

Eyes on the aliens: citizen science contributes to research, policy and management of biological invasions in Europe

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Academic editor: John Ross Wilson | Received 31 January 2022 | Accepted 12 October 2022 | Published 11 November 2022

Citation: Price-Jones V, Brown PMJ, Adriaens T, Tricarico E, Farrow RA, Cardoso AC, Gervasini E, Groom Q, Reyserhove L, Schade S, Tsinaraki C, Marchante E (2022) Eyes on the aliens: citizen science contributes to research, policy and management of biological invasions in Europe. NeoBiota 78: 1–24. https://doi.org/10.3897/neobiota.78.81476

Abstract

Invasive alien species (IAS) are a key driver of global biodiversity loss. Reducing their spread and impact is a target of the Sustainable Development Goals (SDG target 15.8) and of the EU IAS Regulation 1143/2014. The use of citizen science offers various benefits to alien species' decision-making and to society, since public participation in research and management boosts awareness, engagement and scientific literacy and can reduce conflict in IAS management. We report the results of a survey on alien species citizen science initiatives within the framework of the European Cooperation in Science and Technology (COST) Action Alien-CSI. We gathered metadata on 103 initiatives across 41 countries, excluding general biodiversity reporting portals, spanning from 2005 to 2020, offering the most comprehensive account of alien species citizen science initiatives on the continent to date. We retrieved information on project scope, policy relevance, engagement methods, data capture, data quality and data management, methods and technologies applied and performance indicators such as the number of records coming from projects, the numbers of participants and publications. The 103 initiatives were unevenly distributed geographically, with countries with a tradition of citizen science showing more active projects. The majority of projects were contributory and were run at a national scale, targeting the general public, alien plants and insects, and terrestrial ecosystems. These factors of project scope were consistent between geographic regions.

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Most projects focused on collecting species presence or abundance data, aiming to map presence and spread. As 75% of the initiatives specifically collected data on IAS of Union Concern, citizen science in Europe is of policy relevance. Despite this, only half of the projects indicated sustainable funding. Nearly all projects had validation in place to verify species identifications. Strikingly, only about one third of the projects shared their data with open data repositories such as the Global Biodiversity Information Facility or the European Alien Species Information Network. Moreover, many did not adhere to the principles of FAIR data management. Finally, certain factors of engagement, feedback and support, had significant impacts on project performance, with the provision of a map with sightings being especially beneficial. Based on this dataset, we offer suggestions to strengthen the network of IAS citizen science projects and to foster knowledge exchange among citizens, scientists, managers, policy-makers, local authorities, and other stakeholders.

Keywords

biological recording, community science, crowdsourcing, non-native species, public engagement, survey

Introduction

The history of citizen science, broadly defined as the practice of involving members of the public in scientific research, can be traced back centuries (Silvertown 2009). However, in recent decades the field of citizen science has grown and transformed with capabilities enhanced by the use of new technologies (e.g., smartphones) (Howard et al. 2022). As citizen science expands and reaches new audiences, its potential for impact and engagement also grows. Large and diverse audiences across the globe now contribute to initiatives carried out on scales ranging from short-term and local, to generational and international. The role of the citizen scientist is equally variable and as a result, the definition of citizen science has been subject to debate (Heigl et al. 2019; Haklay et al. 2021). In many ecological projects, citizen scientists merely collect and submit field observations to be analysed by professional scientists (Bonney 1996); however, in this paper we also consider more in-depth involvement of citizen scientists, such as collecting experimental data. The definition of citizen science that we will use, based on the definition by Wiggins and Crowston (2011), is the active involvement of citizens in scientific inquiry generating new knowledge or understanding.

One area in which citizen science has seen an increase in contributions is the domain of alien species science and policy (Adriaens et al. 2015; Roy et al. 2018; Schade et al. 2019; Johnson et al. 2020). Alien species are defined as species introduced into a new geographic range by human intervention, either intentionally or accidentally (Blackburn et al. 2011). While alien species may have a positive, neutral, or negative impact on their new environment (Cox and Lima 2006; Goodenough 2010), the term invasive alien species (IAS) refers to species whose introduction and spread has been found to threaten or adversely impact global biodiversity, ecosystem services, society and the economy (Seebens et al. 2017, 2020; IPBES 2019; EU Regulation 1143/2014). Concerns over the impacts of IAS have led to policy responses

internationally, nationally, and locally. For example, as well as being an important target (Target 9) in the Convention on Biological Diversity (United Nations 1992), the UN's Sustainable Development Goals (SDGs) include a specific target on IAS (target 15.8). Similarly, in 2014, in response to the CBD target, the European Union published the EU Regulation 1143/2014 (European Union 2014) to control the spread of IAS in all Member States through prevention, early detection, rapid eradication, and management. This Regulation identifies a list of IAS of Union Concern which pose a threat to biodiversity and related ecosystem services, and require concerted action at the European Union level. Accessible information on these IAS and implementation of associated policies is provided by the European Alien Species Information Network (EASIN; Katsanevakis et al. 2015; Schade et al. 2019). The core function of this system is to gather and integrate data on alien species occurring in Europe from data partners and scientific literature (Katsanevakis et al. 2012). Data originate from official monitoring programmes and research projects, but also from several IAS-focused citizen science projects active throughout Europe. These projects either deliver data to EASIN directly or publish to open data repositories like the Global Biodiversity Information Facility (GBIF), where they are harvested by EASIN.

The data gathered through IAS-focussed projects are eminently actionable, as they hold potential for use in early warning and rapid response, control programmes at various spatial scales, and policy implementation. Citizen science is especially valuable in an IAS context since tackling the spread of these species necessitates upscaled recording both temporally and geographically, improved understanding of the IAS problem, and increased awareness at all levels of society, objectives for which citizen science is well suited (Roy et al. 2018). Ultimately, citizens who become involved in IAS citizen science projects gain a voice in promoting decision-making and policy implementation, thereby supporting the development of IAS policies (Groom et al. 2019). However, there is no updated and systematic analysis of IAS citizen science projects across Europe. This would allow a better understanding of the potential reach and gaps of such projects for European science and policy. Here, we present the first comprehensive overview of European IAS citizen science initiatives. Unlike earlier work (Johnson et al. 2020), we focus on European alien species-specific citizen science projects and journal publication is not used as a criterion for inclusion. Since Europe adopted a common Regulation on IAS (the above mentioned EU Regulation 1143/2014) we wanted to assess the policy relevance of projects with a particular emphasis on the implementation of this Regulation.

In addition to developing a database of European alien species citizen science projects, we were interested in determining if there were geographic differences in three parameters of project scope (target taxon, target audience and environment type), as an indicator for international cooperation. We further evaluated the performance of projects considering their numbers of participants, number of alien species records they yielded and the publications derived from them, in order to understand how various engagement, feedback and support parameters contributed to project performance.

Materials and methods

Data collection

This survey was developed within the scope of the European Cooperation in Science & Technology (COST) Action CA17122 – "Increasing understanding of alien species through citizen science (Alien-CSI)", which includes participants from all EU Member States and a few neighboring countries. This COST Action sets out six research coordination objectives, to be first approached through a European wide analysis of existing IAS citizen science initiatives (Roy et al. 2018).

The first version of the survey was tested, revised and validated in a COST Action workshop in Akrotiri, Cyprus, 25 – 28 February 2019. Representatives from 25 countries in the COST Action attended. The survey (Price-Jones et al. 2021) was shared as a Google Form with all COST Action participants, and disseminated online. Responses were collected from June 27, 2019 to April 6, 2020. For each country, existing citizen science projects involving alien species were contacted and requested to complete the survey. All projects are/were active in EU Member States and/or neighbouring countries. A list of projects was compiled, including from a web search and previously available lists of European citizen science projects (e.g., EASIN, Kus Veenvliet et al. 2019), and the missing projects in the survey database were contacted. Finally, to increase reach, the survey was also disseminated through the European Citizen Science Association (ECSA) newsletter and mailing list and respondents were asked to share it with colleagues and local networks via snowball sampling.

Survey questions and attribute values were developed using JRC metadata standards for citizen science projects (European Commission, Directorate-General for Environment 2018) and the project metadata model of PPSR Core, a set of global, transdisciplinary data and metadata standards for Public Participation in Scientific Research (PPSR Core). The survey included 62 questions (Price-Jones et al. 2021), in nine sections: 1) Contact information of the respondent (a project coordinator); 2) General characterization of the project, including a brief summary, geographical scope, time scale, hosting entities, funding, etc.; 3) Information on project scope, including target audience, taxonomic and environmental scope, project aims, type of data collected, etc.; 4) Policy-related information, i.e., policy relevance and inclusion of species listed in the EU IAS Regulation; 5) Information on engagement, such as type of involvement of the general public in the design of the project, engagement methods and social media used, skills needed to participate and frequency of contributions; 6) Information on feedback and support provided to participants by the project, e.g., if the project provides materials for species identification, guidelines, training activities, information on how data from the project are used, feedback mechanisms and support; 7) Data quality and data management, namely validation mechanism for records, registration type, methods of recording, whether data are open and accessible to the general public, data form used to store data, data standards and data licence used, whether a public

data management plan was in place; 8) Performance indicators of projects, namely usage of smartphone applications, number of participants and number of records, whether learning is assessed, number and type of publications using data from the project; and 9) Notes and remarks.

Preprocessing

Only projects that simultaneously fulfilled the following criteria were included in the analyses: 1) a clearly citizen science-focused project; 2) alien species included in the main scope; and 3) projects developed in Europe (even if not exclusively). As such, national biodiversity networks and portals collecting data on all species were only considered if they had a clear alien species focus. Projects needed to have specific forms of public engagement related to alien species, so projects solely devoted to improving IAS policies but without a typical citizen science component (e.g., data collection using target groups, interaction with volunteers) were not considered. However, projects where data gathering was less relevant, but which had clear educational and outreach goals on IAS, were included.

Due to response rates below 100% for particular questions and the prevalence of responses "Unknown" or "Not applicable", the number of projects that provided a definite response was determined and used for calculations of percentages for each question.

Statistical analysis

Exploratory analysis of project parameters

Of the nine survey sections, six asked for information about project parameters, or characteristics. These sections are: General characterisation of the project, Information on project scope, Policy-related information, Information on engagement, Information on feedback and support, and Data quality and data management strategies. To explore the parameters of all surveyed projects, the frequency of each multiple choice or written answer was determined for each question within the above sections. Additionally, we were interested in determining if an association existed between target audience and target taxonomic group, or between target audience level of 0.05 to test for these associations.

Geographic differences in project scope

In these series of analyses, we were interested in whether there were geographic differences in the distribution of projects, and whether project scope had a geographic component. For this, we divided Europe into five regions: Northern Europe, Eastern Europe, Southern Europe, Western Europe, and the United Kingdom and the Republic of Ireland (the UK and ROI). The UK is considered as a separate region with the ROI due to an extensive history with citizen science (Silvertown 2009). These divisions are commonly used in ecology, with minor variability in the countries in each region (e.g., Bilton et al. 1998). To normalise the quantity of projects according to the different number of inhabitants per region, the number of projects was expressed per million inhabitants using population data from the United Nations (United Nations 2019). Project distribution maps were created using ESRI ArcGIS Pro 2.7. Then, for each of three project scope parameters (target taxon, target audience and environment type), a two-way chi square test was conducted to test for association with geographical region. The tests were carried out with a significance level of 0.05.

Impact of engagement, feedback and support on project performance

To test whether parameters which related to engagement, feedback and support had an effect on project performance, we selected 11 explanatory variables (project duration, four variables related to engagement, and six variables related to feedback/support) and defined three project performance indicators: the number of participants taking part in the project, the number of species records (observations) gathered by the project and the number of publications related to the project reported by the respondent (Table 1). Three cumulative link models (CLMs) were conducted in RStudio version 3.3.3 using the package "ordinal" (Christensen 2018) in R version 4.2.0 (R Core Team 2022) to determine if engagement, feedback/support and project duration had a significant effect on performance indicators. Each of the three tests used a different performance indicator - number of participants, records and publications - as a response variable. All models were carried out with a significance level of 0.05. R code for these tests is published on Zenodo (Price-Jones et al. 2021).

Results

Exploratory analysis of selected project parameters

General characterisation of the project

In total, 129 projects/initiatives completed data for the survey and, of these, 103 fitted the criteria for inclusion and were considered for analysis. Of the 26 that were excluded, 17 were not alien species-focused, seven had no specific forms of public engagement on alien species and two were duplicate entries.

The number of new projects has increased over the past fifteen years with the oldest project recorded beginning in 2005 (Brown et al. 2008) while 21 began in 2019 (Fig. 1). Most projects (76 of 103 projects) are still ongoing. A total of 42 countries were represented in the survey. A majority of projects (66%, 68 of the 103 respondents to this question) were run at the national level, and 85% (87/103) were active in a single country. However, one project, a survey of alien species of Union Concern on iNaturalist, was active in 38 countries. In four countries (Estonia, Malta, Montenegro, and North Macedonia) this represented the sole project.

Table 1. Variables used in the Cumulative Link Model	ls
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Explanatory variables	Response variables
Project duration	Number of participants
Project design (collaborative/contributory; engagement factor)	Number of records
Use of social media (engagement factor)	Number of publications
Level of skill/knowledge required (none/low/advanced; engagement factor)	
Expected contribution frequency (one-off/irregular/regular; engagement factor)	
Provision of guidelines (feedback and support factor)	
Provision of training (feedback and support factor)	
Provision of sightings map (feedback and support factor)	
Provision of active informing (feedback and support factor)	
Provision of feedback (feedback and support factor)	
Provision of support (feedback and support factor)	



Figure 1. Number of new citizen science projects per year on alien species in Europe according to responses to the survey.

The type of organisation responsible for the projects varied between governmental (29%, 30/103) and non-governmental organisations (22%, 23/103), universities (28%, 29/103), public research organisations (22%, 23/103), and private companies, non-profit organisations and individual persons (12%, 12/103). Most projects were fully (54%, 56/103) or partially (19%, 20/103) funded, but 26% (27/103) reported having no funding. Governments were the largest source of funding, although only 36% of projects (28/78) report governments as being their sole source of funding. Otherwise, funding was provided by public entities, the EU LIFE program, NGOs or private sources, or a combination of the above.

Project scope

Plants were the most common target taxonomic group (30%, 31/103; Fig. 2a), the general public was the most common target audience (89%, 92/103; Fig. 2b), and terrestrial habitats the most common environment considered in the projects (57%, 59/103). There was no association between target audience and taxon (p = 0.2779), but an association was observed between target audience and environment (p = 0.0049). Two trends in the data included the prevalence of terrestrial projects aimed at land managers, and freshwater and marine projects aimed at fishers. The marine environment was also the environment type most frequently involving scientists and students.

84% of projects (87/103) focused solely on alien species and 9% (9/103) focused partially on alien species; 7% (7/103) responded that alien species were not the main focus, yet alien species data were collected and received some emphasis. Most projects had multiple aims, the most common being mapping of alien species distribution (Fig. 2c). Most projects also collected more than one type of data, with species presence and/or abundance being the most common.

Policy-related information

75% of projects (59/79) claimed to have policy relevance, with 79% (77/97) including species on the list of IAS of Union concern (EU Regulation 1143/2014), whether exclusively or partially.

Information on engagement

In terms of project design, 39% of projects (41/97) were categorised as collaborative (citizen scientist input was possible in project design) and 53% (56/97) as contributory (projects were designed only by scientists). The top three ways to engage citizens with the projects were through websites (83%, 83/99), social media (64%, 64/99) and live training (41%, 41/99). Newsletters, school engagement, exhibitions, bioblitzes and gaming were also common methods, each used by six or more projects. Of the projects that used social media and stated the platform, Facebook was the most popular platform, used by 65% of projects (63/96), but Twitter, Instagram and YouTube were also used. Almost 95% of projects (94/99) responded that participants needed "None" or "Limited" prior skills or knowledge to participate.

Information on feedback and support

The number of projects that provided species identification materials, guidelines, training, sighting maps, active informing, feedback and support is shown in Table 2. Of the 67% of projects (64/95) that offered training, 47% (45/95) offered group training, 31% (30/95) offered online training, and 7% (7/95) provided training through bioblitzes.



Figure 2. Percentage (indicated by numbers on radar plots) of projects that gave selected responses to project scope questions: **a** target taxon **b** target audience, and **c** stated project aim.

Factor	Percentage of projects		
-	Yes	No	Partial (if applicable)
Provision of species identification materials	76% (74/98)	7% (7/98)	17% (17/98)
Provision of guidelines	87% (85/98)	13% (13/98)	-
Provision of training	67% (64/95)	33% (31/95)	-
Provision of sightings map	86% (78/91)	14% (13/91)	-
Provision of active informing	69% (64/93)	7% (7/93)	24% (22/93)
Provision of feedback	89% (71/80)	11% (9/80)	_
Provision of support	93% (85/91)	7% (6/91)	-

Table 2. Responses to survey questions concerning various feedback and support factors.

Data quality and data management

The large majority (86%, 89/103) of the projects surveyed had validation systems in place, and 6% (6/103) had partially implemented validation systems. An additional 6% (6/103) of respondents indicated that the validation system was unknown to them and only 2%

(2/103) responded they did not have validation in place. Within the subset of projects implementing validation procedures, expert validation was most commonly used, by at least 93% (93/100) of projects. Validation was either performed solely by experts (77%, 77/100), aided by automated systems (3%, 3/100) or peer validation (9%, 9/100), or a combined approach was used (3%, 3/100). Peer validation and automated validation without expert validation were only used by a minority of projects (2%, 2/100).

For data storage, projects used national repositories (38%, 34/89), hard drives (34%, 30/89), GBIF (30%, 27/89) and institutional repositories (23%, 21/89). 58 projects offered participants direct access to their own data. Excel (65%, 44/68) was the most common data form and Darwin Core (50%, 18/36) the most popular data standard. The license Creative Common Attribution (CC BY; 57%, 16/29) was the most common, followed by CC0 licence waiver (10%, 3/29) and Creative Commons Non-Commercial licence (7%, 2/29). Finally, most projects did not draft a data management plan (73%, 40/55).

Project performance

The usage of applications, number of participants, number of records and number of publications all show a distribution of responses that peaked in lower numbers and fell off quickly at higher numbers (Fig. 3), but these values were often unknown or not applicable. Only 33% of projects (21/63) assessed learning of the participants. A similar number of projects produced scientific peer-reviewed publications (94%, 33/37) and science communication publications aimed at the general public (85%, 30/37). Most of these publications directly presented data from the project (47%, 24/51) or were descriptive in nature (43%, 22/51).

Geographic differences in project scope

According to responses to our survey, the UK had more alien species citizen science projects (21) than any other country, followed by Italy (13), Portugal (9) and France (9) (Fig. 4a). However, when project counts per region are normalised by population, Northern Europe has the highest ratio, followed by the UK and ROI, Southern Europe, Eastern Europe and Western Europe (Fig. 4b). There was no association between geographic region and the target audience (p = 0.51), taxon (p = 0.41) or environment (p = 0.16).

Engagement methods and performance

Project duration had a significant, positive impact on the three performance indicators tested, i.e., number of participants (z = 2.78, df = 1, p = 0.0054), publications (z = 3.38, df = 1, p = 0.00073) and records (z = 3.01, df = 1, p = 0.0026). Projects that provided a map also outperformed projects that did not in number of participants (z = 2.13, df = 2, p = 0.033), publications (z = 2.77, df = 2, p = 0.0056) and records (z = 2.84, df = 2, p = 0.0045).



Figure 3. Survey responses concerning project performance, participation and publication. **a** number of participants (n = 72) **b** number of records (n = 73) **c** number of publications (n = 62) and **d** percentage of usage of App (n = 17).

Provision of training was positively related to the number of publications (z = 2.85, df = 1, p = 0.044), as was use of social media (z = 2.35, df = 1, p = 0.019) and provision of guidelines (z = 2.01, df = 1, p = 0.045). Projects that required advanced prior knowledge resulted in more publications than projects that required limited (z = -2.80, df = 2, p = 0.0052) or no (z = -2.74, df = 2, p = 0.0061) prior knowledge. The same result was seen in terms of number of records, with projects that required limited (z = -2.47, df = 2, p = 0.014) or no (z = -2.02, df = 2, p = 0.043) prior knowledge.

Provision of feedback positively impacted the number of publications (z = 2.01, df = 1, p = 0.044) but negatively impacted the number of records (z = -2.01, df = 1, p = 0.044). Provision of support negatively impacted the number of publications (z = -2.59, f = 1, p = 0.0096).



Figure 4. Geographic distribution of projects: **a** number of projects by country and **b** number of projects per million inhabitants by region.

Discussion

Project scope and regional variation

The dominance of national projects in our results is consistent with that observed for other research on management of biological invasions (Hulme et al. 2008). Several factors contribute to this tendency, including nationally-derived funding, differing degrees to which countries are exposed to or aware of alien species, species alien in one country being native to another, logistical convenience (Hulme et al. 2008) and uneven distribution of expertise (Hulme et al. 2009). However, international coordination is necessary to better protect native ecosystems from IAS (Perrings et al. 2010; Katsanevakis et al. 2013), as reflected by international agreements, from the Convention on Biological Diversity (United Nations 1992) to EU Regulation 1143/2014. There was a small degree of international coordination evident in our dataset, with most multinational initiatives being active in six or fewer geographically clustered countries.

Most projects target the general public, which is logical given our inclusion criteria. This strategy aligns with the philosophy of informing (Genovesi et al. 2015), engaging with and inspiring a passion for nature in as many participants as possible (Roy et al. 2015). Plants and insects are the most common target-taxa of projects, possibly because both are broad and speciose groups and are among the taxa containing the most invasive species with recorded ecological and/or economic impacts (Vilà et al. 2010; Haubrock et al. 2021). Furthermore, they can be easily accessible, with many urban species. The under-representation of groups such as birds is notable, and is likely a result of the concentration of data specific to these groups on large crowd-sourcing platforms that did not fit our criteria.

The prevalence of projects in the terrestrial environment similarly reflects convenience for the public, as reported for other citizen science projects (Aceves-Bueno et al. 2017). It is also highlighted by the association we found between target audience and target environment.

The most common aim is mapping of alien species, and participants are often asked to submit species presence and/or abundance data. Species presence is easy to observe, report and validate (Hyder et al. 2015), and works well in conjunction with mapping (e.g., Malek et al. 2018; Dissanayake et al. 2019; Kumar et al. 2019). Nonetheless, presenceonly data have limitations: for example, the lack of absence reporting and the assumption that species were not present because they were not observed (Johnson et al. 2020).

The region with the most recorded projects is the United Kingdom and the Republic of Ireland, reflecting a long history of citizen science in ecology (Silvertown 2009). After this region, more projects in Western and Southern Europe may reflect a higher level of IAS awareness in these regions due to a relatively higher number of funded IAS projects (e.g., LIFE projects in Italy). On the other hand, a low number of projects, e.g., in the Netherlands and Belgium, may be explained by the presence of single, dominant national biodiversity portals not being included in our survey. For example, in Belgium, biodiversity recording is dominated by the general reporting portal www.waarnemingen.be which has a dedicated app and upon which early warning tools for IAS are built (Vanderhoeven et al. 2015; Swinnen et al. 2018). Additionally, there is a possible language bias, if projects from non-English speaking countries were not reached or not motivated to participate in the survey, which was only available in English.

Data quality and management

Studies evaluating data quality and management in citizen science projects sometimes have contradictory conclusions (Crall et al. 2011). Overall, volunteer contributions have been regarded favourably by scientists, e.g., 73% of papers positively described in analysis by Aceves-Bueno et al. (2017). Various tangible benefits have been noted, such as increases in the predicted spatial distribution of IAS by models trained with data from citizen science (César de Sá et al. 2019). However, Aceves-Bueno et al. (2017) also concluded that differences between volunteer data and professional data were significant in 38.4% of projects. In addition, some projects use their data for removal or management of regulated IAS, and as such correct species identification is of utmost importance. The most prominent approaches to validate citizen science data are peer and expert validation, often aided by automatic filtering techniques (Balázs et al. 2021). This is clearly also the case for IAS citizen science in Europe (Adriaens et al. 2021), with our results showing that most projects use expert validation.

Data generated by citizen science are often referred to as dark data: unreproducible, becoming more valuable over time, and at high risk of being lost (Costello and Wieczorek 2014). The implementation of a well-defined data management plan (DMP) can be used to prevent such loss of data. Nonetheless, few of the surveyed projects claimed to have a DMP and our survey did not assess adherence to the DMP for projects that had one. Many citizen science projects are relatively small scale and probably lack experience and/or access to tools for data management planning (Schade et al. 2017). Data management planning could improve the accessibility of data, an important component of FAIR (Findability, Accessibility, Interoperability, and Reusability) data management (Wilkinson et al. 2016; Reyserhove et al. 2020).

Opening alien species data is important to unlock their full potential for science, policy and management (Groom et al. 2015, 2017a,b). However, although some projects deposit their data on national or institutional repositories, less than one third make them freely available on an open data repository, e.g., through GBIF publication. We also found that most alien species citizen science projects produced peer-reviewed papers, but these were not necessarily open access; however, most also produced scientific communications aimed at the public. Reasons for avoiding open data may be multiple, including licensing issues, funding limitations, technical barriers or the privacy of the participants (Ganzevoort et al. 2017). Ganzevoort et al. (2017) found that half of the citizen scientists they surveyed believed that data collected by the citizen science organisation was a public good, but only 12% supported unconditional use. The question of data ownership is complex and can be addressed in legal terms by choice of license. We found that 92% of projects that provided licence information had a licence

allowing public use. Overall, these parameters around data accessibility are consistent with the findings of Wiggins and Crowston (2011) and Schade and Tsinaraki (2016), indicating a willingness to provide access to data.

Optimisation of engagement

We anticipated that higher levels of feedback, support and engagement would improve performance, e.g., the number of participants, records and publications, through the generation of commitment and empowerment. As expected, provision of maps, training and guidelines related positively to one or more of the performance indicators. Unexpectedly, provision of feedback related positively to the number of publications, and negatively to the number of records; provision of support also negatively related to the number of publications. While citizen scientists often claim that receiving feedback is important to their continued participation (Geoghegan et al. 2016; Anđelković et al. 2022), a reduced sample size and the fact that many projects are relatively new (17 started in 2017 and 21 in 2019), may have influenced the results. In addition, the number of publications may be influenced by several other factors such as the publishing dynamic of the project team, or the level of knowledge of the survey respondent on the publications stemming from the project. Also, the many missing values in the survey responses might partly explain some of these results.

Only 39% of the projects were designed collaboratively, thus in most cases citizens were contributing in a predetermined way (usually data collection). Even so, *a priori* fewer projects were expected to be collaborative (e.g., Pocock et al. 2017 analysed more than 500 ecological and environmental citizen science projects and only 4% were collaborative) and so we suspect that this question may have been misunderstood. We define a collaboratively-designed project as a project with citizen scientist participation in the initial conception of the project and all subsequent steps. However, respondents may have considered other roles, such as feedback from participants on project design, as collaboration.

Surveyed projects mostly required low levels of time commitment for learning and participation, possibly recognising that most citizen scientists are amateur observers (Bonney et al. 2016). However, from the authors' own experience, even though many projects target the general public, in reality many of the participants do have some level of expertise in the taxonomic group they report. Another unexpected result was that projects with limited or no skill requirements were related with a significantly lower number of records and publications. Possibly, participants with advanced skill levels, having already invested the time in learning, have a stronger commitment to contributing. Nevertheless, encouraging anyone to participate is highly relevant to the goal of reconnecting people with nature (Devictor et al. 2010) and increasing the chances of prevention and early detection of IAS. Furthermore, if a contributor can both learn and teach (e.g., through peer validation), knowledge is transferred without the need for training or prior expertise. It should be noted, however, that the lessons that can be drawn from this result are limited by how the levels 'none,' 'low' and 'advanced' skills/ knowledge were not defined in our survey, and so may have been interpreted differently by different respondents.

Although it depends on project goals, besides engaging participants, projects often encourage continued participation (Penner 2002). The positive relation between provision of maps, training and guidelines and some of the performance indicators suggest that these investments may encourage participation. It has been shown that publicly displayed maps allow recognition of citizen scientists' efforts (Williams and DeSteno 2008; Crowston and Prestopnik 2013).

Finally, the majority of projects used an internet-based engagement method, such as a website or social media, reflecting the ubiquity of these technologies in Europe (Kemp 2021).

Applications and recommendations

Several lessons can be drawn from the results of our survey. First, sustainability of projects is key to their performance in terms of the number of records they gather, participants they involve and publications derived from them. Second, many citizen science projects apparently have not yet opened their data. Open data publication maximises the use of the data in policy processes, such as their use by EASIN in the implementation of the EU IAS Regulation (Schade et al. 2019) and provides better return to citizen scientists on their contribution and value of their data.

One partial solution to openness and data management issues might be the drafting of DMPs, which are missing in many projects, despite these facilitating better storage, maintenance, and use of data. Although small projects may struggle to create their own, they may take advantage of existing plans, and strategies can be designed to make data openly accessible, for example on the platform GBIF. Few respondents provided information about their scientific outputs, and there is often no information on project web pages about where they publish their datasets.

To further improve outreach and onboarding of new citizen scientists, and sustained participation, our results suggest that the provision of maps with sightings and the provision of training are important. Future work could also be undertaken to compare the performance of different validation procedures and provide recommendations to new projects to improve data quality (Probert et al. 2022).

Our results show an increasing number of new alien species citizen science projects in the last few years that contribute to IAS mapping and policy implementation, but some regions still hold untapped potential for new citizen science initiatives related to alien species. Existing projects may be made accessible to new audiences through language translation or simplification, and through tailoring of aims and species lists to geographic regions (e.g., Invasive Alien Species in Europe application; Trichkova et al. 2021).

The UN's SDGs provide an excellent model for how citizen science can be relevant to setting and achieving goals at a global level. Although SDGs were not initially developed with citizen science in mind, data gathered through citizen science can be used directly for feeding SDG indicators (Fritz et al. 2019; Bishop et al. 2020), can increase the temporal and spatial scale of data collection (Schade et al. 2019) and can engage people with science and the environment (Pocock et al. 2014). Nonetheless, Fraisl et al. (2020) noted poor alignment of citizen science initiatives with target 15.8 on IAS.

Conclusions

The number of citizen science projects dedicated to alien species has been on the rise in Europe in the last decade, yet some regions in Europe still hold untapped potential for new initiatives. Citizen science initiatives often yield data on policy-relevant species, including species of the list of IAS of Union concern, and the data generated by these projects are used for science and management. Despite this, many projects face sustainability problems and only a minority of the data finds its way to open data repositories. Future work could explore the added value of specific alien species projects as compared to general citizen science biodiversity reporting portals, as well as the actual relevance of citizen science data in decision making on IAS. Also, the value of alien species citizen science in terms of increased engagement, learning outcomes and environmental awareness, needs to be further explored. To further foster active alien species citizen science across the continent, we suggest that strategies could be developed i) to support regions where alien species citizen science is currently only emerging and ii) to strengthen the links between projects and entities around the EU IAS Regulation. One way to do so is to provide networking opportunities where projects can exchange experiences.

Acknowledgements

This work was supported by Action CA17122 Increasing understanding of alien species through citizen science (Alien-CSI), supported by COST (European Cooperation in Science and Technology www.cost.eu) through workshops and a short term scientific mission (ECOST-STSM-CA17122-42402). We thank Tim Woods (ECSA), and the Flemish Knowledge centre for Citizen Science (SCivil) for disseminating the survey through their networks. We are grateful to all the project coordinators for filling the survey on citizen science initiatives: Alex Richter-Boix, Andrew Salisbury, Anna Gazda, Anne Goggin, Antonina dos Santos, Antonios Geropoulos, Ariadna Just, Balázs Károlyi, Baudewijn Odé, Benoit Lagier, Bernardo Duarte, Christian Ries, Christophe Bornand, Colette O'Flynn, Conrad Altmann, Daniel Dörler, Dave Kilbey, Diemer Vercayie, Dinka Matosevic, Elsa Quillery, Esra Per, Esther Hughes, Ferenc Lakatos, Francesco Tiralongo, Fredrik Dahl, Gabor Pozsgai, Hanna Koivula, Helen Roy, Helene Hennig, Hélia Marchante, Henk Groenewoud, Hugo Renato Marques Garcia Calado, Inês Correia Rosa, Ioannis Giovos, Jan Marcin Weslawski, Jean-Louis Chapuis, Jirislav Skuhrovec, Jitka Svobodová, Jo Clark, João Encarnação, João Loureiro, John Wilkinson, Jessica Thevenot, Julie Bailey, Jurga Motiejūnaitė, Karel Chobot, Karel Schoonvaere, Karolina Bacela-Spychalska, Katharina Dehnen-Schmutz, Katharina Lapin, Katrin Schneider, Kelly Martinou, Ladislav Pekarik, Luciana Zedda, Luís González Rodríguez, Luís Reino, Maarten de Groot, Malin Strand, Marija Smederevac-Lalić, Marina Golivets, Markus Seppälä, Michael Pocock, Michelle Cleary, Milica Jaćimović, Milvana Arko-Pijevac, Mirela Uzelac, Mónica Moura, Negin Ebrahimi, Nicole Nöske, Niki Chartosia, Nir Stern, Noé Ferreira Rodriguez, Ofer Steinitz, Ondřej Zicha, Paolo

Balistreri, Pavel Pipek, Pedro Anastácio, Periklis Kleitou, Philippe Jourde, Quentin Rome, Rafal Maciaszek, Rigers Bakiu, Rory Putman, Rosa Olivo del Amo, Rui Botelho, Rumen Tomov, Sándor Nagy, Sandro Bertolino, Sofie De Smedt, Sonja Desnica, Tamas Komives, Tarso Costa, Tatsiana Lipinskaya, Tom Evans, Toril L. Moen, Valeria Lencioni, Victor Zamfir. We thank Craig Hilborn for advice on the statistical analysis.

References

- Aceves-Bueno E, Adeleye AS, Feraud M, Huang Y, Tao M, Yang Y, Anderson SE (2017) The accuracy of citizen science data: A quantitative review. Bulletin of the Ecological Society of America 98(4): 278–290. https://doi.org/10.1002/bes2.1336
- Adriaens T, Sutton-Croft M, Owen K, Brosens D, van Valkenburg J, Kilbey D, Groom Q, Ehmig C, Thürkow F, Hende PV, Schneider K (2015) Trying to engage the crowd in recording invasive alien species in Europe: Experiences from two smartphone applications in northwest Europe. Management of Biological Invasions 6(2): 215–225. https://doi. org/10.3391/mbi.2015.6.2.12
- Adriaens T, Tricarico E, Reyserhove L, Cardoso AC, Lopez Canizares C, Mitton I, Schade S, Spinelli F, Tsiamis K (2021) Data-validation solutions for citizen science data on invasive alien species: Tailoring validation tools for the JRC app "Invasive Alien Species in Europe", EUR 30857 EN. Publications Office of the European, Luxembourg, JRC126140.
- Andelković AA, Lawson Handley L, Marchante E, Adriaens T, Brown PMJ, Tricarico E, Verbrugge LNH (2022) A review of volunteers' motivations to monitor and control invasive alien species. NeoBiota 73: 153–175. https://doi.org/10.3897/neobiota.73.79636
- Balázs B, Mooney P, Nováková E, Bastin L, Arsanjani JJ (2021) Chapter 8: Data Quality in Citizen Science. In: Vohland K, Land-Zandstra A, Ceccaroni L, Lemmens R, Perelló J, Ponti M, Samson R, Wagenknecht K (Eds) The Science of Citizen Science: 139–158. https://doi.org/10.1007/978-3-030-58278-4
- Bilton DT, Mirol PM, Mascheretti S, Fredga K, Zima J, Searle JB (1998) Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards post-glacial colonization. Proceedings of the Royal Society B-Biological Sciences 265(1402): 1219–1226. https://doi.org/10.1098/rspb.1998.0423
- Bishop IJ, Warner S, van Noordwijk TCGE, Nyoni FC, Loiselle S (2020) Citizen Science Monitoring for Sustainable Development Goal Indicator 6.3.2 in England and Zambia. Sustainability 12(24): 10271. https://doi.org/10.3390/su122410271
- Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JR, Richardson DM (2011) A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26(7): 333–339. https://doi.org/10.1016/j.tree.2011.03.023
- Bonney R (1996) Citizen science: A lab tradition. Living Bird 15: 7-15.
- Bonney R, Phillips TB, Ballard HL, Enck JW (2016) Can citizen science enhance public understanding of science? Public Understanding of Science 25(1): 2–16. https://doi. org/10.1177/0963662515607406

- Brown PMJ, Roy HE, Rothery P, Roy DB, Ware RL, Majerus MEN (2008) Harmonia axyridis in Great Britain: Analysis of the spread and distribution of a non-native coccinellid. BioControl 53(1): 55–67. https://doi.org/10.1007/s10526-007-9124-y
- César de Sá N, Marchante H, Marchante E, Cabral JA, Honrado JP, Vicente JR, de Sá NC de (2019) Can citizen science data guide the surveillance of invasive plants? A modelbased test with *Acacia* trees in Portugal. Biological Invasions 21(6): 2127–2141. https:// doi.org/10.1007/s10530-019-01962-6
- Christensen R (2018) (Submitted in) Cumulative link models for ordinal regression with the R package ordinal. Journal of Statistical Software, 1–40.
- Costello MJ, Wieczorek J (2014) Best practice for biodiversity data management and publication. Biological Conservation 173: 68–73. https://doi.org/10.1016/j.biocon.2013.10.018
- European Union (2014) Regulation (EU) No. 1143/2014 of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species. Official Journal of the European Union L 317, 4 November 2014, 35–55.
- Cox JG, Lima SL (2006) Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. Trends in Ecology & Evolution 21(12): 674–680. https://doi. org/10.1016/j.tree.2006.07.011
- Crall AW, Newman GJ, Stohlgren TJ, Holfelder KA, Graham J, Waller DM (2011) Assessing citizen science data quality: An invasive species case study. Conservation Letters 4(6): 433– 442. https://doi.org/10.1111/j.1755-263X.2011.00196.x
- Crowston K, Prestopnik NR (2013) Motivation and Data Quality in a Citizen Science Game: A Design Science Evaluation. 46th Hawaii International Conference on System Sciences. IEEE, 450–459. https://doi.org/10.1109/HICSS.2013.413
- Devictor V, Whittaker RJ, Beltrame C (2010) Beyond scarcity: Citizen science programmes as useful tools for conservation biogeography. Diversity & Distributions 16(3): 354–362. https://doi.org/10.1111/j.1472-4642.2009.00615.x
- Dissanayake RB, Stevenson M, Allavena R, Henning J (2019) The value of long-term citizen science data for monitoring koala populations. Scientific Reports 9(1): 1–12. https://doi. org/10.1038/s41598-019-46376-5
- European Commission, Directorate-General for Environment (2018) Citizen science for environmental policy: development of an EU-wide inventory and analysis of selected practices, Publications Office. https://data.europa.eu/doi/10.2779/961304
- Fraisl D, Campbell J, See L, Wehn U, Wardlaw J, Gold M, Moorthy I, Arias R, Piera J, Oliver JL, Masó J, Penker M, Fritz S (2020) Mapping citizen science contributions to the UN sustainable development goals. Sustainability Science 15(6): 1735–1751. https://doi.org/10.1007/s11625-020-00833-7
- Fritz S, See L, Carlson T, Haklay M, Oliver JL, Fraisl D, Mondardini R, Brocklehurst M, Shanley LA, Schade S, Wehn U, Abrate T, Anstee J, Arnold S, Billot M, Campbell J, Espey J, Gold M, Hager G, He S, Hepburn L, Hsu A, Long D, Masó J, McCallum I, Muniafu M, Moorthy I, Obersteiner M, Parker AJ, Weisspflug M, West S (2019) Citizen science and the United Nations Sustainable Development Goals. Nature Sustainability 2(10): 922–930. https://doi.org/10.1038/s41893-019-0390-3

- Ganzevoort W, van den Born RJG, Halffman W, Turnhout S (2017) Sharing biodiversity data: Citizen scientists' concerns and motivations. Biodiversity and Conservation 26(12): 2821– 2837. https://doi.org/10.1007/s10531-017-1391-z
- Genovesi P, Carboneras C, Vilà M, Walton P (2015) EU adopts innovative legislation on invasive species: A step towards a global response to biological invasions? Biological Invasions 17(5): 1307–1311. https://doi.org/10.1007/s10530-014-0817-8
- Geoghegan H, Dyke A, Pateman R, West S, Everett G (2016) Understanding motivations for citizen science. Final report on behalf of UKEOF, University of Reading, Stockholm Environment Institute (University of York) and University of the West of England. http:// www.ukeof.org.uk/resources/citizen-science-resources/MotivationsforCSREPORTFI-NALMay2016.pdf
- Goodenough AE (2010) Are the ecological impacts of alien species misrepresented? A review of the "native good, alien bad" philosophy. Community Ecology 11(1): 13–21. https://doi. org/10.1556/ComEc.11.2010.1.3
- Groom Q, Desmet P, Vanderhoeven S, Adriaens T (2015) The importance of open data for invasive alien species research, policy and management. Management of Biological Invasions 6(2): 119–125. https://doi.org/10.3391/mbi.2015.6.2.02
- Groom Q, Weatherdon L, Geijzendorffer IR (2017a) Is citizen science an open science in the case of biodiversity observations? Journal of Applied Ecology 54(2): 612–617. https://doi.org/10.1111/1365-2664.12767
- Groom Q, Adriaens T, Desmet P, Simpson A, Wever AD, Bazos I, Cardoso AC, Charles L, Christopoulou A, Gazda A, Helmisaari H, Hobern D, Josefsson M, Lucy F, Marisavljevic D, Oszako T, Pergl J, Petrovic-Obradovic O, Prévot C, Ravn HP, Richards G, Roques A, Roy HE, Rozenberg M-AA, Scalera R, Tricarico E, Trichkova T, Vercayie D, Zenetos A, Vanderhoeven S (2017b) Seven Recommendations to Make Your Invasive Alien Species Data More Useful. Frontiers in Applied Mathematics and Statistics 3: 13. https://doi.org/10.3389/fams.2017.00013
- Groom Q, Strubbe D, Adriaens T, Davis AJS, Desmet P, Oldoni D, Reyserhove L, Roy HE, Vanderhoeven S (2019) Empowering Citizens to Inform Decision-Making as a Way Forward to Support Invasive Alien Species Policy. Citizen Science: Theory and Practice 4(1): 1–11. https://doi.org/10.5334/cstp.238
- Haklay M, Fraisl D, Tzovaras BG, Hecker S, Gold M, Hager G, Ceccaroni L, Kieslinger B, Wehn U, Woods S, Nold C, Balázs B, Mazzonetto M, Ruefenacht S, Shanley LA, Wagenknecht K, Motion A, Sforzi A, Riemenschneider D, Dorler D, Heigl F, Schaefer T, Lindner A, Weißpflug M, Mačiulienė M, Vohland K (2021) Contours of citizen science: A vignette study. Royal Society Open Science 8(8): 202108. https://doi.org/10.1098/rsos.202108
- Haubrock PJ, Turbelin AJ, Cuthbert RN, Novoa A, Taylor NG, Angulo E, Ballesteros-Mejia L, Bodey TW, Capinha C, Diagne C, Essl F, Golivets M, Kirichenko N, Kourantidou M, Leroy B, Renault D, Verbrugge L, Courchamp F (2021) Economic costs of invasive alien species across Europe. NeoBiota 67: 153–190. https://doi.org/10.3897/neobiota.67.58196
- Heigl F, Kieslinger B, Paul KT, Uhlik J, Dörler D (2019) Opinion: Toward an international definition of citizen science. Proceedings of the National Academy of Sciences of the United States of America 116(17): 8089–8092. https://doi.org/10.1073/pnas.1903393116

