compendium datasheets, peer-reviewed journal articles and reviews and grey literature (conference papers and proceedings; dissertations and theses; government documents and reports and newspaper articles). The SMEs also relied on their existing knowledge for assessing the species. The likely pathway of arrival and associated confidence levels were used to help focus discussions on the possibility of entry and establishment, but did not contribute to the overall score. Risk is a product

of likelihood of an event occurring and the impact associated with that likelihood. Therefore, the overall risk score was obtained by the following formula: *Likelihood of entry* × *likelihood of establishment* ×

(magnitude of socio-economic impact + magnitude of impact on biodiversity)

Scores below three were considered low risk because of their low impact on the likelihood of entry, establishment, economic and biodiversity damage; scores of three were considered moderate, while scores above 3 (4 and 5) presented a high risk because they had an opposite effect from the low scores. The overall risk score was used to rank species according to their potential threat to Zambia. A minimum score of 54 was considered as the cut-off for further consideration because such a species scored an average of three for all the assessable attributes or more than a three in at least three or more attributes. A score of three suggested a situation that was skewed towards the possibility of entry, establishment and higher impact (social-economic or biodiversity). For all assessed species, recommendations on the next course of action was made.

Results

The initial search yielded a total of 306 plant pathogenic bacteria and 10 protists. However, following a cleaning process to remove pests represented only by genus names, the list was narrowed down to 283 bacterial and 10 Protista species that were eligible for assessment (Suppl. material 2). The cleaned list comprised of 43 species reported as invasive, all of which were bacterial species. The list was further refined to focus on pests that damage value chains relevant to Zambia which resulted in a list of 137 bacteria (Suppl. material 3) and eight Protista (Suppl. material 4) species resulting in a total of 145 pests. It is this list that was subjected to rapid risk assessment using the guidelines presented in Suppl. material 1, but also briefly described in the methodology. In addition, species, not yet reported as present in Zambia, but adjudged to be of phytosanitary concern, were added to each respective pest category although this was only possible for the bacterial species. The additional pests are highlighted in the column named "From horizon scanning" (Suppl. materials 3, 4) particularly those indicated as "N" (for NO) in the list, denoting that the given pest was not part of the original scanning process. Vectors that have been reported to transmit the assessed pest species, especially for the bacteria species were also assessed to establish their associated level of risk (Suppl. material 5). For both categories (Bacteria and Protista), 53% (n = 77 of 145) were reported in Africa. Of the 53% reported in Africa, 60% (n = 46 of 77) were reported for neighbouring countries to Zambia (Suppl. materials 3, 4). Such pests had very high overall risk scores because of their increased likelihood of entry.

Bacteria

The final bacterial list for assessment comprised 137 species as indicated above. Of these, 77 species representing a proportion of 53% were reported in Africa, with 42 of the 77 species (55%) reported in countries neighbouring Zambia. Of the 137 species, 132 (96%) species were identified through the horizon scanning process and five species (4%) were added because they presented a phytosanitary risk to agriculture and, therefore, the economy of Zambia. Sixteen percent (n = 21 of 132) of the species were recorded as invasive in some countries. The highest overall risk score was 140 recorded for *Candidatus Phytoplasma pini*, *Dickeya zeae*, *Leifsonia xyli* subsp. *Xyli* and *Xanthomonas axonopodis* pv. *vasculorum* and the lowest was 5 recorded for *Candidatus Arsenophonus phytopathogenicus*. A proportion of 66% (n = 90) could be introduced as contaminants, 24% (n = 33) either as contaminants or stowaways or both, while the least, 10% (n = 14) as stowaways. The contaminant pathway mainly comprised introduction as seed, plants for planting or plant parts, while stowaways mainly comprised vectors. Introduction through the unaided pathway was not considered likely for this group of pests.

Three of the four of the species (*Pectobacterium parvum*, *P. peruviense* and *P. punjabense*) added to the horizon scanning results belonged to the family Pectobacteriaceae (Soft Rot Pectobacteriaceae or SRP), while one, *Xanthomonas citri* pv. *aurantifolii* belonged to the family Lysobacteraceae. All added SRPs recorded an overall risk score below the suggested cut-off of 54, while the xanthomonad recorded an overall risk score above the suggested cut-off of 54 (75). Eleven percent (n = 15 of 137) of the assessed bacterial species belonged to the Phylum Tenericutes which comprises the phytoplasmas. A proportion of 54% (n = 74 of 137) of the species had full (enhanced) datasheets available in the CABI Compendium which provided access to detailed information for assessment. However, various sources of literature were used to assess the remaining 46% with only basic datasheets. Twenty-one (15%) of the assessed bacterial species are vectored, all of which were phytoplasmas, except for *C. Arsenophonus phytopathogenicus, Candidatus Liberibacter africanus, Candidatus Liberibacter asiaticus, Candidatus Liberibacter africanus, Pantoea stewartii, Spiroplasma citri, Xylella fastidiosa subsp. fastidiosa* and Xylella fastidiosa subsp. pauca.

At the considered cut-off overall score of 54 as suggested by Mulema et al. (2022), sixty-two (47%, n = 137) of the species were classified as high-scoring and hence prioritised for action (Table 1). The high-scoring species were all reported as present in Africa (57 species, 92%), except Sugarcane grassy shoot phytoplasma, Sugarcane white leaf phytoplasma, *X. citri* pv. *aurantifolii*, *X. fastidiosa* subsp. *fastidiosa*, *Xylella fastidiosa* subsp. *Multiplex* and *Xylella fastidiosa* subsp. *pauca* (*Xfp*) (Table 1, Suppl. material 3). A proportion of 70% (40 of 57 pest species) were reported as present in the neighbouring countries.

Protista

Only eight species were assessed, all of which were identified using the Horizon Scanning Tool with no protist of phytosanitary concern added from other sources. All except one, *Physarum cinereum*, had full (enhanced) datasheets available in the CABI Compendium and none had been reported as invasive in any country. Four of the species were reported as present in Africa with only two reported in the neighbouring countries of Angola, Malawi, Mozambique, Tanzania and Zimbabwe (Suppl. material 4). Considering a cut-off of 54 for the overall risk score, only three species *Plasmodiophora brassicae* (125), *Spongospora subterranea* (100) and *Polymyxa graminis* (60) had the highest overall risk score (Suppl. material 4). Although none of the assessed species could be introduced in Zambia through the unaided pathway, six of the species could be introduced through the stowaway pathway and two could be introduced through the contaminant and stowaway pathways.