- Howard L, van Rees CB, Dahlquist Z, Luikart G, Hand BK (2022) A review of invasive species reporting apps for citizen science and opportunities for innovation. NeoBiota 71: 165– 188. https://doi.org/10.3897/neobiota.71.79597
- Hulme P, Roy D, Cunha T, Larsson T (2008) A pan-European inventory of alien species: rationale, implementation and implications for managing biological invasions. Handbook of Alien Species in Europe. Springer, Dordrecht, Netherlands, 1–14. https://doi. org/10.1007/978-1-4020-8280-1_1
- Hulme PE, Nentwig W, Pyšek P, Vilà M (2009) Common market, shared problems: Time for a coordinated response to biological invasions in Europe? NeoBiota 8: 3–19.
- Hyder K, Townhill B, Anderson LG, Delany J, Pinnegar JK (2015) Can citizen science contribute to the evidence-base that underpins marine policy? Marine Policy 59: 112–120. https://doi.org/10.1016/j.marpol.2015.04.022
- IPBES (2019) Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. In: Díaz S, Settele J, Brondízio ES, Ngo HT, Guèze M, Agard J, Arneth A, Balvanera P, Brauman KA, Butchart SHM, Chan KMA, Garibaldi LA, Ichii K, Liu J, Subramanian SM, Midgley GF, Miloslavich P, Molnár Z, Obura D, Pfaff A, Polasky S, Purvis A, Razzaque J, Reyers B, Chowdhury RR, Shin YJ, Visseren-Hamakers IJ, Willis KJ, Zayas CN (Eds) IPBES secretariat, Bonn, Germany. 56 pp. https://doi.org/10.5281/zenodo.3553579
- Johnson BA, Mader AD, Dasgupta R, Kumar P (2020) Citizen science and invasive alien species: An analysis of citizen science initiatives using information and communications technology (ICT) to collect invasive alien species observations. Global Ecology and Conservation 21: e00812. https://doi.org/10.1016/j.gecco.2019.e00812
- Katsanevakis S, Bogucarskis K, Gatto F, Vandekerkhove J, Deriu I, Cardoso AC (2012) Building the European Alien Species Information Network (EASIN): A novel approach for the exploration of distributed alien species data. BioInvasions Records 1(4): 235–245. https://doi.org/10.3391/bir.2012.1.4.01
- Katsanevakis S, Genovesi P, Gaiji S, Hvid HN, Roy H, Nunes AL, Aguado FS, Bogucarskis K, Debusscher B, Deriu I, Harrower C, Josefsson M, Lucy F, Marchini A, Richards G, Trichkova T, Vanderhoeven S, Zenetos A, Cardoso AC (2013) Implementing the European policies for alien species networking, science, and partnership in a complex environment. Management of Biological Invasions 4(1): 3–6. https://doi.org/10.3391/mbi.2013.4.1.02
- Katsanevakis S, Deriu I, D'Amico F, Nunes AL, Sanchez SP, Crocetta F, Arianoutsou M, Bazos I, Christopoulou A, Curto G, Delipetrou P, Kokkoris Y, Panov VE, Rabitsch W, Roques A, Scalera R, Shirley SM, Tricarico E, Vannini A, Zenetos A, Zervou S, Zikos A, Cardoso AC (2015) European Alien Species Information Network (EASIN): Supporting European policies and scientific research. Management of Biological Invasions 6(2): 147–157. https://doi.org/10.3391/mbi.2015.6.2.05
- Kemp S (2021) Digital 2021: Global Digital Overview. https://datareportal.com/reports/digital-2021-global-digital-overview
- Kumar A, Sinha A, Kanaujia A (2019) Using citizen science in assessing the distribution of Sarus Crane (Grus antigone antigone) in Uttar Pradesh, India. International Journal of Biodiversity and Conservation 11(2): 58–68. https://doi.org/10.5897/IJBC2018.1245

- Kus Veenvliet J, Veenvliet P, de Groot M, Kutnar L [Eds] (2019) A Field Guide to Invasive Alien Species in European Forests. Nova vas, Institute Symbiosis, so. e.; The Silva Slovenica Publishing Centre, Slovenian Forestry Institute, Ljubljana, 1–132.
- Malek R, Tattoni C, Ciolli M, Corradini S, Andreis D, Ibrahim A, Mazzoni V, Eriksson A, Anfora G (2018) Coupling Traditional Monitoring and Citizen Science to Disentangle the Invasion of *Halyomorpha halys*. ISPRS International Journal of Geo-Information 7(5): 171. https://doi.org/10.3390/ijgi7050171
- Penner LA (2002) Dispositional and Organizational Influences on Sustained Volunteerism: An Interactionist Perspective. The Journal of Social Issues 58(3): 447–467. https://doi. org/10.1111/1540-4560.00270
- Perrings C, Burgiel S, Lonsdale M, Mooney H, Williamson M (2010) International cooperation in the solution to trade-related invasive species risks. Annals of the New York Academy of Sciences 1195(1): 198–212. https://doi.org/10.1111/j.1749-6632.2010.05453.x
- Pocock MJ, Chapman D, Sheppard L, Roy H (2014) Choosing and Using citizen science: a guide to when and how to use citizen science to monitor biodiversity and the environment. NERC/Centre for Ecology & Hydrology, Wallingford, 1–24.
- Pocock MJO, Tweddle JC, Savage J, Robinson LD, Roy HE (2017) The diversity and evolution of ecological and environmental citizen science. PLoS ONE 12(4): e0172579. https://doi. org/10.1371/journal.pone.0172579
- Price-Jones V, Brown PMJ, Adriaens T, Tricarico E, Farrow RA, Cardosa AC, Gervasini E, Groom Q, Reyserhove L, Schade S, Tsinaraki C, Marchante E (2021) Citizen Science projects on Alien Species in Europe. https://doi.org/10.5281/zenodo.4719259 [dataset]
- Probert AF, Wegmann D, Volery L, Adriaens T, Bakiu R, Bertolino S, Essl F, Gervasini E, Groom Q, Latombe G, Marisavljevic D, Mumford J, Pergl J, Preda C, Roy HE, Scalera R, Teixeira H, Tricarico E, Vanderhoeven S, Bacher S (2022) Identifying, reducing, and communicating uncertainty in community science: A focus on alien species. Biological Invasions 24: 3395–3421. https://doi.org/10.1007/s10530-022-02858-8
- R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- Reyserhove L, Desmet P, Oldoni D, Adriaens T, Strubbe D, Davis AJS, Vanderhoeven S, Verloove F, Groom Q (2020) A checklist recipe: Making species data open and FAIR. Database (Oxford) 2020: 1–12. https://doi.org/10.1093/database/baaa084
- Roy HE, Rorke SL, Beckmann B, Booy O, Botham MS, Brown PMJ, Harrower C, Noble D, Sewell J, Walker K (2015) The contribution of volunteer recorders to our understanding of biological invasions. Biological Journal of the Linnean Society. Linnean Society of London 115(3): 678–689. https://doi.org/10.1111/bij.12518
- Roy H, Groom Q, Adriaens T, Agnello G, Antic M, Archambeau A-S, Bacher S, Bonn A, Brown P, Brundu G, López B, Cleary M, Cogălniceanu D, de Groot M, Sousa TD, Deidun A, Essl F, Pečnikar ŽF, Gazda A, Gervasini E, Glavendekic M, Gigot G, Jelaska S, Jeschke J, Kaminski D, Karachle P, Komives T, Lapin K, Lucy F, Marchante E, Marisavljevic D, Marja R, Torrijos LM, Martinou A, Matosevic D, Mifsud C, Motiejūnaitė J, Ojaveer H, Pasalic N, Pekárik L, Per E, Pergl J, Pesic V, Pocock M, Reino L, Ries C, Rozylowicz L, Schade S, Sigurdsson S, Steinitz O, Stern N, Teofilovski A, Thorsson J, Tomov R, Tricarico

E, Trichkova T, Tsiamis K, van Valkenburg J, Vella N, Verbrugge L, Vétek G, Villaverde C, Witzell J, Zenetos A, Cardoso AC (2018) Increasing understanding of alien species through citizen science (Alien-CSI). Research Ideas and Outcomes 4: e31412. https://doi.org/10.3897/rio.4.e31412

- Schade S, Tsinaraki C (2016) Survey report: data management in Citizen Science projects. Publication Office of the European Union, Luxembourg. Technical report, 1–56.
- Schade S, Tsinaraki C, Roglia E (2017) Scientific data from and for the citizen. First Monday 22. https://doi.org/10.5210/fm.v22i8.7842
- Schade S, Kotsev A, Cardoso AC, Tsiamis K, Gervasini E, Spinelli F, Mitton I, Sgnaolin R (2019) Aliens in Europe. An open approach to involve more people in invasive species detection. Computers, Environment and Urban Systems 78: 101384. https://doi. org/10.1016/j.compenvurbsys.2019.101384
- Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jäger H, Kartesz J, Kenis M, Kreft H, Kühn I, Lenzner B, Liebhold A, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, van Kleunen M, Walker K, Weigelt P, Yamanaka T, Essl F (2017) No saturation in the accumulation of alien species worldwide. Nature Communications 8(1): 1–9. https://doi.org/10.1038/ncomms14435
- Seebens H, Bacher S, Blackburn TM, Capinha C, Dawson W, Dullinger S, Genovesi P, Hulme PE, Kleunen M, Kühn I, Jeschke JM, Lenzner B, Liebhold AM, Pattison Z, Pergl J, Pyšek P, Winter M, Essl F (2020) Projecting the continental accumulation of alien species through to 2050. Global Change Biology 27(5): 97–982. https://doi.org/10.1111/gcb.15333
- Silvertown J (2009) A new dawn for citizen science. Trends in Ecology & Evolution 24(9): 467–471. https://doi.org/10.1016/j.tree.2009.03.017
- Swinnen K, Vercayie D, Vanreusel W, Barendse R, Boers K, Bogaert J, Dekeukeleire D, Driessens G, Dupriez P, Jooris R, Steeman R, Van Asten K, Van Den Neucker T, Van Dorsselaer P, Van Vooren P, Wysmantel N, Gielen K, Desmet P, Herremans M (2018) Waarnemingen. be: Non-native plant and animal occurrences in Flanders and the Brussels Capital Region, Belgium. BioInvasions Records 7(3): 335–342. https://doi.org/10.3391/bir.2018.7.3.17
- Trichkova T, Paunović M, Cogălniceanu D, Schade S, Todorov M, Tomov R, Stănescu F, Botev I, López-Cañizares C, Gervasini E, Hubenov Z, Ignatov K, Kenderov M, Marinković N, Mitton I, Preda C, Spinelli FA, Tsiamis K, Cardoso AC (2021) Pilot Application of 'Invasive Alien Species in Europe' Smartphone App in the Danube Region. Water (Basel) 13(21): 2952. https://doi.org/10.3390/w13212952
- United Nations (1992) Convention on Biological Diversity 28(1-4): 88-103. https://doi. org/10.7882/AZ.1992.018
- United Nations (2019) World Population Prospects 2019, Online Edition. Rev. 1. https:// population.un.org/wpp/Download/Standard/Population/
- Vanderhoeven S, Adriaens T, D'hondt B, Van Gossum H, Vandegehuchte M, Verreycken H, Cigar J, Branquart E (2015) A science-based approach to tackle invasive alien species in Belgium – the role of the ISEIA protocol and the Harmonia information system as de-

cision support tools. Management of Biological Invasions 6(2): 197–208. https://doi.org/10.3391/mbi.2015.6.2.10

- Vilà M, Basnou C, Pyšek P, Josefsson M, Genovesi P, Gollasch S, Nentwig W, Olenin S, Roques A, Roy D, Hulme PE (2010) How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. Frontiers in Ecology and the Environment 8(3): 135–144. https://doi.org/10.1890/080083
- Wiggins A, Crowston K (2011) From Conservation to Crowdsourcing: A Typology of Citizen Science. 44th Hawaii International Conference on System Sciences. IEEE, 1–10. https:// doi.org/10.1109/HICSS.2011.207
- Wilkinson MD, Dumontier M, Aalbersberg IJJ, Appleton G, Axton M, Baak A, Blomberg N, Boiten J-W, da Silva Santos LB, Bourne PE, Bouwman J, Brookes AJ, Clark T, Crosas M, Dillo I, Dumon O, Edmunds S, Evelo CT, Finkers R, Gonzalez-Beltran A, Gray AJG, Groth P, Goble C, Grethe JS, Heringa J, 't Hoen PAC, Hooft R, Kuhn T, Kok R, Kok J, Lusher SJ, Martone ME, Mons A, Packer AL, Persson B, Rocca-Serra P, Roos M, van Schaik R, Sansone S-A, Schultes E, Sengstag T, Slater T, Strawn G, Swertz MA, Thompson M, van der Lei J, van Mulligen E, Velterop J, Waagmeester A, Wittenburg P, Wolstencroft K, Zhao J, Mons B (2016) The FAIR Guiding Principles for scientific data management and stewardship. Scientific Data 3(1): 1–9. https://doi.org/10.1038/sdata.2016.18
- Williams LA, DeSteno D (2008) Pride and perseverance: The motivational role of pride. Journal of Personality and Social Psychology 94(6): 1007–1017. https://doi.org/10.1037/0022-3514.94.6.1007



The invasiveness of Guinea grass (Megathyrsus maximus) is characterized by habitat and differing herbivore assemblages in its native and invaded range

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Academic editor: Matt Hill | Received 28 May 2022 | Accepted 26 October 2022 | Published 11 November 2022

Citation: Rhodes AC, Plowes RM, Martins DJ, Ng'Iru I, Gilbert LE (2022) The invasiveness of Guinea grass (*Megathyrsus maximus*) is characterized by habitat and differing herbivore assemblages in its native and invaded range. NeoBiota 78: 25–44. https://doi.org/10.3897/neobiota.78.87069

Abstract

A significant challenge of global change is the human-mediated movement of pasture grasses and their subsequent impact on ecosystem processes when they become invasive. We must understand invasive grass ecology and their natural enemies in native and introduced ranges to mitigate these impacts. Guinea grass (*Megathyrsus maximus*) is a pantropically introduced pasture grass that escapes intended areas and invades native ecosystems – threatening biodiversity and ecosystem function. The success of invasive plants has often been attributed to ecological release from stressors, including natural enemies and resource availability. Our objective was to assess Guinea grass functional traits across three different habitat types in native and invaded ranges by documenting ungulate and arthropod abundance, diversity, and feeding guilds. Guinea grass functional traits were assessed in three habitat types: grassland, riparian, and woody thickets around nitrogen-fixing *Prosopis glandulosa* in its introduced range in Texas, USA, and *Senegalia mellifera* in its native range in Kenya. We characterized Guinea grass functional traits by measuring plant height, cover, biomass, root-to-shoot ratios, and reproductive traits. We then examined the phytophagous arthropod and ungulate abundance and feeding guild diversity across the three habitat types. We hypothesized that functional trait expression related to invasiveness would be associated with Guinea grass in its introduced range. Also, we hypothesized that the abundance and diversity of phytophagous arthropods and ungulates

would be lower in the invaded range. Finally, we hypothesized that Guinea grass functional traits would differ between the three habitat types, given the habitat types' innate differences in resource availability. We found that Guinea grass was 2.5 times taller and 3.3 times more productive and covered 2.5 times more area in its invaded versus native ranges. Introduced Guinea grass had higher reproduction rates with 2.5 times more reproductive tillers, while habitat type drove vegetative reproduction with 15 times more stoloniferous establishment in wooded and riparian sites than grasslands. Texan ungulate communities were less species-rich, less functionally diverse, and less abundant than the Kenyan ungulate community. The phytophagous arthropod diversity on plants was twice as high on Kenyan Guinea grass than on Texan Guinea grass. Total arthropod family richness was nearly double, with 15 families represented in Kenya and 8 in Texas. These results suggest that Guinea grass has escaped a rich assemblage of arthropods and ungulates and likely explains some of its spread in introduced ranges. This study demonstrates how the invasive success of Guinea grass can be understood in terms of its competitive ability and interaction with natural enemies in the introduced and native ranges and may inform future biological control.

Keywords

arthropod diversity, biological control, ecological stressor release, function, invasion, natural enemy, ungulate

Introduction

Introducing perennial grasses for rangeland improvement has led to the pantropical distribution of highly invasive grasses (Marshall et al. 2012; Rhodes et al. 2021). Alarmingly, the traits beneficial to higher livestock yields are associated with invasiveness (Jank et al. 2014) and lead to human-mediated dispersal (Parsons 1972; Pyšek and Richardson 2007). Dozens of hypotheses have successfully explained biological invasions, each with its level of support and interconnectedness (Catford et al. 2009; Jeschke et al. 2012). Two prevalent hypotheses relevant to invasive grasses are the Enemy Release Hypothesis (Keane and Crawley 2002) and the Evolution of Increased Competitive Ability (Blossey and Notzold 1995). The mechanisms underlying these hypotheses are likely not mutually exclusive (Jeschke et al. 2012), and each mechanism leads to the expansion of realized niche spaces in invaded ranges. When released from biotic and abiotic factors that limit their productivity and reproductivity, nonnative species may become invasive (Cox and Ricklefs 1977; Torchin et al. 2003; Canavan et al. 2019). For example, the Enemy Release Hypothesis posits that specialist enemies will be absent from the new system and that generalists will prefer native plants (Keane and Crawley 2002). However, these hypotheses are often context-dependent, and generalities may not apply to particular species (Pyšek and Richardson 2007). Therefore, describing the mechanisms leading to invasion by focal organisms of management or agricultural interest is critical for understanding the invasion process and mitigating the negative consequences.

Life histories and functional traits are important indicators of plant success and often correlate with their invasiveness and dominance in a community (Cornwell and Ackerly 2010). Invasive species outcompete native plants for resources (Blossey and Notzold 1995; Davis et al. 2000) and win apparent competition by losing natural

enemies (Mlynarek et al. 2017). Several traits are associated with invasiveness: high reproductive output, reproductive mode (sexual vs. asexual), tolerance to herbivory, and height. Plant height often predicts invasiveness (Pyšek and Richardson 2007) through its impact on resource competition, correlation with biomass, and higher competitive ability to exploit resources (Canavan et al. 2019). Traits correlated to plant size are indicators of plant competition through their ability to disproportionately acquire resources such as light, water, and soil nutrients, which drive increased competitive ability and subsequent plant community composition (Lavorel et al. 2007; Dirks et al. 2017). Reproductive traits like clonality, increased flowering quantity, and increased flowering duration are indicators of invasiveness (Cadotte et al. 2006). Resource availability can drive interspecific differences in plant responses to herbivores (Coley et al. 1985). Likewise, intraspecific susceptibility to herbivores varies across resource avail-

ability (Fine et al. 2004). While a perennial grass in both its native and invaded range may have similar strategies, the release from natural enemies could alter these functional traits.

Guinea grass (Megathyrsus maximus syn. Panicum maximum (Jacq.) B.K. Simon & S.W.L. Jacobs) is consistently ranked among the most ecologically damaging invasive plant species globally (Best 2005; Randall 2017; CABI 2022). Guinea grass was first introduced to South Texas in the 1950s and has spread rapidly since the 1970s across a semi-tropical savanna dominated by the nitrogen-fixing trees honey mesquite Prosopis glandulosa Torr (Zitzer et al. 1996; Best 2005). Tree-grass interactions in these savannas include facilitation and competition for water and nutrient resources (Scholes and Archer 1997), and these primary associations mediate invasion at local scales (Colautti et al. 2006). Nitrogen-fixing trees promote improved nutrient availability, shade, and soil moisture (Zitzer et al. 1996; Scholes and Archer 1997) and facilitate Guinea grass invasion (Rhodes et al. 2022). Guinea grass is a tall-statured grass, often apomictic and polyploid, and is highly invasive pantropically (Kaushal et al. 2015; Canavan et al. 2019; Lambertini 2019). Tall-statured invasive grasses generally produce culms over 1.5 m in height and are noted for associating with invasiveness. They are commonly invasive outside their range (Lambert et al. 2010) and characterized by monodominance, high productivity, and reproductive rates (Canavan et al. 2019). Clonality, high flower count, and flowering throughout the season offer flexible life-history traits for invading Guinea grass (Rhodes et al. 2022). Comparing functional traits between naturally occurring invasive and native Guinea grass populations would improve our understanding of its invasion.

This dichotomy of Guinea grass is its economic success and ecological damage, which can be attributed to several core traits; its ability to grow in variable precipitation (400 mm – 1700 mm), high tolerance to herbivory (Sukhchain 2010), high reproductive rates (Rhodes et al. 2022) and strong competitive ability in its invaded range (Ho et al. 2016). Much of our current understanding of Guinea grass reproduction, production, and function is through its development as a pasture grass (Jank et al. 2014; Euclides et al. 2018; Maciel et al. 2018) or as a noxious weed (Alves and Xavier 1986; Best 2005; Ammondt and Litton 2012). However, few studies leverage

an understanding of Guinea grass characteristics and interactions with natural enemies and stressors to understand the success of Guinea grass as an invasive species. Given that resource availability is a driver of invasion processes (Davis et al. 2000; Hui et al. 2016), understanding how resources alter Guinea grass production, reproduction, and interaction with the herbivore community is essential for mitigating Guinea grass invasion and evaluating prospective biological control programs.

Invasive plants alter the structure and function of arthropod communities (Van der Colff et al. 2015). Generally, invasive plants reduce arthropod diversity and abundance, leading to the collapse of trophic links (Herrera and Dudley 2003). However, the response of arthropod functional groups to invasive plants is mixed. Litt et al. (2014) found that abundance and taxonomic richness decreased in around half of the 87 studies reviewed, with the most significant increases in detritivores in 67% of the studies and herbivorous arthropods reduced by 48%. Plant community phylogenetic, phytochemical, and species richness can all impact an insect's ability to feed on plants (Salazar and Marquis 2022). Therefore, many phytophagous arthropods may fail when plant communities change to invasive dominants, which may promote invasion.

Ungulates may be used to manage invasive plants, improving ecological restoration and maintenance outcomes with the added benefit of contributing to livestock yields (Frost and Launchbaugh 2003; Bailey et al. 2019). Grasses escaping from highly abundant and diverse ungulate communities will likely have higher productivity and reproductivity, competing well in invaded ranges. However, ungulates have differential impacts on plant species depending on their feeding guild and the physiology of the plant, such that the functional diversity of herbivores reduces plant establishment success (Rhodes et al. 2018). Targeted grazing of palatable grasses has shown promise for reducing the invasiveness of plants (Gaskin et al. 2021), but sustainable management is expected to entail arthropod associates. Thus, characterizing the arthropod and ungulate diversity in the native range of an invasive grass plays a critical role in understanding the ecological importance of those arthropods and developing efficient biological control programs.

The primary objective of this project was to evaluate Guinea grass functional traits across three habitat types in the native and invaded ranges and document the arthropod and ungulate herbivore community assemblages. To achieve this objective, we; first quantified Guinea grass functional traits and reproductive output across three habitat types. Second, we measured the richness and diversity of phytophagous arthropods and ungulate herbivores on Guinea grass in native ranges versus the invaded range. Third, we characterized these communities by their relative abundance and feeding guild diversity. Fourth, we developed a list of the arthropod community attached to Guinea grass to understand their impact. We hypothesized that functional trait expression related to invasiveness would be associated with Guinea grass in its introduced range. We hypothesized that functional traits would differ between the three habitat types, given the habitat types' innate differences in resource availability. Finally, we hypothesized that the abundance and diversity of phytophagous arthropods and ungulates would be lower in the invaded range, consistent with the Enemy Release Hypothesiss.

Methods

We established field sites to examine Guinea grass occurring naturally in two savanna systems; 1) the native range in Laikipia Province, Kenya, and 2) the invaded range in South Texas, where Guinea grass is spreading rapidly. Ten locations were selected in both Kenya and Texas. Each location had three habitat types: woody mottes (thickets), open grassland, and riparian zones. Three 1 × 1 m subplots were randomly established within each habitat type for 180 subplots. Mottes were under woody legumes with higher soil moisture and soil nitrogen. We selected two major tree species, each native to the study area; *Senegalia mellifera* (Benth.) Seigler & Ebinger, common in the red soils of Laikipia, and *P. glandulosa* in Texas a common species. The grassland sites for each grouping were approximately halfway between the thicket (motte) and riparian habitat types. Riparian areas were defined as the upland portion of ephemeral creeks that fed into the main waterway (Los Olmos Creek in Texas and Ewaso Ng'iro River in Kenya).

In Kenya, field sites were selected along the Ewaso Ng'iro River. West of the river is Mpala Research Centre, an active cattle ranch with approximately 3000 livestock grazing at low to moderate stocking intensities on 19,500 hectares, including Zebu/ Boran mix-breed of cattle, camel, goat, and sheep. Mottes were maintained naturally by large ungulate and fire disturbances. In Texas, the study was conducted at two ranches with active brush and cattle management activities in the semi-arid mesquite thorn tree savanna of the South Texas Plains ecoregion. A 1600-ha ranch pasture in Kenedy, Kleberg & Brooks Counties on Palobia loamy fine sand was brush-chained in the 1970s and then partly root-plowed in 1998 to form residual stands of *P. glandulosa* in a grassland matrix and a 1000-ha ranch pasture in Brooks County, on Padrones fine sand. Cattle regularly graze both sites and feed on Guinea grass. Stocking densities were generally low to moderate using resident longhorn cattle (*Bos primigenius*) and common stocker cattle (*Bos taurus*).

Guinea grass productivity, functional traits, and reproduction

Within each 1×1 m subplot, we measured Guinea grass height to the highest leaf and visually estimated the percent foliar cover at each site to understand how functional traits vary across habitat types and invaded vs. native range. We used the height as a functional indicator that provides a relative indicator of the competitive potential of Guinea grass in its native and invaded range across habitat types. We uprooted one Guinea grass clump from each subplot by tossing the quadrat and selecting the central individual to estimate productivity and resource allocation to above and belowground tissues (three in total from each site). The plants were cut to separate aboveground tissues (leaves, culms, and inflorescences) from the belowground tissues (roots and rhizomes). Belowground tissue was soaked in water overnight and then washed over a 1 mm sieve to remove soil and soil organic matter. The aboveground tissue was dried in a drying oven for 72 hours at 50 °C. Root clumps were broken up over a sieve to remove

the remaining soil. The above and belowground tissue was summed to calculate total biomass, and then root tissue biomass (mg) was divided by shoot biomass (mg) to calculate root to shoot ratio. Finally, in each of the three 1 m² plots, the total numbers of seed heads and stolons were recorded. The mean of the three plots was then used in the analyses. Seedheads were defined as panicles with mature seeds, noted by the ease of dislodging seeds. Stolons were characterized by lateral shoots that had established a rooted node with at least five leaves and a height of 20 cm. These definitions ensured that the stolon had been successfully established.

Ungulate associates

Six camera trap locations along the south, central, and northern portions of Mpala Research Centre and ten camera trap locations were used at the Texas ranch. The camera survey was conducted from January 2019 to June 2019 until the Guinea grass sampling was completed. At each study site, we placed a trail camera (HyperFire 2 Professional Covert Camera Trap) (Reconyx, Holmen, WI, USA) to estimate relative use by ungulate species (O'Brien 2011) within an approximate 30 m detection range and 40° field of view. Cameras were set at very high sensitivity to capture three photos at five-second intervals with a five-minute rest period between triggers. Each ungulate that appeared at least once in the three-photo set was counted, and these counts were summed and divided by the number of active camera days. Counts of each ungulate species were scored and converted into an index of ungulates per camera day. Ungulate species were then grouped into feeding guilds based on their behavior and physiology to grazer, mixed-feeder, and browser, following Kingdon (2015) in Kenya or based on physiology following Hanley (1982) and Holechek et al. (1989) in Texas. These data represent general patterns of ungulate activity (ungulate camera⁻¹ day⁻¹) and are presented as descriptive data.

Arthropod associates

Three Guinea grass plants from each site were uprooted and transported back to the lab. Three culms and the associated roots from each grass clump were examined for arthropods under a microscope, the stem was dissected, and the leaf, inflorescence, culm, and roots. We assumed that arthropods still attached to the plant after this process were more likely to be associated with this plant rather than transient. Arthropod abundance was estimated by recording the number of arthropods per gram of wet plant tissue measured for each morphospecies recognized by morphological differences. Specimens were collected, cataloged, and stored in ethanol in a 2 ml microtube. Each morphospecies was barcoded at the CO1 locus. A region of approximately 450 bp targeting the CO1 locus was amplified with degenerate primers (ZBJ-ArtF1c, ZBJ-ArtR2c (Alberdi et al. 2018), followed by Sanger sequence analysis. Sequence similarity searches were conducted against the NCBI GenBank database to score the family taxonomic assignments. The family level of each morphospecies was used to estimate feeding guild assignments using a standard reference (Simpson 2013). Feeding guilds

were defined as: leaf mining, leaf chewing, stem boring, fruit boring, granivorous, sap-sucking, gallobionts, rhizophagous, detritivorous, and saprophagous. Arthropods with no association with phytophagous families were not used in the analysis but were submitted to GenBank.

Statistics

The height of the tallest culm, total biomass, foliar cover (%), root-to-shoot ratio, stolon count, and reproductive tiller counts were analyzed using mixed-effects regressions. Arthropod morphospecies counts were also analyzed using a mixed-effects regression. For all regressions, the fixed effects were the habitat type and invasion status and their interaction. The heterogeneity of variance was assessed by visually assessing the distribution of model residuals. Invasion status (native vs. invaded) was used as an identity variance structure to deal with variance heterogeneity and site as a random effect to account for spatial autocorrelation. Normality was assessed by visually inspecting a histogram of model residuals and was analyzed as normally distributed data. Feeding guilds for ungulates and arthropods are reported as the raw averages with their standard error. Ungulate camera data is presented as descriptive, given that no direct correlation could be made between ungulate abundance and specific use of Guinea grass. All regression analyses were done in R (R Core Team 2021) using the nlme package for mixed-effects modeling (Pinheiro et al. 2021).

Results

Guinea grass productivity and functional traits

Guinea grass in its invaded range had productivity and functional traits consistent with a successful invader. Guinea grass in its invaded range was taller, had 3.3 times higher biomass per plant, covered 2.5 times more area, and had twice as much shoot production over root production (Fig. 1). Guinea grass height was 2.5 times taller in its invaded range ($F_{(1.10)}$ = 42, p < 0.001) and interacted with habitat ($F_{(2.44)}$ = 4.67, p = 0.014), such that Guinea grass growing in riparian zones of its native range was statistically similar to its invaded range (Fig. 1A). Similarly, Guinea grass plants had higher biomass production in its invaded range ($F_{(1,10)} = 78$, p = < 0.001) and an interactive effect was driven by the low biomass in Kenyan grassland habitats ($F_{(2,44)} = 7.0$, p = .002) (Fig. 1B). Anecdotally, Kenyan grassland Guinea grass had more evidence of grazing than other habitats. Foliar cover (%) was 2.5 times higher in the invaded range $(F_{(1,10)} = 14, p = 0.004)$ and the motte habitat type $(F_{(1,44)} = 12, p < 0.001)$. Further, invasion status had a significant interaction, driven by high cover in invaded mottes $(F_{(1,10)} = 30 p = < 0.001)$ (Fig. 1C). The root-to-shoot ratio in the invaded range was half that of the native range ($F_{(1,10)} = 7.3$, p = 0.02) (Fig. 1D) but did not vary significantly by habitat type, nor was there an interaction ($F_{(1,42)} = 0.99$, and $F_{(2,42)} = 0.92$). Guinea grass produced twice as much aboveground biomass in the invaded range.



Figure 1. Guinea grass's morphological and physiological traits across three habitat types in native and invaded ranges. The morphological and physiological traits of Guinea grass are presented in four panels **A** height (cm) **B** Biomass dry weight (g) **C** percent foliar cover, and **D** root-to-shoot ratio. The bar's colors represent the ephemeral riparian, grassland, and motte portions of the matrix. Habitat type is nested within invaded (Texas) or native range (Kenya). The bar heights are calculated from the model predictions, and the error bars are the standard error.

Reproduction – Seed heads and stolons

Seedborne reproduction by Guinea grass, measured by seedhead count, was nearly three times as abundant in the invaded range ($F_{(1,10)} = 14$, p = 0.004). Still, it did not vary significantly across habitat types or the interaction between native and invaded provenance and by habitat type ($F_{1,42} = 1.3$ and $F_{2,42} = 0.6$, respectively) (Fig. 2A). Stolon production was no different in the invaded range compared to the native range, nor was there an interaction between habitat type or invasion status. However, stolon production differed significantly by habitat type, with the grassland habitat type having around $1/10^{\text{th}}$ to $1/20^{\text{th}}$ the mean number of stolons (Fig. 2B) compared to mottes or riparian sites.



Figure 2. Guinea grass's reproductive output and mode across three habitat types in native and invaded ranges. The reproductive outputs by seedhead and stolon counts of Guinea grass are presented in two panels **A** seedhead count and **B** stolon count. The bar's colors represent the ephemeral riparian, grassland, and motte (woody portion) for each vegetation type, which is nested within invaded (Texas) or native range (Kenya). The bar heights are calculated from the model predictions, and the error bars are the standard error.

Ungulate associates

In Texas, we observed five ungulate species: Bos taurus Linnaeus, 1758, Odocoileus virginianus Zimmermann, 1780, Pecari tajacu Linnaeus, 1758, Sus scrofa Linnaeus, 1758, Boselaphus tragocamelus Pallas, 1766. According to their behavior and physiology, these species were classified into their respective feeding guilds, one grazer, three mixed feeders, and one browser (Hanley 1982; Holechek et al. 1989). In Kenva, we observed 18 ungulate species (in order of abundance): Bos taurus Linnaeus, 1758, Equus quagga Boddaert, 1785, Aepyceros melampus Lichtenstein, 1812, Loxodonta africana Blumenbach, 1797, Giraffa camelopardalis reticulata De Winton, 1899, Equus quagga burchellii Gray, 1824, Syncerus caffer Sparrman, 1779, Equus grevyi Oustalet, 1882, Ovis aries Linnaeus, 1758, Madoqua guentheri Thomas, 1894, Kobus ellipsiprymnus Ogilby, 1833, Tragelaphus strepsiceros Pallas, 1766, Taurotragus oryx Pallas, 1766, Phacochoerus africanus Gmelin, 1788, Capra hircus Linnaeus, 1758, Nanger granti Brooke, 1872, Raphicerus campestris Thunberg, 1811, and Hippopotamus amphibius Linnaeus, 1758. According to their behavior and physiology, nine grazers, six mixed-feeders, and four browsers were assigned to their respective feeding guilds (Kingdon 2015). The native range presented a similar abundance of grazers yet considerably more abundant mixed-feeders and browsers. Total ungulate relative abundance across feeding guilds was 1.5 (ungulate camera⁻¹ day⁻¹) in the native range versus 1.1 (ungulate camera⁻¹ day⁻¹) in the invaded range (Fig. 3).



Figure 3. Ungulate feeding guild abundances in Texas and Kenya. Ungulate counts per camera day¹ are divided into feeding guilds and presented in their invaded (South Texas) and native (Kenya) range. The data are raw means and standard errors.

Arthropod associates

Guinea grass from Texas had less than half the phytophagous morphospecies of arthropods, with an average of 1.01 morphospecies per sample. In contrast, Guinea grass samples from Kenya had 2.14 ($F_{(1,10)} = 12 p = 0.006$). However, there was no significant association between habitat types or interaction between habitat type and invasion status ($F_{(1,42)} = 0.19$, $F_{(2,42)} = 1.46$, respectively) (Fig. 4). When standardized by the mass of sampled Guinea grass, there were on average 0.28 ± 0.06 morphospecies per gram of wet material in Kenya and 0.169 ± 0.03 morphospecies per gram in Texas. In Kenya, we described 27 unique morphospecies across 15 families whose members comprise nine feeding guilds: leaf mining, leaf chewing, stem-boring, fruit-boring, granivorous, sap-sucking, gallobionts, rhizophagous, detritivorous, saprophagous. Ten morphospecies from 8 families and six potential feeding guilds were identified in Texas. In Texas, three morphospecies of oribatid mites were the most abundant, and they primarily decompose organic material (Fig. 5) (Table 1).

Discussion

In South Texas, Guinea grass has pronounced functional and reproductive traits associated with invasiveness. Our results show that Guinea grass in South Texas was taller, dominated a larger area, produced more biomass (especially aboveground), and



Figure 4. Guinea grass-associated arthropod morphospecies in three habitat types in the invaded and native ranges. The bar's colors represent the ephemeral riparian, grassland, and motte (woody) vegetation types. The habitats are nested within invaded (Texas) or native ranges (Kenya). The bar heights are calculated from the model predictions, and the error bars are the standard error.



Figure 5. The potential feeding guilds from the total morphospecies in native and invaded ranges. Panel A compares potential feeding guilds given a given family's known feeding guild types. Panel B sums the total morphospecies count. The bar's colors represent native (dark) or invaded (Texas). The bar heights are the total sum of morphospecies that potentially belong to a particular feeding guild.

Kenya families	Species count*	Texas families	Species count*
Agaonidae	1	Cecidomyiidae	1
Cecidomyiidae	3	Chloropidae	1
Chloropidae	6	Cicadellidae	1
Chrysomelidae	2	Haplozetidae	3
Crambidae	1	Mordellidae	1
Curculionidae	1	Nymphalidae	1
Geometridae	2	Oppiidae	1
Hydraenidae	1	Pyralidae	1
Lygaeidae	1	-	
Noctuidae	2		
Phlaeothripidae	1		
Phycitinae	1		
Pyralidae	3		
Tenebrionidae	1		
Tortricidae	1		
Total	27		10

Table 1. Diversity of arthropod morphospecies collected from Guinea grass in Kenya and Texas.

*Species count is the number of morphospecies in that family.

reproduced sexually at higher rates than in its native range. These differences likely have cascading impacts on Guinea grass's competition with native species (Ammondt and Litton 2012; Ho et al. 2016; Espinosa-Garcia and Villasenor 2017; CABI 2022). Guinea grass's release from ecological stressors and enemies has likely contributed to the highly successful expansion of Guinea grass in the three different habitat types in South Texas. In contrast, the three habitat types in Kenya corresponded to heterogeneous and lower productivity and reproduction capacity compared to the heterogeneity of its invaded range. Likewise, Guinea grass's escape from abundant and functional diverse phytophagous arthropods and ungulates has likely contributed to its success across these variable environments. This study represents an essential step in describing differences in the breadth of Guinea grass's natural enemies in its native and invaded ranges and the niches it can occupy, which improves our understanding of Guinea grass invasion.

The morphology and productivity of invasive plants are functional traits that can predict invasiveness (Pyšek and Richardson 2007). Greater height and biomass influence resource competition and invasiveness (Canavan et al. 2019), indicating faster growth rates, resource acquisition, and reproductive potential. Our results suggest that Guinea grass is becoming dominant in its invasive range by producing taller plants, higher biomass per plant, and higher foliar cover (Fig. 1) (Canavan et al. 2019). This productivity correlates to Guinea grass's ability to compete for light and soil resources which have cascading effects on nutrient cycling and fire cycles (D'Antonio and Vitousek 1992). Tall-statured grasses (Canavan et al. 2019). Reproductive output and mode are associated with the invasiveness of plants (Pyšek 1997; Pyšek and Richardson
2007). In Guinea grass, seed-borne and vegetative reproduction likely leads to an effective invasion across different habitat types and resource availability (Rhodes et al. 2022).

Vegetative reproduction is associated with a higher competitive ability (Pyšek 1997), and the reproductive mode plays a critical role in sustaining local populations and dispersing propagules (Benson and Hartnett 2006; Simberloff 2009). Thus, reproductive traits play an important role in plant community assembly and subsequent invasion (Dirks et al. 2017). Guinea grass's propagule pressure and reproductive plasticity are critical factors for its establishment in novel and resource-available habitat types in its expanding range (Rhodes et al. 2022). In an established perennial grass community, asexual reproduction can represent up to 99% of the population turnover, while sexually reproduced individuals can be near 1% (Benson and Hartnett 2006). The higher reproduction through seedheads in the invaded range and stoloniferous reproduction was important in mottes in the native and invaded range. Guinea grass will likely have more successful establishment events in the invaded range without significant herbivore pressure.

In savanna systems, woody vegetation and riparian patches integrate with a grassland matrix creating gradients of light environment, nutrient turnover, and water availability that have vital impacts on grass growth (Zitzer et al. 1996; Ludwig et al. 2004). The interaction between herbivores and resources significantly impacts community structure and plant production (Olff and Ritchie 1998; Fine et al. 2004; Wan et al. 2014). Plant species in different habitat types may express variable intraspecific traits (Pyšek 1997; Cadotte and Lovett-Doust 2001). Phenotypic plasticity may improve outcomes for invasiveness, and grasses invading multiple habitat types often achieve similar negative impacts on each habitat, despite their different resource availability (Richards et al. 2006). Our study found that in its native range, Guinea grass had different morphometrics and reproductive characteristics across habitat types. In Kenya, Guinea grass height in riparian areas was similar to the height in habitat types of Texas (Fig. 1A). These differences are partly due to increased water resources in riparian environments and nutrients and shade in mottes (Zitzer et al. 1996) that improve outcomes for germination and growth in the critical early stages of development (Rhodes et al. 2022).

Losing natural enemies can increase biomass compared to native populations (Torchin et al. 2003). Guinea grass's escape from a comprehensive assemblage of natural enemies is correlated with these increases in primary production and reproductive capacity. Further, in this study, higher productivity translated into higher seedhead production, which could further accelerate the invasion of Guinea grass in South Texas savannas through enhanced dispersal and propagule pressure (Rhodes et al. 2022). The increase in productivity correlates to the lower number of arthropod herbivores in the invaded range, which is indicative of a reduction in natural enemies (Torchin et al. 2003; Lucero et al. 2020). Aggregated with this loss of phytophagous arthropods is the abundance and diversity of ungulate herbivores, which may contribute to lower productivity in the invaded range. The feeding guilds of both groups of organisms may differentially impact invasion success.

The differences between Kenya and Texas's arthropod and ungulate communities were stark. Several arthropod species encountered in Kenya are from feeding guilds known to tend toward specialization, including stem borers and mites (Rhodes et al. 2021). While invasive species can acquire novel enemies in their invaded range (Torchin et al. 2003; Rhodes and St. Clair 2018), they do so to a lesser degree. Therefore, the acquisition of generalist herbivores in Texas has likely not replaced the top-down impact on Guinea grass dominance and height in its native range, similar to the general results of enemy release (Lucero et al. 2020). However, Kenyan Guinea grass was similar to Texas's in resource-rich environments (riparian areas and mottes). This pattern may be due to increased tolerance to herbivory through resource availability (Coley et al. 1985) or growth-defense trade-offs (Lind et al. 2013). In addition, we documented that the phytophagous arthropod community varies drastically between the native and invaded ranges. In the case of invasive species that are important for forage and livestock yields, breeding programs select to heighten resistance to pathogens (Savidan et al. 1989; Maciel et al. 2018). Together, the arthropod and ungulate communities likely restrict Guinea grass to high-resource habitats and represent a barrier to its spread in Kenya. Yet, when released from these enemies, Guinea grass spreads well in a broader ecological niche in South Texas.

Utilizing natural enemies has formed the basis for classic biological control. The potential for classic biological control has been known for decades (Dodd 1940) as a self-sustaining management practice. However, invasive species management is also context-dependent, and a single organism may not be sufficient to contain an invasive organism. Therefore, more recent biological control programs often include a functionally diverse introduction of natural enemies, grasses such as *Arundo donax* (Goolsby et al. 2011; Goolsby et al. 2016), or control of invasive ants (Porter and Gilbert 2004). A renewed interest in controlling invasive grasses through biological control may lead to substantive improvements in the mitigation of grass invasion (Sutton et al. 2019).

Conclusion

This study represents an essential step in describing the productivity and reproduction of Guinea grass in three resource environments and its association with natural enemies in its native and invaded ranges. Guinea grass achieved high productivity and reproductivity across the range of habitat types in its invaded range when released from native enemies. However, in its native range, when subjected to natural enemies, the potential of Guinea grass was significantly reduced. In addition, the diversity of specialist and generalist arthropods in Kenya is high compared to the few generalist arthropods in Texas. This information improves our understanding of opportunities to develop impactful and sustainable biological control agents (Sutton et al. 2019). Further, since release from a single enemy may not drive increased invasiveness, using a suite of organisms across feeding guilds may be more appropriate in biological control (Porter and Gilbert 2004; Goolsby et al. 2011; Goolsby et al. 2016). Grasses are a critical group of organisms that make up most food crops and are translocated for pasture development, yet are also among the most pervasive invaders (Linder et al. 2018; Sutton et al. 2019). Given these two competing interests, an important goal is to search for sustainable biological control specific to the problematic grass species (Rhodes et al. 2021). Understanding how Guinea grass's ecology and biology differ in its native and introduced range may open opportunities for directed management. A troubling predicament is an enormous effort spent controlling Guinea grass as a weed and a similar effort developing Guinea grass as a pasture grass, encouraging invasive traits (Rhodes et al. 2021). Guinea grass represents an economically valuable forage species for livestock globally (Jank et al. 2014) yet often escapes to degrade ecosystem function and diversity. While traditional means of controlling invasive grasses may not be effective at landscape scales (Rhodes et al. 2021), a renewed focus on biological control for grasses could prove critical for controlling invasive grasses globally (Sutton et al. 2019).

Acknowledgements

We thank the skilled and knowledgeable technicians Godfrey Amoni, Kamukunji Katero from the Mpala Research Centre, and the Centre itself for its logistical support of this research. In Texas, Jason Lawson was our lead technician and was instrumental in assisting Texas's collection and processing of materials. We also thank Kaitlyn Williams and Shellsea Miller for their molecular contributions. The authors have declared that no competing interests exist.

This research was funded by the Lee and Ramona Bass Foundation.

References

- Alberdi A, Aizpurua O, Gilbert MTP, Bohmann K (2018) Scrutinizing key steps for reliable metabarcoding of environmental samples. Methods in Ecology and Evolution 9(1): 134– 147. https://doi.org/10.1111/2041-210X.12849
- Alves A, Xavier FE (1986) Major perennial weeds in Brazil: *Panicum maximum* Jacq., *Paspalum maritimum* Trin., *Brachiaria purpurascens* Hens., *Senecio brasiliensis* Less.
- Ammondt SA, Litton CM (2012) Competition between Native Hawaiian Plants and the Invasive Grass *Megathyrsus maximus*: Implications of Functional Diversity for Ecological Restoration. Restoration Ecology 20(5): 638–646. https://doi.org/10.1111/j.1526-100X.2011.00806.x
- Bailey DW, Mosley JC, Estell RE, Cibils AF, Horney M, Hendrickson JR, Walker JW, Launchbaugh KL, Burritt EA (2019) Synthesis Paper: Targeted Livestock Grazing: Prescription for Healthy Rangelands. Rangeland Ecology and Management 72(6): 865–877. https://doi. org/10.1016/j.rama.2019.06.003
- Benson EJ, Hartnett DC (2006) The role of seed and vegetative reproduction in plant recruitment and demography in tallgrass prairie. Plant Ecology 187(2): 163–178. https://doi. org/10.1007/s11258-005-0975-y

- Best C (2005) Fighting weeds with weeds: battling invasive grasses in the Rio Grande delta of Texas. In: Van Devender T, Espinosa-García, FJ, Harper-Lore, BL, Hubbard T (Eds) Invasive plants on the move: controlling them in North America. Tucson, Arizona, 307–317.
- Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigienous plants - A hypothesis. Journal of Ecology 83(5): 887–889. https://doi. org/10.2307/2261425
- CABI (2022) Megathyrsus maximus In: Invasive Species Compendium. Wallingford, UK: CAB International. www.cabi.org/isc
- Cadotte M, Lovett-Doust J (2001) Ecological and taxonomic differences between native and introduced plants of southwestern Ontario. Ecoscience 8(2): 230–238. https://doi.org/10.1080/11956860.2001.11682649
- Cadotte MW, Murray BR, Lovett-Doust J (2006) Evolutionary and ecological influences of plant invader success in the flora of Ontario. Ecoscience 13(3): 388–395. https://doi.org/10.2980/i1195-6860-13-3-388.1
- Canavan S, Meyerson LA, Packer JG, Pyšek P, Maurel N, Lozano V, Richardson DM, Brundu G, Canavan K, Cicatelli A, Čuda J, Dawson W, Essl F, Guarino F, Guo W-Y, van Kleunen M, Kreft H, Lambertini C, Pergl J, Skálová H, Soreng RJ, Visser V, Vorontsova MS, Weigelt P, Winter M, Wilson JRU (2019) Tall-statured grasses: A useful functional group for invasion science. Biological Invasions 21(1): 37–58. https://doi.org/10.1007/ s10530-018-1815-z
- Catford JA, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Diversity & Distributions 15(1): 22–40. https://doi.org/10.1111/j.1472-4642.2008.00521.x
- Colautti RI, Grigorovich IA, MacIsaac HJ (2006) Propagule pressure: A null model for biological invasions. Biological Invasions 8(5): 1023–1037. https://doi.org/10.1007/s10530-005-3735-y
- Coley PD, Bryant JP, Chapin III FS (1985) Resource availability and plant antiherbivore defense. Science 230(4728): 895–899. https://doi.org/10.1126/science.230.4728.895
- Cornwell WK, Ackerly DD (2010) A link between plant traits and abundance: Evidence from coastal California woody plants. Journal of Ecology 98(4): 814–821. https://doi.org/10.1111/j.1365-2745.2010.01662.x
- Cox GW, Ricklefs RE (1977) Species diversity and ecological release in Caribbean land bird faunas. Oikos 28(1): 113–122. https://doi.org/10.2307/3543330
- D'Antonio CM, Vitousek PM (1992) Biological invasions by exotic grasses, the grass fire cycle, and global change. Annual Review of Ecology and Systematics 23(1): 63–87. https://doi. org/10.1146/annurev.es.23.110192.000431
- Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: A general theory of invasibility. Journal of Ecology 88(3): 528–534. https://doi.org/10.1046/ j.1365-2745.2000.00473.x
- Dirks I, Dumbur R, Lienin P, Kleyer M, Grünzweig JM (2017) Size and reproductive traits rather than leaf economic traits explain plant-community composition in species-rich annual vegetation along a gradient of land use intensity. Frontiers in Plant Science 8: 891. https://doi.org/10.3389/fpls.2017.00891

- Dodd AP (1940) The biological campaign against Prickly-Pear. Commonwealth Prickly Pear Board, Brisbane, [ii +] 117 pp.
- Espinosa-Garcia FJ, Villasenor JL (2017) Biodiversity, distribution, ecology and management of non-native weeds in Mexico: a review. Revista Mexicana De Biodiversidad 88: 76–96. https://doi.org/10.1016/j.rmb.2017.10.010
- Euclides VPB, Carpejani GC, Montagner DB, Nascimento D, Barbosa RA, Difante GS (2018) Maintaining post-grazing sward height of *Panicum maximum* (cv. Mombaca) at 50 cm led to higher animal performance compared with post-grazing height of 30 cm. Grass and Forage Science 73(1): 174–182. https://doi.org/10.1111/gfs.12292
- Fine PVA, Mesones I, Coley PD (2004) Herbivores promote habitat specialization by trees in Amazonian forests. science 305: 663–665. https://doi.org/10.1126/science.1098982
- Frost RA, Launchbaugh KL (2003) Prescription grazing for rangeland weed management. Rangelands 25(6): 43–47. https://doi.org/10.2458/azu_rangelands_v25i6_frost
- Gaskin JF, Espeland E, Johnson CD, Larson DL, Mangold JM, McGee RA, Milner C, Paudel S, Pearson DE, Perkins LB, Prosser CW, Runyon JB, Sing SE, Sylvain ZA, Symstad AJ, Tekiela DR (2021) Managing invasive plants on Great Plains grasslands: A discussion of current challenges. Rangeland Ecology and Management 78: 235–249. https://doi. org/10.1016/j.rama.2020.04.003
- Goolsby JA, Kirk AA, Moran PJ, Racelis AE, Adamczyk JJ, Cortés E, Marcos García M, Martinez Jimenez M, Summy KR, Ciomperlik MA (2011) Establishment of the armored scale, *Rhizaspidiotus donacis*, a biological control agent of *Arundo donax*. The Southwestern Entomologist 36(3): 373–374. https://doi.org/10.3958/059.036.0314
- Goolsby JA, Moran PJ, Racelis AE, Summy KR, Jimenez MM, Lacewell RD, Perez de Leon A, Kirk AA (2016) Impact of the biological control agent *Tetramesa romana* (Hymenoptera: Eurytomidae) on *Arundo donax* (Poaceae: Arundinoideae) along the Rio Grande River in Texas. Biocontrol Science and Technology 26(1): 47–60. https://doi.org/10.1080/09583157.2015.1074980
- Hanley TA (1982) The nutritional basis for food selection by ungulates. Rangeland Ecology & Management/Journal of Range Management Archives 35: 146–151. https://doi. org/10.2307/3898379
- Herrera A, Dudley TL (2003) Invertebrate community reduction in response to Arundo donax invasion at Sonoma Creek. Biological Invasions 5(3): 167–177. https://doi. org/10.1023/A:1026190115521
- Ho CY, Tsai MY, Huang YL, Kao WY (2016) Ecophysiological factors contributing to the invasion of *Panicum maximum* into native *Miscanthus sinensis* grassland in Taiwan. Weed Research 56(1): 69–77. https://doi.org/10.1111/wre.12186
- Holechek JL, Pieper RD, Herbel CH (1989) Range management. Principles and practices. Prentice-Hall.
- Hui C, Richardson DM, Landi P, Minoarivelo HO, Garnas J, Roy HE (2016) Defining invasiveness and invasibility in ecological networks. Biological Invasions 18(4): 971–983. https://doi.org/10.1007/s10530-016-1076-7
- Jank L, Barrios SC, do Valle CB, Simeao RM, Alves GF (2014) The value of improved pastures to Brazilian beef production. Crop & Pasture Science 65(11): 1132–1137. https://doi. org/10.1071/CP13319

- Jeschke J, Aparicio LG, Haider S, Heger T, Lortie C, Pyšek P, Strayer D (2012) Support for major hypotheses in invasion biology is uneven and declining. NeoBiota 14: 1–20. https:// doi.org/10.3897/neobiota.14.3435
- Kaushal P, Paul S, Saxena S, Dwivedi KK, Chakraborti M, Radhakrishna A, Roy AK, Malaviya DR (2015) Generating higher ploidies (7× and 11×) in guinea grass (*Panicum maximum* Jacq.) utilizing reproductive diversity and uncoupled apomixis components. Current Science 109: 1392–1395.
- Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17(4): 164–170. https://doi.org/10.1016/S0169-5347(02)02499-0
- Kingdon J (2015) The Kingdon field guide to African mammals. Bloomsbury Publishing.
- Lambert AM, Dudley TL, Saltonstall K (2010) Ecology and impacts of the large-statured invasive grasses Arundo donax and Phragmites australis in North America. Invasive Plant Science and Management 3(4): 489–494. https://doi.org/10.1614/IPSM-D-10-00031.1
- Lambertini C (2019) Why are tall-statured energy grasses of polyploid species complexes potentially invasive? A review of their genetic variation patterns and evolutionary plasticity. Biological Invasions 21(10): 3019–3041. https://doi.org/10.1007/s10530-019-02053-2
- Lavorel S, Díaz S, Cornelissen JHC, Garnier E, Harrison SP, McIntyre S, Pausas JG, Pérez-Harguindeguy N, Roumet C, Urcelay C (2007) Plant functional types: are we getting any closer to the Holy Grail? Terrestrial ecosystems in a changing world. Springer, 149–164. https://doi.org/10.1007/s10530-019-02053-2
- Lind EM, Borer E, Seabloom E, Adler P, Bakker JD, Blumenthal DM, Crawley M, Davies K, Firn J, Gruner DS, Stanley Harpole W, Hautier Y, Hillebrand H, Knops J, Melbourne B, Mortensen B, Risch AC, Schuetz M, Stevens C, Wragg PD (2013) Life-history constraints in grassland plant species: A growth-defence trade-off is the norm. Ecology Letters 16(4): 513–521. https://doi.org/10.1111/ele.12078
- Linder HP, Lehmann CER, Archibald S, Osborne CP, Richardson DM (2018) Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. Biological Reviews of the Cambridge Philosophical Society 93(2): 1125– 1144. https://doi.org/10.1111/brv.12388
- Litt AR, Cord EE, Fulbright TE, Schuster GL (2014) Effects of Invasive Plants on Arthropods. Conservation Biology 28(6): 1532–1549. https://doi.org/10.1111/cobi.12350
- Lucero JE, Arab NM, Meyer ST, Pal RW, Fletcher RA, Nagy DU, Callaway RM, Weisser WW (2020) Escape from natural enemies depends on the enemies, the invader, and competition. Ecology and Evolution 10(19): 10818–10828. https://doi.org/10.1002/ece3.6737
- Ludwig F, Dawson TE, Prins HHT, Berendse F, de Kroon H (2004) Below-ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift. Ecology Letters 7(8): 623–631. https://doi.org/10.1111/j.1461-0248.2004.00615.x
- Maciel GA, Braga GJ, Guimaraes Jr R, Ramos AKB, Carvalho MA, Fernandes FD, Fonseca CEL, Jank L (2018) Seasonal Liveweight Gain of Beef Cattle on Guineagrass Pastures in the Brazilian Cerrados. Agronomy Journal 110(2): 480–487. https://doi.org/10.2134/agronj2017.05.0262
- Marshall VM, Lewis MM, Ostendorf B (2012) Buffel grass (*Cenchrus ciliaris*) as an invader and threat to biodiversity in arid environments: A review. Journal of Arid Environments 78: 1–12. https://doi.org/10.1016/j.jaridenv.2011.11.005

- Mlynarek JJ, Moffat CE, Edwards S, Einfeldt AL, Heustis A, Johns R, MacDonnell M, Pureswaran DS, Quiring DT, Shibel Z, Heard SB (2017) Enemy escape: A general phenomenon in a fragmented literature? Facets 2(2): 1015–1044. https://doi.org/10.1139/facets-2017-0041
- O'Brien TG (2011) Abundance, density and relative abundance: a conceptual framework. In: O'Connell AF, Nichols JD, Karanth KU (Eds) Camera Traps in Animal Ecology. Springer, Tokyo, 71–96. https://doi.org/10.1007/978-4-431-99495-4_6
- Olff H, Ritchie ME (1998) Effects of herbivores on grassland plant diversity. Trends in Ecology & Evolution 13(7): 261–265. https://doi.org/10.1016/S0169-5347(98)01364-0
- Parsons JJ (1972) Spread of African pasture grasses to the American tropics. Rangeland Ecology & Management/Journal of Range Management Archives 25: 12–17. https://doi. org/10.2307/3896654
- Porter SD, Gilbert LE (2004) Assessing host specificity and field release potential of fire ant decapitating flies (Phoridae: *Pseudacteon*). Assessing host ranges for parasitoids and predators used for classical biological control: a guide to best practice: 152–176.
- Pyšek P (1997) Clonality and plant invasions: can a trait make a difference. The ecology and evolution of clonal plants, 405–427.
- Pyšek P, Richardson DM (2007) Traits associated with invasiveness in alien plants: where do we stand? Biological Invasions. Springer, 97–125. https://doi.org/10.1007/978-3-540-36920-2_7
- Randall RP (2017) A global compendium of weeds. RP Randall, Perth, Australia.
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- Rhodes AC, St. Clair SB (2018) Measures of browse damage and indexes of ungulate abundance to quantify their impacts on aspen forest regeneration. Ecological Indicators 89: 648–655. https://doi.org/10.1016/j.ecolind.2018.02.013
- Rhodes AC, Larsen RT, Clair SBS (2018) Differential effects of cattle, mule deer, and elk herbivory on aspen forest regeneration and recruitment. Forest Ecology and Management 422: 273–280. https://doi.org/10.1016/j.foreco.2018.04.013
- Rhodes AC, Plowes RM, Goolsby JA, Gaskin JF, Musyoka B, Calatayud P-A, Cristofaro M, Grahmann ED, Martins DJ, Gilbert LE (2021) The dilemma of Guinea grass (*Megathyrsus maximus*): A valued pasture grass and a highly invasive species. Biological Invasions 23(12): 1–17. https://doi.org/10.1007/s10530-021-02607-3
- Rhodes AC, Plowes RM, Lawson JR, Gilbert LE (2022) Guinea grass establishment in South Texas is driven by disturbance history and savanna structure. Rangeland Ecology and Management 83: 124–132. https://doi.org/10.1016/j.rama.2022.04.003
- Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9(8): 981–993. https://doi.org/10.1111/j.1461-0248.2006.00950.x
- Salazar D, Marquis RJ (2022) Testing the role of local plant chemical diversity on plant–herbivore interactions and plant species coexistence. Ecology 103(9): e3765. https://doi. org/10.1002/ecy.3765
- Savidan YH, Jank L, Costa JCG, Dovalle CB (1989) Breeding *Panicum maximum* in Brazil 1. Genetic-resources, modes of reproduction and breeding procedures. Euphytica 41(1–2): 107–112. https://doi.org/10.1007/BF00022419

- Scholes RJ, Archer SR (1997) Tree-grass interactions in savannas. Annual Review of Ecology and Systematics 28(1): 517–544. https://doi.org/10.1146/annurev.ecolsys.28.1.517
- Simberloff D (2009) The role of propagule pressure in biological invasions. Annual Review of Ecology, Evolution, and Systematics 40(1): 81–102. https://doi.org/10.1146/annurev. ecolsys.110308.120304
- Simpson SJ (2013) Mouthparts and feeding. In: Simpson SJ, Douglas AE (Eds) The Insects. Structure and Function, 5th edn. Cambridge University Press, Cambridge, 15–45. https:// doi.org/10.1017/CBO9781139035460.005
- Sukhchain (2010) Breeding Guinea grass A review. Range Management and Agroforestry 31: 109–115.
- Sutton GF, Canavan K, Day MD, Den Breeyen A, Goolsby JA, Cristofaro M, McConnachie A, Paterson ID (2019) Grasses as suitable targets for classical weed biological control. BioControl 64(6): 605–622. https://doi.org/10.1007/s10526-019-09968-8
- Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. Nature 421(6923): 628–630. https://doi.org/10.1038/nature01346
- Van der Colff D, Dreyer LL, Valentine A, Roets F (2015) Invasive plant species may serve as a biological corridor for the invertebrate fauna of naturally isolated hosts. Journal of Insect Conservation 19(5): 863–875. https://doi.org/10.1007/s10841-015-9804-3
- Wan HY, Rhodes AC, St. Clair SB (2014) Fire severity alters plant regeneration patterns and defense against herbivores in mixed aspen forests. Oikos 123: 1479–1488. https://doi. org/10.1111/oik.01521
- Zitzer SF, Archer SR, Boutton TW (1996) Spatial variability in the potential for symbiotic N-2 fixation by woody plants in a subtropical savanna ecosystem. Journal of Applied Ecology 33(5): 1125–1136. https://doi.org/10.2307/2404692

REVIEW ARTICLE



Impact of non-native tree species in Europe on soil properties and biodiversity: a review

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Academic editor: Johannes Kollmann | Received 10 June 2022 | Accepted 17 October 2022 | Published 21 November 2022

Citation: Wohlgemuth T, Gossner MM, Campagnaro T, Marchante H, van Loo M, Vacchiano G, Castro-Díez P, Dobrowolska D, Gazda A, Keren S, Keserű Z, Koprowski M, La Porta N, Marozas V, Nygaard PH, Podrázský V, Puchałka R, Reisman-Berman O, Straigytė L, Ylioja T, Pötzelsberger E, Silva JS (2022) Impact of non-native tree species in Europe on soil properties and biodiversity: a review. NeoBiota 78: 45–69. https://doi.org/10.3897/neobiota.78.87022

Abstract

In the context of global change, the integration of non-native tree (NNT) species into European forestry is increasingly being discussed. The ecological consequences of increasing use or spread of NNTs in European forests are highly uncertain, as the scientific evidence is either constraint to results from case studies with limited spatial extent, or concerns global assessments that lack focus on European NNTs. For either case, generalisations on European NNTs are challenging to draw. Here we compile data on the impacts of seven important NNTs (Acacia dealbata, Ailanthus altissima, Eucalyptus globulus, Prunus serotina, Pseudotsuga menziesii, Quercus rubra, Robinia pseudoacacia) on physical and chemical soil properties and diversity attributes in Europe, and summarise commonalities and differences. From a total of 103 publications considered, studies on diversity attributes were overall more frequent than studies on soil properties. The effects on soil properties varied greatly among tree species and depended on the respective soil property. Overall, increasing (45%) and decreasing (45%) impacts on soil occurred with similar frequency. In contrast, decreasing impacts on biodiversity were much more frequent (66%) than increasing ones (24%). Species phylogenetically distant from European tree species, such as Acacia dealbata, Eucalyptus globulus and Ailanthus altissima, showed the strongest decreasing impacts on biodiversity. Our results suggest that forest managers should be cautious in using NNTs, as a majority of NNT stands host fewer species when compared with native tree species or ecosystems, likely reflected in changes in biotic interactions and ecosystem functions. The high variability of impacts suggests that individual NNTs should be assessed separately, but NNTs that lack European relatives should be used with particular caution.

Keywords

biodiversity, biogeography, forest management, pairwise stand comparisons, soil impacts

Introduction

Many non-native tree (NNT) species were introduced to Europe, particularly after the 16th century (Brundu and Richardson 2016). Some of these species have been deliberately favoured across Europe through cultivation, mostly because of the different goods and services they provide to societies (Castro-Díez et al. 2019; Brundu et al. 2020; Pötzelsberger et al. 2020a; Castro-Díez et al. 2021). After their initial introductions, some of the NNTs have spread without further human intervention, profiting from suitable soil and climate, competitive superiority and/or habitat disturbance, eventually becoming naturalised, or even invasive (Dodet and Collet 2012). NNTs in Europe include species that are planted for timber (e.g. *Eucalyptus globulus, Robinia pseudoacacia, Pseudotsuga menziesii, Picea sitchensis*; Brundu and Richardson 2016; Brus et al. 2019; Spiecker et al. 2019; Øyen and Nygaard 2020) or once were used for ornamental purposes and have spread since (e.g. *Ailanthus altissima* and *Acacia dealbata*). Overall, NNTs cover an area of approximately 8.54 million ha, of which *R. pseudoacacia* (2.44 million ha) and *E. globulus* (1.46 million ha) are the most abundant (Fig. 1), corresponding to about 4% of the forest cover in Europe (Brus et al. 2019). This overall percentage hides considerable disparities among European regions and countries. For example, the most abundant tree species in Portugal (*E. globulus*), Hungary (*R. pseudoacacia*) and the UK (*P. sitchensis*) are non-native, while in most other European countries NNTs have a minor importance compared to native tree species (Hasenauer et al. 2016).

The pros and cons of economically valuable NNTs is a topic of lively debate because of the possible detrimental impacts on the ecosystems that may result from the expansion of these species (Campagnaro et al. 2018; Castro-Díez et al. 2019; Pötzelsberger et al. 2020b; Wagner et al. 2021; Wohlgemuth et al. 2021). In addition, NNTs are increasingly discussed in the frame of global change (Brundu and Richardson 2016) and the consequences of climate change on biological invasions and adequate management practices (e.g. Walther et al. 2009). Because of this concern, a large and diverse body of legislation has been created in many European countries, aiming at regulating the establishment of NNTs (Pötzelsberger et al. 2020b). One of the most important pieces of legislation is Regulation (EU) No 1143/2014, the core of which is a list of invasive alien species of concern to the EU, including some NNTs (e.g. A. altissima and Acacia saligna). Among other provisions, the regulation refers the obligation of the different EU Member States to have in place effective management measures for invasive alien species of EU concern that are widespread. In addition, guidelines on the management of NNTs have been proposed by several authors, aiming at minimising their possible detrimental impacts (Brundu and Richardson 2016; Sitzia et al. 2016; Campagnaro et al. 2018; Brundu et al. 2020).

Among the ecological impacts commonly attributed to NNTs, those related to soil and biodiversity are feasible to measure, functionally important and therefore particularly attractive for research (Hulme et al. 2013). The impacts of NNTs on soil properties can have long-term ecological consequences given the importance of soil as a basis for ecosystem functioning. Among the impacts on soil properties and processes most frequently attributed to NNTs are those related to changes in nitrogen content (Castro-Díez et al. 2009) and other nutrients (Medina-Villar et al. 2016), decomposition rate of organic matter (Godoy et al. 2010), pH (Cremer and Prietzel 2017) and organic carbon (Jackson et al. 2002; Zerva et al. 2005). Soil changes induced by NNTs may be viewed as either beneficial or detrimental, depending on the perspective. For example, an increase in soil nitrogen originated by a leguminous species may be considered beneficial from a farmer's perspective, but detrimental from a conservationist's perspective. It may be detrimental if it alters ecosystem functions and processes of a site or preventing the survival of species naturally-adapted to nutrient-poor soils such as sand dune species (Huston and Smith 1987). However, it may be beneficial if we consider the improvement of soil fertility, for example when rehabilitating mined areas (Dutta and Agrawal 2003; Vlachodimos et al. 2013; Yuan et al. 2018) or improving the conditions for nitrophilous weed species (Yelenik et al. 2004). As to biodiversity, there is certainly a solid argument for the detrimental impacts of NNTs in situations where they reduce richness and diversity of native taxa, or the abundance of native animal or plant populations. A large body of literature has been produced on the

impacts of European NNT species on biodiversity, ranging from microbes (Krevš and Kučinskienė 2017) to plant communities (Chabrerie et al. 2007), invertebrates (Gossner 2016) or birds (Calviño-Cancela 2013).

A large number of papers on the impacts of NNTs has accumulated steadily during the previous century and more rapidly after the launching of the Millennium Ecosystem Assessment (Hassan et al. 2005). Researchers have been particularly keen in conducting comparisons between ecosystems populated by NNTs and native vegetation (NV; mostly native tree (NT) species or native treeless or open ecosystems (OE)), using similar site and climate conditions, to quantify the impact on specific ecosystem properties. Such studies are highly valuable as they often generate robust results based on sound statistical designs. However, the impact of NNTs can be highly contextdependent (Castro-Díez et al. 2019; Sapsford et al. 2020; Castro-Díez et al. 2021), and may vary, e.g. according to the management history of the studied stands, the soil and climate characteristics of the sites, or to the NV to which it is compared. Europeanwide or global assessments have so far focused on the impact of NNTs on ecosystem services (Castro-Díez et al. 2019), on the impact of mainly invasive plant species on communities and ecosystems (Pyšek et al. 2012), or on the impact of five major NNTs in Natura 2000 sites in Europe (Campagnaro et al. 2018). Nevertheless, a standardised analysis on the impact of NNTs on soil properties and biodiversity based on the existing literature is lacking.



Time since first introduction [yrs]

Figure 1. Current forest area cover of 18 selected NNTs in Europe and year since first introduction (based on Sanz-Elorza et al. 2004; Hasenauer et al. 2016; Badalamenti et al. 2018; Brus et al. 2019). The surface of each square is proportional to the surface covered by each NNT in Europe. The coordinates correspond to the centroid of the square. For NNTs with insufficient information, the symbol '+' is used.

To fill this knowledge gap, here we select seven important NNTs and compile data from a large body of literature on their impacts on soil and biodiversity in Europe, to summarise their commonalities and differences. Specifically, in this study we aim to: (a) assess the relative importance of the different NNTs and their impacts based on published papers, dissertations and reports; (b) assess the impacts of NNTs on soil properties and diversity attributes of different taxa in forests of Europe, based on pairwise comparisons against NV; (c) analyse the commonalities and differences in the impacts of selected NNTs; and (d) discuss the factors that may explain similar or contrasting responses based on available information on NNT traits, biogeography and management.

Materials and methods

NNT selection and workflow

This study was initiated in the frame of the COST Action Non-Native Tree Species for European Forests – Experiences, Risks and Opportunities (FP 1403; 2014–18). From the more than 150 NNTs growing in European forests and forestry trials (Brus et al. 2019), we initially selected the 18 most important ones (Table 1), with their importance assessed according to their forest area cover (if available), and the presence in numerous European countries (Europe defined geographically, but excluding Russia and including Turkey), and/or their rapid spread. We assumed that for the species with these three characteristics, there are likely to be more studies and publications on the impacts. A species is defined as being non-native to Europe if its native range is wholly outside of Europe. Thus, tree species native to Europe but planted outside of their regional distributional range, such as *Pinus nigra, Larix decidua* or *Picea abies* were not considered as being non-native even when planted outside of their native range.

The workflow was divided into three phases. In the first phase, we searched the Web of Science (WOS) using the name of the NNT species (e.g. *Prunus serotina*; see Table 1; Fig. 2) as search string. The search covered all papers published until August 2021. Publications retrieved from the search were filtered in order to only retain those featuring pairwise comparisons of NNTs vs. NV regarding effects on physical and chemical soil properties and diversity attributes (abundance, species richness, diversity) of different taxonomic groups studied in European countries. We extended the comparisons with NTs to non-forest ecosystems (OE), as long as they represented a reference for naturalness in the study area. In this phase, four species (*Acacia longifolia, A. saligna, Eucalyptus camaldulensis* and *Populus × canadensis*) were excluded from further examination due to the low number of studies (n < 20). To increase the number of studies, we extended the search in the second phase to (i) scientific literature with no restriction on language, and (ii) PhD and MSc theses, or other studies published in non-WOS journals or books. Then, the statistical design of pairwise comparisons (NNTs vs. NV) of the selected studies was checked, and the results were examined for analysis of statistical significance, be

it based on tests, figures with error bars, data tables allowing for calculating, e.g. t-tests, or reporting significance. Because of the great variety of indicators used in different studies for assessing differences in soil properties and diversity attributes, the parameters were aggregated, according to Tables 2, 3. Three other species (*Fraxinus pennsylvanica, Abies grandis* and *Pinus contorta* var. *latifolia*) were excluded from the analysis because of the low numbers of soil properties or taxa groups concerning these species.

In the third phase, we focused on NNTs having >150 comparisons (cases), where a comparison of NNTs vs. NV regarding one soil property or one species group is one case. As a result, *Acer negundo* (n = 21), *P. sitchensis* (n = 23), *Pinus radiata* (n = 2) and *Pinus strobus* (n = 8) were excluded, leaving seven species: *A. dealbata, R. pseudoacacia, Quercus rubra, E. globulus, P. serotina, A. altissima* and *P. menziesii*. For the final seven NNTs selected from the 18 focal ones, a total of 103 scientific publications (Suppl. material 1: table S1, fig. 2) with pairwise comparisons regarding soil properties and diversity attributes were included in the analysis. The number of papers found for the NNTs positively correlates with the area cover, with most studies concerning *R. pseudoacacia* (n = 32), *P. menziesii* (n = 27) and *E. globulus* (n = 22) (Suppl. material 1: fig. S1). For three species the number of publications was too low (*P. sitchensis*, n = 4; *P. radiata*, n = 1; and *P. strobus*, n = 1), even though these species are among the most widely planted NNTs in Europe.

Table 1. Non-native tree species (NNTs) in Europe considered for literature searches (phase 1), the number of European countries where the species is present (Hasenauer et al. 2016; Brus et al. 2019; gbif.org), total area cover (if indicated, otherwise NA), and selection for final comparisons in regard to a sufficient number of pairwise comparisons. Only NNTs that reached the end of phase 3 had a sufficient number of papers on their impacts.

Family	Species	Origin	Presence in European countries		Considered in	
	_	-	Countries #	Area [ha]	study phase	
Broadleaves						
Fabaceae	Acacia dealbata	Australia	5	NA	3	
Fabaceae	Acacia longifolia	Australia	5	NA	1	
Fabaceae	Acacia saligna	Australia	10	NA	1	
Fabaceae	Robinia pseudoacacia	North America	29	2.437.600	3	
Fagaceae	Quercus rubra	North America	24	345.333	3	
Myrtaceae	Eucalyptus camaldulensis	Australia	4	20.000	1	
Myrtaceae	Eucalyptus globulus	Australia	6	1.458.000	3	
Oleaceae	Fraxinus pennsylvanica	North America	10	NA	2	
Rosaceae	Prunus serotina	N or C America	14	NA	3	
Salicaceaae	Populus × canadensis		14	162.274	1	
Sapindaceae	Acer negundo	N or C America	16	4.724	2	
Simaroubaceae	Ailanthus altissima	Asia	18	7.142	3	
Conifers						
Pinaceae	Abies grandis	North America	11	10.459	2	
Pinaceae	Picea sitchensis	North America	13	1.160.400	2	
Pinaceae	Pinus radiata	North America	3	257.000	2	
Pinaceae	Pinus contorta var. latifolia	North America	11	736.000	2	
Pinaceae	Pinus strobus	North America	19	70.382	2	
Pinaceae	Pseudotsuga menziesii	North America	32	830.707	3	



Figure 2. Flowchart of the selection of publications and non-native tree species (NNT). Studies on the effects of NNTs in European forests on soil properties and diversity attributes of different taxonomic groups were considered.

Data analysis

Aggregated soil properties and diversity attributes were counted according to increasing (+1), neutral (0) or decreasing (-1) effects (p < 0.05) for the final seven NNTs. The presence of an NNT was considered to have an increasing or decreasing effect if the average values of an attribute reported for NNT stands/individuals were significantly higher or lower when compared with NV stands/individuals. The terms increasing and decreasing relate to the direction of change rather than any judgement about whether the effect on the ecosystem is beneficial/detrimental. While for diversity attributes, increasing effects translate to an increase of abundance- or diversity related attributes, increasing effects with respect to soil properties can be, for some examples, interpreted as having an adverse effect on an ecosystem. For example, an increase in C:N ratio indicates a reduction of N availability, e.g. reduced soil activity.

Due to the great variety of soil properties and diversity attributes used in the studies, comparable traits were aggregated. Cases of increasing, decreasing and neutral effects were counted and used to display differences among NNTs. The numbers then served for transformations to percentages. As these balances reflect all cases found for

Soil properties,	Soil properties, original	
aggregated		
Ν	N, N floor, N foliar, N litter, N mineral, N soil, N stock, N topsoil, N topsoil stock, N total, N	223
	total floor, N total topsoil, NH_4^+ , NH_4^+ topsoil, NH_4^+ , NO_2^- , NO_3^- , NO_3^- topsoil, NO_3^-/NH_4^+	
pН	pH floor, pH A, pH B, pH H ₂ O, pH-H ₂ O floor, pH H ₂ O topsoil, pH KCl, pH KCL floor, pH	149
	KCL litter, pH KCL topsoil, pH L, pH litter, pH soil, pH topsoil	
C:N	C:N, C:N A, C:N B, C:N floor, C:N foliar, C:N litter, C:N organic, C:N soil, C:N topsoil	93
Ca	Ca+, Ca+ floor, Ca+ litter, Ca+ soil, Ca+ topsoil, Ca ₂ +, Ca ₂ + exchangeable	70
К	K, K available, K floor, K topsoil, K topsoil available, K total floor, K soil, K total topsoil, K+,	67
	K+ floor, K ₂ O	
Mg	Mg, Mg floor, Mg soil, Mg total floor, Mg total topsoil, Mg ₂ +, Mg ₂ + floor, MgO	60
Р	P, P available, P available topsoil, P exchangeable, P foliar, P total, P total floor, P total topsoil, P	60
	total, P_2O_2 , P_2O_3 , P_2O_4 , P_2O_5 , P_2O_6 , P_2O_7 , PO_4	
CEC	Cation exchange capacity: CEC, CEC floor, CEC litter, CEC topsoil	58

Table 2. Most frequently analysed soil properties collected from 103 papers, aggregated and by original description, including number of cases (No); for a complete list of all properties mentioned in the references, see Suppl. material 1: table S1.

soil properties and diversity attributes, irrespective of whether these cases refer to similar soil properties or closely related taxonomic groups in a specific reference, possible nested cases may lead to biased results. Therefore, averages of cases per aggregated soil property and diversity attribute were also calculated reference-wise and balances were re-calculated accordingly. For example, Buchholz et al. (2015) compared different insect taxonomic groups regarding abundance, species richness and beta-diversity in *R. pseudoacacia* and *Betula pendula* stands in the city of Berlin, Germany. From 17 cases, four were significantly decreasing (-1), and in 13 cases no significant differences were found (0). For this reference, the average effect on insects was calculated as -0.24 (-4/17). Three other references reported all decreasing effects of *R. pseudoacacia* on insects (1× in Reif et al. 2016, 1× in Hejda et al. 2017, and 2× in Kadlec et al. 2018). Averaging for all cases affecting insects, the total effect of *R. pseudoacacia* was calculated as -0.38 (-8/21) for this diversity attribute. In contrast, if the average effect on insects was calculated separately for the four references (-0.24, -1, -1, -1), and then the average total effect was calculated, then the total effect was -0.81 (-3.24/4).

To summarise our results of the effects of the final seven NNTs on soil properties and diversity attributes, we used a Principal Components Analysis (PCA). Effect scores for each NNT are based on total averages. Only the effects with data available for all NNTs were considered in this analysis. All analyses and graphs were developed using the statistical software R version 4.1.3 (R Development Core Team, 2022) and the packages dplyr, ggplot2, rgdal and raster.

Data availability

The data underpinning the analysis reported in this paper are deposited at https://doi. org/10.16904/envidat.350.

Coarse taxa	Taxa groups mentioned in the references	Biodiversity measures	No
group			
Vascular	Garden natives, geophytes, hemicryptophytes, nemoral plant species, ni-	Abundance, biomass,	720
plants	trophilous species, rare plant species, road natives, shrubs, small herbs, tall	cover, alpha-, beta-,	
	herbs, therophytes, tree regeneration, trees, vascular plants, wood natives	gamma- diversity	
Microorgan-	Ammonification, ammonification rate, acid phosphotase (AP) activity,	Abundance, activity	229
isms	bacteria, beta-glucosidase (BG) activity, decomposition, fungi, enzyme	rates, alpha-diversity	
	activity, glycine aminopeptidase (GAP) activity, geometric mean of enzy-		
	matic activities (GMEA), microbes, mineralisation, mineralisation rate,		
	N mineralisation, nitrification rate, soil species		
Insects	Blattodea; Coleoptera: taxonomic: Carabidae, Staphylinidae, Scolytidae,	Abundance, biomass,	193
	functional: phytophagous, xylophagous, zoophagous, aphidophagous,	alpha-, beta-, gamma-	
	mycetophagous, copro-/sapro-/necrophagous, omniphagous, saproxylic;	diversity	
	Dermaptera; Diptera: Brachycera (all, Syrphidae), Nematocera;		
	Hemiptera: Sternorhyncha – Aphidina, Psyllidae; Auchenorryncha;		
	Heteroptera; Hymenoptera: Formicidae, others; Lepidoptera (all,		
	moths, Heterocera, larvae); Neuroptera; Psocoptera; Raphidioptera;		
	Thysanoptera; holometabolic larvae; other insects or not further		
	distinguished		
Other arthro-	Arachnida: Acari (Acaridida, Actinedida, Gamasina, Oribatidae:	Abundance, biomass,	165
pods	Gymnonota, Macropylina, Poronota), Araneae, Opiliones; Collembola	alpha-, beta-diversity	
	(Entomobryomorpha, Poduromorpha, Symphypleona); Myriapoda:		
	Chilopoda, Diplopoda; Isopoda; Entognatha: Protura Functional		
	arthropod groups: aerial, micro-/macro, mycetophagous, polyphagous,		
	saprophagous, soil-dwelling		
Bryophytes	Bryophytes	Abundance, alpha-,	78
		beta-diversity	
Birds	Bird species	Abundance, alpha-	70
		diversity	
Mammals	Bats, carnivores, mammals	Abundance	24
Lichens	Lichens	Abundance, alpha-	17
		diversity	

Table 3. Most frequent taxa groups (aggregated) from 103 papers, with original taxa groups, diversity attributes, and number of cases (No).

Results

The majority of the selected studies were conducted in Central Europe and the Western Mediterranean region, while studies on NNTs in the British Isles, North and East Europe (e.g. *P. sitchensis* or *A. negundo*) were excluded because of the low numbers of cases (Fig. 3). Among the NNTs, *P. menziesii* (n = 615), *R. pseudoacacia* (n = 391) and *A. dealbata* (n = 360) accumulated most cases, followed by *P. serotina* (n = 315), *Q. rubra* (n = 230), *E. globulus* (n = 207) and *A. altissima* (n = 158).

In general, the different NNTs were compared with the NV that was dominant in each study region (Suppl. material 1: table S3). For *A. dealbata* and *E. globulus*, Mediterranean pine (e.g. *Pinus pinaster*) and oak species (e.g. *Quercus ilex* and *Q. suber*) but also Mediterranean shrubland types were used for the comparisons. *Ailanthus altissima* was mostly studied in floodplains characterised by the presence of poplar (*Populus* spp.) species. For *P. serotina* and *Q. rubra*, both growing mostly in Central Europe, *Pinus*



Figure 3. Geographic distribution of studies with pairwise comparisons between tree species non-native to Europe (NNTs; countries considered for this study in grey) and native vegetation (NV), and number of cases for each of the seven NNTs with in total sufficiently high numbers of cases (>150).

sylvestris, oak species (*Quercus* spp.) and *Fagus sylvatica* were mostly the native references. For *P. menziesii*, the native references were mostly *Picea abies* and *F. sylvatica*, and for *R. pseudoacacia* the native references were mostly pine and oak species.

Soil properties

From 780 soil property comparisons collected for the seven NNTs, the aggregated properties N (n = 223), pH (n = 152), and C:N (n = 93) were the most frequently considered properties in the studies (Fig. 4, Suppl. material 1: table S2). Except for cases regarding *P. menziesii*, the other soil properties received little attention, in particular cation exchange capacity (CEC), Mg, K, P and Ca.

The number of cases per species and per soil property was uneven (Fig. 4). Among the NNTs, the most studied was *P. menziesii* with the highest number of overall soil property case studies (n=364), followed by *A. dealbata* (n=135) and *R. pseudoacacia* (n=108). The lowest number of cases was found for *Q. rubra* (n=32). Of all soil property cases considered, 16.9% were decreasing, 61.5% neutral, and 21.5% increasing. Out of 56 possible combinations of eight aggregated soil properties for each of the seven NNTs, the literature review retrieved information on 49 combinations. Of these, impacts were decreasing in 19 cases (39%), neutral in eight (16%) and increasing in 22 (45%).