Vectors and vectored species

Two of the assessed protists species, Spongospora subterranea and Polymyxa graminis, are reported vectors of Potato mop-top virus (Chikh-Ali and Karasev 2023) and various diseases of wheat, barley and groundnut viruses, respectively (Kanyuka et al. 2003). A total of eighty species were reported to vector the assessed bacterial species. Of these, 11 (18%) had been reported in Africa and were Anguina agrostis, Bactericera trigonica, Diaphorina citri, Neoaliturus tenellus, Nephotettix nigropictus, Orosius albicinctus, Orosius orientalis, Pentastiridius leporinus, Philaenus spumarius and Trioza erytreae (Table 2, Suppl. material 5). Two of these species have been reported as present in neighbouring countries, D. citri in Malawi and T. erytreae in DR Congo, Malawi, Tanzania and Zimbabwe, while T. erytreae has been reported as present in Zambia (Table 2, Suppl. material 5). The highest overall risk score was 125 for D. citri, while the lowest was 2 scored for Aphrodes bicinctus, Colladonus montanus, Euscelis lineolatus, Helochara delta, Neoaliturus pulcher, Zeoliarus atkinsoni and Zeoliarus oppositus. Trioza erytreae was not scored because it was already reported as present in Zambia as indicated above (Aidoo 2023). The assessed vectors were likely to be introduced mainly through the contaminant pathway, especially for those reported outside Africa or in Africa, but not in neighbouring countries, although the stowaway pathway was also possible for those reported outside Africa as eggs or young adults. Further, those reported in neighbouring countries were likely to be introduced as contaminant or stowaways or they could spread unaided.

including l	ikelihood	and impact sco	ores hav	ve not been included in th	s Table; however,	they are	presented in Sı	1ppl. materials 3, 4.			
Pest species (Preferred name)	Kingdom	Family	Invasiv	e Host species	Vectored by Vi	sctor Afric of count wid repoi	an Neighbouring ries countries with a reports ts	Where the pathogenic organism has been reported in Africa	Likely C pathway of arrival (CO, UN, ST)	Dverall risk score	Suggested actions
avenae avenae	Bacteria	Comamonadaceae		Main hosts: Oryza sativa, Saccharum officinarum, Sorghum bicolor, Zat mays		Y	Y	Burkina Faso, Comoros, Côte d'Ivoire, DR Congo, Egypt, Ethiopia, Gabon, Kenya, Madagaseat, Malawi, Mauritus, Mozambique, Niger, Nigeria, Réunion, Siterra Loone, South Africa, Sudan, Tanzania, Uganda, and Zimbabwe	00	100	Detection surveillance
Candidatus Liberibacter africanus	Bacteria	Phyllobacteriaceae	Y	Main hosts: Calodendrum capense, Girns aurantifolia, Girns innon, Girns sobilis, Girns reticulata, Cirns sinensis, Cirns paradisi, and Poncirus trifoliata	Trinza erytreae	Y	Υ	Angola, Burnudi, Cameroon, Central African Republic, Conoross, Eswatni, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Nigeria, Réunion, Rwanda, Somila, Uganda, South Africa, Tauzania, Zimbabwe, and Saint Helena	CO, ST	96	Detection surveillance
Candidatus Liberibacter asiaticus	Bacteria	Phyllobacteriaceae	Υ	Main host: Citrus reticulata and Citrus sinensis	Diaphorina citri	Υ	Z	Ethiopia, Kenya, Mauritius, and Réunion	CO, ST	72	A pest-initiated PRA to advise on import requirements.
Candidatus Liberibacter solanacearum	Bacteria	Phyllobacteriaceae	Y	Main hosts: Capsicum annuum, Datura stramonium, Solanum lycopersicum, Solanum tuberosum	Bactericena cockerelli, Bactericena trigonica, Trioza	Y	Z	Morocco and Tunisia	ST	72	No action is suggested for now.

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Table 1. Presents bacteria and protist species identified through horizon scanning that recorded an overall score of 54 and above. The overall score is derived from the product of likelihood and impact scores. Three likely pathways; contaminant (CO), stowaway (ST), and unaided (UN) were considered. These pathways are defined by Hulme et al. (2008) under the three mechanisms through which alien species may enter a new geographical or political region. Most of the assessed parameters

Deet en oci oc	Kinadom	Family	Inviena	Hast snacias	Vactored hv	Vactor	African	Neichhouring	When the notherenic	I iboly	Owner	Successful actions
Preferred name)	and a second secon	Ánna -			6	of	countries with reports	countries with reports	w not up anogene organism has been reported in Africa	pathway of arrival (CO, UN, ST)	risk score	
ndidatus top lasma ris	Bacteria	Acholeplasmaaceae	×	Main hosts: Allium cepa, Auronne cornancia armania, Anethum Busasia napus, Bunsia a oleracaa subsp. aapitata, Bussia a oleracaa subsp. italica, Bussia a oleracaa subsp. italica, Bussia a oleracaa argentea, Chrysanthernum fritacens, Chrysanthernum gratecens, Chrysanthernum fritacens, Chrysanthernum grates, Chrysanthernum grates, Chrysanthernum grates, Chrysanthernum grates, Thomas a oleraca, lagers area, Tagets erecta, Tagers patula, Trifolium tyens, and Za mays Trifolium tepens, and Za mays	Aphrodes bicinctus, Colladonnus geminatus, geminatus, Euceliaiu airrigatus, Eucedis, Euseclis jimolatus, Euseclis graderas, Hishinonoides Relatiformis, Macrostels ateris, Macrostels ateris, Macrostels strijforus, Macrostels seconotatus, Macrostels seconotatus, Macrostels seconotatus, Macrostels seconotatus, Scaphyropius acutus		×	Z	South Africa	CO, ST	105	No action is suggested for now. This is advised by the absence of all the reported vectors in Africa.
ıdidatus toplasma antifolia	Bacteria	Acholeplasmataceae		Main hosts: Citrus aurantiifolia	Hishimonus phycitis		Y	z	Ethiopia, South Africa, Sudan, and Uganda	CO, ST	54	No action is suggested for now.
didatus toplasma zae	Bacteria	Acholeplasmataceae		Main host: Oryza satiua	Nephotettis cinciterys, Nephotettis nigropictus, Nephotettis viressens		Y	z	Kenya	ST	72	With less evidence of transmission in seed, a pest-initiated PRA may not be appropriate at the a detection to establish the status of the pest.
didatus toplasma	Bacteria	Acholeplasmataceae		Main hosts. Pinus halepensis, Pinus yluestris	Unknown		Y	Y	Mozambique	ST	140	Detection surveillance to guide on other phyrosanitary measures

Pest species (Preferred name)	Kingdom	Family	Invasive	Host species	Vectored by	Vector of c	African countries with reports	Neighbouring countries with reports	Where the pathogenic organism has been reported in Africa	Likely pathway of arrival (CO, UN, ST)	Overall risk score	Suggested actions
Candidatus Phytoplasma solani	Bacteria	Acholeplasmataceae	×	Main hosts: Capsian amuum, Lavandula argustifolia, Solanum lyeopersicum, Sulanum tuberosum, Vitis vinifera, Zaa majs	Anaceratagallia ribauti, Hyalesthes obsoletus Signoret; Reptalus panzeri		¥	z	Niger	CO, ST	06	No action is necessary for now. A pest-initiated PRA is also not necessary because the pest is not naturally seed- transmitted yet the rectors have not been reported in Africa.
Cassava witches' broom	Bacteria	Acholeplasmataceae		Main host: Manihot esculenta	Unknown		Y	z	Côte d'Ivoire	CO, ST	84	No action is suggested for now
Dickeya chrysanthemi	Bacteria	Pectobacteriaceae		Main hosts. Chrystarthemum morifoltum and Dianthus caryophyllus			Y	¥	Algeria, Comoros, Cote d'Ivoire, Egypt, Morocco, Republic of the Congo, Reunion, South Africa, Sudan, and Zimbabwe	8	120	Detection surveillance
Dickeya dadantii	Bacteria	Pectobacteriaceae		Main host: Solanum tuberosum			Y	Υ	Comoros and Zimbabwe	CO	72	Detection surveillance
Dickeya dianthicola	Bacteria	Pectobacteriaceae		Main host: Solanum tuberosum			Y	z	Morroco and South Africa	8	54	Detection surveillance
Dickeya zeae	Bacteria	Pectobacteriaceae		Main host: Zea mays			Y	γ	Comoros, Egypt, Mauritius, Réunion, South Africa, Sudan, and Zimbabwe	CO	140	Detection surveillance
Herbasp irillum rubrisubalbicans	Bacteria	Oxalobacteraceae		Main hosts. Sacebarum officinarum, Sorghum halepense, Zea maps. Other host. Sorghum bicelor			Y	X	Angola, Benin, Burundi, Central African Republic, Côte d'Ivoire, Madagascar, Malawi, Mauritius, Nigeria, Réunion, Tanzania, and Togo	00	120	Detection surveillance
<i>Leifsonia xyli</i> subsp. <i>xyli</i>	Bacteria	Microbacteriaceae	×	Main host: Sachurum officinarum			×	Y	Burkina Faso, Cameroon, Comocos, Djibouri, DR Comoros, Djibouri, DR Ethiopia, Kenya, Madagascar, Makawi, Mali, Mauritus, Mozambique, Nigeria, Republic of the Congo, Republic of the Congo, Returion, Sychelles, Somalia, South Africa, Sudan, Tarazaid, Uganda, and Zimbalwe	ST	140	Detection surveillance

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<u>g</u> <u>B</u>	шордини	ramuy	IIIVasive	rrost species	vectored by	of co	with eports	veignoouring countries with reports	w nere ue parnogenic organism has been reported in Africa	Likely pathway of arrival (CO, UN, ST)	Uverau risk score	ouggested actions
anatis	Bacteria	Erwiniaceae		Main hosts: Allium cepa, Ananas comosus, Brassica rapa subsp. pekinensis, Cirrus sinensis, Cauamis mulo, Cucamis satitus, Fragaria ananasa, Oryza satitu, Pranus persia, Zat map	Diabrotica virgifera virgifera		×	X	Benin, Burkina Faso, Egypt, Morocco, Nigeria, South Africa, Togo, and Zimbabwe	8	120	Detection surveillance
trea	Bacteria	Erwiniaceae		Main host: Ananas comosus			Y	Y	Tanzania	00	80	Detection surveillance
ewartii vartii	Bacteria	Erwiniacae		Main hosts: Zea mays subsp. mays. Zea mays subsp. meriatana. Zea mays subsp. Parviglumises, Triticum aestirum	Chaetoenena pulicaria Melsheimer		¥	z	Benin and Togo	ST	105	No action is necessary for now because the pathogen has only been reported in Benin and Togo while the vector has only been reported in Cameroon.
rium m	Bacteria	Pectobacteriaceae		Main host: Solanum tuberosum			Y	Y	Algeria, Egypt, Mauritius, Morocco, Mozambique, South Africa, Tanzania, Tunisia, and Zimbabwe	8	80	Detection surveillance
rium lorum	Bacteria	Pectobacteriaceae		Main host: Beta vulgaris var. saccharifera, Solanum tuberosum			¥	z	Egypt	8	60	A detection surveillance followed pest-initiatiated PRA
<i>rium</i> al.	Bacteria	Pectobacteriaceae		Main host: Solanum tuberosum			¥	γ	Algeria, Egypt, Kenya, Morocco, Réunion, South Africa, and Zimbabwe	00	80	Detection surveillance
rium un	Bacteria	Pectobacteriaceae		Main host: Solanum tuberosum			Y	Y	Algeria, Central African Republic, Egype, Ethiopia, Libya, Mauritus, Morocco, Republic of the Congo, South Africa, Sudan, and Zimbabwe	0	100	Detection surveillance
rium ri	Bacteria	Pectobacteriaceae	Υ	Main host : Solamum tuberosum			Y	Υ	South Africa, and Zimbabwe	CO	60	Detection surveillance
phora	Protista	Plasmodiophoraceae	0	Main hosts: Brassica napus, Brassica oleracea subsp. capitata, Brassica oleracea subsp. gorgylodes, Brassica olevacea subsp. gorgylodes,			Y	Y	Angola, Malawi, São Tomé and Príncipe, and South Africa	ST	125	Detection surveillance