Figure 4. Proportion of cases with significant increasing (green), significant decreasing (red) or neutral (grey; non-significant) effects of the seven tree species non-native to Europe (NNTs) on soil properties (aggregations listed in Table 2) found for each NNT in comparison to stands of native vegetation (NV). Numbers of cases are shown next to the NNTs names, below the soil properties and above the bars. Increasing, decreasing and neutral effects were based on statistical significance (p<0.05).

The following clear trends could be observed: *A. dealbata* increased nitrogen and phosphorus and decreased pH in soils. C:N ratio decreased, e.g. soil activity became higher, in stands of *P. serotina* and *R. pseudoacacia*. In many cases 'no changes' was the most common outcome per species and soil property; in particular, this was observed for *A. altissima* for nitrogen and pH, *P. menziesii* for nitrogen, pH, C:N, calcium, potassium, magnesium and CEC, and *R. pseudoacacia* for pH and, to some extent, also for nitrogen.

Diversity attributes

Of all cases considered, the occurrence of NNTs was recorded as having a decreasing effect in 22.4% of cases, a neutral effect in 65.4% of cases, and an increasing effect in 12.1% of cases.

The number of cases per species and per diversity attribute was more even than for soil properties (Fig. 5). The highest numbers of cases were recorded for *R. pseudoacacia* (n=283), *P. serotina* (n=269) and *P. menziesii* (n=251); whereas the lowest numbers of cases were recorded for *E. globulus* (n=156) and *A. altissima* (n=114). The category vascular plants was the most frequently studied taxonomic group (n=720), while several other groups were rarely studied (birds, bryophytes, mammals, lichens). Most decreasing effects were reported for *E. globulus* and – to a considerable extent – also for *A. altissima*. While in a majority of cases, *P. serotina* presented increasing effect balances, *R. pseudoacacia* had one increasing effect out of seven combinations, *P. menziesii* two increasing effects out of five combinations, and *A. dealbata* two increasing effects out of six combinations. In contrast to the reviewed effects on soil properties,



Figure 5. Proportion of cases with increasing (green), decreasing (red) or non-significant (grey) effects of tree species non-native to Europe (NNTs) on diversity attributes (abundance, species richness or diversity) of different taxonomic groups in comparison to native vegetation (NV). Numbers of cases are shown next to the NNTs names, below the diversity attributes and above the bars. Increasing, decreasing and neutral effects were based on statistical significance (p < 0.05).

the consistency of the effects on taxa groups was greater. *Acacia dealbata, A. altissima, E. globulus* and *Q. rubra* had clearly decreasing effect balances on vascular plant species diversity when compared to native counterparts.

Out of 56 possible combinations, the literature review retrieved information on 38. Out of these balances of NNTs occurrences, 25 (65.8%) had a decreasing effect, 9 (23.7%) an increasing effect, and 4 (10.5%) a neutral effect.

Effects of diversity attributes were finally compared between the two approaches of averaging cases (Fig. 6). Averages using only one value for a taxa group per reference (grey bars) corresponded quite well with averages over all diversity attributes, e.g. taxa groups per NNT (black bars). Only in a few cases, such as vascular plants and bryophytes on *P. serotina* and lichens on *R. pseudoacacia*, did the use of subordinate groups contrast with the averages per reference.

Consistently available soil properties and diversity attributes were used to analyse the different effects of NNTs by Principal Components Analysis (PCA). While cases for all NNTs were available for the soil variables N, P, C:N ratio and pH, three taxa groups (insects, other arthropods and vascular plants) served for comparisons of all NNTs (Suppl. material 1: fig. S2). In the soil biplot, *E. globulus* and *Q. rubra* tended to increase C:N ratio, e.g. decreased soil activity, and increased pH in the case of *Q. rubra*. In the opposite direction, *A. dealbata*, *R. pseudoacacia* and *P. serotina* corresponded to increased N- and P-contents as well as decreased C:N ratio, indicating increased soil activity. Equally, the presence of NNTs (except for *Q. rubra* and *A. altissima*) tended to decrease pH. While *P. menziesii* seemed to slightly decrease soil activity (e.g. increase C:N) and slightly deplete N and P, *A. altissima* corresponded to an increased P content in soils.



Figure 6. Averaged effects (increasing=1, decreasing= -1, none=0) of tree species non-native to Europe (NNTs) on the most frequently mentioned taxonomic groups. Grey bars indicate averaged effects using all cases (e.g. subordinate groups) found in the references; black bars indicate average values of one value for each reference and taxonomic group (e.g. subordinate groups are averaged per reference).

In contrast to the soil biplot, the biodiversity biplot resulted in complex patterns of taxa groups and NNTs that are mainly driven by the strongest signals of diversity × species interactions and distorting weaker signals (Suppl. material 1: fig. S2). While *A. altissima* and *A. dealbata* clearly decreased vascular plant diversity, most NNTs decreased both insect and arthropod diversity.

Discussion

Most studied NNTs and most studied impacts

The number of comparisons between tree species non-native to Europe (NNTs) and native vegetation (NV) are an indicator of the effort that has been invested by researchers in the study of different impacts of these NNTs on native ecosystems. This effort may give us information on the importance of each combination of species impact for the scientific community (e.g. Pyšek et al. 2020). Our analyses demonstrate that the most abundant studies found on pairwise comparisons between NNTs and NV matched the widespread NNTs P. menziesii, R. pseudoacacia, E. globulus and Q. rubra, but also three NNTs (A. altissima, A. dealbata and P. serotina) with comparatively small area cover. In contrast, although the conifers P. sitchensis, P. strobus and P. contorta var. latifolia have been quite widely planted, there were too few studies with pairwise comparisons to be considered in our analyses. Most likely, the reason for the disparity between the area occupied and the number of studies (or comparisons) is the invasive status of A. altissima, A. dealbata and P. serotina in several countries, the spread of these species and their impact on native ecosystems during the last decades. Many papers dealing with these species mention their invasiveness in the respective introduction sections, serving as a justification for the study. On the other hand, for the four most studied species (P. menziesii, R. pseudoacacia, E. globulus and Q. rubra) there is no

apparent relationship between the area occupied by each species and the number of cases. For example, *P. menziesii*, which is an economically important species in terms of timber production particularly in France and Germany, is by far the most studied species in our database with 615 cases but it occupies only one third of the area of *R. pseudoacacia* (Brus et al. 2019), with 391 cases.

According to our database, the number of comparisons between NNTs and NV was higher for diversity in taxonomic groups than for soil properties. There may be various reasons for this. Researchers can assess a large number of taxa groups in the same study, sometimes using the same plot, as is the case for plant studies. On the other hand, there is a much larger number of taxa to be studied than soil properties. Within the universe of different soil properties, soil nitrogen, pH and C:N, were the most studied, probably because of their ecophysiological relevance for plants and ecosystems, but also because their assessment is relatively easy affordable. As for taxonomic groups, the variation in the abundance of vascular plants was more studied than the variation of all other groups. Methodological reasons, including high costs for sorting and identifying species-rich groups such as insects can explain this imbalance. In contrast, mammals and lichens were the least studied groups of our selection, with the lowest number of cases and the lowest number of NNTs. The difficulties associated with mammal censuses at the scale most NNTs were planted is probably the main reason for the dearth of studies. As for lichens, only a few available studies point to an underrepresentation in such comparisons of NNTs and NV, a phenomenon that may produce bias in the interpretation of NNT impacts (Hulme et al. 2013).

Impacts of NNTs on soil properties

Our results show inconsistent impacts on soil properties. Most studies show no significant effects on soil properties, indicating that in many conditions, other intrinsic local factors, namely parent bedrock, soil type or topography may be more important than the tree species. However, some soil impacts seem to be strongly related to particular tree species. This is the case of nitrogen, which is increased by the two Fabaceae species (*A. dealbata* and *R. pseudoacacia*). This is in line with the findings by Castro-Díez et al. (2019) who found a strong phylogenetic signal in the effect of NNT on soil fertility mostly because of N-fixing species. However, soil fertility comes at the cost of soil acidification as a direct or indirect consequence of nitrogen fixation (Tang et al. 1999).

We would expect fast-growing species, such as *E. globulus*, to produce an increasing effect on nitrogen content due to increased productivity, which could contribute to increase the organic matter by stronger root growth and increased litter input (Evans 2009). However, this was not the case in the studies assessed here. In fact, the short-rotation silviculture (Tomé et al. 2021), and the slow litter decomposition (e.g. Pozo et al. 1998) in *E. globulus* stands, probably leads to lower soil nitrogen content and a concomitant increase in C:N (Castro-Díez et al. 2012; Mallen-Cooper et al. 2022).

Impacts of NNT on biodiversity attributes

The different taxonomic groups were, in a majority of cases negatively impacted by the studied NNTs when compared with the status of local NV. However, there are remarkable exceptions among the eight taxonomic groups examined and among the seven NNTs. With respect to microorganisms, for instance, there were two times more studies showing an increasing rather than a decreasing biodiversity. Most of these studies refer to *A. dealbata*. The results for this NNT may be linked to the results found for soil. The higher nutrient concentration found in most comparisons translates into a higher microbial activity, as found for example by Souza-Alonso et al. (2014). Bryophytes were also increasingly impacted, particularly by *P. menziesii*. Apparently, in this case the moist environment provided by closed and dense canopies of *P. menziesii* stands are likely to cause an increase of bryophytes, while the less shade-tolerant vascular plants declined (Finch and Szumelda 2007). Furthermore, several NNTs had no effect on biodiversity attributes of different taxonomic groups, e.g. regarding vascular plants under *R. pseudoacacia* (Sitzia et al. 2012; Vítková et al. 2017) and *P. serotina* (Chabrerie et al. 2010).

As for vascular plants, the most studied taxonomic group, different reasons may explain the increasing or decreasing biodiversity responses to NNTs, found in our review. A. dealbata, A. altissima and E. globulus were associated with marked detrimental impacts on plant diversity and abundance. In the case of A. dealbata, the reasons for the decrease have been related to light competition (Lorenzo et al. 2012), allelopathic effects (Lorenzo and Rodríguez-Echeverría, 2012), and changes in soil nutrients and microbial composition (Rodríguez-Echeverría et al. 2013). Similar reasons were referred to the decreasing plant diversity in stands of A. altissima, namely direct competition and allelopathic effects (Motard et al. 2015). The reasons behind the decreasing biodiversity response to *E. globulus*, may be related to intensive management practices. *Eucalyptus* globulus stands are usually coppiced every 10-12 years along three rotations, and the understory is often removed (Tomé et al. 2021). These frequent disturbances may contribute to the decrease of plant diversity (Lomba et al. 2011; Vaz et al. 2019). Other authors, however, point to intrinsic characteristics of *E. globulus* that may be associated to the impact on plant diversity, including the amount of light that reaches the soil, preventing the establishment of shade-tolerant species or the lack of seed-dispersing birds (Calviño-Cancela 2013). Allelopathy, which inhibits germination and root growth of understory plants, has been referred to by many authors (e.g. Souto et al. 2001; Becerra et al. 2018), while a more recent study did not find evidence of allelopathic effects of E. globulus on Californian native species (Nelson et al. 2021). This latter work suggests that other mechanisms, such as changes in osmotic potential and water or light acquisition, may better explain the suppression of understory in *E. globulus* plantations. As for *P. menziesii*, the reasons for lowered plant diversity and abundance are normally related with the dense cover of plantations before thinning, strongly shading the ground (Augusto et al. 2002; Finch and Szumelda 2007). In contrast, according to Budde (2006) and Podrázský et al. (2014), species richness and abundance can be increased in comparison to native stands of *P. sylvestris, P. abies, F. sylvatica* and *Quercus* spp., which obviously strongly depends on management intervention applied to *P. menziesii* stands aimed to support continuous growth and equally increase light transmission. Comparisons of *R. pseudoacacia* stands with NV resulted mostly in non-significant effects, i.e. vascular plant diversity did not differ in the paired stands. There were, however, more cases with increasing than decreasing responses of biodiversity, e.g. Buchholz et al. (2015). Examples with reduced biodiversity highlight the dominance of nitrophilous species in *R. pseudoacacia* stands in contrast to herbaceous vegetation in native forests (Benesperi et al. 2012), or the lack of native plant species adapted to low pH and nitrogen levels in favour of exotic and ubiquituous species in *R. pseudoacacia* vs. NV may explain why this NNT does not seem to have a general cascade effect on vascular plant diversity.

The higher numbers of increasing vs. decreasing biodiversity responses to *P. serotina* are surprising and reflect the context of the studies considered in the analyses. For *P. serotina*, many increasing cases originate from two studies by Dyderski and Jagodziński (2021a, 2021b), in which several diversity attributes are listed. Most of them refer to *P. serotina* growing in *Pinus sylvestris* stands or plantations and were compared mostly with dense and species-poor *Fagus sylvatica* stands. In contrast, the paper-balanced score in Fig. 6 (in black; only one average value per taxonomic group per paper), results in a distinct decreasing response in vascular plant diversity. This corresponds with many studies that report a reduction in light levels caused by the presence of *P. serotina* (e.g. Starfinger et al. 2003; Chabrerie et al. 2010; Dyderski and Jagodziński 2019).

In summary, it is challenging to disentangle the different factors responsible for a certain impact and to ascertain which factors are more important when it comes to cultivated NNTs (Augusto et al. 2002; Tomé et al. 2021). Similarly to E. globulus, plantations of other NNTs are often intensively managed in relatively short rotations to maximise timber production, which results in specific disturbance and light availability regimes across the cultivation cycle (Augusto et al. 2002). These disturbance regimes may include pruning, thinning, understory removal and a clear cut at the end of a rotation. Therefore, cultivation history needs to be considered to better understand the long-term impacts of NNTs on biodiversity (e.g. Carneiro et al. 2008). However, the control for management influence, allowing its separation from the intrinsic species characteristics, is not included in most studies that compared NNTs and NV. The discussion on this topic becomes even more problematic with cultivated NNTs that spread spontaneously and mix with both native and non-native species, such as in the case for E. globulus in the Iberian Peninsula (Tomé et al. 2021) and R. pseudoacacia in Central Europe (Vítková et al. 2017). More sound conclusions can be drawn from those species that are currently not cultivated and which are considered noxious weeds by national legislations, such as A. altissima or A. dealbata (Pötzelsberger et al. 2020b). Given their invasive behaviour, there is a strong probability that most stands have been naturally-regenerated and that no management operations have influenced the impacts they cause.

According to our results, the NNTs that cause the strongest impact on biodiversity are those that are phylogenetically distant from European plant species. This is in line with other studies showing the importance of congeneric plant species in the establishment and survival of other living organisms that are part of the ecosystem (Harvey et al. 2012; Spafford et al. 2013). A. dealbata, E. globulus and A. altissima are associated with the lowest biodiversity in comparison to NV. While, as previously stated, the role of management should be taken into account in the case of *E. globulus*, this role is not a significant factor in *A. dealbata* and *A. altissima*. Therefore, one possible explanation is the lack of eco-evolutionary history with local native species, making it more difficult to establish ecological interactions, such as pollination, herbivory, seed dispersal and entire food webs, in particular when the planted area is small and thus interactions with NV are rare. Nonetheless, there are cases of strong ecological integration of NNTs, such as the case reported for the pollination of *E. globulus* in Galicia, Spain (Calviño-Cancela and Neumann 2015). However, this might occur at the expense of reproductive success and maintenance of native plant populations (Arceo-Gómez and Ashman 2016). The development of these NNT-based novel ecosystems should therefore be taken into consideration when assessing and analysing the impact of NNTs on native species (Hobbs et al. 2006). Besides intrinsic factors of NNTs related to the phylogenetic relatedness (e.g. secondary plant compounds) also structural properties (bark, canopy architecture) as well as co-introductions of associated species can be important in some cases as shown for *P. menziesii* (Gossner et al. 2005; Gossner and Ammer 2006; Gossner 2016).

Conclusions

Our review provides an overview of current knowledge of the effects of NNTs on selected soil properties and diversity attributes and thus a general basis for the discussion on planting and favouring of NNTs in Europe in the face of global change. It shows that despite its relevance, information on the ecological impacts of NNTs is still limited for most species. Our results for seven NNTs with sufficient data suggest that overall impacts on soil properties are low, and in some cases NNTs may even increase soil fertility. However, nutrient enrichment that facilitates the spreading of ruderal or expansive species needs to be carefully assessed, especially in naturally nutrient-poor environments that are particularly important for biodiversity conservation. Significant negative impacts on biodiversity—in particular on vascular plants, insects, and other ar-thropods—are observed more frequently and suggest a cautious use of NNTs, especially for species that have no close relatives in Europe. In addition to these general trends, our results suggest a strong context-dependency of impacts, especially with respect to focal taxa mainly occurring in different regions and structural properties of the managed stands.

Acknowledgements

This article is based upon work from COST Action FP1403 (NNEXT) 'Non-native tree species for European forests – experiences, risks and opportunities' supported by COST (European Cooperation in Science and Technology) (www.cost.eu). We thank Daniel Scherrer for support in producing Fig. 3.

References

- Arceo-Gómez G, Ashman TL (2016) Invasion status and phylogenetic relatedness predict cost of heterospecific pollen receipt: Implications for native biodiversity decline. Journal of Ecology 104(4): 1003–1008. https://doi.org/10.1111/1365-2745.12586
- Augusto L, Ranger J, Binkley D, Rothe A (2002) Impact of several common tree species of European temperate forests on soil fertility. Annals of Forest Science 59(3): 233–253. https://doi.org/10.1051/forest:2002020
- Badalamenti E, Cusimano D, La Mantia T, Pasta S, Romano S, Troia A, Ilardi V (2018) The ongoing naturalisation of *Eucalyptus* spp. in the Mediterranean Basin: New threats to native species and habitats. Australian Forestry 81(4): 239–249. https://doi.org/10.1080/00 049158.2018.1533512
- Becerra PI, Catford JA, Inderjit Luce McLeod M, Andonian K, Aschehoug ET, Montesinos D, Callaway RM (2018) Inhibitory effects of *Eucalyptus globulus* on understorey plant growth and species richness are greater in non-native regions. Global Ecology and Biogeography 27(1): 68–76. https://doi.org/10.1111/geb.12676
- Benesperi R, Giuliani C, Zanetti S, Gennai M, Lippi MM, Guidi T, Nascimbene J, Foggi B (2012) Forest plant diversity is threatened by *Robinia pseudoacacia* (black-locust) invasion. Biodiversity and Conservation 21(14): 3555–3568. https://doi.org/10.1007/s10531-012-0380-5
- Brundu G, Richardson DM (2016) Planted forests and invasive alien trees in Europe: A Code for managing existing and future plantings to mitigate the risk of negative impacts from invasions. NeoBiota 30: 5–47. https://doi.org/10.3897/neobiota.30.7015
- Brundu G, Pauchard A, Pyšek P, Pergl J, Bindewald AM, Brunori A, Canavan S, Campagnaro T, Celesti-Grapow L, Dechoum MDS, Dufour-Dror J-M, Essl F, Flory SL, Genovesi P, Guarino F, Guangzhe L, Hulme PE, Jäger H, Kettle CJ, Krumm F, Langdon B, Lapin K, Lozano V, Le Roux JJ, Novoa A, Nuñez MA, Porté AJ, Silva JS, Schaffner U, Sitzia T, Tanner R, Tshidada N, Vítková M, Westergren M, Wilson JRU, Richardson DM (2020) Global guidelines for the sustainable use of non-native trees to prevent tree invasions and mitigate their negative impacts. NeoBiota 61: 65–116. https://doi.org/10.3897/neobiota.61.58380
- Brus R, Pötzelsberger E, Lapin K, Brundu G, Orazio C, Straigyte L, Hasenauer H (2019) Extent, distribution and origin of non-native forest tree species in Europe. Scandinavian Journal of Forest Research 34(7): 533–544. https://doi.org/10.1080/02827581.2019.1676464
- Buchholz S, Tietze H, Kowarik I, Schirmel J (2015) Effects of a major tree invader on urban woodland arthropods. PLoS ONE 10(9): e0137723. https://doi.org/10.1371/journal. pone.0137723

- Budde S (2006) Auswirkungen des Douglasienanbaus auf die Bodenvegetation im nordwestdeutschen Tiefland. Cuvillier Verlag, Göttingen, 111 pp.
- Calviño-Cancela M (2013) Effectiveness of eucalypt plantations as a surrogate habitat for birds. Forest Ecology and Management 310: 692–699. https://doi.org/10.1016/j.foreco.2013.09.014
- Calviño-Cancela M, Neumann M (2015) Ecological integration of eucalypts in Europe: Interactions with flower-visiting birds. Forest Ecology and Management 358: 174–179. https://doi.org/10.1016/j.foreco.2015.09.011
- Campagnaro T, Brundu G, Sitzia T (2018) Five major invasive alien tree species in European Union forest habitat types of the Alpine and Continental biogeographical regions. Journal for Nature Conservation 43: 227–238. https://doi.org/10.1016/j.jnc.2017.07.007
- Carneiro M, Fabião A, Martins MC, Fabião A, Abrantes Da Silva M, Hilário L, Lousá M, Madeira M (2008) Effects of harrowing and fertilisation on understory vegetation and timber production of a *Eucalyptus globulus* Labill. plantation in Central Portugal. Forest Ecology and Management 255(3–4): 591–597. https://doi.org/10.1016/j.foreco.2007.09.028
- Castro-Díez P, González-Muñoz N, Alonso A, Gallardo A, Poorter L (2009) Effects of exotic invasive trees on nitrogen cycling: A case study in Central Spain. Biological Invasions 11(8): 1973–1986. https://doi.org/10.1007/s10530-008-9374-3
- Castro-Díez P, Fierro-Brunnenmeister N, Gonzalez-Munoz N, Gallardo A (2012) Effects of exotic and native tree leaf litter on soil properties of two contrasting sites in the Iberian Peninsula. Plant and Soil 350(1–2): 179–191. https://doi.org/10.1007/s11104-011-0893-9
- Castro-Díez P, Vaz AS, Silva JS, van Loo M, Alonso Á, Aponte C, Bayón Á, Bellingham PJ, Chiuffo MC, DiManno N, Julian K, Kandert S, La Porta N, Marchante H, Maule HG, Mayfield MM, Metcalfe D, Monteverdi MC, Núñez MA, Ostertag R, Parker IM, Peltzer DA, Potgieter LJ, Raymundo M, Rayome D, Reisman-Berman O, Richardson DM, Roos RE, Saldaña A, Shackleton RT, Torres A, Trudgen M, Urban J, Vicente JR, Vilà M, Ylioja T, Zenni RD, Godoy O (2019) Global effects of non-native tree species on multiple ecosystem services. Biological Reviews of the Cambridge Philosophical Society 94(4): 1477–1501. https://doi.org/10.1111/brv.12511
- Castro-Díez P, Alonso Á, Saldaña-López A, Granda E (2021) Effects of widespread non-native trees on regulating ecosystem services. The Science of the Total Environment 778: 146141. https://doi.org/10.1016/j.scitotenv.2021.146141
- Chabrerie O, Hoeblich H, Decocq G (2007) Determinism and ecological consequences of the invasive dynamics of late cherry (*Prunus serotina* Ehrh.) on plant communities in Compiègne forest. Acta Botanica Gallica 154: 383–394. https://doi.org/10.1080/12538078.2007.10516071
- Chabrerie O, Loinard J, Perrin S, Saguez R, Decocq G (2010) Impact of *Prunus serotina* invasion on understory functional diversity in a European temperate forest. Biological Invasions 12(6): 1891–1907. https://doi.org/10.1007/s10530-009-9599-9
- Cremer M, Prietzel J (2017) Soil acidity and exchangeable base cation stocks under pure and mixed stands of European beech, Douglas fir and Norway spruce. Plant and Soil 415(1–2): 393–405. https://doi.org/10.1007/s11104-017-3177-1
- Dodet M, Collet C (2012) When should exotic forest plantation tree species be considered as an invasive threat and how should we treat them? Biological Invasions 14(9): 1765–1778. https://doi.org/10.1007/s10530-012-0202-4

- Dutta RK, Agrawal M (2003) Restoration of opencast coal mine spoil by planting exotic tree species: A case study in dry tropical region. Ecological Engineering 21(2–3): 143–151. https://doi.org/10.1016/j.ecoleng.2003.10.002
- Dyderski MK, Jagodziński AM (2019) Context-dependence of urban forest vegetation invasion level and alien species' ecological success. Forests 10(1): 26. https://doi.org/10.3390/ f10010026
- Dyderski MK, Jagodziński AM (2021a) How do invasive trees impact shrub layer diversity and productivity in temperate forests? Annals of Forest Science 78(1): 20. https://doi. org/10.1007/s13595-021-01033-8
- Dyderski MK, Jagodziński AM (2021b) Impacts of invasive trees on alpha and beta diversity of temperate forest understories. Biological Invasions 23(1): 235–252. https://doi. org/10.1007/s10530-020-02367-6
- Evans J (2009) The multiple roles of planted forests. In: Evans J (Ed.) Planted forests: uses, impacts, and sustainability. CABI, Wallingford, 61–90. https://doi. org/10.1079/9781845935641.0061
- Finch O-D, Szumelda A (2007) Introduction of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) into Western Europe: Epigaeic arthropods in intermediate-aged pure stands in northwestern Germany. Forest Ecology and Management 242(2–3): 260–272. https://doi. org/10.1016/j.foreco.2007.01.039
- Godoy O, Castro-Díez P, Van Logtestijn RSP, Cornelissen JHC, Valladares F (2010) Leaf litter traits of invasive species slow down decomposition compared to Spanish natives: A broad phylogenetic comparison. Oecologia 162(3): 781–790. https://doi.org/10.1007/s00442-009-1512-9
- Gossner MM (2016) Introduced tree species in central Europe consequences for arthropod communities and species interactions. In: Krumm F, Vítková L (Eds) Introduced tree species in European forests: Opportunities and challenges. European Forest Institute, 264–282.
- Gossner M, Ammer U (2006) The effects of Douglas-fir on tree-specific arthropod communities in mixed species stands with European beech and Norway spruce. European Journal of Forest Research 125(3): 221–235. https://doi.org/10.1007/s10342-006-0113-y
- Gossner M, Gruppe A, Simon U (2005) Aphidophagous insect communities in tree crowns of the neophyte Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and Norway spruce (*Picea abies* L.). Journal of Applied Entomology 129(2): 81–88. https://doi.org/10.1111/j.1439-0418.2005.00937.x
- Harvey KJ, Nipperess DA, Britton DR, Hughes L (2012) Australian family ties: Does a lack of relatives help invasive plants escape natural enemies? Biological Invasions 14(11): 2423– 2434. https://doi.org/10.1007/s10530-012-0239-4
- Hasenauer H, Gazda A, Konnert M, Mohren G, Pötzelsberger E, Spiecker H, Van Loo M (Eds) (2016) Non-native tree species for European forests: Experiences, risks and opportunities. University of Natural Resources and Life Sciences, Vienna, 427 pp.
- Hassan R, Scholes R, Ash N (2005) Ecosystems and human well-being: Current state and trends. Island Press, Washington, 917 pp.

- Hejda M, Hanzelka J, Kadlec T, Štrobl M, Pyšek P, Reif J (2017) Impacts of an invasive tree across trophic levels: Species richness, community composition and resident species' traits. Diversity & Distributions 23(9): 997–1007. https://doi.org/10.1111/ddi.12596
- Hobbs RJ, Arico S, Aronson J, Baron JS, Bridgewater P, Cramer VA, Epstein PR, Ewel JJ, Klink CA, Lugo AE, Norton D, Ojima D, Richardson DM, Sanderson EW, Valladares F, Vilà M, Zamora R, Zobel M (2006) Novel ecosystems: Theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography 15(1): 1–7. https://doi.org/10.1111/j.1466-822X.2006.00212.x
- Hulme PE, Pyšek P, Jarošik V, Pergl J, Schaffner U, Vilà M (2013) Bias and error in understanding plant invasion impacts. Trends in Ecology & Evolution 28(4): 212–218. https://doi. org/10.1016/j.tree.2012.10.010
- Huston M, Smith T (1987) Plant succession Life-history and competition. American Naturalist 130(2): 168–198. https://doi.org/10.1086/284704
- Jackson RB, Banner JL, Jobbágy EG, Pockman WT, Wall DH (2002) Ecosystem carbon loss with woody plant invasion of grasslands. Nature 418(6898): 623–626. https://doi. org/10.1038/nature00910
- Kadlec T, Štrobl M, Hanzelka J, Hejda M, Reif J (2018) Differences in the community composition of nocturnal Lepidoptera between native and invaded forests are linked to the habitat structure. Biodiversity and Conservation 27(10): 2661–2680. https://doi.org/10.1007/ s10531-018-1560-8
- Krevš A, Kučinskienė A (2017) Influence of invasive Acer negundo leaf litter on benthic microbial abundance and activity in the littoral zone of a temperate river in Lithuania. Knowledge and Management of Aquatic Ecosystems 418(418): 26. https://doi.org/10.1051/kmae/2017015
- Lomba A, Vicente J, Moreira F, Honrado J (2011) Effects of multiple factors on plant diversity of forest fragments in intensive farmland of Northern Portugal. Forest Ecology and Management 262(12): 2219–2228. https://doi.org/10.1016/j.foreco.2011.08.014
- Lorenzo P, Rodríguez-Echeverría S (2012) Influence of soil microorganisms, allelopathy and soil origin on the establishment of the invasive *Acacia dealbata*. Plant Ecology & Diversity 5(1): 67–73. https://doi.org/10.1080/17550874.2012.713404
- Lorenzo P, Pazos-Malvido E, Rubido-Bará M, Reigosa MJ, González L (2012) Invasion by the leguminous tree Acacia dealbata (Mimosaceae) reduces the native understorey plant species in different communities. Australian Journal of Botany 60(8): 669–675. https://doi. org/10.1071/BT12036
- Mallen-Cooper M, Atkinson J, Xirocostas ZA, Wijas B, Chiarenza GM, Dadzie FA, Eldrige DJ (2022) Global synthesis reveals strong multifaceted effects of eucalypts on soils. Global Ecology and Biogeography 31(8): 1667–1678. https://doi.org/10.1111/geb.13522
- Medina-Villar S, Rodríguez-Echeverría S, Lorenzo P, Alonso A, Pérez-Corona E, Castro-Díez P (2016) Impacts of the alien trees *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L. on soil nutrients and microbial communities. Soil Biology & Biochemistry 96: 65–73. https://doi.org/10.1016/j.soilbio.2016.01.015
- Motard E, Dusz S, Geslin B, Akpa-Vinceslas M, Hignard C, Babiar O, Clair-Maczulajtys D, Michel-Salzat A (2015) How invasion by *Ailanthus altissima* transforms soil and litter

communities in a temperate forest ecosystem. Biological Invasions 17(6): 1817–1832. https://doi.org/10.1007/s10530-014-0838-3

- Nelson KM, Bisbing S, Grossenbacher DL, Ritter M, Yost JM (2021) Testing an invasion mechanism for *Eucalyptus globulus*: Is there evidence of allelopathy? American Journal of Botany 108(4): 607–615. https://doi.org/10.1002/ajb2.1635
- Øyen BH, Nygaard PH (2020) Impact of Sitka spruce on biodiversity in NW Europe with a special focus on Norway–evidence, perceptions and regulations. Scandinavian Journal of Forest Research 35(3–4): 117–133. https://doi.org/10.1080/02827581.2020.1748704
- Piwczyński M, Puchałka R, Ulrich W (2016) Influence of tree plantations on the phylogenetic structure of understorey plant communities. Forest Ecology and Management 376: 231–237. https://doi.org/10.1016/j.foreco.2016.06.011
- Podrázský V, Martiník A, Matějka K, Viewegh J (2014) Effects of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) on understory layer species diversity in managed forests. Journal of Forest Science 60(7): 263–271. https://doi.org/10.17221/49/2014-JFS
- Pötzelsberger E, Spiecker H, Neophytou C, Mohren F, Gazda A, Hasenauer H (2020a) Growing non-native trees in European forests brings benefits and opportunities but also has its risks and limits. Current Forestry Reports 6(4): 339–353. https://doi.org/10.1007/s40725-020-00129-0
- Pötzelsberger E, Lapin K, Brundu G, Adriaens T, Andonovski V, Andrašev S, Bastien J-C, Brus R, Čurović M, Čurović Ž, Cvjetković B, Đodan M, Domingo-Santos JM, Gazda A, Henin J-M, Hernea C, Karlsson B, Keča L, Keren S, Keserű Z, Konstantara T, Kroon J, La Porta N, Lavnyy V, Lazdina D, Lukjanova A, Maaten T, Madsen P, Mandjukovski D, Marín Pageo FJ, Marozas V, Martinik A, Mason WL, Mohren F, Monteverdi MC, Neophytou C, Neville P, Nicolescu V-N, Nygaard PH, Orazio C, Parpan T, Perić S, Petkova K, Popov EB, Power M, Rédei K, Rousi M, Silva JS, Sivacioğlu A, Socratous M, Straigytė L, Urban J, Vandekerkhove K, Wąsik R, Westergren M, Wohlgemuth T, Ylioja T, Hasenauer H (2020b) Mapping the patchy legislative landscape of non-native tree species in Europe. Forestry 93(4): 567–586. https://doi.org/10.1093/forestry/cpaa009
- Pozo J, Basaguren A, Elósegui A, Molinero J, Fabre E, Chauvet E (1998) Afforestation with *Eucalyptus globulus* and leaf litter decomposition in streams of northern Spain. Hydrobiologia 373/374: 101–109. https://doi.org/10.1023/A:1017038701380
- Pyšek P, Jarosik V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vila M (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: The interaction of impact measures, invading species' traits and environment. Global Change Biology 18(5): 1725–1737. https://doi.org/10.1111/j.1365-2486.2011.02636.x
- Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kühn I, Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, van Kleunen M, Vilà M, Wingfield MJ, Richardson DM (2020) Scientists' warning on invasive alien species. Biological Reviews of the Cambridge Philosophical Society 95(6): 1511–1534. https://doi.org/10.1111/brv.12627
- R Development Core Team (2022) R: A language and environment for statistical computing. Vienna, Austria.
- Reif J, Hanzelka J, Kadlec T, Strobl M, Hejda M (2016) Conservation implications of cascading effects among groups of organisms: The alien tree *Robinia pseudoacacia* in the Czech

Republic as a case study. Biological Conservation 198: 50–59. https://doi.org/10.1016/j. biocon.2016.04.003

- Rodríguez-Echeverría S, Afonso C, Correia M, Lorenzo P, Roiloa SR (2013) The effect of soil legacy on competition and invasion by *Acacia dealbata* Link. Plant Ecology 214(9): 1139–1146. https://doi.org/10.1007/s11258-013-0238-2
- Sanz-Elorza M, Dana Sánchez ED, Sobrino Vesperinas E (2004) Atlas de las Plantas Alóctonas Invasoras en España, Madrid.
- Sapsford SJ, Brandt AJ, Davis KT, Peralta G, Dickie IA, Gibson RD II, Green JL, Hulme PE, Nuñez MA, Orwin KH, Pauchard A, Wardle DA, Peltzer DA (2020) Towards a framework for understanding the context dependence of impacts of non-native tree species. Functional Ecology 34(5): 944–955. https://doi.org/10.1111/1365-2435.13544
- Sitzia T, Campagnaro T, Dainese M, Cierjacks A (2012) Plant species diversity in alien black locust stands: A paired comparison with native stands across a north-Mediterranean range expansion. Forest Ecology and Management 285: 85–91. https://doi.org/10.1016/j.foreco.2012.08.016
- Sitzia T, Campagnaro T, Kowarik I, Trentanovi G (2016) Using forest management to control invasive alien species: Helping implement the new European regulation on invasive alien species. Biological Invasions 18(1): 1–7. https://doi.org/10.1007/s10530-015-0999-8
- Souto XC, Bolano JC, Gonzalez L, Reigosa MJ (2001) Allelopathic effects of tree species on some soil microbial populations and herbaceous plants. Biologia Plantarum 44(2): 269– 275. https://doi.org/10.1023/A:1010259627812
- Souza-Alonso P, Novoa A, González L (2014) Soil biochemical alterations and microbial community responses under *Acacia dealbata* Link invasion. Soil Biology & Biochemistry 79: 100–108. https://doi.org/10.1016/j.soilbio.2014.09.008
- Spafford RD, Lortie CJ, Butterfield BJ (2013) A systematic review of arthropod community diversity in association with invasive plants. NeoBiota 16: 81–102. https://doi.org/10.3897/neobiota.16.4190
- Spiecker H, Lindner M, Schuler J (2019) Douglas-fir an option for Europe. What Science Can Tell Us 9, European Forest Institute, Joensuu, Finland, 1–124.
- Starfinger U, Kowarik I, Rode M, Schepker H (2003) From desirable ornamental plant to pest to accepted addition to the flora? The perception of an alien tree species through the centuries. Biological Invasions 5(4): 323–335. https://doi.org/10.1023/ B:BINV.0000005573.14800.07
- Tang C, Unkovich MJ, Bowden JW (1999) Factors affecting soil acidification under legumes. III. Acid production by N₂-fixing legumes as influenced by nitrate supply. The New Phytologist 143(3): 513–521. https://doi.org/10.1046/j.1469-8137.1999.00475.x
- Tomé M, Almeida MH, Barreiro S, Branco MR, Deus E, Pinto G, Silva JS, Soares P, Rodríguez-Soalleiro R (2021) Opportunities and challenges of *Eucalyptus* plantations in Europe: The Iberian Peninsula experience. European Journal of Forest Research 140(3): 489–510. https://doi.org/10.1007/s10342-021-01358-z
- Vaz AS, Honrado JP, Lomba A (2019) Replacement of pine by eucalypt plantations: Effects on the diversity and structure of tree assemblages under land abandonment and implications for landscape management. Landscape and Urban Planning 185: 61–67. https://doi. org/10.1016/j.landurbplan.2019.01.009

- Vítková M, Müllerová J, Sádlo J, Pergl J, Pyšek P (2017) Black locust (*Robinia pseudoacacia*) beloved and despised: A story of an invasive tree in Central Europe. Forest Ecology and Management 384: 287–302. https://doi.org/10.1016/j.foreco.2016.10.057
- Vlachodimos K, Papatheodorou EM, Diamantopoulos J, Monokrousos N (2013) Assessment of *Robinia pseudoacacia* cultivations as a restoration strategy for reclaimed mine spoil heaps. Environmental Monitoring and Assessment 185(8): 6921–6932. https://doi.org/10.1007/ s10661-013-3075-9
- Wagner V, Večeřa M, Jiménez-Alfaro B, Pergl J, Lenoir J, Svenning JC, Pyšek P, Agrillo E, Biurrun I, Campos JA, Ewald J, Fernández-González F, Jandt U, Rašomavičius V, Šilc U, Škvorc Ž, Vassilev K, Wohlgemuth T, Chytrý M (2021) Alien plant invasion hotspots and invasion debt in European woodlands. Journal of Vegetation Science 32(2): e13014. https://doi.org/10.1111/jvs.13014
- Walther GR, Roques A, Hulme PE, Sykes MT, Pyšek P, Kühn I, Zobel M, Bacher S, Botta-Dukát Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarošik V, Kenis M, Klotz S, Minchin D, Moora M, Nentwig W, Ott J, Panov VE, Reineking B, Robinet C, Semenchenko V, Solarz W, Thuiller W, Vilà M, Vohland K, Settele J (2009) Alien species in a warmer world: Risks and opportunities. Trends in Ecology & Evolution 24(12): 686–693. https://doi. org/10.1016/j.tree.2009.06.008
- Wohlgemuth T, Moser B, Pötzelsberger E, Rigling A, Gossner MM (2021) Über die Invasivität der Douglasie und ihre Auswirkungen auf Boden und Biodiversität. Schweizerische Zeitschrift für Forstwesen 172(2): 118–127. https://doi.org/10.3188/szf.2021.0118
- Yelenik S, Stock W, Richardson D (2004) Ecosystem level impacts of invasive Acacia saligna in the South African fynbos. Restoration Ecology 12(1): 44–51. https://doi.org/10.1111/ j.1061-2971.2004.00289.x
- Yuan Y, Zhao Z, Niu S, Li X, Wang Y, Bai Z (2018) Reclamation promotes the succession of the soil and vegetation in opencast coal mine: A case study from *Robinia pseudoacacia* reclaimed forests, Pingshuo mine, China. Catena 165: 72–79. https://doi.org/10.1016/j. catena.2018.01.025
- Zerva A, Ball T, Smith KA, Mencuccini M (2005) Soil carbon dynamics in a Sitka spruce (*Picea sitchensis* (Bong.) Carr.) chronosequence on a peaty gley. Forest Ecology and Management 205(1–3): 227–240. https://doi.org/10.1016/j.foreco.2004.10.035

Supplementary material I

Supplementary information

Authors: Thomas Wohlgemuth, Martin M. Gossner, Thomas Campagnaro, Hélia Marchante, Marcela van Loo, Giorgio Vacchiano, Pilar Castro-Díez, Dorota Dobrowolska, Anna Gazda, Srdjan Keren, Zsolt Keserű, Marcin Koprowski, Nicola La Porta, Vitas Marozas, Per Holm Nygaard, Vilém Podrázský, Radosław Puchałka, Orna Reisman-Berman, Lina Straigytė, Tiina Ylioja, Elisabeth Pötzelsberger, Joaquim S. Silva Data type: tables and figuees (docx. file)

Explanation note: table S1: references and number of comparisons per NNT used from these references: Ps.me=Pseudotsuga menziesii, Ro.ps=Robinia pseudoacacia, dealbata, Pr.se=Prunus serotina, Eu.gl=Eucalyptus globulus, Ac.de=Acacia Qu.ru=Quercus rubra, Ai.al=Ailanthus altissima; table S2: all collected soil traits from 103 papers, aggregated and by original description, including number of cases (No), alphabetically ordered; table S3: non-native tree species (NNTs) and percentage of native trees (NT) or open ecosystems (OS) to which the cases compare; figure S1: area cover of eleven non-native tree species (NNTs; phase 3, see Fig. 2; + indicates that the species are present on a relatively small area, the threshold being set at 500 ha for this analysis) vs. number of papers with pairwise comparisons meeting standards; figure S2: biplots of Principal Components Analysis (PCA) using the mean effect of NNTs on four soil properties (left) and three taxa groups (right). Only those variables with a complete set of values for all NNTs were considered for building the PCA.