Rapid risk assessment of plant pathogenic bacteria and protists

Pest species (Preferred name)	Kingdom	Family	Invasive	Host species	Vectored by	Vector of c	African ountries with reports	Neighbouring countries with reports	Where the pathogenic organism has been reported in Africa	Likely pathway of arrival (CO, UN, ST)	Overall risk score	Suggested actions
Polymyxa graminis	Protista	Plasmodiophoraceae		Main hosts: Arachis hypogaes, Avena sativa, Hordeum vulgare, Oryza sativa, Secale cereale, Triticum aestivum		Streak mosaic of wheat	Y	Z	Burkina Faso, Côte d'Ivoire, Mali, Niger, and Senegal	ST	60	A pest-initiated PRA to advise on import requirements.
Pseudomonas cichorii	Bacteria	Pseudomonadaceae	Y	Main hosts: Apium guteolens, Onysanthemun nerointinn, Onysanthemun nerointinn, Onysanthemun nersition, Chyorium endivia subsp. endivia, Gaborium endivia subsp. erispum, Gaborium endivia subsp. erispum, Latenta satist, and Vigna Latenta satist, and Vigna			Y	Y	Burundi, Egypt, South Africa, and Tauzania	CO	120	Detection surveillance
Pseudomonas corrugata	Bacteria	Pseudomonadaceae		Main host: Solanum lycopersicum			Y	Y	Egypt, South Africa, and Tanzania	00	120	Detection surveillance
Pseudomonas marginalis pv. marginalis	Bacteria	Pseudomonadaceae		Main host: Lactuca sativa			Y	Υ	Egypt, Ethiopia, Kenya, Nigeria, South Africa, Tanzania, and Uganda	CO, ST	60	Detection surveillance
Pseudomonas mediterranea	Bacteria	Pseudomonadaceae		Main host: Solamm lycopersicum			Υ	Υ	Egypt, South Africa, and Tanzania	CO	80	Detection surveillance
Pseudomonas syringae pv. atrofaciens	Bacteria	Pseudomonadaceae		Main host: Triticum aestivum			Y	Z	Morocco, South Africa, and Zimbabwe	0	60	Detection surveillance
Pseudomonas syringae pv. coronafaciens	Bacteria	Pseudomonadaceae		Main host : Avena fatua, Avena sativa, Secale cereale			Y	Y	Ethiopia, Kenya, Morocco, Zimbabwe	0	96	Detection surveillance
Pseudomonas syringae pv. garrae	Bacteria	Pseudomonadaceae		Main host: Coffea arabica			Y	Z	Kenya	CO	60	No action is suggested for now.

Pest species (Preferred name)	Kingdom	Family	Invasive	Host species	Vectored by	Vector of	African countries with reports	Neighbouring countries with reports	Where the pathogenic organism has been reported in Africa	Likely pathway of arrival (CO, UN, ST)	Overall risk score	Suggested actions
Psendomonus syringae pv. maculicola	Bacteria	Pseudomonadaceae		Main hosts: Brassica juncar vat. juncat, Brassica nigra, Brassica oleracer vat: borptis, Brassica oleracear vat: optingta, Brassica oleracear vat. gongylodes, Brassica oleracear vat. inidite, Brassica oleracear vat. inidite, Brassica oleracear vat. inidite, Brassica subsp. pekinentis, Brassica rapa subsp. rapa, Raphanus saturas			×	Y	Algeria, Mauritius, Mozambique, South Africa, Zimbabwe	CO	80	Detection surveillance
Pseudomonas syringae pv. mellea	Bacteria	Reudomonadaccae		Main hosts: Atriplex bortensis, Arropa belladomu, Datura stratmonium, Hyoscyamus niger, Nicotiana data, Nicotiana Vicotiana adata, Nicotiana Nicotiana adata, Nicotiana Solanum byopersium, Cannabis sativa			X	X	Tanzania	0	80	Detection surveillance
Pseudomonas syringae pv. pisi	Bacteria	Pseudomonadaceae		Main hos t: Pisum sativum			¥	Y	Kenya, Malawi, Tanzania, Zimbabwe, and South Africa	CO	60	Detection surveillance
Pseudomonas syringae pv. sesami	Bacteria	Pseudomonadaceae		Main hosts: Sexamun indicum			Y	Y	Egypt, South Africa, Tarzania, and Uganda	0	60	Detection surveillance
Pseudomonas syringae pv. striafaciens	Bacteria	Pseudomonadaceae		Main hosts : Avena sativa, Hordeum vulgare, Zea mays			X	Y	South Africa and Zimbabwe	0	100	Detection surveillance
Pseudomonas syringae pv. tomato	Bacteria	Pseudomonadaceae		Main host: Solanum lycopersicum			Y	Y	Morocco, South Africa, Tanzania, and Tunisia	CO	80	Detection surveillance
Ralstonia solanacearum (Phylotype II)	Bacteria	Burkholderiaceae	Y	Main host: Musa Spp.			Y	z	Ethiopia, Libya, Nigeria, and Senegal	CO, ST	72	A pest-initiated PRA to advise on import requirements.
Spongospora subterranea	Protista	Plasmodiophoraceae		Main host Solanum tuberosum		Potato Mop Top Virus.	¥	×	Algeria, Burundi, Egypt, Kenya, Madagascar, Mauritius, Morocco, Mozambique, Rwanda, South Africa, Tanzania, Tunisia, and Tanzania, Tunisia, and	CO, ST	100	Detection surveillance

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Pest species (Preferred name)	Kingdom	Family	Invasive	Host species	Vectored by	vector of c	African ountries with reports	Neighbouring countries with reports	Where the pathogenic organism has been reported in Africa	Likely pathway of arrival (CO, UN, ST)	Overall risk score	Suggested actions
Streptomyces scabiei	Bacteria	Streptomyces		Main host: Solanum tuberosum			Y	z	South Africa	CO, ST	54	Detection surveillance
Sugarcane grassy shoot phytoplasma	Bacteria	Acholeplasmataceae		Main hosts. Saccharum officinarum, Saccharum spontaneum	Deltocephalus vulgaris		z			CO, ST	70	A pest-initiated PRA to advise on import requirements.
Sugarcane white leaf phyroplasma	Bacteria	Acholeplasmataceae		Main hosts: Saccharum officinarum, Saccharum spontaneum, Other hosts: Saccharum edule, Saccharum nobustum	Matsummatettix hirogophicus, Yamatoettix flavovittatus		z			CO, ST	20	A pest-initiated PRA to advise on import requirements.
Sugarcane yellow leaf phytoplasma	Bacteria	Acholeplasmataceae		Main hosts: Sacharum officinarum	Sacchanoydne saccharivora, Masurumarettix hiroglophicus, Dedrocephalus udgaris, Yamatorettix flavovittatus		А	z	Могоссо	CO, ST	105	A pest-initiated PRA to advise on import requirements.
Xanthomonas axonopodis pv. cajani	Bacteria	Lysobacteraceae		Main host: Cajamus cajam			Y	Y	Malawi and Sudan	CO	72	Detection surveillance
Xanthomonus axonopodis pu manihotis	Bacteria	Lysobacteraceae	Y	Main host: Manihos esculenta			Y	A	Benin, Burkina Faso, Burtundi, Cameroon, Carntal African Republic, Connors, Gôte d'Ivoire, DR Congo, Ghana, Kenya, Madagascar, Malawi, Mali, Mauritius, Mayotte, Mali, Mauritius, Mayotte, Mali, Mauritius, Mayotte, Niger, Nigeria, Republic of the Congo, Reunion, Rwanda, South Africa, Sudan, Tiazania, Togo, and Uganda	0	80	Detection surveillance
Xanthomonas axonopodis pv. vasculorum	Bacteria	Lysobacteraceae	¥	Main host: Sacoharum officinarum			¥	Y	Eswatini, Ghana, Madagascar, Malawi, Mauritius, Mozambique, Réunion, South Africa, and Zimbabwe	8	140	Detection surveillance
Xanthomonas axonopodis pv. vignicola	Bacteria	Lysobacteraceae		Main host: Vigna unguiculata			Y	X	Botswana, Egypt, Nigeria, South Africa, Sudan, Tanzania, and Zimbabwe	8	60	Detection surveillance

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Suggested actions	Detection surveillance	Detection surveillance	Detection surveillance	Detection surveillance	Detection surveillance	Although this pest has not been reported in Africa, a detection surveillance is suggested before additional measures are instituted.
Overall risk score	60	60	60	80	100	75
Likely pathway of arrival (CO, UN, ST)	00	8	00	8	8	0
Where the pathogenic organism has been reported in Africa	Zimbabwe	Algeria, Angola, Ethiopia, Ghana, Kenya, Libya, Malawi, Maurius, Morocco, Mozambique, Seychelles, Somalia, Tanzania, Togo, Uganda, and Zimbabwe	Ghana, Malawi, South Africa, and Zimbabwe	Burundi, DR Congo, Kenya, Malawi, Rwanda, Tanzania, and Uganda	Benin, Burkina Faso, DR Congo, Cóte d'Ivoire, Ethiopia, Gabon, Madagascar, Mali, Mauritius, Réunion, Serregal, Scychelles, Somalia, Sudan, and Tanzania	
Neighbouring countries with reports	Y	К	Y	Y	Y	
African countries with reports	Y	Y	Y	Y	¥	z
Vector of						
Vectored by						
Host species	Main host: Armoracia rusticana, Brassica oleracea var. botrytis, Brassica oleracea var. gemmifera, Brassica oleracea var. italica	Main hosts: Brasica junca var, junca., Brasica junca var, iunca., Brasica oteraca var. obrytis, Brasica oteraca var. capitata, Brasica oteraca var. grapjode, Brasica oteraca var. grapjode, Brasica oteraca var. wiridis, Brasica oteraca var. wiridi	Main host: Tagetes erecta, Zinnia elegans	Main host: Manihot exculenta	Main hosts: Citrus sinensis, Citrus paradasi, Citrus limon, and Gitrus auramiifolita	Main hosts: Citrus sinensis, Citrus paradisi, Citrus limon, and Girus aurumijolita
Invasive					¥	
Family	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraccae
Kingdom	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria
Pest species (Preferred name)	Xanthomonas campestris pv. armoraciae	Xanthomonus camportris camportris camportris	Xanthomonas campestris pv. zimniae	Xanthomonas cassavae	Xanthomonas citri pv. citri	Xanthomonas citri subsp. aurantiifolia

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Suggested actions	Detection surveillance	Detection surveillance	Detection surveillance	Detection surveillance	Detection surveillance	Detection surveillance	Detection surveillance	Detection surveillance	A detection surveillance followed by a pest-initiated PRA to advise on import requirements of key of host species.
Overall risk score	80	80	60	80	60	75	60	100	56
Likely pathway of arrival (CO, UN, ST)	8	8	8	8	0	8	CO	8	CO, ST
Where the pathogenic organism has been reported in Africa	Comoros, Mauritius, Nigeria, Réunion, Seychelles, and Tanzania	Comoros, Ethiopia, Mauritius, Seychelles, and Tanzania	Nigeria, Sudan, and Tanzania	Benin, Burkina Faso, Burundi, Cameroon, Egypt, Gabon, Gambia, Guinea, Mali, Niger, Nigeria, Senegal, Tanzania, Togo, and Uganda	Côte d'Ivoite, Kenya, Madagascat, Nigeria, Senegal, Burkina Faso, Burundi, Mali, and Uganda	Côte d'Ivoire, Ethiopia, Gambia, Madagascar, Niger, South Africa, and Togo	Burundi, DR Congo, Ethiopia, Kenya, Rwanda, Tanzania, and Uganda	Madagascar, South Africa, and Zimbabwe	
Neighbouring countries with reports	Y	Υ	Y	Y	z	z	Y	Υ	
African countries with reports	Y	Y	Y	Y	Y	X	Y	Y	z
Vector of									
Vectored by									
Host species	Main hosts: Capsicum amuum, Capsicum frutescens, Solanum bycopersicum	Main hosts: Capsicum annuum, Solanum lycopersicum	Main hosts: Sexamum indicum	Main host: Oryza sativa	Main host: Oyza satiw; Wild host: Zizamia aquatica	Main hosts: Panicum miliaceum, Searia italica, Songhum dmum, Sorghum bicolor, Songhum halepense, Songhum sudanense, Zea mays	Main hosts: Ensete ventricosum, Musa sp.	Main hosts : Eucalyptus grandis, Saccharum officinarum, Zea mays	Main hosts: Cistus monspeliensis, Coffea sp. Erysimum sp., Juglans regia, Nerium oleander, Polygala myrtifolia, Pruna anium, Pruna ducis, Sutvia reomarinus, Semotorennos, Vereinius,
Invasive					Y		Y	7	
Family	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae	Lysobacteraceae
Kingdom	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria
Pest species (Preferred name)	Xanthomonas euvesicatoria pv. euvesicatoria	Xanthomonas euvesicatoria pv. perforans	Xanthomonas euvesicatoria pv. sesami	Xanthomonas oryzae pv. oryzae	Xanthomonas oryzae pv. oryzicola	Xanthomonas vasicola pv. holcicola	Xanthomonas vasicola pv. musacearum	Xanthomonas vasicola pv. vasculorum	<i>Xylella fastidiosa</i> subsp. <i>fastidiosa</i>