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Link: https://doi.org/10.3897/neobiota.78.87022.suppl1

RESEARCH ARTICLE



Small but voracious: invasive generalist consumes more zooplankton in winter than native planktivore

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Academic editor: Jonathan Jeschke | Received 20 May 2022 | Accepted 13 October 2022 | Published 23 November 2022

Citation: Ogorelec Ž, Brinker A, Straile D (2022) Small but voracious: invasive generalist consumes more zooplankton in winter than native planktivore. NeoBiota 78: 71–97. https://doi.org/10.3897/neobiota.78.86788

Abstract

In recent years, Lake Constance has experienced an invasion and domination of three-spined stickleback (*Gasterosteus aculeatus*) in the pelagic zone, which has coincided with a decline in the native whitefish (*Coregonus wartmanni*) population. Similar massive invasions of sticklebacks into pelagic zones have been recognized also in marine areas or small lakes worldwide. However, their diet overlaps with native species is rarely evaluated, especially in the winter season, which often presents a bottleneck for fish survival. In this study, we compared the diet of pelagic sticklebacks with the diet of the substantially larger native whitefish in different seasons, to evaluate the threat of the recent stickleback invasion on whitefish populations. By monthly sampling of zooplankton and both fish species diets, we could demonstrate that sticklebacks select similar prey throughout most of the year and consume more prey than whitefish during the winter. With relations between prey availability and prey selection, interspecific and intraspecific seasonal diet variability and indices like a prey-specific index of relative importance, we discuss the importance of zooplankton species traits and abundance for whitefish and stickleback predation. This study shows that sticklebacks, despite their small size, represent a serious potential diet competitor to native planktivorous fish. Sticklebacks quickly adapt to new environments, and thus we advocate precautions regarding their introduction into similar lakes as Lake Constance, as this could cause irreversible ecological changes.

Keywords

Bythotrephes longimanus, Lake Constance, overwintering strategy, pelagic whitefish diet, planktivory, predator size, seasonal prey selection, stickleback invasion

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Introduction

The spread of invasive species can cause irreversible changes in ecosystems because it often affects many biological organisational levels, from genes to ecosystems (Ellender and Weyl 2014). Native species with an analogous ecological role can be especially affected if the invader is superior in resource utilisation (Dick et al. 2017). Dietary overlap is an important factor that can be used to predict the likelihood of competition when resources are limited (Sale 1974). Furthermore, understanding seasonal changes in the abundance of prey and the selection of prey by predators is fundamental for understanding the interactions between native and invasive species as well as energy transfer within ecosystems (Baxter et al. 2004). Therefore, studying the diets of invaders *in situ* and comparing them with natives is valuable for assessing possible ecological consequences.

Lake Constance is facing a new fish invasion, and besides a pilot study (Bretzel et al. 2021), no study to date has investigated the seasonal diet of the invader - the threespined stickleback (Gasterosteus aculeatus Linnaeus, 1758; henceforth referred to as "stickleback"). Sticklebacks were first reported in the lake in the 1950s (Muckle 1972; Roch et al. 2018); however, for decades, their presence was limited to the littoral zone. Then, in 2012/2013, sticklebacks suddenly invaded the pelagic zone in high numbers (-2300 per ha) (Rösch et al. 2018; Eckmann and Engesser 2019). In September 2014, they represented more than 95% of the number and 25% of the biomass of all pelagic fish (Alexander and Vonlanthen 2016). Except for the Baltic Sea, which has brackish water, sticklebacks rarely appear in the pelagic zone in such abundance (Jakubavičiute et al. 2016). The impact of sticklebacks on freshwater zooplankton, which is by far the most important invertebrate food in the pelagic zone, is rarely investigated and mostly restricted to smaller shallow lakes (Campbell 1991; Sanchez-Gonzales et al. 2001; Jakobsen et al. 2003). Furthermore, the effects of increased predation pressure from sticklebacks on the diet of whitefish (Coregonus wartmanni Bloch, 1784), which was historically and until recently a keystone pelagic fish in Lake Constance (Eckmann and Rösch 1998) has not yet been studied.

Although Lake Constance is among the most studied lakes globally, the diet of whitefish was analysed only sporadically. The first study was done almost 100 years ago during the initial oligotrophic state of the lake (Auerbach et al. 1924; Elster 1944), while during the eutrophic state of the lake only two sampling campaigns of whitefish diet were made (Hartmann 1983; Becker and Eckmann 1992; Eckmann et al. 2002). Afterwards, the lake underwent re-oligotrophication, which decreased the yield of whitefish (Baer et al. 2016). In parallel with the invasion of sticklebacks, whitefish yield additionally declined and whitefish growth was further reduced (Rösch et al. 2018). Probably, these reductions are related to increased competition for food caused by the stickleback invasion; however, functional studies are lacking. Additionally, during the invasion, the zooplankton community underwent significant changes, e.g. a sudden increase in the numbers of a small daphniid, *Daphnia cucullata* (IGKB 2020), whose role in fish diets is unknown.

The final sizes of sticklebacks and whitefish greatly differ (Kottelat and Freyhof 2007). According to Kleiber's law (Kleiber 1947), larger whitefish are expected to
consume more food per individual but less per biomass than smaller sticklebacks. As both fish species also differ in their spawning and juvenile growing seasons (Kottelat and Freyhof 2007), differences in their feeding behaviours and diet preferences might be more pronounced during certain seasons. As body size plays an important role in overwintering feeding strategies in some species (van Deurs et al. 2011), differences between both fish species could result in their predation differences in winter. Except for young and small fish, which are limited by their gape size (Hartmann 1983; Branstrator and Lehman 1996; Makrakis et al. 2008), zooplanktivorous fish generally select larger, more conspicuous, and more abundant prey (Lazzaro 1987; Gliwicz and Pijanowska 1989). Laboratory data show that already 2-cm-long sticklebacks can consume prey in the same size range as larger and older first-year-of-life (0+) whitefish (Ogorelec et al. 2022). However, whitefish and sticklebacks have different sizes, morphologies, and feeding strategies, and thus their predation success on various types of zooplankton may differ in situ. Sticklebacks are small fish and considered feeding generalists, consuming a wide range of littoral and pelagic food (Wootton 1984). By contrast, the pelagic whitefish is a specialised zooplankton feeder with morphological and behavioural adaptations for effective zooplanktivory (Lazzaro 1987) and can selectively pick large quantities of the larger zooplankton species, e.g. Bythotrephes longimanus (Eckmann et al. 2002). Therefore, they might feed differently and more selectively than sticklebacks.

To better understand the diets and feeding relations of whitefish and sticklebacks *in situ*, we conducted a 1-year seasonal diet study, sampling fish using gillnetting and trawling, and assessing their stomach content. We aimed to assess 1) whether sticklebacks consume more zooplankton biomass per body weight than whitefish, 2) diet differences depending on prey availability and season, 3) whether zooplanktivorous whitefish are more selective than generalist sticklebacks, and 4) the implications of the zooplankton consumption of sticklebacks on whitefish.

Methods

Study site

Upper Lake Constance is located in the south of Germany and represents the main basin of Lake Constance. It is a lake with an area of 476 km² and an average depth of 101 m. With increased human population and eutrophication, the concentration of phosphorus peaked at around 90 μ g/L in the late 1970s. Afterwards, building wastewater treatments and the phosphorus ban in detergents started to show effects on phosphorus concentration, which gradually decreased and in the 2000s approached values recorded in the early 1950s (below 10 μ g/L). Nowadays, the lake is oligotrophic, average chlorophyll-a concentrations are around 2–3 μ g/L, diatoms are the dominating algae, and the density of zooplankton is low (dry weight in the upper 20 m = 80 μ g/L) (IGKB 2018). The lake contains around 30 fish species, among which only whitefish,

sticklebacks, perch (*Perca fluviatilis* Linnaeus, 1758), lake trout (*Salmo trutta* Linnaeus, 1758), arctic char (*Salvelinus* cf. *umbla* Linnaeus, 1758), and deepwater char (*Salvelinus profundus* Schillinger, 1901) occupy the pelagic zone in the adult stage of life (Eckmann et al. 2006; Alexander and Vonlanthen 2016).

Sampling

From April 2017 to May 2018, monthly fishing and zooplankton sampling took place in the pelagic zone of Upper Lake Constance. Gillnet fishing for whitefish was performed with 7-m-high net panels of different lengths and mesh sizes (14, 17, 20, 26, 32, 36, 38, and 40 mm) combined into one 420-m-long fleet. The net panels were set up 2 h before sunset and picked up 1.5 h after sunset, resulting in only 3-4 h of fishing, which prevented significant digestion of fish stomach content. As whitefish depth distribution is temperature dependent, mean fishing depth is changing with the season (Thomas et al. 2010). Surface water temperature during the study period in Lake Constance ranged from 5.1 °C in February 2018 to 22.9 °C in August 2017, when the thermocline extended from 10 to 15 m depth (IGKB 2018, 2020). Therefore, the depth at which nets were set ranged from 5-12 m in May to 32-39 m in January to ensure a sufficient number of samples. Caught fish were handled according to the German Animal Protection Law (§ 4) and the ordinance on slaughter and killing of animals (Tierschutzschlachtverordnung § 13). Immediately afterwards, they were put on ice and transported to the laboratory, where they were weighed (to the nearest 0.01 g and 1 g for sticklebacks and whitefish, respectively), measured (to the nearest 1 mm), and sexed. Stomachs (oesophagus to pylorus) were preserved in 70% EtOH for subsequent content analysis. In parallel to whitefish fishing, zooplankton was sampled. A standard Clarke Bumpus sampler with a 16-cm-wide opening and net mesh of 100 μ m was used at different depths (0–10, 10–20, 20–30, 30–40, and 40–60 m), and then all depths were pooled together to obtain an average density for the entire water column (0-60 m). Zooplankton strongly migrates around dusk, and thus sampling was performed twice: immediately after setting up the gillnets and just before picking them up, i.e. before and after sunset (May and July 2017 were without day samples due to issues with the net). The average densities of both (day and night) samplings were used for further analysis.

Sticklebacks were caught by trawling with a 3-m-high and 2-m-wide trawl with a mesh size of 6 mm. The mesh size of the codend was 4 mm. Trawling was conducted after sunset at depths of 0–3, 3–6, 9–12, 15–18, and 21–24 m; the process is described in detail by Gugele et al. (2020). Caught fish were killed with an overdose of clove oil and put into 70% EtOH for stomach (oesophagus to pylorus) content analysis. To ensure comparable data between whitefish and sticklebacks, we aimed to catch both fish species on similar dates. However, due to technical difficulties and weather conditions, sampling dates for both fish species differ by an interval of 0–15 days. For comparing fish diets for each month, the dates July 31, 2017, and November 2, 2017, were denoted as August and October, respectively.

Zooplankton identification

Samples of zooplankton from the lake and stomachs were divided for identification and counted into aliquots of at least 300 individuals using a sedimentation tube with two equal chambers at its bottom. Eight zooplankton taxa were identified: Eudiaptomus gracilis Sars, 1862, Cyclopoida Burmeister, 1834, Bosmina spp. Baird, 1845, Daphnia cucullata Sars, 1862, Daphnia longispina O. F. Müller, 1776, Daphnia galeata Sars, 1864, Leptodora kindtii Focke, 1844, and Bythotrephes longimanus Leydig, 1860. Other taxa, including Diaphanosoma brachyurum Liévin, 1848 and flying insects or benthic invertebrates, represented less than 0.2% of the stomach content and were thus excluded from the analysis. Damaged zooplankton from stomachs was identified from the remaining fragments as described by Stich and Maier (2006). Data regarding zooplankton species-specific average sizes were obtained from routine zooplankton sampling of the Limnological Institute, University of Konstanz, from May 2017 to April 2018 to complete one full year. If size measurements of certain species were missing (B. longimanus and L. kindtii), our measurements from the lake samples were used. In both cases, body measurements were done on animals, fixed with 4% formaldehyde (routine sampling) or with 70% EtOH (our measurements).

Data analysis: consumed zooplankton biomass and predator selectivity

Zooplankton dry weight was calculated from species-specific body length, and the dry weight correlations were obtained from multiple authors to gather information for all zooplankton species (Geller 1989; Hälbich 1997; Michaloudi 2005; Bledzki and Rybak 2016). From the counted zooplankton from each fish stomach and the average dry weight of each zooplankton species, the total consumed zooplankton dry weight was calculated for each fish. Dividing this value with fish wet weight yielded the prey:predator ratio (mg/g). The difference in consumed zooplankton dry weight and the prey:predator ratio was compared with the non-parametric Wilcoxon-Mann-Whitney test for two periods during which both fish were present: May 2017 to September 2017 and October 2017 to January 2018.

Fish selectivity for zooplankton species was expressed as the Chesson Index, which considers not only the percentage of prey in the stomach but also in the environment (Chesson 1978): $\alpha_i = (r_i / p_i) / (\sum_{i=1}^m r_i / p_i)$; where $r_i = \text{proportion of food}$ item i in the stomach, $p_i = \text{proportion of food}$ item i in the environment, m = number of food items in the environment. In our case, m was considered the average number of every zooplankton species per m³ in the entire sampling depth profile (0–60 m) and was calculated from the average values of the first (day) and second (night) samplings. Values that were equal to zero (i.e. no prey species present) were excluded from the Chesson Index calculation. The index values vary between 0 and 1, where $\alpha > 1 / m$ indicates a preference, and $\alpha < 1 / m$ indicates avoidance of prey species by the predator.

Seasonal Bray-Curtis similarities

Non-metric multidimensional scaling (NMDS) ordination plots based on Bray-Curtis similarities was used to identify seasonal changes in the diet of sticklebacks and whitefish. All analyses were conducted in PRIMER (v.7.0.13, PRIM-ER-e, Quest Research Limited, Albany, New Zealand). Stomach content data was fourth-root transformed and averaged for each time point (sampling month), and a Bray-Curtis resemblance matrix was created. Time points with less than five individuals were omitted from the analysis. Subsequently, NMDS was performed with 1000 repeats (Kruskal fit scheme = 1, minimum stress = 0.01; Clarke and Warwick 2001).

In order to compare the seasonal patterns of the diet composition between the two fish species, stomach content data of five individuals were pooled (for each species and time point) and subsequently standardized (by total) to improve comparability between the two species. Pooling of five individuals was done to decrease the number of zero values in each category of prey species (Hourston et al. 2004). An overview of the number of replicates for each time point, season, and species after pooling is available in Suppl. material 1: table S2. The data was then transformed (square-root), and a Bray-Curtis resemblance matrix was created. Next, averages were bootstrapped based on species and season (n = 33 bootstraps per group, minimum rho = 0.99, bootstrap regions = 95%) and plotted in an NMDS orientation plot (1000 repeats, Kruskal fit scheme = 1, minimum stress = 0.01). Analysis of similarities (ANOSIM) was used to test for significant differences between the diets of whitefish and sticklebacks in each season (one-way, 999 permutations; Clarke 1993). If ANOSIM revealed statistically significant differences between sticklebacks and whitefish in a season, a similarity percentages procedure (SIMPER) was used to identify the contribution of individual prey species to the differences between the two fish species (one-way, 70% cut-off; Clarke 1993).

As both adult and first-year-of-life (0+) sticklebacks were sampled in July and September of 2017, permutational multivariate analysis of variance (PERMANO-VA; Anderson 2001) was used to examine whether the stomach content differed between the two age groups. A Bray-Curtis resemblance matrix (Clarke et al. 2006) was created from the dataset (n = 15 adults, n = 22 0+ fish), and PERMANOVA (two-way, 999 permutations, sums of squares: type III (partial), unrestricted permutation of raw data) was conducted, taking into account the factors "age", "time point", and their interaction ("age × time point"). The month of August was excluded from the analysis as only 0+ sticklebacks were caught. In case of statistically significant differences between 0+ and adult sticklebacks, the analysis of seasonal changes in diet and patterns between sticklebacks and whitefish was repeated excluding 0+ sticklebacks. For this purpose, stomach content data of four individuals were pooled (for each fish species and sampling point), standardized (by total), and analysed as described above.

Beta regression and prey-specific index of relative importance

The dependence of the relative abundance in the stomach on \log_{10} transformed relative abundance *in situ* was analysed for each fish and zooplankton species using beta regression, which is an appropriate regression method when dependent variables range between 0 and 1 (Cribari-Neto and Zeileis 2010). To avoid pseudoreplication, beta regression was performed with median relative abundances of each zooplankton species for each fish species sample on each sampling date. As for NMDS, beta regression analysis was performed with and without 0+ sticklebacks (Fig. 5, Suppl. material 1: fig. S2, respectively).

The importance of each prey taxa for the predator diets was expressed with the preyspecific index of relative importance (%PSIRI). It is similar to the commonly used index of relative importance, which uses occurrence, numerical abundance, and biomass of each prey taxon in predator diets, and provides a balance between all three parameters in calculating the index metric (Cortés 1997; Liao et al. 2001). However, %PSIRI is a recommended replacement due to less erroneous behaviour and more balanced treatment of prey quantity measures (Brown et al. 2012): %PSIRI = %FOi * (%PWi + %PNi) / 2; where %FO_i = frequency of occurrence (present/not present in stomachs), %PW_i = prey-specific weight, and %PN_i = prey-specific number in the predator's diet. PWi = Σ %Wi / ni and PNi = Σ %Ni / ni; where %W_i = prey i weight (relative to all prey weight in an individual stomach), %N_i = prey i number (relative to all prey number in an individual stomach), and n_i = number of stomachs containing prey i.

Data were analysed and plotted with statistical software R (R Core Team 2018), using the packages tidyverse (Wickham et al. 2019), lubridate (Grolemund and Wickham 2011), Rmisc (Hope 2013), rstatix (Kassambara 2021), ggpubr (Kassambara 2020), and betareg (Cribari-Neto and Zeileis 2010). Bray-Curtis similarities and all related analyses and plotting were done with PRIMER v.7.0.13 and JMP Pro 15.2.1 (SAS Institute).

Data availability

The raw data are available via Zenodo at https://doi.org/10.5281/zenodo.6523369 (after 1.1.2023).

Results

We analysed the stomach contents of 221 whitefish and 144 sticklebacks obtained from monthly fishing in the pelagic zone of Upper Lake Constance between April 2017 and May 2018. Caught whitefish were 180–461 mm long (42–898 g), and sticklebacks were 20–81 mm long (0.05–7.3 g). From July to September, we identified two size groups (0+ and 1+ and older) of sticklebacks. The group of smaller sticklebacks

(0+) measured 20–30 mm in length in July, increased in size through summer, and merged with the group of older sticklebacks in the very beginning of November. The contribution of this group (0+) to all sampled sticklebacks was 55% in July, 100% in August, and 68% in September (Fig. 1A, Suppl. material 1: table S1). Diets could be compared from May 2017 to January 2018, when both fish species were caught in sufficient quantities, i.e. at least 16 each, except for May and January, when only five sticklebacks were caught (see Suppl. material 1: table S1). Both fish species foraged almost exclusively on pelagic zooplankton. Only 18 and 15 whitefish' and sticklebacks' stomachs, respectively, contained other prey (e.g. Diptera imago, Chironomidae pupae, roe) and from these only 4 and 8 whitefish' and sticklebacks' stomachs, respectively, contained for the littoral or benthic zone (e.g. Chironomidae larvae or *Gammarus* sp.). Only two sticklebacks' stomachs contained larger amounts (n = 11 and n = 74) of this type of food.

Consumed prey biomass

From May to September, whitefish had a significantly higher total dry weight consumption per fish than sticklebacks (whitefish, n = 80; sticklebacks, n = 100, median difference = 12.0 mg, W = 385, p < 0.0001). However, from October to January, sticklebacks surpassed whitefish in zooplankton consumption (whitefish, n = 64; sticklebacks, n = 76, median difference = 1.03 mg, W = 3687, p < 0.0001) (Fig. 1B). In terms of dry weight consumption per weight of fish, sticklebacks consumed more in both mentioned periods (whitefish, n = 80; sticklebacks, n = 100, median difference = 1.02 mg/g, W=6912, p < 0.0001, and whitefish, n = 64; sticklebacks, n = 76, median difference = 0.53 mg/g, W= 4596, p < 0.0001 respectively) (Fig. 1C).

Interspecific and intraspecific seasonal diet variability

The smallest crustacean zooplankton in the lake was *Bosmina* spp., followed by copepods, daphniids and both predatory zooplankton species (Fig. 2A). The lowest dry weight had cyclopoid copepods and the highest had *B. longimanus* (Fig. 2B). Small zooplankton had the highest relative abundance, especially copepods, which dominated throughout most of the season (Fig. 2C). However, when larger prey was available their contribution to the fish diet was low. Zooplankton contribution to the diet of each fish individual differed, resulting in high interspecific and intraspecific variability in whitefish and stickleback diets throughout the year (Fig. 2D, E). Both fish diets followed the trend of absolute abundance of zooplankton species. In May 2017, they consumed mostly *Bosmina* spp., which was by far the most abundant species in this month. In summer, they consumed larger and more diverse prey like *B. longimanus*, *L. kindtii* and *D. longispina*. In winter, when other prey was less abundant, they relied mostly on copepods and *D. longispina* (Fig. 3A). From May 2017 to January 2018, the selection of *B. longimanus* strongly prevailed in both fish (Fig. 3B).



Figure 1. Seasonal changes of whitefish and sticklebacks between 2017 and 2018. Fish mass (empty dots represent sticklebacks that can be identified as a separate, 0+ group; **A**), zooplankton dry weight (DW) consumption (average ± 1 SD; **B**) and zooplankton DW consumption per gram of fish wet weight (WW) (average ± 1 SD; **C**). Note that sticklebacks were caught from May until January and that in (**A**), (**C**) values for April are missing due to missing measurements of whitefish weight. Large SD values in some months are due to small sample numbers, non-feeding, or a mixture of 0+ and 1+ fish in the case of July sticklebacks (see Suppl. material 1: table S1).

The results of NMDS indicate seasonal changes in the diets of sticklebacks and whitefish (Fig 4A, B). The stress values of the NMDS ordination plots were 0.1 and 0.09 for sticklebacks and whitefish, respectively. The seasonal changes in the diets of both fish species were compared with bootstrapped averages (Fig. 4C). The stress value of the NMDS ordination plot was 0.14. ANOSIM revealed statistically significant dif-



Figure 2. Crustacean zooplankton taxa of Lake Constance and their contribution to fish diets. Zooplankton average body size (**A**), dry weight (**B**), seasonal composition (depth: 0-60 m; **C**) and seasonal contribution to diet of individual whitefish (**D**) and sticklebacks (**E**). Empty slots represent missing data (fish not caught or fish with empty stomachs).

ferences between species (R = 0.549, P = 0.001). Comparing diets of sticklebacks and whitefish per season with the posthoc tests revealed statistically significant differences in summer and winter (see Suppl. material 1: table S3). However, when 0+ sticklebacks were excluded from the analysis, statistical differences remained only in winter (see Suppl. material 1: fig. S1). The similarity percentages procedure revealed an average



Zooplankton species

Figure 3. Zooplankton consumption by whitefish and sticklebacks. Seasonal zooplankton consumption (**A**) and Chesson's prey selectivity index from May 2017 to January 2018 (**B**). Lines with black squares represent average zooplankton density (N/m³) in the lake (depth: 0–60 m). The period in which our samples contained both fish species is delimited by dashed vertical lines. Zooplankton is ordered from the smallest to the largest species. Chesson's Index values above and below the red line (a = 1/m) represent preference and avoidance, respectively, for each zooplankton species over the compared period.



Figure 4. Seasonal changes in the number of consumed prey species for sticklebacks and whitefish in Lake Constance. Non-metric multidimensional scaling (NMDS) ordination plots for whitefish (**A**) and stickleback (**B**) data based on Bray-Curtis similarities. An NMDS ordination plot of bootstrapped averages for both species (**C**). Vectors indicate the direction and strength of individual prey species on orientation (Pearson correlation).

dissimilarity between sticklebacks and whitefish of 46.97%. Cyclopoida and *D. long-ispina* contributed most to the dissimilarity, with 20.81% and 18.73%, respectively (see Suppl. material 1: table S4). Likewise we found statistically significant differences in diet composition between the two age groups of sticklebacks (df = 1, pseudo F = 6.5429, p = 0.001), the two time points (df = 1, pseudo F = 9.8253, p = 0.001), and their interactions (df = 1, pseudo F = 4.5848, p = 0.001).

Feeding on large or abundant prey

Fish intensively preyed on large zooplankton species (*D. longispina*, *B. longimanus*, and *L. kindtii*) already at low relative abundances, whereas they consumed smaller zooplankton species only when these species were the most dominant prey (Figs 2, 5).



Figure 5. Relationships between the percentages of zooplankton species in diets versus *in situ* for whitefish and sticklebacks. Small dots represent the diet contributions in individual fish, and large dots represent the median diet contribution at the various sampling dates. The lines show the fits from beta regression based on the median diet contributions.

The consumption of all species, except *E. gracilis*, cyclopoid copepods and *D. galeata*, increased with increasing relative densities of the species *in situ*. *Bosmina* spp. was significantly more consumed by sticklebacks than by whitefish. A significant interaction between fish species and relative zooplankton density was observed for *D. longispina* and *L. kindtii* due to strong increases in diet with increasing relative abundances of these zooplankters for whitefish, but not for sticklebacks (Fig. 5, Suppl. material 1: table S5).

The importance of various prey species in fish diets

Prey-specific indices of relative importance (%PSIRI) (Fig. 6) indicate that *D. galeata* was never important in the fish diets (%PSIRI always < 0.5%). *D. cucullata* was (except in September) more important prey for sticklebacks than for whitefish, whereas *Bosmina* spp. was very important for both fish species in May (%PSIRI > 58%), and for sticklebacks also during summer (%PSIRI > 21% until the August). Cyclopoid copepods had the highest %PSIRI values for both fish species in November and



Figure 6. The seasonal prey-specific index of relative importance (%PSIRI) for each zooplankton species in whitefish and sticklebacks. The period in which our samples contained both fish species is delimited by dashed vertical lines. Zooplankton species are ordered from the smallest to the largest species.

December (%PSIRI between 17% and 77%), whereas *E. gracilis* had the highest %PSIRI values for whitefish in December (%PSIRI = 38%) and for sticklebacks in September (%PSIRI = 31%). Among all zooplankton species, *D. longispina* had the most persistent %PSIRI values for all seasons (%PSIRI always > 1%) and was especially important prey for whitefish in winter (%PSIRI up to 95%). *L. kindtii* had high %PSIRI values only for whitefish in some summer and autumn months (%PSIRI up to 57%). The most important prey for both fish species from May 2017 to January 2018 was *B. longimanus* (with an average %PSIRI values in this period of 27% for whitefish and 23% for sticklebacks). Throughout this period, average %PSIRI values above 10% were observed for *D. longispina* for both fish species, *Bosmina* spp. and Cyclopoida for sticklebacks, and *L. kindtii* for whitefish. The importance of *D. longispina* strongly

increased (%PSIRI = 40%) when the entire year was considered (May 2017 to April 2018; data available only for whitefish) instead of the compared period (see Suppl. material 1: table S6).

During the compared period (May 2017 to January 2018), both large predatory species (*B. longimanus* and *L. kindtii*), *Bosmina* spp., and Cyclopoida appeared in approximately equal numbers and occurrences in the whitefish diet, whereas *D. longispina* dominated in numeric contribution and *B. longimanus* in biomass contribution (see Suppl. material 1: fig. S3A). In the stickleback diet, *B. longimanus* contributed the most in biomass, whereas Cyclopoida contributed the most in number (see Suppl. material 1: fig. S3B).

Discussion

Invasive species often present a threat to native species because of competition for the same food resources. This study demonstrates that invasive sticklebacks, which weigh 100-fold less than native whitefish, had a higher food consumption per body weight and even consumed more food per individual fish in some autumn and winter months. Despite many morphological, behavioural, and size differences between the two fish species, the number of consumed prey species overlapped during most of the year and differed only in winter; in summer, their diets differed only when 0+ sticklebacks were included in the analysis. Moreover, similar zooplankton species were of high importance for both fish species, with rare, large, and conspicuous *B. longimanus* being the most preferred and important prey. This could lead to food competition, especially for highly selected prey items during periods of limited resources.

Consumed prey biomass

As assumed according to Kleiber's law, sticklebacks had higher consumption per body weight than whitefish. Surprisingly, in late autumn and winter, sticklebacks consumed even more zooplankton per individual. To the best of our knowledge, our study is the first one to demonstrate that in the winter season, small fish consumed more food than the large cold-water fish species. During this time, zooplankton density generally dropped, large zooplankton species, e.g. L. kindtii and B. longimanus, disappeared and adult whitefish consumed less prey. With lower temperatures, body metabolism drops, and many fish species reduce their feeding activities (Johnston and Dunn 1987). During longer periods of hibernation or low food intake, larger and fatter organisms have an advantage over smaller organisms because of their higher ratio between reserve size and basal metabolism (Ultsch 1989; van Deurs et al. 2011). However, regardless of size, fish mortality is lower if they acquire food during winter (Thompson et al. 1991; Heermann et al. 2009; Geissinger et al. 2021), especially for cold-water fish species, which are adapted to be active at low temperatures (Sullivan 1986). Whitefish are coldwater fish species (Kottelat and Freyhof 2007) that also actively feed during winter if there is enough food (Hayden et al. 2022).

Winter anorexia was shown mostly for fish for which the risk of predation is high. In such cases, fish prefer to reduce their activity and hide, unless they risk death from starvation (Farley et al. 2011; van Deurs et al. 2011). In the pelagic zone of Lake Constance, piscivorous fish are rare (Alexander and Vonlanthen 2016). Thus, feed-ing is predicted to be a more successful strategy, provided more energy is gained from prey than is spent to catch prey. As shown in an aquaria study, small fish feed on small zooplankton at higher rates than large fish (Ogorelec et al. 2022). It is unclear whether larger fish have lower catching abilities or ignore small prey due to the relatively smaller energy income per small zooplankter.

Interspecific and intraspecific seasonal diet variability

Besides relatively high amounts of consumed prey in certain months, sticklebacks also consumed similar prey species as whitefish throughout most of the year. Winter was an exception, during which whitefish relied on larger available prey (*D. longispina* and *E. gracilis*) or stopped feeding (see above), whereas sticklebacks continued to consume a large amount of smaller but more abundant cyclopoid copepods. Differences in summer were only observed when the predominant 0+ sticklebacks were included in the analysis: although these sticklebacks also preferred large zooplankton, they consumed a lower proportion of large zooplankton than adults (e.g. from July to September, *B. longimanus* represented 33% and 4% of prey abundance in the diets of adult and 0+ sticklebacks, respectively). Of note, 0+ whitefish were not present in our samples due to their efficient avoidance of gillnetting (Sandlund and Næsje 1989) and trawling. However, they have similar feeding rates as those of 0+ sticklebacks (Ogorelec et al. 2022), which suggests that including 0+ whitefish in the analysis would decrease, rather than increase observed dietary differences between species.

The interspecific differences might have been obscured because of certain methodological and biological issues. i) The intraspecific diet variability was high, which is in line with reports on zooplankton patchiness (Wiebe 1971) and fish specialisation on a few prey species that visually match the fish searching image concept (Lazzaro 1987). The small sample numbers in some months thus might have resulted in poor representations of the entire population. ii) Although fish sampling was planned to occur on as similar dates as possible, dates differed by up to 15 days because of lack of manpower and poor weather conditions. Especially in springtime, zooplankton composition can change within this time frame (Seebens et al. 2013), most likely affecting fish diets. iii) Finally, gillnet depths were selected according to the preferences of harder-to-catch whitefish, whereas trawling for sticklebacks always occurred at the same depths. To account for the high variability within species and between sampling dates and efficiencies, monthly data were grouped into seasons. Assessing the depths of occurrence and their relation to prey selection was beyond the scope of this study, however, as both fish species occupy similar water column depths (Thomas et al. 2010; Gugele et al. 2020), the difference in fish diets between species should not be affected much by depth and temperature.

Feeding on large or abundant prey

Despite their large size differences, both fish species equally favoured large and conspicuous zooplankton, especially *B. longimanus*. This species is among the most preferred prey by whitefish both in Lake Constance (Becker and Eckmann 1992) and in other pre-Alpine and Alpine lakes (Mookerji et al. 1998; Gerdeaux et al. 2002; Müller et al. 2007). Although many authors have reported that small fish avoid *B. longimanus* because of its spine (Barnhisel 1991; Barnhisel and Harvey 1995; Jarnagin et al. 2000), we observed that *B. longimanus* was not only consumed by sticklebacks longer than 25 mm but was also their most preferred prey (this preference further increased with increasing fish size). Although *L. kindtii* had the largest body size among the sampled zooplankton, it was not the most selected, probably due to its transparency and, consequently, low conspicuousness. By contrast, the most selected species, *B. longimanus*, has a large and conspicuous eye, which is important for attracting fish (Lazzaro 1987).

Although large prey was positively selected, it was not the most abundant (especially predatory L. kindtii and B. longimanus) and therefore not necessarily the most consumed. In the spring of 2017, when densities of other zooplankton taxa were much lower, fish consumed high amounts of Bosmina spp., even though it was the smallest crustacean zooplankton species in the lake. In this year, densities of Bosmina spp. were exceptionally high (almost as high as the maximum observed during eutrophic conditions; Straile and Geller 1998), but already next year their numbers were lower in the lake and in the fish diets. Similar results of whitefish preying on *Bosmina* spp. were also found in Lake Lucerne (Mookerji et al. 1998). Other studies also showed that predation on smaller zooplankton is high only when the abundance of larger, and thus preferred, zooplankton is low (Ivlev 1961; Lazzaro 1987). When prey appears at very high densities, the energy and time required for searching for prey are significantly reduced (Holling 1959). The profitability of prey is its energy value subtracted by the predator's energy requirements to find and consume prey per time unit (Sinervo 2007). Thus, the abundance of Bos*mina* spp. might compensate for its presumably low energy value by reducing the energy required for searching. Furthermore, Bosmina spp. is not as evasive as copepods, thus predators have a higher attack efficiency and lower handling time (Lazzaro 1987).

The importance of various prey species in fish diets

Although *B. longimanus* represented less than 0.1% of the number of all zooplankton in the lake, it was the most important prey and contributed the highest biomass to the diets of both fish species from late spring to autumn. It was absent in colder months, and thus its importance in the annual whitefish diet was surpassed by *D. longispina*, which was the largest zooplankton species during winter. Among zooplankton, *Daphnia* is one of the most important and most selected prey items for fish because of its abundance, size, nutritional value, and low evasiveness (Lazzaro 1987). In our study, this high importance was indicated only for larger *D. longispina*, but not for *D. cucullata*, even though the latter has become the most abundant cladoceran since 2016 (IGKB 2020). Likewise in a mesocosm study,

both, whitefish and sticklebacks strongly suppressed *D. longispina*, whereas *D. cucullata* was less affected by fish predation (Ogorelec et al. 2021). In general, *D. cucullata* is less prone to fish predation because of its smaller size and narrower body (Gliwicz 2001). Even though it did not migrate and was present mostly in the epilimnion, which should make it more vulnerable to fish predation compared to migratory *D. longispina*, it contributed more to the whitefish diet only in September, when its density was 25-fold higher than that of *D. longispina*. The third daphniid species in our samples, *D. galeata*, was not present in Lake Constance in pre-eutrophic times (Auerbach et al. 1924); however later, in eutrophic times, it became very abundant in the lake and important in the whitefish diet (Becker and Eckmann 1992). Our study was performed during the oligotrophic state of the lake and revealed a low abundance and importance of *D. galeata* for fish, indicating the reversibility of its role (which became insignificant again) after re-oligotrophication.

Bosmina spp. and cyclopoid copepods were of high importance in fish diets in spring and autumn, respectively. Although they had the lowest mass among crustacean zooplankton (Fig. 2B), their occurrence and numbers were high in fish diets (see Suppl. material 1: fig. S3) when other prey was mostly absent, which resulted in high %PSIRI values for those months. Although larger and also very abundant, *E. gracilis* was not an important prey item, which is probably linked to its evasiveness (Lazzaro 1987). Its low contribution to fish diets was also observed in many other lakes (Mookerji et al. 1998; Mehner et al. 2008). The higher consumption of *L. kindtii* by whitefish than by sticklebacks could be due to different foraging strategies. Whereas sticklebacks use the hover search strategy, whitefish rely more on the swim search strategy (Ogorelec et al. 2022), which enables searching through a larger volume of water and (in the case of sinusoidal swimming) can help detect prey due to changing light conditions that increase the contrasts (and shadows) of inconspicuous prey, e.g. *L. kindtii* (Jarolim et al. 2010).

The effects of sticklebacks on zooplankton and planktivorous fish

Comparing our study with previous findings regarding whitefish in Lake Constance in eutrophic times and without sticklebacks in the pelagic zone (Becker and Eckmann 1992) indicates that the whitefish diet has declined quantitatively (decreased numbers of zooplankton in stomachs) and qualitatively (smaller zooplankton species in stomachs). As whitefish growth is most strongly related to standing stock biomass followed by phosphorus concentrations (Thomas and Eckmann 2007), both a new food competitor and re-oligotrophication can decrease whitefish growth (Deweber et al. 2022). Similar diets do not directly indicate competition; when resources are abundant, diets may overlap to any degree without competition for resources. However, when resources are limited, diet similarity and competition can be directly related (Sale 1974), which is most probably the case in oligotrophic Lake Constance.

An aquarium experimental study demonstrated no differences in the feeding rates between co-occurring sticklebacks and 0+ whitefish, whereas similar-sized sticklebacks had larger feeding rates than those of whitefish (Ogorelec et al. 2022). However, these findings cannot account for the advantage of the swim-search strategy of whitefish when not spatially limited. In the field, at least large whitefish seem to have some advantage, as they can search through larger volumes of water, have a larger stomach capacity, and can thus consume higher numbers of large zooplankton species when they are abundant. However, when food becomes scarcer (in late autumn and winter), the competition presumably increases. Studies from Lake Constance have shown that in the eutrophic and oligotrophic state of the lake, most whitefish fed abundantly also during winter (Auerbach et al. 1924; Becker and Eckmann 1992). However, our current study has shown that now, after the stickleback invasion, many whitefish (up to 50%; see Suppl. material 1: table S1) had empty stomachs during winter.

In contrast to (pre-eutrophic) studies of whitefish (Auerbach et al. 1924) and recent studies of sticklebacks (Roch et al. 2018), our study did not find any fish larvae in the diets of either fish. This suggests that fish larvae might only be important prey for sticklebacks in the littoral zone or on specific occasions, e.g. during whitefish larvae stocking (Roch et al. 2018). Due to rapid evolution, sticklebacks may now diverge into groups occupying littoral, pelagic or profundal habitats (Hudson et al. 2021), albeit, up to now only subtle genetic differences between individuals occupying different habitats were observed (Dahms et al. 2022). Our study showed that the pelagic sticklebacks have already specialised in feeding on zooplankton. All sticklebacks were caught offshore (>100 m water depth), and the proportion of fish that had any littoral/ benthic prey in their stomachs was low (5.5%).

High numbers of sticklebacks (Alexander and Vonlanthen 2016), and their high biomass consumption throughout most of the year are very likely exerting strong effects on the zooplankton community. In contrast to whitefish, whose multiple generations inhabit the pelagic zone throughout the entire year (Eckmann et al. 2007; DeWeber et al. 2021), sticklebacks shift habitat in May, migrating to the littoral zone to spawn (Gugele et al. 2020). This might reduce the predation pressure on pelagic zooplankton in a lake dominated by sticklebacks during this period. However, in summer, adult sticklebacks returned to the pelagic zone together with large numbers of rapidly growing 0+ sticklebacks. Therefore, predation on large zooplankton increased, especially in September when sticklebacks tend to appear in vast densities (Gugele et al. 2020) and, according to our data, consume high amounts of zooplankton. In winter, when zooplankton densities in the lake were low, stickleback consumption remained high and thus presumably strongly affected zooplankton community abundance and structure. Stickleback invasion is thus the most probable reason for recent zooplankton changes, especially the increased proportion of small zooplankton species, e.g. D. cucullata (IGKB 2020).

To date, few reports have investigated sticklebacks invading the pelagic zone and interacting with other pelagic fish or zooplankton. The exception is the Baltic Sea, where numerous studies tried to reveal the causes and consequences of stickleback increase (Olin et al. 2022). One study showed that sticklebacks are potential competitors for herring and sprat due to similar diets and prey selection (Jakubavičiute et al. 2016), whereas others showed that sticklebacks suppress native fish by preying on their larvae (Ljunggren et al. 2010; Byström et al. 2015; Eklöf et al. 2020). This is in accordance with the findings of Roch et al. (2018) and our current study for Lake Constance, which together demonstrate that sticklebacks most likely suppress whitefish populations and force predator

shifts by consuming prey and juveniles of native predators. Due to the rapid colonisation of new areas and the invasiveness of sticklebacks (Fang et al. 2018; Hudson et al. 2021), this small fish could present a large threat to indigenous aquatic species.

Conclusions

This study has contributed to our understanding of the diets of both whitefish and sticklebacks, and has provided insights into the interplay between both small and large as well as native and invasive fish species. It has shown that sticklebacks successfully fed all year round, also in winter, when some whitefish stopped feeding. Owing to their small size, sticklebacks also have lower absolute metabolic demands than whitefish, and thus their energy acquisition in winter is distinctively higher. Further bioenergetics research is needed to evaluate whether larger fish are less successful in capturing small and evasive zooplankton or whether they ignore this prey due to negative profitability. Such information could provide important insights into global invasions of small pelagic fish species. When 0+ sticklebacks and the winter season were excluded, no seasonal differences in the number of consumed prey species were observed. Furthermore, our findings do not indicate that specialised whitefish are more selective predators than sticklebacks. Similar prey preference and importance, especially for conspicuous *B. longimanus* and other large prey, indicate a high probability of interspecific competition between both fish species. The high numbers and effective and persistent feeding of invasive sticklebacks, as indicated in this study, affect not only whitefish populations, but presumably also zooplankton communities. This may explain the appearance and numerical dominance of small and less preferred zooplankton species, e.g. D. cucullata, and the reduced growth and yield of whitefish after the invasion of sticklebacks. As Lake Constance is similar to many other pre-Alpine lakes in this region, potential invasions of pelagic stickleback populations could cause drastic and irreversible changes in the food webs and ecosystem functioning of such lakes.

Acknowledgements

We would like to thank Andreas Revermann for help catching the whitefish in almost all weather conditions, Andreas Revermann and Sarah Maria Gugele for providing stickleback samples, Carsten Wunsch for his unselfish help with sampling, Ingabritta Hormann for her patient help with counting zooplankton, Samuel Roch for preparing the NMDS figures, and Eva Lasič for editing a draft of this manuscript. This work was funded by the Deutsche Forschungsgemeinschaft (German Research Foundation; 298726046/GRK2272; RTG R3), the Bavarian State Ministry of the Environment and Consumer Protection, the Public Scholarship, Development, Disability and Maintenance Fund of the Republic of Slovenia (Ad futura scholarship 11013-8/2021), and the grant "SeeWandel: Life in Lake Constance - the past, present and future" within the framework of the Interreg V programme "Alpenrhein-Bodensee-Hochrhein (Germany/Austria/Switzerland/Liechtenstein)", to which funds are provided by the European Regional Development Fund as well as the Swiss Confederation and cantons. The funders had no role in study design, data collection and analysis, the decision to publish, or preparation of the manuscript.