Rapid risk assessment of plant pathogenic bacteria and protists

scores. M	ost of t <u>l</u>	ne assessed	parameters	including likelihood and im	pact scores ha	ave not b	een include	d in this	Table; however, they	are presen	ted in S	suppl. material 5.
Vector species	Class	Order	Family	Known host plant species	Vectored of	African countries with reports	Neighbouring countries with reports	Reports in Zambia	Distribution in Africa	Likely pathway of arrival (CO, ST, UN)	Overall risk score	Suggested action
Anguina agrostis	Chroma- dorea	- Rhabditida	Anguinidae	Main hosts. Agratis cantint, Agratis capillaris, Agratis cantata, Agratis stonnifera, Bronna eventus, Datrylis geomerata, Festuca ruforu var. commuta, ovina, Festuca ruforu var. commuta, laiom multiflorum , Duluom rigidum, Phlaum putenee, Poa amuta, Poa memorik, Pou palairis,	Rathayibucter taxicus	Y	Z	z	South Africa	00	45	No action is suggested for now because the risk score is very low and the pest is not reported in Africa.
Bactericera trigonica	Insecta	Hemiptera	Triozidae	Main hosts : Apium graveolens and Daucus carota subsp. sativus	Candidatus Liberibacter solanacearum	Y	Z	z	Algeria, Egypt, Morocco, and Tunisia	CO, ST	15	No action is suggested for now because the risk score is very low and the pest is not reported in Africa.
Diaphorina citri	Insecta	Hemiptera	Liviidae	Main hosts: Cirrus aurantifolia, Cirrus limon, Murraya koenigii	Candidatus Liberibacter asiaticus	Y	Y	z	Burundi, Cameroon, Central African Republic, Comoros, Eswatini, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Réunion, and Rwanda	CO, ST, UN	125	Since the pest is reported in a neighbouring country, a detection surveillance is needed to establish its stautus
Neoaliturus tenellus	Insecta	Hemiptera	Cicadellidae	Main host. Armonucia natitana, Beta vulgaris	Candidatus Phytoplasma trifolii; Spiroplasma citri	Y	Z	Z	Algeria, Egrpt, Libya, Morocco, Namibia, South Africa, Sudan, and Tunisia	CO, ST, UN	80	Since the pest is reported in a key vading pattner (South Africa), a detection surveillance is needed to establish its status. This action is also underscored by the high score.
Nephotettix nigropictus	Insecta	Hemiptera	Cicadellidae	Main hosts: Cyperus eculentus, Oryza sativa	Candidatus Phytoplasma oryzae	Y	Z	z	Cameroon	CO, ST, UN	80	A detection surveillance is suggested because of the high score. This is underscored by the importance of the value chain and the pathogenic organism vectored by the pest.

Table 2. Rapid risk assessment of vectors reported to transmit bacterial pathogenic organisms identified through horizon scanning. Only vectors reported in Africa are presented. Three likely pathways; contaminant (CO), stowaway (ST) and unaided (UN) were considered. These pathways are defined by Hulme et al. (2008) under the three mechanisms through which alien species may enter a new geographical or political region. The overall score is derived from the product of likelihood and impact

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Vector	Class	Order	Family	Known host plant species	Vectored of	African	Neighbouring	Reports	Distribution in Africa	Likely	Overall	Suggested action
species				c c		countries	countries with	.ц		pathway of	risk	}
4						with	reports	Zambia		arrival (CO,	score	
Orosius	Insecta	Hemiptera	Cicadellidae	Main host: Sesamum indicum	Pigeon pea	Y	z	z	Sudan, and Tunisia	CO, ST	80	This pest needs regulation
albicinctus					witches' broom phytoplasma							because of the likely source of planting materials.
Orosius	Insecta	Hemiptera	Cicadellidae	Main host: Sesamum indicum	Candidatus	Υ	z	z	Egypt	CO, ST	20	No action is suggested for
orientalis					Phytop lasma							now because the risk score
					<i>trifolii</i> ; Soybean							is very low and the pest is
					phyllody phytoplasma							not reported in Africa.
Pentastiridius	Insecta	Hemiptera	Cixiidae	Main hosts: Prunus dulcis	Candidatus	Υ	z	z	Algeria and Tunisia	CO, ST,	12	No action is suggested for
leporinus					Arsenophonus					ND		now because the host is
					phytopathogenicus							not likely to be present in Zambia.
Philaenus	Insecta	Hemiptera	Cicadellidae	Main hosts: Onobrychis vicitfolia,	Xylella fastidiosa	Y	z	z	Algeria and Tunisia	CO, ST	36	No action is suggested for
spumarius				Prunus avium, Prunus dulcis, Prunus	subsp. fastidiosa;							now because the risk score
				persica, Rubus fruticosus, Rubus	Xylella fastidiosa							is very low and the pest is
				idaeus, Vitis vinifera	subsp. multiplex							not reported in Africa.
Philaenus	Insecta	Hemiptera	Aphrophoridae	Main host: Artemisia sp.,	Xylella fastidiosa	Υ	z	z	Algeria, Morocco, and	CO, ST	100	Since the pest is reported
spumarius				Onobrychis viciifolia, Prunus avium,	subsp. Pauca				Tunisia			in Africa, and with a
				Prumus dulcis, Prumus persica, Rubus								high score, a detection
				fruticosus, Rubus idaeus, Vitis vinifera								surveillance is needed
												to establish its status is
												suggested and possibly
												a pest-initiated PRA
												to advise on import
												requirements.
Trioza	Insecta	Hemiptera	Triozidae	Main hosts: Citrus aurantiifolia,	Candidatus	Υ	Υ	Υ	DR Congo, Eritrea,			Not assessed because
erytreae				Citrus deliciosa, Citrus jambhiri,	Liberibacter				Eswatini, Ethiopia, Gabon,			the vector is present
				Gitrus limon, Citrus maxima,	africanus				Kenya, Madagascar, Malawi,			in Zambia. The only
				Citrus medica, Citrus paradisi,					Mauritius, Reunion,			possible action could
				Citrus reticulata, Citrus sinensis,					Rwanda, Saint Helena, Sao			be a delimiting survey
				Citrus x nobilis, Fortunella sp., x					Tome & Principe, South			to determine extent of
				Citrofortunella microcarpa					Africa, Sudan, Tanzania,			spread.
									Uganda, Zambia, and			
									Zimbahaa			

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Suggested actions

For all the assessed pests, one of three actions was suggested to guide next steps which included conducting a detection surveillance or pest-initiated pest risk analysis (PRA) or taking no action. A detection surveillance was recommended when the pest had been reported as present in a country or countries neighbouring Zambia or a country or countries with high trade traffic to Zambia, such as South Africa. A pest-initiated PRA was suggested when the pest was affecting a value chain key to the economy of Zambia. Such a pest could be introduced as a contaminant especially through seed if it were seed-borne or seed-transmitted. However, in some situations where the pest had not been reported in Zambia, but was present in neighbouring countries, the suggested actions were a detection surveillance followed by a pest-initiated PRA. The rationale behind this was to ensure phytosanitary measures are only instituted after establishing the pest status in the country. A case in point is Candidatus Liberibacter africanus, which was indicated as absent in Zambia, based on available information in the CABI Compendium, yet it was reported in the neighbouring countries of Malawi, Tanzania and Zimbabwe along with the vector (Trioza erytreae) which is also reported as present in Zambia. For some bacterial and Protista species, a "no action" recommendation was made especially when the likelihood of entry and establishment was very low. However, for some pests, the "no action" recommendation was followed by periodic monitoring of the status of the pests especially where the low overall risk score was occasioned by a low likelihood of entry, but the likelihood of establishment, socioeconomic and environmental impact where medium (three) or high (above three) and the risk of this pest could increase with a change in likelihood of entry.

Discussion

Horizon scanning was utilised to select pest species not yet reported as present in the region at risk (Zambia) followed by an assessment of their likelihood of introduction, establishment and potential impacts on the economy and biodiversity. The approach has been used in several countries to avail key information about potential biological invasions to risk managers (Sutherland et al. 2008; Gassó et al. 2009; Roy et al. 2014; Bayón and Vilà 2019; Peyton et al. 2019), Spain (Gassó et al. 2009; Bayón and Vilà 2019) and United Kingdom (Sutherland et al. 2008). This information has enabled prevention of introduction through increased awareness to support early-warning and rapid response and contingency planning (Peyton et al. 2020). For some of the pest species provided by the Horizon Scanning Tool, only basic datasheets were available. This affected assessment of risk associated with likelihood of introduction, establishment and potential pathways of introduction. In addition, for most pest species, information on potential socio-economic and environmental impacts is lacking even in enhanced datasheets or completely unavailable. Lastly, information about some of the vectors reported to transmit some of the assessed pathogenic organisms is lacking.

For instance, *Xylella fastidioda* subspecies have been reported to be transmitted by a multitude of vectors, but information on these vectors is not available in SSA. This is why assessment of risk associated with pest species identified through horizon scanning was conducted by SMEs.

The pests that recorded high scores were those reported in Africa and mainly in neighbouring countries or countries with high traffic of trade, such as South Africa, demonstrating that the likelihood of entry is key in determining the overall risk score. More than half of the pests reported as present in Africa were reported in neighbouring countries. This indicates that Zambia needs to ensure that the status of the pests reported as absent in Zambia, but present in neighbouring countries, is correctly established. This will require collaboration of the Plant Quarantine and Phytosanitary Service (PQPS), which is the National Plant Protection Organisation (NPPO), with other key actors, such as public and private research institutions, international research organisations, academia, public and private extension delivery organisations and regional NPPOs.

Soft Rot Pectobacteriaceae (SRP) are one of the most devastating phytopathogenic organisms known to affect a wide range of crops, especially in Solanum tuberosum, Zea mays and a multitude of horticultural crops (Gallois et al. 1992; Adeolu et al. 2016; van der Wolf et al. 2021; Van Gijsegem et al. 2021). The SRPs identified through horizon scanning and assessed included Dickeya chrysanthemi, D. dadantii, D. dianthicola, D. fangzhongdai, D. paradisiaca, D. solani, D. zeae, Pectobacterium aroidearum, P. atrosepticum, P. betavasculorum, P. brasiliense, P. carotovorum, P. cypripedii, P. odoriferum, P. parmentieri and P. polaris, all of which affect S. tuberosum, except, D. zeae, P. cypripedii and P. odoriferum. All these SRPs recorded overall risk scores above 54, except D. fangzhongdai, D. paradisiaca, D. solani, P. aroidearum, P. cypripedii, P. odoriferum and P. polaris majorly because they had not been reported in Africa with the exception of *P. cypripedii*, which has been reported as present in South Africa. The SRPs that recorded scores above 54 have all been reported in neighbouring countries, except D. dianthicola and P. betavasculorum. It is on this basis that there was a suggestion for detection surveillance to be conducted for these pests before any phytosanitary measure is instituted. However, for the SRPs not recorded in neighbouring countries, detection surveillance was still suggested to confirm pest status, followed by a pestinitiated PRA.

The SRPs that were added because they presented a phytosanitary risk to *S. tubero*sum value chain included *D. oryzae*, *P. parvum*, *P. punjabense* and *P. peruviense*. *Pecto*bacterium punjabense is a new species which was recently isolated from *S. tuberosum* (Sarfraz et al. 2018). This species was added because it is closely related to *P. parmentieri*, a species that was highlighted through horizon scanning. *Pectobacterium parmentieri* was reported in the neighbouring country of Zimbabwe and also highlighted as invasive. Both *P. parvum* and *P. punjabense* were recently elevated from *P. carotovorum*, a species highlighted by horizon scanning and reclassified into new species (Waleron et al. 2018; Pasanen et al. 2020). *Pectobacterium carotovorum* was reported in a number of countries and in the neighbouring country of Zimbabwe. *Dickeya oryzae* was recently elevated from *D. zeae*, hence this elevation from a strain that had been highlighted through horizon scanning dictated the inclusion of *D. oryzae* in the risk assessment process. All the added SRPs recorded low overall risk score because they have not yet been reported in Africa. However, because they have been elevated from SRPs already reported in Africa and more so in neighbouring countries, detection surveillance was suggested to establish pest status.