References

- Alexander TJ, Vonlanthen P (2016) Artenvielfalt und Zusammensetzung der Fischpopulation im Bodensee. Kastanienbaum, 1–68. http://www.ibkf.org/wp-content/uploads/2018/03/ ProjetLac_Bodensee_2014_fin_web.pdf
- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46. https://doi.org/https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x
- Auerbach M, Maerker W, Schmalz J (1924) Hydrographisch-biologische Bodensee-Untersuchungen. I. Ergebnisse der Jahre 1920–1922. Archiv für Hydrobiologie Supplement 3: 597–738.
- Baer J, Eckmann R, Rösch R, Arlinghaus R, Brinker A (2016) Managing Upper Lake Constance Fishery in a multi-sector policy landscape: beneficiary and victim of a century of anthropogenic trophic change. In: Song AM, Bower SD, Onyango P, Cooke SJ, Chuenpagdee R (Eds) Inter-Sectoral Governance of Inland Fisheries. St. John's, Canada, 1–15.
- Barnhisel DR (1991) Zooplankton spine induces aversion in small fish predators. Oecologia 88: 444–450. https://doi.org/10.1007/BF00317591
- Barnhisel DR, Harvey HA (1995) Size-specific fish avoidance of the spined crustacean Bythotrephes: field support for laboratory predictions. Canadian Journal of Fisheries and Aquatic Sciences 52: 768–775. https://doi.org/10.1139/f95-076
- Baxter CV, Fausch KD, Murakami M, Chapman PL (2004) Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. Ecology 85: 2656–2663. https://doi.org/10.1890/04-138
- Becker M, Eckmann R (1992) Plankton selection by pelagic European whitefish in Lake Constance: dependency of season and time of day. Polskie Archiwum Hydrobiologii 39: 393–402.
- Bledzki LA, Rybak JI (2016) Freshwater crustacean zooplankton of Europe. Springer International Publishing Switzerland, 918 pp. https://doi.org/10.1007/978-3-319-29871-9
- Branstrator DK, Lehman JT (1996) Evidence for predation by young-of-the-year alewife and bloater chub on *Bythotrephes cederstroemi* in Lake Michigan. Journal of Great Lakes Research 22: 917–924. https://doi.org/10.1016/S0380-1330(96)71012-2
- Bretzel JB, Geist J, Gugele SM, Baer J, Brinker A (2021) Feeding Ecology of Invasive Three-Spined Stickleback (*Gasterosteus aculeatus*) in Relation to Native Juvenile Eurasian Perch (*Perca fluviatilis*) in the Pelagic Zone of Upper Lake Constance. Frontiers in Environmental Science 9: 1–14. https://doi.org/10.3389/fenvs.2021.670125
- Brown SC, Bizzarro JJ, Cailliet GM, Ebert DA (2012) Breaking with tradition: Redefining measures for diet description with a case study of the Aleutian skate *Bathyraja aleutica* (Gilbert 1896). Environmental Biology of Fishes 95: 3–20. https://doi.org/10.1007/s10641-011-9959-z
- Byström P, Bergström U, Hjälten A, Ståhl S, Jonsson D, Olsson J (2015) Declining coastal piscivore populations in the Baltic Sea: Where and when do sticklebacks matter? Ambio 44: 462–471. https://doi.org/10.1007/s13280-015-0665-5

- Campbell CE (1991) Prey selectivities of threespine sticklebacks (*Gasterosteus aculeatus*) and phantom midge larvae (*Chaoborus* spp.) in Newfoundland lakes. Freshwater Biology 25: 155–167. https://doi.org/10.1111/j.1365-2427.1991.tb00481.x
- Chesson J (1978) Measuring Preference in Selective Predation. Ecology 59: 211–215. https:// doi.org/10.2307/1936364
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117–143. https://doi.org/10.1111/j.1442-9993.1993. tb00438.x
- Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E, Plymouth.
- Clarke KR, Somerfield PJ, Chapman MG (2006) On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray–Curtis coefficient for denuded assemblages. Journal of Experimental Marine Biology and Ecology 330: 55–80. https://doi.org/10.1016/j.jembe.2005.12.017
- Cortés E (1997) A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. Canadian Journal of Fisheries and Aquatic Sciences 54: 726–738. https://doi.org/10.1139/cjfas-54-3-726
- Cribari-Neto F, Zeileis A (2010) Beta Regression in R. Journal of Statistical Software 34: 1–24. https://doi.org/10.18637/jss.v034.i02
- Dahms C, Roch S, Elmer KR, Ros A, Brinker A, Jacobs A (2022) Rapid intralacustrine evolution of an invasive pelagic three-spined stickleback. bioRxiv: 1–47. https://doi. org/10.1101/2022.09.01.506194
- van Deurs M, Hartvig M, Steffensen JF (2011) Critical threshold size for overwintering sandeels (*Ammodytes marinus*). Marine Biology 158: 2755–2764. https://doi.org/10.1007/ s00227-011-1774-8
- Deweber JT, Baer J, Brinker A (2022) Turning summer into winter: nutrient dynamics, temperature, density dependence and invasive species drive bioenergetic processes and growth of a keystone coldwater fish. Oikos, 1–14. https://doi.org/10.1111/oik.09316
- DeWeber JT, Rösch R, Baer J, Brinker A (2021) Long term changes in body condition and gillnet selectivity in Lake Constance pelagic spawning whitefish *Coregonus wartmanni*. Canadian Journal of Fisheries and Aquatic Sciences 78: 1–43. https://doi.org/10.1139/cjfas-2020-0231
- Dick JTA, Laverty C, Lennon JJ, Barrios-O'Neill D, Mensink PJ, Robert Britton J, Médoc V, Boets P, Alexander ME, Taylor NG, Dunn AM, Hatcher MJ, Rosewarne PJ, Crookes S, MacIsaac HJ, Xu M, Ricciardi A, Wasserman RJ, Ellender BR, Weyl OLF, Lucy FE, Banks PB, Dodd JA, MacNeil C, Penk MR, Aldridge DC, Caffrey JM (2017) Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology 54: 1259–1267. https://doi.org/10.1111/1365-2664.12849
- Eckmann R, Rösch R (1998) Lake Constance fisheries and fish ecology. Archiv für Hydrobiologie, Special Issues: Advances in Limnology 53: 285–301. http://nbn-resolving.de/ urn:nbn:de:bsz:352-opus-40075
- Eckmann R, Engesser B (2019) Reconstructing the build-up of a pelagic stickleback (*Gasterosteus aculeatus*) population using hydroacoustics. Fisheries Research 210: 189–192. https://doi.org/10.1016/j.fishres.2018.08.002

- Eckmann R, Becker M, Schmid M (2002) Estimating food consumption by a heavily fished stock of zooplanktivorous *Coregonus lavaretus*. Transactions of the American Fisheries Society 131: 946–955. https://doi.org/10.1577/1548-8659(2002)131%3C0946:EFCBAH %3E2.0.CO;2
- Eckmann R, Gerster S, Kraemer A (2006) Yields of European perch from Upper Lake Constance from 1910 to present. Fisheries Management and Ecology 13: 381–390. https://doi. org/10.1111/j.1365-2400.2006.00516.x
- Eckmann R, Kugler M, Ruhle C (2007) Evaluating the success of large-scale whitefish stocking at Lake Constance. Archiv fur Hydrobiologie, Special Issues Advances in Limnology 60: 361–368. http://nbn-resolving.de/urn:nbn:de:bsz:352-opus-39700
- Eklöf JS, Sundblad G, Erlandsson M, Donadi S, Hansen JP, Eriksson BK, Bergström U (2020) A spatial regime shift from predator to prey dominance in a large coastal ecosystem. Communications Biology 3: 1–9. https://doi.org/10.1038/s42003-020-01180-0
- Ellender BR, Weyl OLF (2014) A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. Aquatic Invasions 9: 117–132. https://doi.org/10.3391/ai.2014.9.2.01
- Elster H-J (1944) Über das Verhältnis von Produktion, Bestand, Befischung und Ertrag sowie über die Möglichkeiten einer Steigerung der Erträge, untersucht am Beispiel der Blaufelchenfischerei des Bodensees. Zeitschrift für Fischerei 42: 169–357.
- Fang B, Merilä J, Ribeiro F, Alexandre CM, Momigliano P (2018) Worldwide phylogeny of three-spined sticklebacks. Molecular Phylogenetics and Evolution 127: 613–625. https:// doi.org/10.1016/j.ympev.2018.06.008
- Farley EV, Starovoytov A, Naydenko S, Heintz R, Trudel M, Guthrie C, Eisner L, Guyon JR (2011) Implications of a warming eastern Bering Sea for Bristol Bay sockeye salmon. ICES Journal of Marine Science 68: 1138–1146. https://doi.org/10.1093/icesjms/fsr021
- Geissinger EA, Gregory RS, Laurel BJ, Snelgrove PVR (2021) Food and initial size influence overwinter survival and condition of a juvenile marine fish (age-0 Atlantic cod). Canadian Journal of Fisheries and Aquatic Sciences 78(4): 472–482. https://doi.org/10.1139/cjfas-2020-0142
- Geller W (1989) The energy budget of two sympatric *Daphnia* species in Lake Constance: productivity and energy residence times. Oecologia 78: 242–250. https://doi.org/10.1007/ BF00377162
- Gerdeaux D, Bergeret S, Fortin J, Baronnet T (2002) Diet and seasonal patterns of food composition of *Coregonus lavaretus* in Lake Annecy: Comparison with the diet of the other species of the fish community. Advances in Limnology 57: 199–207.
- Gliwicz ZM (2001) Species-specific population-density thresholds in cladocerans? Hydrobiologia 442: 291–300. https://doi.org/10.1023/A:1017590207759
- Gliwicz ZM, Pijanowska J (1989) The role of predation in zooplankton succession. In: Plankton Ecology; Succession in Plankton Communities. Springer, Berlin, Heidelberg, 253–296. https://doi.org/10.1007/978-3-642-74890-5
- Grolemund G, Wickham H (2011) Dates and Times Made Easy with lubridate. Journal of Statistical Software 40: 1–25. https://doi.org/10.18637/jss.v040.i03
- Gugele SM, Baer J, Brinker A (2020) The spatiotemporal dynamics of invasive three-spined sticklebacks in a large, deep lake and possible options for stock reduction. Fisheries Research 232: 105746. https://doi.org/10.1016/j.fishres.2020.105746

- Hälbich A (1997) Populationsdynamik von *Bythotrephes longimanus* (LEYDIG) und *Leptodora kindtii* (Focke) im Bodensee und mögliche Mechanismenihrer Koexistenz.
- Hartmann J (1983) Two feeding strategies of young fishes. Archiv fur hydrobiology 96: 497–509.
- Hayden B, Harrod C, Thomas S, Kahilainen KK (2022) Winter ecology of specialist and generalist morphs of European whitefish, *Coregonus lavaretus*, in subarctic northern Europe. Journal of Fish Biology101(2): 1–11 [389–399]. https://doi.org/10.1111/jfb.14999
- Heermann L, Eriksson L-O, Magnhagen C, Borcherding J (2009) Size-dependent energy storage and winter mortality of perch. Ecology of Freshwater Fish Skip slideshow 18(4): 560– 571. https://doi.org/10.1111/j.1600-0633.2009.00371.x
- Holling CS (1959) The Components of Predation as Revealed by a Study of Small-Mammal Predation of the European Pine Sawfly. The Canadian Entomologist 91: 293–320. https:// doi.org/10.4039/Ent91293-5
- Hope RM (2013) Rmisc: Ryan Miscellaneous. https://cran.r-project.org/package=Rmisc
- Hourston M, Platell M, Valesini F, Potter I (2004) Factors influencing the diets of four morphologically divergent fish species in nearshore marine waters. Journal of the Marine Biological Association of the UK 84: 805–817. https://doi.org/10.1017/S0025315404009981h
- Hudson CM, Lucek K, Marques DA, Alexander TJ, Moosmann M, Spaak P, Seehausen O, Matthews B (2021) Threespine Stickleback in Lake Constance: The Ecology and Genomic Substrate of a Recent Invasion. Frontiers in Ecology and Evolution 8: 1–22. https://doi. org/10.3389/fevo.2020.611672
- IGKB (2018) Bericht Nr. 42 Bericht Nr. 42: Limnologischer Zustand des Bodensees, 1–140. https://www.igkb.org/fileadmin/user_upload/dokumente/publikationen/gruene_berichte/42_gb42gesamtbericht.pdf
- IGKB (2020) 43 Bericht Nr. 43 Bericht Nr. 43: Limnologischer Zustand des Bodensees.
- Ivlev V (1961) Experimental ecology of the feeding of fishes. Yale University Press, New Haven.
- Jakobsen TS, Hansen PB, Jeppesen E, Grønkjær P, Søndergaard M (2003) Impact of three-spined stickleback *Gasterosteus aculeatus* on zooplankton and chl a in shallow, eutrophic, brackish lakes. Marine Ecology Progress Series 262: 277–284. https://doi.org/10.3354/meps262277
- Jakubavičiute E, Casini M, Ložys L, Olsson J (2016) Seasonal dynamics in the diet of pelagic fish species in the southwest Baltic Proper. ICES Journal of Marine Science 74: 750–758. https://doi.org/10.1093/icesjms/fsw224
- Jarnagin ST, Swan BK, Kerfoot WC (2000) Fish as vectors in the dispersal of *Bythotrephes cederstroemi*: Diapausing eggs survive passage through the gut. Freshwater Biology 43: 579–589. https://doi.org/10.1046/j.1365-2427.2000.00547.x
- Jarolim O, Kubecka J, Martin Č, Vašek M, Peterka J, Matena J (2010) Sinusoidal swimming in fishes: The role of season, density of large zooplankton, fish length, time of the day, weather condition and solar radiation. Hydrobiologia 654: 253–265. https://doi.org/10.1007/ s10750-010-0398-1
- Johnston IA, Dunn J (1987) Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. Symposia of the Society for Experimental Biology 41: 67–93.
- Kassambara A (2020) ggpubr: "ggplot2" Based Publication Ready Plots. https://cran.r-project. org/package=ggpubr

- Kassambara A (2021) rstatix: Pipe-Friendly Framework for Basic Statistical Tests. https:// cran.r-project.org/package=rstatix
- Kleiber M (1947) Body size and metabolic rate. Physiological Reviews 27: 511–541. https:// doi.org/10.1152/physrev.1947.27.4.511
- Kottelat M, Freyhof JJ (2007) Copeia Handbook of European freshwater fishes. Publications Kottelat, Cornol, Switzerland. https://doi.org/10.1643/OT-08-098a.1
- Lazzaro X (1987) A review of planktivorous fishes: their evolution, feeding behaviours, selectivities, and impacts. Hydrobiologia 146: 97–167. https://doi.org/10.1007/BF00008764
- Liao H, Pierce CL, Larscheid JG (2001) Empirical Assessment of Indices of Prey Importance in the Diets of Predacious Fish. Transactions of the American Fisheries Society 130: 583–591. https://doi.org/10.1577/1548-8659(2001)130%3C0583:EAOIOP%3E2.0.CO;2
- Ljunggren L, Sandstrom A, Bergstrom U, Mattila J, Lappalainen A, Johansson G, Sundblad G, Casini M, Kaljuste O, Eriksson BK (2010) Recruitment failure of coastal predatory fish in the Baltic Sea coincident with an offshore ecosystem regime shift. ICES Journal of Marine Science 67: 1587–1595. https://doi.org/10.1093/icesjms/fsq109
- Makrakis MC, Nakatani K, Bialetzki A, Gomes LC, Sanches PV, Baumgartner G (2008) Relationship between gape size and feeding selectivity of fish larvae from a Neotropical reservoir. Journal of Fish Biology 72: 1690–1707. https://doi.org/10.1111/j.1095-8649.2008.01845.x
- Mehner T, Padisak J, Kasprzak P, Koschel R, Krienitz L (2008) A test of food web hypotheses by exploring time series of fish, zooplankton and phytoplankton in an oligo-mesotrophic lake. Limnologica 38: 179–188. https://doi.org/10.1016/j.limno.2008.05.001
- Michaloudi E (2005) Dry weights of the zooplankton of Lake Mikri Prespa (Macedonia, Greece). Belgian Journal of Zoology 135: 223–227.
- Mookerji N, Heller C, Meng HJ, Burgi HR, Muller R (1998) Diel and seasonal patterns of food intake and prey selection by *Coregonus* sp. in re-oligotrophicated Lake Lucerne, Switzerland. Journal of Fish Biology 52: 443–457. https://doi.org/10.1111/j.1095-8649.1998.tb02009.x
- Muckle R (1972) Der Dreistachlige Stichling (*Gasterosteus aculeatus* L.) im Bodensee. Schriften des Vereins für Geschichte des Bodensees und seiner Umgebung 124: 249–257.
- Müller R, Breitenstein M, Bia MM, Rellstab C, Kirchhofer A (2007) Bottom-up control of whitefish populations in ultra-oligotrophic Lake Brienz. Aquatic Sciences 69: 271–288. https://doi.org/10.1007/s00027-007-0874-5
- Ogorelec Ž, Rudstam LG, Straile D (2022) Can young-of-the-year invasive fish keep up with young-of-the-year native fish? A comparison of feeding rates between invasive sticklebacks and whitefish. Ecology and Evolution 12(1): 1–10. https://doi.org/10.1002/ece3.8486
- Ogorelec Ž, Wunsch C, Kunzmann AJ, Octorina P, Navarro JI (2021) Large daphniids are keystone species that link fish predation and phytoplankton in trophic cascades. Fundamental and Applied Limnology 194: 297–309. https://doi.org/10.1127/fal/2020/1344
- Olin AB, Olsson J, Eklöf JS, Eriksson BK, Kaljuste O, Briekmane L, Bergström U (2022) Increases of opportunistic species in response to ecosystem change: the case of the Baltic Sea three-spined stickleback. ICES Journal of Marine Science 79(5): 1–16. https://doi.org/10.1093/ icesjms/fsac073
- R Core Team (2018) R: A language and environment for statistical computing. http://www.rproject.org

- Roch S, von Ammon L, Geist J, Brinker A (2018) Foraging habits of invasive threespined sticklebacks (*Gasterosteus aculeatus*) – impacts on fisheries yield in Upper Lake Constance. Fisheries Research 204: 172–180. https://doi.org/10.1016/j. fishres.2018.02.014
- Rösch R, Baer J, Brinker A (2018) Impact of the invasive three-spined stickleback (*Gasterosteus aculeatus*) on relative abundance and growth of native pelagic whitefish (*Coregonus wartmanni*) in Upper Lake Constance. Hydrobiologia 824: 243–254. https://doi.org/10.1007/s10750-017-3479-6
- Sale PF (1974) Overlap in Resource Use, and Interspecific Competition. Oecologia, Berlin, 17: 245–256. [2 tables] https://doi.org/10.1007/BF00344924
- Sanchez-Gonzales S, Ruiz-Campos G, Contreras-Balderas S (2001) Feeding ecology and habitat of the threespine stickleback, *Gasterosteus aculeatus microcephalus*, in a remnant population of northwestern Baja California, Mexico. Ecology of Freshwater Fish 10: 191–197. https://doi.org/10.1034/j.1600-0633.2001.100401.x
- Sandlund OT, Næsje TF (1989) Impact of a pelagic gill-net fishery on the polymorphic whitefish (*Coregonus lavaretus* L. s.l.) population in Lake Femund, Norway 7: 85–97. https://doi.org/10.1016/0165-7836(89)90009-X
- Seebens H, Einsle U, Straile D (2013) Deviations from synchrony: spatio-temporal variability of zooplankton community dynamics in a large lake. Journal of Plankton Research 35: 22–32. https://doi.org/10.1093/plankt/fbs084
- Sinervo BR (2007) Optimal Foraging Theory: Constraints and Cognitive Processes.
- Stich HB, Maier G (2006) Enumeration of prey items in stomachs of European whitefish (*Coregonus lavaretus* L.) which contain digested fragments. Limnologica 36: 138–142. https://doi.org/10.1016/j.limno.2006.02.002
- Straile D, Geller W (1998) Crustacean zooplankton in Lake Constance from 1920 to 1995: response to eutrophication and re-oligotrophication. Archiv für Hydrobiologie, Special Issues: Advances in Limnology 53: 255–274. http://nbn-resolving.de/urn:nbn:de:bsz:352-opus-39858
- Sullivan KM (1986) Physiology of feeding and starvation tolerance in overwintering freshwater fishes. In: Developments in environmental biology of fishes, 259–268. https://doi. org/10.1007/978-94-017-1158-6_22
- Thomas G, Eckmann R (2007) The influence of eutrophication and population biomass on common whitefish (*Coregonus lavaretus*) growth – the Lake Constance example revisited. Canadian Journal of Fisheries and Aquatic Sciences 64: 402–410. https://doi.org/10.1139/f07-019
- Thomas G, Rösch R, Eckmann R (2010) Seasonal and long-term changes in fishing depth of Lake Constance whitefish. Fisheries Management and Ecology 17: 386–393. https://doi. org/10.1111/j.1365-2400.2010.00734.x
- Thompson JM, Bergersen EP, Carlson CA, Kaeding LR (1991) Role of size, condition, and lipid content in the overwinter survival of age-0 colorado squawfish. Transactions of the American Fisheries Society 120: 346–353. https://doi.org/10.1577/1548-8659(1991)120%3C0346 :ROSCAL%3E2.3.CO;2
- Ultsch GR (1989) Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles, and snakes. Biological Reviews 64: 435–515. https://doi.org/https://doi. org/10.1111/j.1469-185X.1989.tb00683.x

- Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the tidyverse. Journal of Open Source Software 4: 1686. https://doi.org/10.21105/joss.01686
- Wiebe PH (1971) a Computer Model Study of Zooplankton Patchiness and Its Effects on Sampling Error. Limnology and Oceanography 16: 29–38. https://doi.org/10.4319/lo.1971.16.1.0029
- Wootton RJ (1984) Feeding. In: Wootton RJ (Ed.) A Functional Biology of Sticklebacks. Springer US, Boston, MA, 32–62. https://doi.org/10.1007/978-1-4615-8513-8_4

Supplementary material I

Supplementary data

Authors: Žiga Ogorelec, Alexander Brinker, Dietmar Straile Data type: docx file

- Explanation note: table S1. Number of sampled fish and fish with empty stomachs for each month. In May 2017, sticklebacks were caught on two occasions: 3 sticklebacks on the 10th and 2 sticklebacks on the 30th of May. Sticklebacks were also classified into a separate first-year-of-life (0+) group according to their sizes. table S2. Number of replicates for each sampling time point after pooling five samples for sticklebacks and whitefish. table S3. Differences between whitefish and stickleback diets in each season. table S4. The contribution of individual prey species to the differences between stickleback and whitefish diets (similarity percentages procedure; one-way, 70% cut-off). table S5. Results of beta regression relating relative zooplankton consumption to relative zooplankton density, fish species, and the interaction between relative density and fish species (see Fig. 5). table S6. Summary of the prey-specific index of relative importance (%PSIRI) for both fish predators for the compared period (May 2017 to January 2018). Asterisks represent calculations made for whitefish for the entire year (May 2017 to April 2018). figure S1. Seasonal changes in the diets of sticklebacks (excluding 0+) and whitefish in Lake Constance. figure S2. Whitefish and stickleback (excluding 0+ fish) relative consumption of zooplankton species depending on the proportional density of zooplankton species. figure S3. Annual averages of the three parameters (% occurrence, body weight and relative abundance (% number)) of the preyspecific index of relative importance for A) whitefish and B) sticklebacks.
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Link: https://doi.org/10.3897/neobiota.78.86788.suppl1

RESEARCH ARTICLE



Population genetics of an invasive mosquito vector, Aedes albopictus in the Northeastern USA

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Academic editor: Deepa Pureswaran | Received 2 May 2022 | Accepted 25 October 2022 | Published 23 November 2022

Citation: Gloria-Soria A, Shragai T, Ciota AT, Duval TB, Alto BW, Martins AJ, Westby KM, Medley KA, Unlu I, Campbell SR, Kawalkowski M, Tsuda Y, Higa Y, Indelicato N, Leisnham PT, Caccone A, Armstrong PM (2022) Population genetics of an invasive mosquito vector, *Aedes albopictus* in the Northeastern USA. NeoBiota 78: 99–127. https://doi.org/10.3897/neobiota.78.84986

Abstract

The Asian tiger mosquito (*Aedes albopictus*) arrived in the USA in the 1980's and rapidly spread throughout eastern USA within a decade. The predicted northern edge of its overwintering distribution on the East Coast of the USA roughly falls across New York, Connecticut, and Massachusetts, where the species has been recorded as early as 2000. It is unclear whether *Ae. albopictus* populations have become estab-

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lished and survive the cold winters in these areas or are recolonized every year. We genotyped and analyzed populations of *Ae. albopictus* from the northeast USA using 15 microsatellite markers and compared them with other populations across the country and to representatives of the major global genetic clades to investigate their connectivity and stability. Founder effects or bottlenecks were rare at the northern range of the *Ae. albopictus* distribution in the northeastern USA, with populations displaying high levels of genetic diversity and connectivity along the East Coast. There is no evidence of population turnover in Connecticut during the course of three consecutive years, with consistent genetic structure throughout this period. Overall, these results support the presence of established populations of *Ae. albopictus* in New York, Connecticut, and Massachusetts, successfully overwintering and migrating in large numbers. Given the stability and interconnectedness of these populations, *Ae. albopictus* has the potential to continue to proliferate and expand its range northward under mean warming conditions of climate change. Efforts to control *Ae. albopictus* in these areas should thus focus on vector suppression rather than eradication strategies, as local populations have become firmly established and are expected to reemerge every summer.

Keywords

Asian tiger mosquito, colonization, container-breeder, invasion genetics, propagule pressure, range expansion

Introduction

The Asian tiger mosquito (*Aedes albopictus*) is a highly invasive species that spread from its native range in East Asia to more than 50 countries on every continent, except Antarctica, during the last 40 years (Sprenger and Wuithiranyagool 1986; Kraemer et al. 2015). The global range expansion and success of this species has been propelled largely by human migration, transportation, and global commerce. *Ae. albopictus* lays desiccation-resistant eggs and develops in artificial water-holding containers, which facilitate its dispersal and establishment in urban and suburban environments (Sprenger and Reiter 1987; Hawley et al. 1987; Parker et al. 2020). Although *Ae. albopictus* feeds opportunistically on a wide range of species (Niebylski et al. 1994; Delatte et al. 2010) it can be an aggressive human biter and a vector of emergent human arboviruses including dengue, chikungunya, and Zika viruses (Metselaar et al. 1980; Gratz 2004; Paupy et al. 2012; Gloria-Soria et al. 2021). This raises the concern that the risk of these arboviruses will increase as this species proliferates and expands its geographic range, as observed in the Indian Ocean Islands, Italy, France, Japan, and Hawaii (Paupy et al. 2009; Grandadam et al. 2011; Rezza 2012).

In the continental USA, *Ae. albopictus* has been detected in 40 states, since the first population was discovered in Houston Texas in 1985 (Sprenger and Wuithiranyagool 1986; Hahn et al. 2017). However, many of these state records could represent transient seasonal introductions rather than established populations. *Aedes albopictus* has become established in southern California and much of the eastern half of the country (Linthicum et al. 2003; Kraemer et al. 2015), with populations continuing to move northward. The northern boundary for overwintering populations has been suggested to be at the isotherm of the coldest month mean temperature of 0 °C based on its distribution in Asia (Nawrocki and Hawley 1987) or isotherms with mean annual

temperatures above 11 °C (Kobayashi et al. 2002). This corresponds roughly to southern New England and New York, where *Ae. albopictus* populations emerge annually, with the boundary expected to shift north due to a warming climate (Rochlin et al. 2013). *Ae. albopictus* was first detected in New York (NY) in 2000, in New York City and neighboring Long Island counties in 2003, and is currently spreading north into the Hudson Valley (Kulasekera et al. 2001; Rochlin et al. 2013; Hahn et al. 2016; Kache et al. 2020). In Connecticut (CT), this species was first detected in 2003 and then in 2006 (Andreadis et al. 2005; Andreadis 2009; Armstrong et al. 2017), and has been reported every year since 2010 during continuous statewide mosquito surveillance (Armstrong et al. 2017). Collections occur primarily along the southern margin of CT and successful overwintering of a local population was documented in 2013, during one of the four winters sampled (Armstrong et al. 2017).

We performed population genetic analyses on Ae. albopictus collected from NY, CT, and Massachusetts (MA), and compared them to established populations from other USA states and countries to better understand the process of mosquito colonization at the northern expansion front. Collections include mosquitoes sampled from 23 locations along the USA eastern seaboard from Florida to MA, one population from California and temporal collections at four locations in CT spanning three consecutive years. In addition, we include collections from Thailand, Japan, and Brazil as representatives of the major global genetic clusters identified in this species (Kotsakiozi et al. 2017). Here, we characterize the genetic diversity and genetic structure of *Ae. albopictus* populations in the Northeast USA, and evaluate the stability of populations in CT as representatives of the northern edge of Ae. albopictus distribution in the USA East Coast; seeking to understand the patterns of *Ae. albopictus* range expansion and establishment in the country. Based on classic invasion theory (Nei et al. 1975; Sakai et al. 2001), we predict low diversity at the northeastern invasion front (CT, NY, MA) relative to the south and the native range, with diversity in the Northeast declining gradually with latitude and evidence of recent bottlenecks consequence of founder events. Furthermore, if these populations have become established we expect stability in their genetic structure over multiple years.

Methods

Collections

A total of 1,342 *Ae. albopictus* mosquitoes were sent to the Connecticut Agricultural Experiment Station from Departments of Public Health, Mosquito Abatement Districts, and collaborators. All individuals were received as adults directly from the field, with the exception of four sampling sites that were collected as larvae. Larvae from Tappan, NY were reared and underwent one generation in the laboratory, larvae from Fire Island and Spring Valley (NY) underwent 6 generations. Vero Beach samples came from field-collected larvae subsequently reared to adulthood. Samples were received as adults in ethanol and silica gel, with the exception of those of Thailand, Japan,



Figure 1. *Aedes albopictus* collection map. Populations of the northeastern USA (NE) are labeled with numbers, corresponding to their ID in Table 1. Outgroups included in this study, representing known genetic clusters are shown in the world map insert in the bottom right corner.

and Brazil which were obtained as DNA aliquots. The samples included 24 locations within the USA (Table 1, Fig. 1 and Suppl. material 1). Temporal samples were collected from Connecticut at four locations every year for three years, with the exception of Norwalk, for which only two years were collected.

DNA extraction and microsatellite genotyping

Individual mosquitoes were homogenized with a sterile plastic pestle and DNA was extracted following the Qiagen (Hilden, Germany) protocol for purifying total DNA from insects with the Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany), with an additional RNAse A step. Samples were stored at -20 °C until further use. Mosquitoes from Connecticut, which had previously been homogenized in 1 ml of PBS-G media (phosphate buffered saline, 30% heat-inactivated rabbit serum, 0.5% gelatin), were processed following the manufacturers protocol for electrically homogenized samples.

Mosquitoes were genotyped at 15 microsatellite loci, including locus A9 from Porretta et al. (2006), 11 loci from Beebe et al. (2013), and three new loci developed for this study (Suppl. material 2). The AG10, AG01, and AG07 loci were identified during a screen for candidate trinucleotide microsatellite markers using QDD v.3.1. (Meglécz et al. 2014) on *Ae. albopictus* genomic data from Palatini et al. (2020). These new loci successfully genotyped across USA populations in a pilot study and were polymorphic across individuals and populations tested (unpublished data). Polymerase chain reactions (PCR) were conducted as loci combinations (Suppl. material 2)

ID	Location	Year	N	Ho	Hs	Gis	AR
1	Bridgeport, CT, USA	2018	48	0.531	0.664	0.119	5.52
1	Bridgeport, CT, USA	2019	35	0.551	0.657	0.199	5.16
1	Bridgeport, CT, USA	2020	47	0.536	0.642	0.162	5.38
2	Milford, CT, USA	2018	48	0.551	0.667	0.165	5.01
3	New Haven, CT, USA	2018	48	0.591	0.673	0.174	5.07
4	Norwalk, CT, USA	2018	48	0.567	0.678	0.122	5.32
4	Norwalk, CT, USA	2020	46	0.518	0.655	0.164	5.04
5	Stamford, CT, USA	2020	48	0.494	0.637	0.21	4.99
6	Stratford, CT, USA	2018	48	0.573	0.657	0.224	4.95
6	Stratford, CT, USA	2019	18	0.506	0.637	0.128	4.83
6	Stratford, CT, USA	2020	48	0.532	0.646	0.205	4.17
7	West Haven, CT, USA	2018	46	0.564	0.649	0.177	4.92
7	West Haven, CT, USA	2019	39	0.545	0.645	0.132	5.25
7	West Haven, CT, USA	2020	46	0.527	0.662	0.156	5.25
8	Lincoln, DE, USA	2015	25	0.532	0.613	0.204	5.49
9	Washington, DC, USA	2018	47	0.513	0.645	0.132	4.70
10	Riverdale, MD, USA	2015	28	0.494	0.610	0.206	5.20
11	New Bedford, MA, USA	2018	39	0.523	0.633	0.038	5.29
12	Mercer, NJ, USA	2018	48	0.511	0.666	0.191	4.77
13	Tappan, NY, USA *	2018	41	0.531	0.641	0.175	4.85
14	Fire Island, NY, USA *	2018	48	0.537	0.657	0.232	5.18
15	Selden, NY, USA	2019	26	0.556	0.669	0.173	4.40
16	Riverhead, NY, USA	2019	45	0.570	0.684	0.182	4.56
17	Bayview, NY, USA	2019	34	0.530	0.647	0.169	5.46
18	Babylon, NY, USA	2018	46	0.555	0.649	0.168	5.62
19	Spring Valley, NY, USA*	2018	28	0.485	0.597	0.180	4.80
20	Harrisburg, PA, USA	2015	25	0.496	0.655	0.145	5.16
21	Philadelphia, PA, USA	2018	48	0.535	0.625	0.188	4.01
22	Fairfax, VA, USA	2018	46	0.499	0.625	0.243	5.04
-	Vero Beach, FL, USA	2018	24	0.658	0.684	0.145	4.95
-	San Gabriel, CA, USA	2018	47	0.593	0.673	0.201	6.88
-	Manaus, Brazil	2017/18	22	0.459	0.622	0.262	4.88
-	Tokyo, Japan	2017/18	42	0.542	0.647	0.162	4.96
-	Chanthaburi, Thailand	2016	20	0.651	0.740	0.121	7.23

Table 1. Population information and genetic diversity based on 15 microsatellite loci.

ID: location identifier in Fig. 1. Locations beyond the focus area are shown in the insert of Fig. 1 and were not assigned an ID; N: number of individuals; H0: observed heterozygosity; Hs: expected heterozygosity; Gis: Inbreeding Coefficient; AR: estimated by rarefaction (N = 30 genes). *underwent 1–6 generations in laboratory.

in 10 µl reactions using the Type-it Microsatellite PCR Master Mix (Qiagen; Hilden, Germany) and 200 nM of each forward and reverse primer pairs. Thermocycler conditions were: 95 °C × 5', 5 touch-down cycles reducing the annealing temperature every cycle by 2 °C from 60 °C to 52 °C (95 °C × 30", Tm × 30", 72 °C × 30"), 25× (95 °C × 30", 50 °C × 30", 72 °C × 30"), and 60 °C× 30' for all loci combos, except for loci set #2 (tri25/AG10), for which we used GoTaq DNA polymerase from Promega (Madison, USA). Primer concentrations were the same for the GoTaq reaction with the thermocycler conditions 95 °C × 2', 5 touch-down cycles reducing Tm every cycle

by 2 °C from 61 °C to 53 °C (95 °C × 45", Tm × 30", 72 °C × 30"), 25× (95 °C × 45", 51 °C × 30", 72 °C × 30"), and 72 °C × 20'.

The resulting products were processed for fragment analysis at the DNA Analysis Facility at Science Hill at Yale University, using GS 500 Liz internal size standard (Applied Biosystems, Waltham MA, USA). Microsatellite alleles were scored using Geneious 11.1.4 (Biomatters Ltd) microsatellite plugin (http://www.geneious.com) using the bins and panels in Suppl. material 3.

Raw allele frequencies are available at VectorBase (www.vectorbase.org), Population Biology Project ID: VBP0000814.

Genetic diversity

Loci were analyzed for within-population deviations from Hardy-Weinberg equilibrium (HWE) using the Weir and Cockerham (1984) exact test as implemented in Genepop v. 4.7.5 (Raymond and Rousset 1995; Rousset 2008). Null allele frequencies and linkage disequilibrium among pairs of loci (LD) were also estimated with this software. HWE and LD tests were run with 10,000 dememorizations, 1000 batches, and 10,000 iterations per batch. Average observed (*Ho*) and expected (*He*) heterozygosities, and inbreeding coefficients (G_{is}) were estimated for each population in GenoDive 3.04 (Meirmans 2020). Allelic richness (*AR*) was calculated in HP-RARE (Kalinowski 2005), which uses rarefaction to correct for unequal sample sizes (N = 30 genes). Bonferroni correction was applied to the appropriate results to account for multiple testing. A regression analysis in R v. 3.2.2. (R Core Team 2018) was used to evaluate if genetic diversity changed with latitude.

Changes in recent population size were evaluated using Bottleneck v. 1.2.02 (Cornuet and Luikart 1997) under the Infinite Allele Model (IAM) (Maruyama and Fuerst 1985) and the two-phase model (TPM) with a proportion of SMM in the TPM = 0.00 and a variance of the geometric distribution for TPM = 0.36, as recommended by the authors when dealing with microsatellite markers (Cornuet and Luikart 1997). The Wilcoxon sign-rank test (Luikart et al. 1998) was used to determine significance, after Bonferroni multiple test correction.

Effective population size (Ne) was calculated for the temporal collections in CT using NeEstimator (Do et al. 2014) with the Waples (1989) method and three options for computing the standardized variance in allele frequency, F [*Fe* (Nei and Tajima 1981); *Fk* (Pollak 1983); and *Fs* (Jorde and Ryman 2007)]; assuming 3 generations per year. *Ne* was also estimated from these populations using a single population sample (as opposed to sampling a population multiple times) with the bias-corrected version of the LD method from Waples and Do (2008). Average *Ne* was estimated using arithmetic and harmonic mean to account for the effect of outliers. Two-sample *Ne* estimates are known to be robust to overlapping generations and can deal with lower levels of polymorphisms (Luikart et al. 2010), but may be affected by changes in allele frequencies occurring during the time lapsed; while single-sample methods are not affected by gene flow and drift but may be biased by overlapping generations and are unable to distinguish from infinite population sizes when not enough polymorphisms are present (Saarman et al. 2017). Kinship within collections was assessed in ML-Relate (Kalinowski et al. 2006), which uses maximum likelihood estimates of relatedness to discriminate between four common pedigree relationships: unrelated (U), half-siblings (HS), full-siblings (FS), and parent-offspring (PO). The program tests every population for an excess in heterozygosity relative to the observed allelic diversity.

Population structure

Bayesian clustering analysis was conducted in STRUCTURE v. 2.3 (Pritchard et al. 2000). STRUCTURE identifies genetic clusters and assigns individuals to these clusters with no *a priori* information of sample location. The most likely number of clusters (K) was determined by conducting 20 independent runs from each K = 1 to 8 for the complete dataset, K = 1 to 11 for Japan + America, K = 1 to 10 for the states at the northeastern invasion front (NY, CT, MA), and K = 1 to 11 for the CT temporal dataset. Each run assumed an admixture model and correlated allele frequencies using a burn-in value of 100,000 iterations followed by 500,000 repetitions. The optimal number of K clusters was determined following the guidelines of Prichard et al. (Pritchard et al. 2000) and the Delta K method (Evanno et al. 2005), as implemented by STRUCTURE HAR-VESTER (Earl and vonHoldt 2012). Results were plotted with the program CLUMPAK (Kopelman et al. 2015) and DISTRUCT v.1.1 (Rosenberg 2004). Discriminant analysis of Principal Components (DAPC) were conducted on allele frequencies using the ADE-GENET package (Jombart 2008) in R v. 3.2.2. (R Core Team 2018) from the same datasets analyzed with STRUCTURE, both using pre-defined populations and with the find.clusters command to identify genetic clusters without a-priori information.

Molecular Analysis of Variance was performed in Genodive 3.04 (Meirmans 2020) with 1000 permutations. Pairwise genetic distances (*Fst*) were calculated in the same software. A geographic distance matrix was produced from geographic coordinates in the Geographic distance matrix generator v. 1.2.3. (Ersts 2016). Correlation between genetic and geographic distance (isolation by distance; IBD) was evaluated for all populations in the Northeast, along I-95 interstate corridor from Virginia (VA) to CT, and across the northeastern invasion front (NY, CT, MA), using a Mantel test and 9999 permutations in the Ade4 package (Dray and Dufour 2007) within R (R Core Team 2018).

Results

Genetic diversity

We genotyped a total of 1,342 individual *Ae. albopictus* mosquitoes from 27 geographic locations at 15 microsatellite loci, for an average of 40 individuals per location (Fig. 1, Table 1). Seventy-nine of the 508 possible population-by-locus comparisons (15.55%) deviate from HWE (p < 0.05) after sequential Bonferroni correction. Putative null alleles were inferred at all loci, except for tri20, with average frequencies across populations between

0.02 - 0.22. Linkage disequilibrium is significant in 37 out of the 3,585 locus-by-locus tests (1.03%) after multiple test correction, consistent with the loci being independent.

There is an average of 13.8 \pm 6.46 alleles per locus, ranging from 8 to 31, with a mean allele richness (*AR*) across populations of *AR* = 5.13 \pm 0.61 (ranging from 4.01 to 7.23; Suppl. material 4). Average observed heterozygosity (*Ho*) is 0.54 \pm 0.42, with a lowest value of 0.46 and a highest of 0.66 observed in Brazil and Florida, respectively (Table 1). The average inbreeding coefficient (*G*_{*i*}) across populations is 0.17 \pm 0.04, with a maximum value of 0.26 in Brazil and a minimum of 0.04 in Florida (Table 1). Regression analysis to establish if genetic diversity decays at the invasion front (higher latitudes) indicates that latitude explains a small part of the variation in *Ho* (adjusted R² = 0.13, F(_{1,27}), p = 0.03; Suppl. material 9: fig. S1A), with *Ho* increasing with latitude rather than decreasing. Latitude does not correlate with changes in *AR* (adjusted R² = 0.04, F(_{1,27}), p = 0.15; Suppl. material 9: fig. S1B). Genetic diversity at the northern front of the invasion (CT, NY, MA) is no different from that from Japan and Thailand (*Ho*: t_{1.02} = -1.0411, p = 0.4843; *AR*: t_{1.01} = -0.9218, p = 0.5248).

Only four populations have evidence of a recent bottleneck. Bottlenecks were inferred for Fire Island and Spring Valley (NY), Mercer County (NJ), and Norwalk (CT), under both the Infinite Allele Model (IAM) (Maruyama and Fuerst 1985) and the two-phase model (TPM) using the Wilcoxon sign-rank test (Cornuet and Luikart 1997) after a Bonferroni multiple test correction (Suppl. material 5). Among them, Fire Island and Spring Valley had been maintained in the laboratory for six generations prior to genotyping, which may explain the bottleneck signature (Table 1).

Local estimates of effective population size across CT using the two-sample method on temporal collections (see Methods) yield mean values of Ne = 94.97 (harmonic mean) and Ne = 121.21 (arithmetic mean), ranging from 37.70 to 317.10 (Suppl. material 10: fig. S2A). Single-sample estimations based on LD yield a harmonic mean of Ne = 126.84 and an arithmetic mean of Ne = 2,337.50, ranging from 47.40 to 23,830 (Suppl. material 10: fig. S2B); with the highest value estimated for West Haven (2020) as an outlier.

Analysis of kinship determined that, on average, 1.97% of the pairwise relationships within a population involved first degree pairs (Parent-offspring and full sibling; Suppl. material 6). Tappan NY, Spring Valley NY, and Vero Beach FL have the highest percentage of first-degree pairwise relationships (>5%). Removing first-degree relatives from these populations did not have a major impact in the genetic diversity estimates ($t_{Ho(4)} = 0$, p = 1; $t_{Gis(4)} = 0$, p = 1; $t_{AR(3.98)} = -0.2964$, p = 0.7817), inference of bottlenecks, or the population structure analysis (data not shown).