The xanthomonad, X. citri pv. Aurantifolii, was added because, along with Xanthomonas citri pv. Citri, both cause Citrus canker disease (CCD) or Asiatic citrus canker (Gottwald et al. 2002; Gabriel et al. 2020; Naqvi et al. 2022). The disease affects several plants in the family Rutaceae particularly Citrus, Fortunella and Poncirus species (da Gama et al. 2018; Nagvi et al. 2022). All known commercial varieties of Citrus have been reported to succumb to the diseases (Gottwald et al. 1989, 2002; Vojnov et al. 2010). The economic impacts due to CCD result from stem die-back, fruit blemishes which affect the quality and eventual price and early fruit drop (Graham 2001; Gottwald et al. 2002). The two pathovars, X. citri pv. aurantifolii and X. citri pv. citri are mainly introduced into new geographical areas through the transportation of infected fruits from infested zones to production areas free of the disease (Gottwald et al. 2002; Nagyi et al. 2022). The two pathovars are considered guarantine organisms in most countries where they have not yet been reported (Schubert et al. 2001; Gottwald et al. 2002; Naqvi et al. 2022), hence the overall risk score of 75 and 100 for X. citri pv. aurantifolii and X. citri pv. Citri, respectively, was enough to instigate a suggestion of surveillance since X. citri py. citri had been recorded in the neighbouring country of Tanzania.

One of the emerging bacterial pathogenic species of economic importance, Xylella fastidiosa that has now been reported in America, Asia, Europe and Oceania, but not yet in Africa, was also assessed (Baldi and La Porta 2017; Rapicavoli et al. 2018). Xy*lella fastidiosa* is divided into three main subspecies, each with a specific host range, X. fastidiosa subsp. fastidiosa which causes Pierce's disease; X. fastidiosa subsp. multiplex which causes almond leaf scorch and phony peach disease; and X. fastidiosa subsp. pauca which causes citrus variegated chlorosis and olive quick decline syndrome (Sanderlin 2017; Rapicavoli et al. 2018; Greco et al. 2021). Three other subspecies, although of limited economic importance and host spectrum, also cause X. fastidiosa disease symptoms. They are X. fastidiosa subsp. morus, X. fastidiosa subsp. sandyi which causes oleander leaf scorch and X. fastidiosa subsp. tashke which causes leaf scorch in Chitalpa tashkentensis (Schuenzel et al. 2005; Randall et al. 2009; Nunney et al. 2014; Rapicavoli et al. 2018). The three major subspecies and X. fastidiosa subsp. sandyi were picked through horizon scanning and assessed. Two of these subspecies, X. fastidiosa subsp fastidiosa and X. fastidiosa subsp. pauca affect crop species (Citrus sinensis and Coffee. arabica) (Marucci et al. 2008; Bergsma-Vlami et al. 2017; Esteves et al. 2020) that are key to the Zambian economy. Xylella fastidiosa has the capacity to rattle the trading capacity of any country. It is a quarantine pest in most of Europe, the destination of agricultural produce from Africa and, therefore, it is essential that it is kept out of Zambia and other African countries.

Based on the results from the rapid risk assessment, the following recommendations are suggested; (1) conduct detection surveillance especially for pests reported in neighbouring countries to establish pest status before any further action, such as developing pest-initiated PRAs is conducted. Where the pest is established as present, a delimiting survey is suggested to establish the boundaries of infestation. Although not yet detected in Africa, periodic surveillance for X. fastidiosa should be conducted. It is also essential for funds to be allocated to conduct research on the likely vectors of this pathogen; (2) Pest-initiated PRA should be conducted for pests that cause high economic damage or may endanger trade in value chains key to the Zambian economy; (3) The risk associated with the assessed pests needs to be reviewed periodically to establish any changes and devise necessary mitigation measures. The suggested periodic review will require the establishment of a pest risk register to which these bacteria and protist species will be added. The risk registers are developed, based on the concept by the United Kingdom's Plant Health Risk Register⁹, Northern Ireland's Plant Health Risk Register¹⁰ or Finland's FinnPRIO-Explorer¹¹. Lastly, the results from this assessment will support the updating of the list of regulated pests. The actions suggested will be implemented by the Zambian NPPO, Plant Quarantine and Phytosanitary Service (PQPS) working with key actors in Extension, Research and Academia.

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⁹ https://planthealthportal.defra.gov.uk/pests-and-diseases/uk-plant-health-risk-register.

¹⁰ https://www.daera-ni.gov.uk/publications/ni-plant-health-risk-register.

¹¹ https://finnprio-explorer.rahtiapp.fi.

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Supplementary material I

All data from horizon scanning for Zambia

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- Explanation note: The table presents the data yield from the Horizon scanning exercise using the Horizon Scanning Tool. The initial search yielded a total of 306 plant pathogenic bacteria and 10 protists. However, following a cleaning process to remove pests represented only by genus names, the list was narrowed down to 283 bacterial and 10 Protista species that were eligible for assessment.
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Link: https://doi.org/10.3897/neobiota.91.113801.suppl1

Supplementary material 2

Guidelines for scoring species

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Data type: xlsx

Explanation note: The documents includes the guildes used in making assessments for the pests.

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Supplementary material 3

Plant pathogenic bacteria assessment for Zambia

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Explanation note: The table presents all the 137 plant pathogenic bacteria prioritised for assessment based on value chains.

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Supplementary material 4

Plant pathogenic protist assessment for Zambia

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- Explanation note: The table presents the 8 plant pathogenic protists prioritised for assessment based on value chains.
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Link: https://doi.org/10.3897/neobiota.91.113801.suppl4

Supplementary material 5

Assessment for vector species

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Explanation note: The table presents assessment scores for vectors known to transmit the assessed plant pathogenic organisms especially the bactria species.

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