Population structure

The optimal number of genetic groups inferred from the complete dataset is K = 3, based on Bayesian clustering analysis and the Delta K method (Evanno et al. 2005). The first cluster consists of Florida, California, Brazil, and Thailand while different degrees of admixture between the second and third cluster are observed throughout the rest of the populations analyzed, including Japan and the northeastern USA (Fig. 2A). This grouping is consistent with the DAPC using predefined populations, except that

in the DAPC plot Florida is placed within the cluster that includes the northeastern USA (Fig. 2B). No clear genetic structure was detected within the genetic cluster that included Japan and eastern North America, despite a suggested K = 3 using the Delta K method (Suppl. material 11). Incipient population structure is suggested by the clustering analysis of the populations at the northeastern invasion front, with Fire Island and Bayview (NY) showing certain differentiation at K = 3 (Suppl. material 12).

Analysis of Molecular Variance (AMOVA) on the complete dataset indicates that most of the variation can be explained at the individual level, with a lower contribution from the population level (Table 2).



В



Figure 2. Population structure on the complete *Aedes albopictus* dataset based on 15 microsatellite markers **A** STRUCTURE plot with each individual represented by a vertical bar. The height of each bar is the probability of assignment to each of K = 3 genetic clusters (indicated by different colors) **B** discriminant analysis of principal components (DAPC).

Source of Variation	Nested in	% var	F-stat	F-value	Std.Dev.	P-value
Within Individual	-	0.792	F_it	0.208	0.049	-
Among Individual	Population	0.160	F_is	0.168	0.050	0.001
Among Population	—	0.047	F_st	0.047	0.004	0.001

Table 2. Analysis of Molecular Variance on all populations genotyped for 15 microsatellite loci.



Figure 3. Geographic genetic differentiation (IBD: isolation by distance) across **A** New York, Connecticut, and Massachusetts; and **B** New York and Connecticut. Genetic distance is given as the linearized F_{st} [$F_{st}/(1/F_{st})$] and geographic distance is provided in kilometers (Km). Statistical significance was evaluated using a Mantel test, yielding a significant positive slope only when Massachusetts is excluded (p = 0.072 and p < 0.000 in A and B, respectively).

We then tested for isolation by distance (IBD) throughout the northeastern USA (Virginia, District of Columbia, New Jersey, NY, CT, and MA) to determine whether genetic distance (F_{sr}) was correlated with geographic distance (Km) and found no correlation (Mantel statistic = -0.0406, p = 0.4368; Suppl. materials 7, 13). Likewise, there was no IBD in populations located along the I-95 corridor from Virginia to CT (Mantel statistic = 0.088, p = 0.295; Suppl. material 7 and Suppl. material 13), or at the northeastern invasion front: CT, NY, MA (Mantel statistic = 0.382, p = 0.072; Suppl. material 7 and Fig. 3A). However, strong IBD was detected when only NY and CT were analyzed (Mantel statistic = 0.727, p = 0.000; Suppl. material 7 and Fig. 3B).

Temporal stability

Bayesian clustering analysis and DAPC across all Connecticut populations indicate weak population structure in CT (Suppl. material 14). Analysis of the temporal series indicates that these population clusters prevail over multiple years, suggesting the development of local populations (Fig. 4). In contrast, there is no support for temporal structure by year of collection (Fig. 4). This result agrees with the AMOVA, with variation mostly explained at the individual and population level rather than by year of collection


Figure 4. Population structure on *Aedes albopictus* samples from the Connecticut temporal series based on 15 microsatellite markers **A** STRUCTURE plot with each individual represented by a vertical bar. The height of each bar is the probability of assignment to each of K = 3 genetic clusters (indicated by different colors) **B** discriminant analysis of principal components (DAPC). Partially overlapping genetic clusters can be distinguished, grouping temporal collections from the same location.

(Table 3; AMOVA_{Time_Points} p = 0.901). When DAPC was used to infer genetic clusters without population priors, three genetic clusters were inferred (Suppl. material 15). However, these clusters include individuals from all collection points and years (Suppl. material 8), with very few individuals assigned to a third cluster, in agreement with the incipient differentiation suggested by the Bayesian clustering analyses.

Source of Variation	Nested in	%var	F-stat	F-value	Std.Dev.	P-value
Within Individual	-	0.801	F_it	0.199	0.044	-
Among Individual	Population	0.183	F_is	0.186	0.044	0.001
Among Population	Series_A	0.018	F_sc	0.018	0.004	0.001
Among Time points	-	-0.002	F_ct	-0.002	0.002	0.901

Table 3. Analysis of Molecular Variance on temporal samples from Connecticut genotyped for 15 microsatellite loci.

Discussion

We find that *Ae. albopictus* from the northeastern USA are related to *Ae. albopictus* from Japan and harbor high genetic diversity with limited geographic structure. This suggests regional gene flow and a northward invasion driven by a combination of multiple local and long-distance dispersal events that has led to the establishment of northern populations overwintering locally.

Discarded tires are preferred breeding sites for container-inhabiting *Aedes* mosquitoes (Yee 2008) and likely explain how this species entered the country. The USA began importing used tires from Japan in 1968, and by the mid-1970's most used tires were imported from countries where *Ae. albopictus* was native, mostly from Japan and Taiwan (Sprenger and Reiter 1987). Our results agree with previous work showing that eastern USA populations most likely originated from northern (temperate) East Asia, based on historical records, phenotypic traits (photoperiod sensitivity and cold-hardiness), and genetic markers (Hawley et al. 1987; Kambhampati et al. 1991; Kotsakiozi et al. 2017). We also find that the population in southern California is genetically distinct from those occupying eastern USA, consistent with reports of an introduction of Chinese origin in 2001 and 2011 (Linthicum et al. 2003, Zhong et al. 2013).

Shortly after its initial detection in Texas in 1985 (Moore 1999, Hahn et al 2016), Ae. albopictus rapidly spread throughout much of eastern USA. Currently the states of CT, MA, and NY represent the northern limit of the distribution. Classic invasive population genetics predicts that populations at the invasion front would have reduced genetic diversity, consequence of founder effects during the colonization process (Nei et al. 1975; Sakai et al. 2001). We find high genetic diversity (H_o) at the Ae. albopictus northern invasion front, equivalent to that in the native range: Japan and Thailand. Furthermore, evidence of recent bottlenecks (founder effects) was restricted to the two collections from New York that spent 6 generations in the laboratory (Spring Valley and Fire Island), and Mercer County (NJ), and Norwalk (CT). Since bottlenecks are common after laboratory colonization (Gloria-Soria et al. 2019), the bottlenecks detected in Spring Valley and Fire Island are likely the result of the colonization process. A growing number of studies have now demonstrated that the genetic diversity patterns following an invasion event are complex and depend on the size of the propagule (number of individuals invading), frequency of introductions, number of sources, admixture events, or a combination of these (Lockwood et al 2005; Dlugosch and Parker 2008; Facon et al. 2008; Handley et al. 2011; Bock et al. 2015; Jaspers et al. 2021). Different invasion scenarios may result in lower, equal, or higher genetic diversity metrics in the non-native range relative to the native range (Jaspers et al. 2021). High H_o values at the invasive range of *Ae. albopictus* have also been reported by others using allozymes (Black et al. 1988), microsatellites (Manni et al. 2017), and genomewide single nucleotide polymorphisms [SNPs] (Kotsakiozi et al. 2017). The observed genetic diversity in the northeastern USA could be explained by expanding propagules that are subjected to drift and then merge (admixture), or by constant input of alleles that restore the original diversity levels and could possibly exceed them (Lockwood et al. 2005; Facon et al. 2008). In *Ae. japonicus*, another Asian container-breeding mosquito that invaded the USA, merging of two genetic groups was reported in Pennsylvania between 1999/2000 and 2004/2005 and resulted in the loss of the original introduction bottleneck signature and high levels of genetic diversity (Fonseca et al. 2010).

The heterozygosity values observed in Ae. albopictus in the Northeast USA are equivalent to those observed in *Ae. aegypti* in the USA (t_{177} = 1.027, p = 0.318; Gloria-Soria et al. 2016). Despite this similarity, estimates of inbreeding are an order of magnitude larger in Ae. albopictus than in Ae. aegypti (Gloria-Soria et al. 2016). High Ae. albopictus inbreeding values have been previously reported in the USA using allozymes (Black et al. 1988) and in populations outside *Ae. albopictus* native range with microsatellites (Beebe et al. 2013), and may reflect the local breeding structure of this container mosquito (Black et al. 1988). Alternatively, the increase in homozygosity relative to the expected Hardy-Weinberg equilibrium diagnostic of inbreeding may also be the result of a Wahlund effect or the presence of null alleles, and distinguishing among those mechanisms is not trivial (Barros et al. 2020). We detected putative null alleles at low frequencies (0.02 - 0.22) at all but one of the 15 loci used in this study. Microsatellite null alleles are frequent in insects (Chapuis and Estoup 2007), and in Ae. albopictus (Beebe et al. 2013; Manni et al. 2017). Studies have shown that at low frequencies (< 0.20), the presence of null alleles does not affect analyses of genetic diversity and population structure (Dakin and Avise 2004; Chapuis and Estoup 2007; Wei et al. 2019). In 2017 De Meeûs (2018) proposed a statistical test to differentiate the null alleles from a Wahlund effect, based on correlations among F-statistics. Null alleles are expected to increase both Fis and Fst, creating a strong positive correlation, while a Wahlund effect will move the values in the opposite direction and generate weak or no correlation. We find no correlation between Gis and Gst (equivalent to Fis and Fst), indicating that either a Wahlund effect or inbreeding (or both), are most likely responsible for the observed heterozygote deficits rather than null alleles (R² = 0.024, $F_{(1-13)}$ = 0.328, p = 0.577). A Wahlund effect arises when genotype proportions are calculated from samples that include individuals belonging to genetically differentiated groups in time or space, for example, subpopulations or cohorts (De Meeûs 2018). It is thus possible that the diversity pattern we observe is the result of substructure within Ae. albopictus collections that goes undetected due to the scale of this work. The latter will be consistent with the small neighborhood size estimated for Ae. albopictus in Connecticut (Ne ~ 100), which is overall lower than those estimated from wild *Ae. aegypti* using 12 microsatellite markers by Saarman et al. (2017).

At the regional scale, Ae. albopictus in the northeastern USA is genetically homogeneous. This lack of population structure is congruent with the findings of Kotsakiozi et al. (2017) using ~58,000 genome-wide SNP and likely reflects the demographic features of these species, rather than a lack of marker resolution. One possibility is that being a relatively new invasion there has not been enough time for detectable genetic differentiation to arise. However, fine scale structure is evident in Ae. aegypti from California just two years after breeding populations were first detected (Gloria-Soria et al. 2014; Pless et al. 2017). The absence of population structure in Ae. albopictus may be better explained by the invasion history of Ae. albopictus, spreading faster than Ae. aegypti in North America due to its biology and propagule size, and the high connectivity within the region. Consistent with this hypothesis, we detect isolation by distance along CT and NY that does not extend to MA or the rest of the East Coast. This is probably a consequence of the proximity of CT and NY, with gene-flow predominantly occurring via neighboring populations through natural and human-aided dispersal (Handley et al. 2011; Medley et al. 2015). Geographic differentiation within this area is observed and suggests that these populations may already be established and had sufficient time to differentiate.

In Connecticut, *Ae. albopictus* has been recorded every year since 2010 (Armstrong et al. 2017) but it has not been determined whether these populations are present yearround or are reintroduced annually. Unlike its congener *Ae. aegypti, Ae. albopictus* is capable of diapausing at the egg stage (Armbruster 2016) and overwintering has been reported in CT after mild winters (Armstrong et al. 2017). If CT was recolonized from the south every year, we would expect that collections from one year will be more similar to each other than between years. We did not find evidence of temporal structure in these collections but rather a weak spatial signature across years, consistent with overwintering. However, at this point we cannot exclude the possibility that these populations are recolonized by a large influx of individuals from the same sources every year.

Conclusions

The overall absence of bottlenecks, lack of genetic structure, patterns of isolation by distance, and temporal stability at the northeastern invasive front suggest that *Ae. albopictus* populations in the northeastern USA may already be established as overwintering populations. Furthermore, the high levels of genetic diversity, signatures of inbreeding and small neighborhood sizes suggest that *Ae. albopictus* populations in the northeast USA experience high propagule pressure, probably as the result of multiple, diverse, and frequent invasion sources from southeastern USA populations and possibly from abroad. We suggest that *Ae. albopictus* in eastern USA behave as a metapopulation, in which genetic variation is consistently introduced to the area via human-aided dispersal, and where local genetic drift and selection lead to differentiated small breeding units interconnected across space and time, with admixture through secondary contact further increasing variability.

Acknowledgements

The authors would like to thank the following collaborators that contributed with samples for this project: M. Hutchinson (Pennsylvania Department of Agriculture); A. Lima, J. Smith, Y. Tan, and the Fairfax County Health Department, Fairfax, VA; M. Helwig, C.N. Boyer, and the Pennsylvania Department of Environmental Protection, Vector Management Program; K. Itokawa (Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo, Japan) and R. Uraki (National Center for Global Health and Medicine); C.R. Courtney and the DFS DC Public Health Laboratory; L.C. Harrington (Cornell U.); M. Doyle and the San Gabriel Mosquito and Vector Control District (California); M.P. Santoriello and C.L. Romano (Suffolk County Department of Health Services); and numerous seasonal staff at the different institutions for assistance with mosquito surveillance, identification and processing.

This publication was funded the Cooperative Agreement U01CK000509, awarded by the Center for Disease Control and Prevention. Its content is solely the responsibility of the authors and do not necessarily represent the official views of the Center for Disease Control and Prevention or the Department of Health and Human Services. The funding agency did not participate in the design of the study, collection, analysis, or interpretation of data. The study was partly funded by the National Science Foundation-Coupled Natural Human Systems award (DEB 1824807). CA was supported by the National Institutes of Health under award number R01AI132409 (PI: Caccone).

References

- Andreadis TG (2009) Failure of *Aedes albopictus* to overwinter following introduction and seasonal establishment at a tire recycling plant in the northeastern USA. Journal of the American Mosquito Control Association 25(1): 25–31. https://doi.org/10.2987/08-5813.1
- Andreadis TG, Thomas MC, Shepard JJ (2005) Identification guide to the mosquitoes of Connecticut. The Connecticut Agricultural Experiment Station, New Haven, CT.
- Armbruster PA (2016) Photoperiodic diapause and the establishment of *Aedes albopictus* (Diptera: Culicidae) in North America. Journal of Medical Entomology 53(5): 1013–1023. https://doi.org/10.1093/jme/tjw037
- Armstrong PM, Andreadis TG, Shepard JJ, Thomas MC (2017) Northern range expansion of the Asian tiger mosquito (*Aedes albopictus*): Analysis of mosquito data from Connecticut, USA. PLoS Neglected Tropical Diseases 11(5): e0005623. https://doi.org/10.1371/journal.pntd.0005623
- Barros J, Winkler FM, Velasco LA (2020) Assessing the genetic diversity in Argopecten nucleus (Bivalvia: Pectinidae), a functional hermaphrodite species with extremely low population density and self-fertilization: Effect of null alleles. Ecology and Evolution 10(9): 3919– 3931. https://doi.org/10.1002/ece3.6080
- Beebe NW, Ambrose L, Hill LA, Davis JB, Hapgood G, Cooper RD, Russell RC, Ritchie SA, Reimer LJ, Lobo NF, Syafruddin D, van den Hurk AF (2013) Tracing the tiger: Population

genetics provides valuable insights into the *Aedes* (*Stegomyia*) *albopictus* invasion of the Australasian Region. PLoS Neglected Tropical Diseases 7(8): e2361. https://doi.org/10.1371/journal.pntd.0002361

- Black IV WC, Ferrari JA, Rai KS, Sprenger D (1988) Breeding structure of a colonizing species: *Aedes albopictus* (Skuse) in the United States. Heredity 60(2): 173–181. https://doi. org/10.1038/hdy.1988.29
- Bock DG, Caseys C, Cousens RD, Hahn MA, Heredia SM, Hübner S, Turner KG, Whitney KD, Rieseberg LH (2015) What we still don't know about invasion genetics. Molecular Ecology 24(9): 2277–2297. https://doi.org/10.1111/mec.13032
- Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24(3): 621–631. https://doi. org/10.1093/molbev/msl191
- Cornuet JM, Luikart G (1997) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144(4): 2001–2014. https://doi.org/10.1093/genetics/144.4.2001
- Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. Heredity 93(5): 504–509. https://doi.org/10.1038/sj.hdy.6800545
- De Meeûs T (2018) Revisiting F₁₅, F₅₇, Wahlund effects, and null alleles. The Journal of Heredity 109(4): 446–456. https://doi.org/10.1093/jhered/esx106
- Delatte H, Desvars A, Bouétard A, Bord S, Gimonneau G, Vourc'h G, Fontenille D (2010) Blood-feeding behavior of *Aedes albopictus*, a vector of Chikungunya on La Réunion. Vector Borne and Zoonotic Diseases (Larchmont, N.Y.) 10(3): 249–258. https://doi. org/10.1089/vbz.2009.0026
- Dlugosch KM, Parker IM (2008) Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17(1): 431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x
- Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: Re- implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14(1): 209–214. https://doi. org/10.1111/1755-0998.12157
- Dray S, Dufour A (2007) The ade4 Package: Implementing the Duality Diagram for Ecologists. Journal of Statistical Software 22(4): 1–20. https://doi.org/10.18637/jss.v022.i04
- Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4(2): 359–361. https://doi.org/10.1007/s12686-011-9548-7
- Ersts P, Geographic Distance Matrix Generator v.1.2.3 (2016) American Museum of Natural History, Center for Biodiversity and Conservation. http://biodiversityinformatics.amnh. org/open_source/gdmg [accessed on 2021-8-16]
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14(8): 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x
- Facon B, Pointier JP, Jarne P, Sarda V, David P (2008) High genetic variance in life-history strategies within invasive populations by way of multiple introductions. Current Biology 18(5): 363–367. https://doi.org/10.1016/j.cub.2008.01.063

- Fonseca DM, Widdel AK, Hutchinson M, Spichiger SE, Kramer LD (2010) Fine-scale spatial and temporal population genetics of *Aedes japonicus*, a new US mosquito, reveal multiple introductions. Molecular Ecology 19(8): 1559–1572. https://doi.org/10.1111/j.1365-294X.2010.04576.x
- Gloria-Soria A, Brown JE, Kramer V, Hardstone Yoshimizu M, Powell JR (2014) Origin of the dengue fever mosquito, *Aedes aegypti*, in California. PLoS Neglected Tropical Diseases 8(7): e3029. https://doi.org/10.1371/journal.pntd.0003029
- Gloria-Soria A, Ayala D, Bheecarry A, Calderon-Arguedas O, Chadee DD, Chiappero M, Coetzee M, Elahee KB, Fernandez-Salas I, Kamal HA, Kamgang B, Khater EIM, Kramer LD, Kramer V, Lopez-Solis A, Lutomiah J, Martins Jr A, Micieli MV, Paupy C, Ponlawat A, Rahola N, Rasheed SB, Richardson JB, Saleh AA, Sanchez-Casas RM, Seixas G, Sousa CA, Tabachnick WJ, Troyo A, Powell JR (2016) Global genetic diversity of *Aedes aegypti*. Molecular Ecology 25(21): 5377–5395. https://doi.org/10.1111/mec.13866
- Gloria-Soria A, Soghigian J, Kellner D, Powell JR (2019) Genetic diversity of laboratory strains and implications for research: The case of *Aedes aegypti*. PLoS Neglected Tropical Diseases 13(12): e0007930. https://doi.org/10.1371/journal.pntd.0007930
- Gloria-Soria A, Payne AF, Bialosuknia SM, Stout J, Mathia N, Eastwood G, Ciota AT, Kramer LD, Armstrong PM (2021) Vector competence of *Aedes albopictus* populations from the Northeastern United States for chikungunya, dengue, and Zika viruses. The American Journal of Tropical Medicine and Hygiene 104(3): 1123. https://doi.org/10.4269/ ajtmh.20-0874
- Grandadam M, Caro V, Plumet S, Thiberge J-M, Souarés Y, Failloux A-B, Tolou HJ, Budelot M, Cosserat D, Leparc-Goffart I, Després P (2011) Chikungunya virus, Southeastern France. Emerging Infectious Diseases 17(5): 910–913. https://doi.org/10.3201/eid1705.101873
- Gratz NG (2004) Critical review of the vector status of *Aedes albopictus*. Medical and Veterinary Entomology 18(3): 215–227. https://doi.org/10.1111/j.0269-283X.2004.00513.x
- Hahn MB, Eisen RJ, Eisen L, Boegler KA, Moore CG, McAllister J, Savage HM, Mutebi JP (2016) Reported distribution of *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* in the United States, 1995–2016 (Diptera: Culicidae). Journal of Medical Entomology 53(5): 1169–1175. https://doi.org/10.1093/jme/tjw072
- Hahn MB, Eisen L, McAllister J, Savage HM, Mutebi JP, Eisen RJ (2017) Updated reported distribution of *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in the United States, 1995–2016. Journal of Medical Entomology 54(5): 1420–1424. https://doi.org/10.1093/jme/tjx088
- Handley LJL, Estoup A, Evans DM, Thomas CE, Lombaert E, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. BioControl 56(4): 409–428. https:// doi.org/10.1007/s10526-011-9386-2
- Hawley WA, Reiter P, Copeland RS, Pumpuni CB, Craig Jr GB (1987) Aedes albopictus in North America: Probable introduction in used tires from northern Asia. Science 236(4805): 1114–1116. https://doi.org/10.1126/science.3576225
- Jaspers C, Ehrlich M, Pujolar JM, Künzel S, Bayer T, Limborg MT, Lombard F, Browne WE, Stefanova K, Reusch TB (2021) Invasion genomics uncover contrasting scenarios of genetic diversity in a widespread marine invader. Proceedings of the National Academy of Sciences of the United States of America 118(51): e2116211118. https://doi.org/10.1073/pnas.2116211118

- Jombart T (2008) adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics 24(11): 1403–1405. https://doi.org/10.1093/bioinformatics/btn129
- Jorde PE, Ryman N (2007) Unbiased estimator for genetic drift and effective population size. Genetics 177(2): 927–935. https://doi.org/10.1534/genetics.107.075481
- Kache PA, Eastwood G, Collins-Palmer K, Katz M, Falco RC, Bajwa WI, Armstrong PM, Andreadis TG, Diuk-Wasser MA (2020) Environmental determinants of *Aedes albopictus* abundance at a northern limit of its range in the United States. The American Journal of Tropical Medicine and Hygiene 102(2): 436–447. https://doi.org/10.4269/ ajtmh.19-0244
- Kalinowski ST (2005) HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5(1): 187–189. https://doi. org/10.1111/j.1471-8286.2004.00845.x
- Kalinowski ST, Wagner AP, Taper ML (2006) ML-Relate: A computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes 6(2): 576– 579. https://doi.org/10.1111/j.1471-8286.2006.01256.x
- Kambhampati S, Black WC IV, Rai KS (1991) Geographic origin of the US and Brazilian Aedes albopictus inferred from allozyme analysis. Heredity 67(1): 85–94. https://doi. org/10.1038/hdy.1991.67
- Kobayashi M, Nihei N, Kurihara T (2002) Analysis of northern distribution of *Aedes albopictus* (Diptera: Culicidae) in Japan by geographical information system. Journal of Medical Entomology 39(1): 4–11. https://doi.org/10.1603/0022-2585-39.1.4
- Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15(5): 1179–1191. https://doi.org/10.1111/1755-0998.12387
- Kotsakiozi P, Richardson JB, Pichler V, Favia G, Martins AJ, Urbanelli S, Armbruster PA, Caccone A (2017) Population genomics of the Asian tiger mosquito, *Aedes albopictus*: Insights into the recent worldwide invasion. Ecology and Evolution 7(23): 10143–10157. https://doi.org/10.1002/ece3.3514
- Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, Hendrickx G, Schaffner F, Elyazar IRF, Teng H-J, Brady OJ, Messina JP, Pigott DM, Scott TW, Smith DL, Wint GRW, Golding N, Hay SI (2015) The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLife 4: e08347. https://doi.org/10.7554/eLife.08347
- Kulasekera VL, Kramer L, Nasci RS, Mostashari F, Cherry B, Trock SC, Glaser C, Miller JR (2001) West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. Emerging Infectious Diseases 7(4): 722–725. https://doi.org/10.3201/ eid0704.017421
- Linthicum KJ, Kramer VL, Madon MB, Fujioka K (2003) Introduction and potential establishment of *Aedes albopictus* in California in 2001. Journal of the American Mosquito Control Association 19(4): 301–308.
- Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. Trends in Ecology & Evolution 20(5): 223–228. https://doi.org/10.1016/j. tree.2005.02.004

- Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. The Journal of Heredity 89(3): 238–247. https://doi.org/10.1093/jhered/89.3.238
- Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010) Estimation of census and effective population sizes: The increasing usefulness of DNA-based approaches. Conservation Genetics 11(2): 355–373. https://doi.org/10.1007/s10592-010-0050-7
- Manni M, Guglielmino CR, Scolari F, Vega-Rúa A, Failloux AB, Somboon P, Lisa A, Savini G, Bonizzoni M, Gomulski LM, Malacrida AR, Gasperi G (2017) Genetic evidence for a worldwide chaotic dispersion pattern of the arbovirus vector, *Aedes albopictus*. PLoS Neglected Tropical Diseases 11(1): e0005332. https://doi.org/10.1371/journal.pntd.0005332
- Maruyama T, Fuerst PA (1985) Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111(3): 675–689. https://doi.org/10.1093/genetics/111.3.675
- Medley KA, Jenkins DG, Hoffman EA (2015) Human-aided and natural dispersal drive gene flow across the range of an invasive mosquito. Molecular Ecology 24(2): 284–295. https:// doi.org/10.1111/mec.12925
- Meglécz E, Pech N, Gilles A, Dubut V, Hingamp P, Trilles A, Grenier R, Martin JF (2014) QDD version 3.1: A user-friendly computer program for microsatellite selection and primer design revisited: Experimental validation of variables determining genotyping success rate. Molecular Ecology Resources 14(6): 1302–1313. https://doi.org/10.1111/1755-0998.12271
- Meirmans PG (2020) Genodive version 3.0: Easy-to-use software for the analysis of genetic data of diploids and polyploids. Molecular Ecology Resources 20(4): 1126–1131. https:// doi.org/10.1111/1755-0998.13145
- Metselaar D, Grainger CR, Oei KG, Reynolds DG, Pudney M, Leake CJ, Tukei PM, d'Offay RM, Simpson DIH (1980) An outbreak of type 2 dengue fever in the Seychelles, probably transmitted by *Aedes albopictus* (Skuse). Bulletin of the World Health Organization 58(6): 937.
- Moore CG (1999) *Aedes albopictus* in the United States: Current status and prospects for further spread. Journal of the American Mosquito Control Association 15(2): 221–227.
- Nawrocki SJ, Hawley WA (1987) Estimation of the northern limits of distribution of *Aedes albopictus* in North America. Journal of the American Mosquito Control Association 3(2): 314–317.
- Nei M, Tajima F (1981) Genetic drift and estimation of effective population size. Genetics 98(3): 625–640. https://doi.org/10.1093/genetics/98.3.625
- Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution; International Journal of Organic Evolution 29(1): 1–10. https:// doi.org/10.1111/j.1558-5646.1975.tb00807.x
- Niebylski ML, Savage HM, Nasci RS (1994) Blood hosts of *Aedes albopictus* in the United States. Journal of the American Mosquito Control Association 0(3): 447–450.
- Palatini U, Masri RA, Cosme LV, Koren S, Thibaud-Nissen F, Biedler JK, Krsticevic F, Johnston JS, Halbach R, Crawford JE, Antoshechkin I, Failloux A-B, Pischedda E, Marconcini M, Ghurye J, Rhie A, Sharma A, Karagodin DA, Jenrette J, Gamez S, Miesen P, Masterson P, Caccone A, Sharakhova MV, Tu Z, Papathanos PA, Van Rij RP, Akbari OS, Powell J, Phil-

lippy AM, Bonizzoni M (2020) Improved reference genome of the arboviral vector *Aedes albopictus*. Genome Biology 21(1): 1–29. https://doi.org/10.1186/s13059-020-02141-w

- Parker AT, McGill K, Allan BF (2020) Container type affects mosquito (Diptera: Culicidae) oviposition choice. Journal of Medical Entomology 57(5): 1459–1467. https://doi. org/10.1093/jme/tjaa045
- Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D (2009) Aedes albopictus, an arbovirus vector: From the darkness to the light. Microbes and Infection 11(14–15): 1177–1185. https://doi.org/10.1016/j.micinf.2009.05.005
- Paupy C, Kassa Kassa F, Caron M, Nkoghé D, Leroy EM (2012) A chikungunya outbreak associated with the vector *Aedes albopictus* in remote villages of Gabon. Vector Borne and Zoonotic Diseases (Larchmont, N.Y.) 12(2): 167–169. https://doi.org/10.1089/ vbz.2011.0736
- Pless E, Gloria-Soria A, Evans BR, Kramer V, Bolling BG, Tabachnick WJ, Powell JR (2017) Multiple introductions of the dengue vector, *Aedes aegypti*, into California. PLoS Neglected Tropical Diseases 11(8): e0005718. https://doi.org/10.1371/journal.pntd.0005718
- Pollak E (1983) A new method for estimating the effective population size from allele frequency changes. Genetics 104(3): 531–548. https://doi.org/10.1093/genetics/104.3.531
- Porretta D, Gargani M, Bellini R, Calvitti M, Urbanelli S (2006) Isolation of microsatellite markers in the tiger mosquito *Aedes albopictus* (Skuse). Molecular Ecology Notes 6(3): 880–881. https://doi.org/10.1111/j.1471-8286.2006.01384.x
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155(2): 945–959. https://doi.org/10.1093/genetics/155.2.945
- R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. The Journal of Heredity 86(3): 248–249. https://doi.org/10.1093/ oxfordjournals.jhered.a111573
- Rezza G (2012) *Aedes albopictus* and the reemergence of Dengue. BMC Public Health 12(1): 1–3. https://doi.org/10.1186/1471-2458-12-72
- Rochlin I, Ninivaggi DV, Hutchinson ML, Farajollahi A (2013) Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in Northeastern USA: Implications for public health practitioners. PLoS ONE 8(4): e60874. https://doi.org/10.1371/journal. pone.0060874
- Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure. Molecular Ecology Notes 4(1): 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x
- Rousset F (2008) Genepop'007: A complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8(1): 103–106. https://doi. org/10.1111/j.1471-8286.2007.01931.x
- Saarman NP, Gloria-Soria A, Anderson EC, Evans BR, Pless E, Cosme LV, Gonzalez-Acosta C, Kamgang B, Wesson DM, Powell JR (2017) Effective population sizes of a major vector of human diseases, *Aedes aegypti*. Evolutionary Applications 10(10): 1031–1039. https://doi. org/10.1111/eva.12508

- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annual Review of Ecology and Systematics 32(1): 305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037
- Sprenger P, Reiter D (1987) The used tire trade: A mechanism for the worldwide dispersal of container breeding mosquitoes. Journal of the American Mosquito Control Association 3: 494.
- Sprenger D, Wuithiranyagool T (1986) The discovery and distribution of *Aedes albopictus* in Harris County, Texas. Journal of the American Mosquito Control Association 2(2): 217–219.
- Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121(2): 379–391. https://doi.org/10.1093/genetics/121.2.379
- Waples RS, Do C (2008) Ldne: A program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8(4): 753–756. https://doi. org/10.1111/j.1755-0998.2007.02061.x
- Wei Y, Wang J, Song Z, He Y, Zheng Z, Fan P, Yang D, Zhou G, Zhong D, Zheng X (2019) Patterns of spatial genetic structures in *Aedes albopictus* (Diptera: Culicidae) populations in China. Parasites & Vectors 12(1): 1–15. https://doi.org/10.1186/s13071-019-3801-4
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution; International Journal of Organic Evolution 38(6): 1358–1370. https:// doi.org/10.1111/j.1558-5646.1984.tb05657.x
- Yee DA (2008) Tires as habitats for mosquitoes: A review of studies within the eastern United States. Journal of Medical Entomology 45(4): 581–593. https://doi.org/10.1603/0022-2585(2008)45[581:TAHFMA]2.0.CO;2
- Zhong D, Lo E, Hu R, Metzger ME, Cummings R, Bonizzoni M, Fujioka KK, Sorvillo TE, Kluh S, Healy SP, Fredregill C, Kramer VL, Chen X, Yan G (2013) Genetic analysis of invasive *Aedes albopictus* populations in Los Angeles County, California and its potential public health impact. PLoS ONE 8(7): e68586. https://doi.org/10.1371/journal.pone.0068586

Detailed collection information

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

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Aedes albopictus microsatellite primers used in this study

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

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Link: https://doi.org/10.3897/neobiota.78.84986.suppl2

Supplementary material 3

Microsatellite bins used to call alleles in Geneious v. 11.1.5 (Biomatters LTD)

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

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Allele numbers and allelic richness at 15 microsatellite loci used in this study

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: table (excel file)

- Explanation note: Allele numbers and allelic richness at 15 microsatellite loci used in this study obtained using rarefaction to correct for unequal sample sizes (N = 30 genes) in HP-RARE (Kalinowski 2005).
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Link: https://doi.org/10.3897/neobiota.78.84986.suppl4

Supplementary material 5

Probability of a recent bottleneck at each *Aedes albopictus* location, under the infinite allele model (IAM) and the two-phase model (TPM) with variance of 0.36 Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: table (excel file)

- Explanation note: Probability of a recent bottleneck at each *Aedes albopictus* location, under the infinite allele model (IAM) and the two-phase model (TPM) with variance of 0.36; as estimated using the software Bottleneck v. 1.2.02 (Cornuet and Luikart, 1997). The Wilcoxon sign-rank test (Luikart et al. 1998) was used to determine significance. Values in bold indicate significant differences after Bonferroni multiple test correction.
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Kinship analysis

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: table (excel file)

- Explanation note: Summary of pedigree relationships within *Aedes albopictus* collections. Unrelated (U), half-siblings (HS), full-siblings (FS), and parent-offspring (PO), as estimated by ML-Relate (Kalinowski et al. 2006). Values in bold highlight collections with the frequency of first-degree relatives above 5%.
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Link: https://doi.org/10.3897/neobiota.78.84986.suppl6

Supplementary material 7

Isolation by distance analyses (IBD)

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: table (excel file)

Explanation note: Matrices of geographic distance in meters and genetic distance as Fst. Results from Mantel tests for *Aedes albopictus* in the northeastern USA; the I-95 interstate corridor from Virginia (VA) to CT; the northeastern expansion front (New York, Connecticut, Massachusetts); and through Connecticut and New York.

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Genetic clusters inferred from all Connecticut collections using discriminant analysis of principal components in the ADEGENET package

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: table (excel file)

- Explanation note: Genetic clusters inferred from all Connecticut collections using discriminant analysis of principal components in the ADEGENET package (Jombart 2008) in R v. 3.2.2. (R Core Team 2018).
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Link: https://doi.org/10.3897/neobiota.78.84986.suppl8

Supplementary material 9

Latitude of each northeastern USA Aedes albopictus location

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: figure (pdf file)

- Explanation note: Latitude of each northeastern USA *Aedes albopictus* location plotted against A its observed heterozygosity (Ho) and B allelic richness estimated by rarefaction (N = 30). Linear regression in R v. 3.2.2. (R Core Team 2018) indicates a correlation between latitude and Ho (adjusted R2 = 0.13, F(1,27), p = 0.03) but not between latitude and allelic richness (adjusted R2 = 0.04, F(1,27), p = 0.15).
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Estimates of effective population size based of Connecticut populations obtained with NeEstimator (Do et al. 2014)

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: figure (pdf file)

- Explanation note: Estimates of effective population size based of Connecticut populations obtained with NeEstimator (Do et al. 2014) using A population pairs using the two-sample Waples (1989) method and three options for computing the standardized variance in allele frequency, F [Fe (Nei and Tajima 1981); Fk (Pollak 1983); and Fs (Jorde and Ryman 2007)]; and B a single population sample using the biascorrected version of the linkage disequilibrium method Waples and Do (2008). Mean effective population size estimates (Ne), 95% confidence intervals (CI) are displayed by locality. The average Ne across all estimates is displayed with a dashed (harmonic mean) and dotted (arithmetic mean) horizontal lines. Note that the arithmetic mean of B is missing as it falls outside the plotted area (Ne = 2,337.5).
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Population structure of *Aedes albopictus* from the United States and Japan based on 15 microsatellite markers

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: figure (pdf file)

- Explanation note: A STRUCTURE plot with each individual represented by a vertical bar. The height of each bar is the probability of assignment to each of K = 3 genetic clusters (indicated by different colors). B Discriminant analysis of principal components (DAPC).
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Link: https://doi.org/10.3897/neobiota.78.84986.suppl11

Supplementary material 12

Population structure of *Aedes albopictus* at the United States northeastern invasion front (New York, Connecticut, Massachusetts) based on 15 microsatellite markers Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: figure (pdf file)

- Explanation note: Population structure of *Aedes albopictus* at the United States northeastern invasion front (New York, Connecticut, Massachusetts) based on 15 microsatellite markers. A Discriminant analysis of principal components (DAPC) and B STRUCTURE plot with each individual represented by a vertical bar. The height of each bar is the probability of assignment to each of K = 3 and K = 6 genetic clusters (indicated by different colors).
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Geographic genetic differentiation (IBD: isolation by distance) across the Northeast USA

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: figure (pdf file)

- Explanation note: Genetic distance is given as the linearized Fst [Fst/(1/Fst)] and geographic distance is provided in kilometers (Km).
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Link: https://doi.org/10.3897/neobiota.78.84986.suppl13

Supplementary material 14

Population structure on *Aedes albopictus* samples from all Connecticut samples (no temporal series) based on 15 microsatellite markers

Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: figure (pdf file)

- Explanation note: A STRUCTURE plot with each individual represented by a vertical bar. The height of each bar is the probability of assignment to each of K = 3 genetic clusters (indicated by different colors). B Discriminant analysis of principal components (DAPC).
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Inferred genetic clusters from *Aedes albopictus* of the Connecticut temporal series Authors: Andrea Gloria-Soria, Talya Shragai, Alexander T. Ciota, Todd B. Duval, Barry W. Alto, Ademir J. Martins, Kathleen M. Westby, Kim A. Medley, Isik Unlu, Scott R. Campbell, Malgorzata Kawalkowski, Yoshio Tsuda, Yukiko Higa, Nicholas Indelicato, Paul T. Leisnham, Adalgisa Caccone, Philip M. Armstrong

Data type: figure (pdf file)

- Explanation note: Inferred genetic clusters from *Aedes albopictus* of the Connecticut temporal series using Discriminant Analysis of Principal Components in ADE-GENET (Jombart 2008)
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Size distribution and reproductive phenology of the invasive Burmese python (Python molurus bivittatus) in the Greater Everglades Ecosystem, Florida, USA

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Academic editor: Wolfgang Rabitsch | Received 22 August 2022 | Accepted 8 November 2022 | Published 23 November 2022

Citation: Currylow AF, Falk BG, Yackel Adams AA, Romagosa CM, Josimovich JM, Rochford MR, Cherkiss MS, Nafus MG, Hart KM, Mazzotti FJ, Snow RW, Reed RN (2022) Size distribution and reproductive phenology of the invasive Burmese python (*Python molurus bivittatus*) in the Greater Everglades Ecosystem, Florida, USA. NeoBiota 78: 129–158. https://doi.org/10.3897/neobiota.78.93788

Abstract

The design of successful invasive species control programs is often hindered by the absence of basic demographic data on the targeted population. Establishment of invasive Burmese pythons (*Python molurus bivittatus*) in the Greater Everglades Ecosystem, Florida USA has led to local precipitous declines (> 90%) of mesomammal populations and is also a major threat to native populations of reptiles and birds. Efforts to control this species are ongoing but are hampered by the lack of access to and information on the expected biological patterns of pythons in southern Florida. We present data from more than 4,000 wild Burmese

^{*} These authors contributed equally to this work.

pythons that were removed in southern Florida over 26 years (1995–2021), the most robust dataset representing this invasive population to date. We used these data to characterize Burmese python size distribution, size at maturity, clutch size, and seasonal demographic and reproductive trends. We broadened the previously described size ranges by sex and, based on our newly defined size-stage classes, showed that males are smaller than females at sexual maturity, confirmed a positive correlation between maternal body size and potential clutch size, and developed predictive equations to facilitate demographic predictions. We also refined the annual breeding season (approx.100 days December into March), oviposition timing (May), and hatchling emergence and dispersal period (July through October) using correlations of capture morphometrics with observations of seasonal gonadal recrudescence (resurgence) and regression. Determination of reproductive output and timing can inform population models and help managers arrest population growth by targeting key aspects of python life history. These results define characteristics of the species in Florida and provide an enhanced understanding of the ecology and reproductive biology of Burmese pythons in their invasive Everglades range.

Keywords

clutch size, ecological timing, Everglades National Park, gonadal development, invasive species, morphometrics, oviposition, reproductive potential, reptile, size at maturity, snake

Introduction

Invasive species cause some of the most ecologically damaging and costly impacts on ecosystems (Fantle-Lepczyk et al. 2022). They can outcompete and consume natives, impacting species across trophic levels. Invasive reptiles, and snakes in particular, comprise some of the most infamous species with documented deleterious trophic impacts in multiple systems. For example, the invasive brown treesnake (Boiga irregularis) in Guam's tropical ecosystem has decimated native vertebrate populations on the island, driving many to extirpation or extinction (Savidge 1987; Wiles 1987; Rodda and Fritts 1992), and leading to seed dispersal loss and potential changes in forest structure (Rogers et al. 2017). Recently, the common wolf snake (Lycodon capucinus) has been linked to the precipitous decline of lizard species on Christmas Island, AUS (Emery et al. 2021). In continental USA, the invasive Burmese python (Python molurus bivittatus) has been linked to severe declines of mammalian prey populations and the accompanying ecological impacts in southern Florida's Greater Everglades Ecosystem (Dorcas et al. 2012; Hoyer et al. 2017; Burkett-Cadena et al. 2021; Taillie et al. 2021). The success of Burmese pythons is likely due, in part, to their size coupled with their reproductive biology (Willson et al. 2011; Card et al. 2018). Though some work has been done describing instances of python demographics and reproduction across its invasive range in southern Florida (Snow et al. 2007b; Krysko et al. 2008), stronger inferences can be made with increased sample sizes, particularly from the wild population.

The vast landscape and inaccessible habitats of the Greater Everglades Ecosystem paired with extremely low detection probabilities of the Burmese python (est. < 5%; Nafus et al. 2020) have stymied the accumulation of robust, long-term ecological and biological data of the species. These large predatory snakes may have been introduced

to, and established in, Everglades National Park by the mid-1980's or even earlier (Willson et al. 2011). Since then, the species has spread across the entire southern portion of Florida and has been implicated in severe population declines of some midsized mammals in the area (Dorcas et al. 2012; McCleery et al. 2015), as well as in changes to vector-borne disease transmission dynamics (Hoyer et al. 2017; Burkett-Cadena et al. 2021), food-web perturbations (Reichert et al. 2017), and spillover of parasites to native species (Miller et al. 2018). However, little is known about much of the Burmese pythons' biology or reproductive phenology in the wild (but see Krysko et al. 2012; Smith et al. 2015). Several detection and removal strategies have been tested and/or implemented over the years (e.g., Reed et al. 2011; Hunter et al. 2015; Falk et al. 2016; Parker et al. 2021), but captures remain low, and population-level information or temporal trends remain limited. However, understanding this species' morphology and reproduction in its entire invasive range which spans southern Florida will provide researchers with a demographic baseline which can aid in the development of new, biologically targeted control tools.

To determine if control efforts are effective, an invasive species' population size needs to be estimable so that measured changes can be documented. Population abundances and survival changes across ontogeny can be estimated using predictive mathematical tools such as stage (size or age class) structured population matrix models (Hanley et al. 2019a) using basic data on phenotypic and reproductive parameters. Yet, for Burmese pythons, no long-term or robust reproductive stage data exist, and the basic questions about population dynamics or growth potential in the wild (e.g., Hanley et al. 2019b; Currylow et al. 2022a) are almost completely unknown. Additionally, inconsistent methods across studies and over time exacerbate issues with defining species size-stage classes through size at maturity or identifying phenotypic variation (Feldman and Meiri 2012). For example, snake studies often measure animal body size using total length (tip of snout to tip of tail; e.g., Burger et al. 1987; Snow et al. 2007b) while others use snout-vent length (SVL; tip of snout to vent; e.g., Reed et al. 2016; Josimovich et al. 2021). This difference is important to note when attempting to understand or compare morphologies between the sexes because male squamates store their hemipenes, inverted, in the ventral tail base, causing males to have generally longer tails (and therefore relatively longer total lengths) than females of the same SVL (Fitch 1960; Shine et al. 1999).

Our objectives were to characterize size at maturity, reproductive status, and temporal trends in wild Burmese python populations distributed within the Greater Everglades Ecosystem. We compiled 26 years of data on a variety of morphometrics collected from more than 4,000 python captures resulting from several invasive species removal efforts and studies across southern Florida between 1995 and 2021 (Currylow et al. 2022b). With these data we aimed to address the following hypotheses: if wild Burmese pythons 1) exhibit sexual dimorphism, then females will be larger than males at sexual maturity; 2) have clutch sizes that depend on maternal body sizes, then large clutches will be associated with larger body sizes; 3) synchronously hatch and disperse from nests, then they will appear on the landscape en masse within one period; and 4) exhibit a regular annual reproductive cycle, then gonadal states of each sex will uniformly differ across months. We were able to use this data compilation to describe morphologies and gonadal states, define size distributions, develop predictive size equations, characterize size-stage classes, identify reproductive status, and infer reproductive potential (size at maturity and potential clutch size). We also investigated those data across time to better understand the seasonality of reproductive trends and hatchling emergence in southern Florida, USA.

Methods

Study site

Our study site included private, state, and federal lands in Florida where invasive Burmese pythons have established. The area encompasses much of the Greater Everglades Ecosystem in Florida, USA from south of Lake Okeechobee through the Florida Keys. From east to west coasts, southern Florida is surrounded by a complex of roads and man-made waterways. The Everglades is composed of limestone bedrock covered by ridge and slough habitats and mangrove complexes (Lodge 2017). These areas are characterized by freshwater marshes, tree islands, tropical hardwood hammocks, pinelands, cypress forests, mixed and mangrove swamps, prairies, coastal lowlands, and estuarine systems (Lodge 2017).

Specimen acquisition and processing

Burmese python specimens used in this study were sourced year-round through nonprofit organization, Federal, and State funded research, removal, volunteer, and management programs and private individuals between 1995 and 2021 (see citations herein). We report morphometric and/or reproductive information from 4,007 specimens from across southern Florida, many of which were collected on linear features such as roadways and levees (Fig. 1). We recorded location of capture (UTM WGS84), date of capture/death, sex, weight (grams), snout vent length (SVL, cm), tail length (cm), tail completeness (intact or broken/incomplete), total length (cm), reproductive status based on gonad developmental state (see below), and number of most developed ovarian structure/oviductal eggs. To allow for future comparisons of our data to other records, we recorded both SVL and tail length when possible, tested for differences between the sexes, and aimed to provide equations for the estimation of one body size measurement using the other.

Reproductive parameters

We recorded reproductive data for pythons during necropsies and classified reproductive status using a visual assessment of the specimens' most developed gonadal structure



Figure 1. Map of removal locations of 4,007 Burmese python (*Python molurus bivittatus*) specimens concentrated along roadways and levees across southern Florida, USA from 1995 through 2021. The Florida base map was compiled from several data providers, including the U.S. Geological Survey, National Oceanic and Atmospheric Administration, National Park Service, Garmin, and Esri to a scale of ca. 1:70 kilometer (ArcGIS Desktop 10.8.1 version 10.8.1.14362).

(following Aldridge 1979; Seigel et al. 2001). Gonad developmental state categories included: undeveloped (no follicles/oviductal eggs or testes categorized or identified), primary follicles (small, preliminary or pre-vitellogenic oocytes), secondary follicles (pre-ovulatory vitellogenic follicles), oviductal eggs (post-ovulatory/ovigerous), flaccid testes (testes distinguished but not turgid), semi-turgid testes, and turgid testes.

We discovered and report information from 13 Burmese python nests laid between 2006 and 2022. Five of these have been partially described previously (see Snow et al. 2007a; Snow et al. 2010; Hanslowe et al. 2016; Wolf et al. 2016; Currylow et al. 2022c), and we therefore cite those with the comparisons to other nests on oviposition phenology, maternal python SVL, and clutch sizes for summary purposes. In six instances, we discovered and monitored oviposition in wild pythons in the field as part of other studies. Five of the nests were found after hatching and clutch sizes were inferred by counting eggshells.

Statistical analyses

Size distribution

To process the raw dataset for analyses, we grouped and averaged all data from any single animal that was measured multiple times within a single month. Where available, we summarized the median sizes (SVL and weights) of python captures and/or separated pythons by sex. We regressed SVL by weight of each sex and fit a second-degree polynomial to visualize the non-linear relationship with an r-squared value.

Size at maturity

To determine if wild Burmese pythons exhibit sexual size dimorphism where females are larger than males at sexual maturity with the non-normal data, we used the nonparametric Wilcoxon method adjusted for multiple pairwise comparisons to test for mean differences of SVL between stages of gonad developmental state (undeveloped, flaccid testes, semi-turgid testes, turgid testes, primary follicles, secondary follicles, or oviductal eggs). Using the reproductive stages that correlated with size ranges, we then categorized size-stage classes (juvenile, sub-adult, adult) to represent general sizes at maturity for each sex. For those individuals with intact tails, we tested if tail length was proportionally different across size-stage classes using a Standard Least Squares Restricted Maximum Likelihood model (REML) with size-stage class as the predictor variable and the proportion of the tail length to total body length for individuals as the response variable with individual identification as a random effect to account for repeated measures. We then tested if the proportion of tail length to total length was different between the sexes overall, as well as within each size-stage class group using REMLs. To determine predictive equations that could be used to standardize and interpret records across studies, we fit a regression line between tail length and SVL or SVL and total length for each sex for those individuals with intact tails and known sexes.

Clutch size

To determine if wild Burmese pythons have larger clutch sizes as they grow to larger body sizes, we evaluated potential clutch size for correlation with maternal body size (SVL). We used females having secondary follicles or oviductal eggs at necropsy, enumerating them as a maximum clutch size proxy. We then performed a regression analysis to determine a predictive equation for the relationship. We further investigated if there was a difference in the potential clutch size estimates using only pre-ovulatory secondary follicles vs only oviductal eggs. We again performed regression analyses for each of these potential clutch size estimates to maternal body size to refine predictive equations. For the nests we discovered from 2006 through 2022 that were associated with known females, we regressed female size (SVL) of maternal pythons with the total number of oviposited eggs and fit a linear regression to determine a predictive equation for the relationship.

Seasonal trends

To evaluate if wild hatchling Burmese pythons synchronously hatch and disperse, we investigated seasonality (monthly variation) of morphometric trends (size and reproductive parameters). We used a standard least squares regression model fit with the restricted maximum likelihood method using SVL as the response variable and month as the predictor variable. We included individual python identification as a random effect to account for repeated measures of some individuals. When investigating juvenile python size classes to identify temporal trends in hatching or emergence seasonality, we again used the above-described model but now included weight (g) as a response variable and followed model tests with a least squares means Tukey HSD post hoc analysis to detect significant differences among months. We used a bivariate nonparametric probability density plot to visualize the spread, correlation, and skewness of the annual juvenile size data. We then used the contour polygons to identify the characteristic annual size distinctions within the juvenile size-stage class as young-of-year (YoY; hatchling pythons emerging before calendar-year end) and older juveniles.

To determine if Burmese pythons follow a regular annual reproductive cycle in southern Florida, we modeled adult reproductive cycles of each sex over time using logistic regression. We performed a chi-square test of independence to assess the relationship between gonadal state and observation month to understand annual cycles in gonadal recrudescence (where females' follicles become markedly and heavily vitellogenic and males' testes become engorged and turgid) and regression. For all other analyses where we compared between two groups, we used pooled t-tests or chi squared analyses depending on data type. For analyses where more than two variables were investigated, we used analyses of variance followed by Tukey HSD post hoc analyses where appropriate.

Sample sizes varied across years and for different analyses depending on available data, therefore both year ranges and sample sizes are specified for each figure and analysis. All analyses were carried out using JMP statistical software (SAS Institute Inc 2021) and significance was determined at $\alpha \leq 0.05$. The data used in this manuscript are available as a USGS data release (Currylow et al. 2022b).

Results

We collected necropsy data from every specimen based on specimen condition, staffing, and data needs over the years (1995–2021; Suppl. material 1). Due to the nature of the collection of the dataset, sample sizes for each analysis are stated and may vary based on the data that were collected at the time. Of the 4,007 individual pythons represented in the dataset, 126 were morphometrically remeasured over multiple months (2–19 months) before euthanasia as part of other projects and constitute 487 rows of the 4,378 total data rows on which we report here. All individual animals have only one set of reproductive status (gonadal state) measurements, as those necessitated destructive sampling (i.e., during necropsies). As the dataset reflects opportunistic captures and surrenders, these data may not represent the entirety of the populations' variation or demographic patterns (e.g., Falk et al. 2016).

Size distribution

Burmese pythons for which SVL was recorded ranged in size from 39.6 to 498.0 cm SVL (median = 167 cm, n = 3,938) and 40–84,800 g (median = 3,270 g, n = 4,191). Grouped by sex, females grew to larger sizes ranging 39.6–498.0 cm SVL (median = 165 cm, n = 1,740) and 50–84,800 g (median = 2,768 g, n = 1,765), males ranged 42.0–399.5 cm SVL (median = 173 cm, n = 2,046) and 50–44,000 g (median = 3,800 g, n = 2,212; Fig. 2) and those for which sex was indeterminable or was not recorded ranged 44.7–424.0 cm SVL (median = 62 cm, n = 148) and 40–63,100 g (median = 179 g, n = 214).

Size at maturity

We confirmed that wild Burmese pythons in Florida indeed exhibit sexual dimorphism whereby females are larger than males at sexual maturity, but also that males have longer tails than females of the same size. We found trends in python size (SVL and weight) by gonadal development state (Fig. 3). We subsequently used gonadal development and associated SVLs to identify size differences associated with reproductive status (non-reproductive or reproductively active; Table 1; overall model $F_{6,2117}$ = 755.1500, p < 0.0001), though some overlap occurs at the extremes or depending on season (see seasonal trends below). The SVLs of reproductive individuals were different between the sexes, where females were longer on average (mean = 297.8 cm SVL, SE = 4.0726, 95% confidence interval (CI) = 285.0–310.5 cm SVL, n = 138) than males (211.0 cm SVL, SE = 2.3882, 95% CI = 206.7–216.1 cm SVL, n = 399; $F_{1.535} = 334.3382$, p < 0.0001).

We used sizes associated with gonad developmental state from Table 1 to designate size-stage classes in each sex (Table 2). Primary follicles can be found in adult females throughout the year, but the smallest females with follicles were 151 cm SVL (see Table 1), so those below this threshold were considered juvenile females. Similarly, males may exhibit flaccid testes when not reproductively active, but the smallest males exhibiting semi-turgid testes were 125 cm SVL; males below this length were considered



Figure 2. Snout-vent lengths (SVL; cm) of Burmese pythons (*Python molurus bivittatus*) from 1995 through 2021 in southern Florida, USA. Females (magenta) grow larger and longer than males (blue). Individuals without sex recorded (grey) are limited to juvenile and subadult size-stage class for clarity. Second-degree polynomial goodness of fit lines shown by sex with sample sizes and R² values in parentheticals.



Figure 3. Female (left) and male (right) gonad developmental state by animal size data from necropsied Burmese pythons (*Python molurus bivittatus*) captured from 2003 through 2021 in southern Florida, USA. Snout-vent lengths (SVL, cm) are displayed as boxplots and mean body weights (g) are represented as the solid blue smoother line with 95% confidence buffer; sample sizes are listed across the top. Mean SVLs and weights were highest in pythons with more progressed gonadal development and were different from the smaller-sized pythons with less- or undeveloped gonadal states (see Table 1).

Table 1. Burmese python (*Python molurus bivittatus*) gonad developmental state (testes, follicles, or oviductal eggs) by quantiles of snout-vent lengths (SVL, cm). The sizes at gonad developmental state not connected by the same letter in the Mean SVL Comparisons column are significantly different (all other *P*-values \leq 0.0181). Data are from specimens collected from 2003 through 2021 in the Greater Everglades Ecosystem, Florida, USA.

Gonad Developmental State	n	Mean SVL Comparisons	Min	10%	25%	Median	75%	90%	Max	Reproductive Status
Undeveloped $\buildrel \otimes \buildrel \otimes$	835	А	42	57	60	67	106	157	283	Non-reproductive
∂ Flaccid Testes	368	В	58	133	165	187	212	232	360	Non-reproductive
∂ Semi-turgid Testes	246	С	125	167	186	205	229	251	347	Reproductive
👌 Turgid Testes	153	D	142	182	194	212	231	265	330	Reproductive
\bigcirc Primary Follicles	384	E	151	178	195	219	260	339	498	Non-reproductive
\bigcirc Secondary Follicles	80	F	184	206	239	276	380	424	478	Reproductive
♀ Oviductal Eggs	56	F	185	216	246	276	307	382	475	Reproductive

Table 2. Estimated size-stage class demarcations (snout-vent length; SVL, cm) and gonad developmental state

 (Table 1) and mean tail lengths by sex for invasive Burmese pythons (*Python molurus bivittatus*) in southern

 Florida, USA. Data are from specimens collected from 1995 through 2021 in the Greater Everglades Ecosystem.

Size-stage Class	$\stackrel{\bigcirc}{_{+}}$ Body Length (cm SVL)	% $\stackrel{\bigcirc}{_{\sim}}$ Tail to Total Length	් Body Length (cm SVL)	% 🕈 Tail to Total Length
Juvenile	39-150.9	12.6	42-124.9	12.5
Sub-adult	151-205.9	12.3	125-181.9	12.8
Adult	206+	11.8	182+	13.1

juvenile males. The smallest 10% of reproductively active females (developing secondary follicles or oviductal eggs) were approximately 206 cm SVL while the smallest 10% of males exhibiting turgid testes were approximately 182 cm SVL, so these thresholds were considered as transitional from sub-adults to adults of each sex (Table 2).

We used individuals with both SVL and complete tail length measurements to calculate the average tail length for each sex across size-stage classes (Table 2). Proportion of tail length to body length did not differ between the size-stage classes but did differ between the sexes ($F_{1,2450} = 204.4130$, p < 0.0001) and between the sexes of each size-stage class (juvenile = $F_{1,1207} = 3.9234$, p = 0.0478; sub-adults = $F_{1,539} = 67.3810$, p < 0.0001; adults = $F_{1,716.6} = 501.2598$, p < 0.0001). Further, we provide two expressions (EQ1: R² = 0.90, n = 287 and EQ2: R² = 0.75, n = 450) to approximate tail lengths using an SVL measurement for adults of each sex:

EQ1: Adult female tail length (cm) = 4.478214 + 0.1165135*SVL (cm)

EQ2: Adult male tail length (cm) = 4.5850061 + 0.1287574*SVL (cm)

For all length-size-stage class relationship equations and graphs by sex, see Suppl. material 2). To estimate sizes of only reproductive adults using only total length measurements (or vice versa with only SVL), we developed two expressions (EQ3: $R^2 > 0.99$, n = 58 and EQ4: $R^2 > 0.99$, n = 186) from the data:

EQ3: Reproductive adult females:

- a) SVL (cm) = -4.25341 + 0.8954965*Total Length (cm)
- b) Total Length (cm) = 4.4790281 + 1.1165112*SVL (cm)

EQ4: Reproductive adult males:

- a) SVL (cm) = -3.287218 + 0.8818756*Total Length (cm)
- b) Total Length (cm) = 4.5850061 + 1.1287574*SVL (cm)

Clutch sizes

We confirmed that wild female Burmese pythons in Florida overall have larger clutch sizes with larger body sizes, but that the number of secondary follicles is likely an overestimate of actual clutch sizes. We excluded individuals exhibiting egg retention (n = 5; see Anderson et al. 2022) in these analyses and results. We found a positive correlation in female body size (SVL) to potential clutch size (secondary follicles or oviductal eggs; $R^2 = 0.81$, n = 115, p < 0.0001; Suppl. material 3) that could be predicted using the following regression equation (EQ5):

EQ5: Potential clutch size = -46.95676 + 0.282554*SVL (cm)

However, potential clutch size was larger when enumerating secondary follicles (mean = 44.5, SE = 2.6641, 95% CI = 39.3–49.9, range = 8–103, n = 70) than oviductal eggs (mean = 30.8, SE = 3.0617, 95% CI = 24.7–36.8, range = 11–72, n = 53; $t_{121} = -3.68069$, p = 0.0004). The regression equations for each state (EQ6: R² = 0.81, n = 65 and EQ7: R² = 0.88, n = 51) are as follows:

EQ6: Potential clutch size of secondary follicles only = -46.46219 + 0.2907525*SVL (cm)

EQ7: Potential clutch size of oviductal eggs only = -35.8948 + 0.2306006*SVL (cm)

In our dataset, eight instances of known females were found associated with nests. We found that female SVL was positively correlated with the total number of oviposited eggs in a clutch, and developed an equation to describe the association (EQ8; $R^2 = 0.81$, DF = 7, *p* = 0.0023):

EQ8: Oviposited eggs (actual clutch size) = -31.9255 + 0.2152397*SVL (cm)

The number of oviposited eggs from 13 wild nests (Table 3) were 22-84 (mean = 49, SD = 22), the largest of which was from a nest discovered after hatching without a known maternal female (Nest 8; and therefore could not be associated with female

body sizes above). In 2021, we monitored two nests (Nests 5 & 6) and found that lay dates were both approximately 15 May (± 3 d; exact dates unknown due to sampling rate). One of those two nests was destroyed by a nest predator (Nest 5; see Currylow et al. 2022c), but the successful nest was ultimately incubated in the laboratory, commenced hatching approximately 63 d after being laid, and took 3 days to hatch completely (19–21 July, 2021). We found that all the known hatching dates were also in July (Table 3). Of the four nests with notes and hatched eggs (Table 3, Nests 6, 7, & 9), Nest 6 comprised 9 inviable of 40 total, Nest 7 comprised 9 inviable eggs of the 79 eggs total, and Nest 9 comprised 2 inviable of the 39 total. We noted that a small proportion of oviposited eggs in two of the nests were discolored, misshapen, and smaller than the rest in the clutch and proved to be inviable (Nest 6 = 6 of 40; Nest 10 = 1 of 24).

Seasonal trends

We found that there were changes in the size (SVL) of pythons captured across months that confirm annual seasonal patterns in both hatchling and adult Burmese pythons in Florida ($F_{11, 3375} = 164.7925$, p < 0.0001). Pythons were captured year-round (see Suppl. material 4), but most annual captures were juveniles between the months of July through October when the YoY emerge (Fig. 4). To see adult python captures separated out by sex across months, see Suppl. material 5.

Hatchling emergence

We confirmed that Burmese pythons synchronously hatch and disperse from nests during a discrete annual time period, but also that the YoY (those emerging before the calendar-year end) can be distinguished from small yearling pythons during this period from summer into the fall months in Florida. Of the nests we discovered dur-

Table 3. Opportunistically recorded parameters of Burmese python (*Python molurus bivittatus*) nests discovered during work in the Greater Everglades Ecosystem, Florida, USA from 2006 through 2022. SVL = Snout-vent length.

	Maternal SVL (cm)	Approx. lay date	Approx. hatch date	# Eggs or shells	Citation
Nest 1	414	5-17-2006	-	46	Snow et al. 2007a
Nest 2	286	-	7-2008	27	Snow et al. 2010
Nest 3	264	-	7-29-2009	22	Wolf et al. 2016
Nest 4	265	5-2015	7-02-2015	25	Hanslowe et al. 2016
Nest 5	396	5-13-2021	NA	64	Currylow et al. 2022c
Nest 6	321	5-18-2021	7-20-2021	40	Current Study
Nest 7	-	-	7-18-2021	79	Current Study
Nest 8	-	-	2020	84 (shells)	Current Study
Nest 9	315	-	7-13-2022	39	Current Study
Nest 10	260	-	7-30-2022	24	Current Study
Nest 11	-	-	-	74 (shells)	Current Study
Nest 12	-	-	-	71 (shells)	Current Study
Nest 13	-	-	-	46 (shells)	Current Study



Figure 4. Density histogram of the proportion of 3,908 Burmese pythons (*Python molurus bivittatus*) across months by size bins (in snout-vent length; SVL; in cm) from southern Florida, USA between 1995 and through 2021. Size bins generally correspond to size-stage class but vary between sexes (see text).

ing this work, all with known hatch dates hatched in July (Table 3). Further, when only looking at the juvenile size-stage class in the larger dataset, we found that the YoY captured during July – October (n = 1,486) were distinctly smaller (SVL) than other juvenile pythons on the landscape ($F_{11,1287} = 72.3710$, p < 0.0001). During these four months, median sizes for YoY hatchlings were 62.9 cm SVL (interquartile range 58.6–71.5 cm) and 125.0 g (interquartile range = 107.0–194.0 g; see Suppl. material 6). By November, juvenile pythons measured 93.5 cm SVL median (interquartile range 84.0–101.0 cm) at 548 g (interquartile range 360.0–680.0 g) and started to become indistinguishable from juvenile pythons from the previous year (Fig. 5).

Reproductive cycles

We also confirmed that adult Burmese pythons exhibit a regular annual reproductive cycle, but that we could further define reproductive seasonality of breeding, oviposition, incubation, and that not all individuals undergo these changes every year. We found that adult pythons in southern Florida exhibited annual reproductive cycles in the average monthly recrudescence and regressive states of the ovaries ($\chi^2_{33,383}$ = 290.435,

p < 0.0001) and testes ($\chi^2_{22,535} = 282.567$, p < 0.0001; Fig. 6). Of the 210 pythons having either turgid testes or preovulatory secondary follicles, 192 (91%) were found between December and March in both sexes (Fig. 6). We found that females may have primary follicles throughout the year (commonly in addition to secondary follicles or



Figure 5. Juvenile Burmese python (*Python molurus bivittatus*) captures (dark dots) across months of the year (scale in Julian days) by snout-vent length (SVL; cm) in southern Florida, USA (1995–2021). Shaded probability polygons represent 25%, 50% ,75%, and 99% data density contours. The separation of the dark lower right set of overlapping polygons from the rest of the points earlier in the year highlights the months of highest juvenile encounters (July through October) and their correlated spread of sizes over those months, helping to distinguish the smaller young-of-year (YoY) hatchlings from other juveniles. Outside the July into October timeframe, YoY and juveniles from the prior year cannot be confidently distinguished.



Figure 6. Annual reproductive cycle probability density contour plots of Burmese python (*Python molurus bivittatus*) females (top; n = 933 snakes) and males (bottom; n = 1,123 snakes) in southern Florida, USA. Python sizes (snout-vent length; SVL, cm) are shown across months of the year (scale in Julian days) and separated by observed gonad developmental state (colored density polygons at 25%, 50%, 75%, and 99% data contours) from necropsied individuals collected from 2003 through 2021. Grey shaded vertical band represents purported breeding season (approximately 100 days December into March) when seasonal gonadal recrudescence peaks (see text). The purple shaded vertical band for females represents hypothesized oviposition timing (e.g., initiation of nesting season) based on presence of oviductal eggs in specimens, field observations, and published accounts. Note: sample sizes are low for gravid females due to low encounter rates during nesting.

oviductal eggs) but exhibited gonadal recrudescence (i.e., vitellogenic/secondary follicles) during that December into March period in 70 of 85 instances. In males, we found individuals to have flaccid testes throughout the year, but we found males exhibited gonadal recrudescence most frequently beginning in November through March (semi-turgid testes in 162 of 189 instances) and turgid testes were found in December and into March (133 of 141 instances) followed by gonadal regression (Fig. 6). Because these time periods during which gonadal recrudescence occurred were correlated in both sexes and with numerous field observations of courtship and breeding (Smith et al. 2015; Smith et al. 2016), we further refine the southern Florida Burmese python breeding season here as lasting approximately 100 days, from early December into mid-March (Fig. 6).

Oviductal eggs were found in females from March through May (52 of 58 instances; Fig. 6). Of the nests we opportunistically encountered during this study (Table 3), all known lay dates were in May (oviposition timing), and all known hatch dates were in July (a two-month incubation period). Due to the presence of eggs in 5 of 58 female pythons outside of those months (e.g., August through December), we determined they had retained oviductal eggs from a prior reproductive season (see Anderson et al. 2022). One of these five females also showed signs of egg resorption in the month of August. Additionally, we recorded gonadal state in 184 adult females during the months of December through May, 67 of which we found to be in gonadal latency (i.e., non-reproductive, having non-developed follicles). Those 67 non-reproductive females (307.7 cm, SE = 6.3735) during this time, but there was no length difference when only looking at animals exceeding the upper 95% of the mean SVL (> 302.5 cm; n = 63) and 24% of those individuals were still not reproductive.

Discussion

The introduction and subsequent spread of invasive species is an enormous management issue that is complex (Vitousek et al. 1996), damaging, and costly (Diagne et al. 2021; Fantle-Lepczyk et al. 2022). Characteristics associated with invasiveness remain elusive (Hayes and Barry 2008) because of complex environment interactions and feedbacks with the invader (Gurevitch et al. 2011), but demographic processes (e.g., survival, growth, reproduction) are fundamental to population structure (Griffith et al. 2016), determining whether invasions proceed or fail. Despite the importance, basic components of reproductive phenology data are limited for many invasives, such as the cryptic Burmese python. In a system like the Greater Everglades Ecosystem which is known for high floral and faunal diversity, including dozens of threatened or endangered faunal species (Brown et al. 2006), knowledge of the biological traits of species can assist with prediction, prevention, and control of invasion (Govindarajulu et al. 2005). Here, we elucidated size distribution, size at maturity, potential clutch sizes, and seasonal trends in emergence and reproductive phenology of the highly invasive Burmese python.
Size distribution

Though there are some limitations (e.g., many of the specimens used in this study were captured while crossing roads or levees and may not be representative of the population as a whole), this is the first time a robust and long-term dataset has been available to describe the seasonal morphometric and reproductive trends of wild Burmese pythons and for the invasive population found in the Greater Everglades Ecosystem, FL, USA. Not all necropsy data were collected from every specimen due to specimen condition, staffing, or data needs, and such incomplete or inconsistent data collection methods can prevent comparisons across studies. Therefore, to facilitate cross-study and future comparisons, we present several equations and reference figures derived from a large sample size to define length relationships (i.e., total, tail, and snout-vent lengths; EQ1–EQ4; Fig. 2; Suppl. material 2).

We found that python size distribution is broader than previously estimated for this invasive population (Reed and Rodda 2009; de Vosjoli and Klingenberg 2012; Krysko et al. 2012) as our smallest hatchlings were under 40 cm SVL and the largest adults reached nearly 500 cm SVL (557 cm total length). In comparison, another dataset on hatchling Burmese python sizes reported a range from 58 to 66 cm SVL (Josimovich and Currylow 2021), though Snow et al. (2007b) reported a hatchling of 38 cm total length, they also stated that it was probably an inaccurate measurement. The average sizes of adult females in our dataset (277 cm SVL, 17,255 g) were 79% longer and nearly 4 times heavier than the average for males (155 cm SVL, 4,394 g). Only one python out of the longest 90 measurements in our dataset (those > 370 cm SVL; 409–557 cm total length; 17,950–84,800 g) was male (400 cm SVL and 44,000 g; no tail or total length was recorded). In comparison, the largest Burmese pythons from their native range have been reported to reach 579-610 cm total length (Wall 1921; Murphy and Henderson 1997; Snow et al. 2007b). Record-breaking female Burmese pythons from across southern Florida appear to incrementally increase the recorded maximum size each year according to media reports, but the largest male on record remains unmatched at 438 cm SVL (493 cm total length, 63,500 g; Easterling and Bartoszek 2019).

Size at maturity

As we hypothesized, our data support the sexual size dimorphism expected in size at maturity as summarized by Reed and Rodda (2009), though, our estimates appear to be somewhat smaller. Additionally, we are aware of only two confirmed accounts of size at maturity described in the literature, both in females (Wall 1921; Willson et al. 2014). We found that minimally-sized mature individuals are slightly shorter than previous estimates for males (ca. 208 cm total length vs. 210 cm reported by de Vosjoli and Klingenberg 2012 but see Reed and Rodda 2009) and for females (ca. 229 cm total length vs. 259 cm total length reported by Pope 1961). There are no other known records of verified minimum size at maturity in wild male Burmese pythons with the closest relative, the Indian python (*P. m. molurus*), only very recently reported from the native range to be 172 cm SVL (198 total length; Vishnu et al. 2021). Likewise

in females, a single prior report noted that the smallest reproductive female Burmese python known was found in May 2013 from the southern Florida population and reached only 210 cm total length with 11 oviductal eggs (Willson et al. 2014), and, very recently, another female of this size was reported to be gravid from the same population (Anderson et al. 2022).

While investigating size at maturity, we also found that there were differences in the proportion of tail length to body length between the sexes in every size-stage class. However, the relatively small difference (0.1%) was barely statistically significant in the juvenile size-stage class when reproductive organs are not yet developed, and the result is likely not biologically meaningful. Subadult differences grew somewhat, but only adult python tail proportions were consistently different in a functional way (i.e., hemipene storage). Adult female tails were shorter, averaging 11.8% of their total lengths compared to males whose tails were, on average, 13.1% of their total length. This proportion and the equations derived from the data (EQ1-EQ4) can aid in the evaluation of data from differing research reports that were previously incomparable due to inconsistent collection methods or measurements. For example, applying our proportion estimates and equations to the largest male in our dataset with only SVL recorded (400 cm SVL), we can estimate that his total length was approximately 452-456 cm. Further, using these equations, probable length can be estimated for individual specimens with damaged (i.e., incomplete) tails that would have otherwise rendered total length measurements indeterminable.

Clutch sizes

A critical factor in understanding population growth potential is the lifetime egg production and survival of females. To parameterize such models, researchers need to start with estimates of annual reproductive potential. We hypothesized that python potential clutch size would correlate with maternal body size, and we found the relationship could be estimated using maternal body size (in cm SVL; see EQ5). Though limited in sample size, we were able to show that true clutch size (oviposited eggs) also increased with maternal body size (in SVL; Table 3). However, we further found that a count of secondary follicles (often used to estimate clutch size at the time of python removal) is likely to overestimate actual clutch sizes (e.g., EQ6 vs EQ7). Reports of necropsied Burmese pythons containing unusually high "egg" counts and inferred as directly removing a high number of pythons from the population warrant cautious interpretation. There are little data on nest success and hatchling survival, adult females may not ovulate all follicles, ovulated follicles may not be otherwise viable (e.g., lack appropriate yolk provisions or not fertilized), follicles or oviductal eggs may be resorbed, or expelled within a clutch at a visible size difference. For example, one adult female python (482 SVL, 74,600 g) from Everglades National Park in 2012 was found to contain 87 oviductal eggs with 2 that appeared to be in the process of being resorbed (Krysko et al. 2012). Similarly, the nests we discovered as part of this study were noted to contain several inviable and misshapen eggs, with some being visibly smaller (though few

were enumerated at the time). The prevalence of these "slugs" is unknown in the wild population but are commonly referenced in the herpetoculture literature (Blackburn 1998). Though some reports include "inviable" eggs as a subset of total clutch sizes, it is not known if smaller inviable eggs are considered in the same way, if most clutches contain them, or if those reports that do not explicitly include inviable counts simply lump them all together, possibly skewing clutch size estimates. There also is evidence that females fail to oviposit their entire clutch, retaining some shelled oviductal eggs beyond normal oviposition timing (Anderson et al. 2022), however the prevalence and implications of this are still unclear.

We found that clutch sizes including only oviductal eggs or laid eggs ranged from 11 to 84 (mean = 34, SD = 18, median = 27, interquartile range = 24-46, *n* = 66). This range is in line with previously reported clutch sizes from this population (21, 27, 29, 35, 37, 46, 79, 85, and 87; Snow et al. 2007a, b; Krysko et al. 2008; Snow et al. 2010; Krysko et al. 2012), which all fall within those thought to be typical clutch sizes from their native range as reported by Wall (1921). Yet, our three highest counts of potential clutch size (those above the highest reported number of oviductal eggs of 87 from southern Florida; Krysko et al. 2012) were all pre-ovulatory follicles numbering 89, 90, and 103 from females found in Feb 2020 (SVL = 475 cm), Dec 2020 (SVL = 430 cm), and Jan 2017 (SVL = 429 cm), respectively. Similarly high numbers of preovulatory follicles have been reported (Rochford et al. 2010), whereas the highest number of oviductal eggs we documented was 72 from a 475 cm SVL female found in Mar 2019. These numbers are somewhat higher than those reported in a short note by Brien et al. (2007) (mean = 36, n = 8, range = 19-46), but those authors did not distinguish the potential clutch sizes between secondary (pre-ovulatory vitellogenic) follicles and oviductal eggs.

Seasonal trends

Hatchlings

The data supported our hypothesis that pythons synchronously hatch and disperse from nests, appearing on the Florida landscape in large numbers during the summer and fall months (July into October) and peaking in August (Fig. 4). This size-stage class constitutes the highest number of captures throughout the year, and the initial surge in July corresponds well with hatch date observations (Table 3). However, from this long-term dataset, we were further able to distinguish the YoY hatchlings from those smaller individuals remaining on the landscape from a prior year during a period between July and October (Fig. 5). This distinction helps us understand variation in size over the first year of life, a parameter vital to life tables used to estimate population growth trends. During the summer months, we can relatively accurately identify a YoY hatchling based on SVL and month of capture. However, more work needs to be done to determine whether estimates can be generated for size-stage classes (i.e., size relative to length of time in days or years), such as radio-tracking wild Burmese pythons long-term from hatching.

Reproductive cycles

A clear understanding of reproductive timing can not only inform population models but also aid managers in targeting times of year when control mechanisms may be most effective in arresting population growth. In this study, we confirmed our hypothesis that adult Burmese pythons exhibit annual reproduction and further refined the biologically significant time periods (i.e., breeding season, oviposition timing, and incubation period). We found that seasonal recrudescence of gonadal structures aligns well with field observations of gregarious behaviors (e.g., Smith et al. 2015; Smith et al. 2016). Using this paired timing among gonadal recrudescence and regression along with the presence or absence of breeding behaviors, we discretely defined the Burmese python breeding season in southern Florida as the approximately 100 days beginning in December and ending mid-March (see Fig. 6). In northern India at a similar latitude but much higher elevation than southern Florida, reports of the closely related P. m. molurus exhibit heightened breeding activity during February and March, but may extend into August (Bhupathy and Vijayan 1989). However, more aligned with observations in southern Florida, there is evidence indicating that pythons breed in Sri Lanka December through February (Wall 1921), and P. m. bivittatus breed in Thailand December to mid-March (Smith et al. 2021).

Breeding season may be the time that adult male Burmese pythons in southern Florida become most vagile annually. Though we cannot account for effort in our dataset, most adult males are captured between the months of November through March, presumably because they are in search of mates. While adult female captures remain relatively steady across months at 2–5% of all captures, adult male captures increase to 7%, 10%, and 12% of total numbers in November through January, decreasing to 6% and 5% in February and March, and then remain under 4% for the remainder of the year (see Suppl. materials 4, 5). Snow et al. (2007b) also reported multiple captures during November through January, 35% of which were from December through January alone, suggesting that the uptick coincides with seasonal behaviors such as breeding.

We found that 87% of reproductive-sized females physiologically prepare for nesting by beginning vitellogenesis (developing secondary follicles) in November and continuing into March (Fig. 6, top). Females are ovigerous (having oviductal eggs) from March into May. This is a shorter period than the Krysko et al. (2008) estimate of January through April but comparable to Snow et al. (2010). We also saw that female pythons oviposit, starting in May (Fig. 6, top). This is concordant with radio-tracked females in Thailand that have been observed to nest between April and June (Smith et al. 2021). However, not all adult females in our dataset were reproductive during these periods. We found that approximately 36% (n = 67) of adult females (n = 184) were non-reproductive (having undeveloped follicles) during the months of December through May (Fig. 6, top). This indicates that approximately a third of all females in any given year may not reproduce, perhaps reproducing every 2–3 years. Literature supports a biennial reproductive cycle in these and other snakes and could be due to lowered body condition and recovery following nesting (Bertona and Chiaraviglio 2003; Reed and Rodda 2009; Willson et al. 2011). Burmese pythons exert effort as they exhibit nest guarding/defense (e.g., Currylow et al. 2022c) and incubate eggs through shivering thermogenesis during brooding (Benedict 1932; Hutchison et al. 1966; Van Mierop and Barnard 1978; Snow et al. 2010).

Our limited dataset on incubation/prehatching period (e.g., Table 3) indicates an approximately two-month development period, which agrees with the current literature estimates and is suggested to be somewhat regulated by temperatures (Pope 1961; Van Mierop and Barnard 1976). There is a paucity of precise data on oviposition timing for wild pythons in southern Florida (Harvey et al. 2008), but radio-tracked females have led to the narrowest windows thus far. We found the two females we tracked in 2021 oviposited very close in time (within 6 days in mid-May; Table 3, Nests 5 & 6). The nests that had hatch date recorded (Table 3, Nests 3, 4, 6, 7, 9 & 10) did so in July, often within days of each other. The nest we monitored throughout 2021 (Nest 6) took about 63 days to incubate and 3 days to complete hatching. This is concordant with the captive literature where oviposition usually happens approximately two months after breeding, incubation lasts anywhere from 53 to 88 days, and hatching is complete after a couple days (Ross and Marzec 1990; Murphy and Henderson 1997).

Management implications

Management of Burmese pythons in Florida is costly due to difficulties associated with low detection and the vast wilderness of the Greater Everglades Ecosystem. Our findings regarding python size distribution and reproductive phenology provide standardized equations for direct cross-study comparisons that can inform population models and help managers target pythons for removal with a greater return on investment. For example, we have determined the size threshold for adult males, which provides managers operating scout snake programs (i.e., Fitzgerald et al. 2021) a reliable metric to easily assess adult males as future scout snakes. Additionally, our data indicate that male Burmese pythons are primarily seeking mates for 100 days from December through March, so that will likely be the most effective window of time for managers to physically visit their scout snakes and survey for associate snakes. When deciding where to direct limited management resources, managers can consider targeting specific size-stage classes by time of year because we defined the above-listed breeding season and found that pythons hatch synchronously and disperse on the landscape from July through October. And because we found that adult females oviposit in May and attend their nests during the subsequent two-month incubation period, that timeframe could be targeted for removal of those individuals with the highest reproductive potential along with their entire clutch of eggs (e.g., using dogs trained to detect pythons). Our finding that secondary follicles do not necessarily represent clutch size can help managers understand the number of total potential pythons removed with each individual reproductive female. Yet we did find that true clutch size increased with female body size, so the removal of fewer but larger females could be considered by managers to be a higher priority over removing sheer numbers of total pythons. We found that YoY can be distinguished from the older juveniles during a July into October annual window, which can afford managers the relative number of hatchlings that naturally survive into a second year in their management unit. As research and management activities continue in concert, detection and control will become more effective with studies that refine our understanding of python demographics, reproduction, and seasonal trends.

Acknowledgements

We thank T.F. Dean, T. Pernas, M.F. McCollister and S. Schulze of the National Park Service (NPS) and N.G. Aumen of the U.S. Geological Survey (USGS) for facilitation of this project in Everglades National Park and Big Cypress National Preserve. Funding for the Everglades work and in-kind support was provided by the USGS Greater Everglades Priority Ecosystems Science (GEPES) Program, NPS, and USGS Biothreats and Invasive Species Program. We thank G.E. Anderson for invaluable assistance with final data preparation, A.L. Fitzgerald, C.J. Robinson, and the many University of Florida research interns, NPS and USGS staff, volunteers, Florida Fish and Wildlife Conservation Commission (FWC) and South Florida Water Management District personnel that were involved with the collection of these data. L. Bonewell provided project management. Some data were collected in association with activities conducted under NPS Scientific Research Permits BICY-00134 & -00159 and EVER-2018-SCI-0063, an interagency agreement between USGS and NPS #P18PG00352, FWC permits EXOT 19–43, -44, -45, and -114; EXOT 20–62, -63, -86, -93, and -184; and EXOT 21–71, -97, -111, and -278. We thank J.C. Guzy and four reviewers for their valuable input on improving earlier versions of this manuscript. No Institutional Animal Care and Use Committee approval was necessary because the invasive pythons were euthanized as part of mitigation management activities, but methods for safe euthanasia were developed in consultation with the NPS Wildlife Health Team. Data used in this manuscript are available at Currylow et al. 2022b. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

- Aldridge RD (1979) Female reproductive cycles of the snakes *Arizona elegans* and *Crotalus viridis*. Herpetologica 35: 256–261. https://www.jstor.org/stable/3891696
- Anderson GE, Ridgley FN, Josimovich JM, Reed RN, Falk B, Yackel Adams AA, Currylow AF (2022) Egg retention in wild-caught *Python bivittatus* in the Greater Everglades Ecosystem, Florida, USA. The Herpetological Journal 32(3): 109–113. https://doi. org/10.33256/32.3.109113
- Benedict FG (1932) The physiology of large reptiles: with special reference to the heat production of snakes, tortoises, lizards and alligators. Carnegie Institution of Washington, Washington, 539.

- Bertona M, Chiaraviglio M (2003) Reproductive biology, mating aggregations, and sexual dimorphism of the Argentine boa constrictor (*Boa constrictor occidentalis*). Journal of Herpetology 37(3): 510–516. https://doi.org/10.1670/122-02A
- Bhupathy S, Vijayan VS (1989) Status, distribution, and general ecology of the Indian python, *Python molurus molurus*. Linn. in Keoladeo National Park, Bharatpur, Rajasthan, India. Journal of the Bombay Natural History Society 86: 381–387.
- Blackburn DG (1998) Resorption of oviductal eggs and embryos in squamate reptiles. The Herpetological Journal 8: 65–71.
- Brien ML, Cherkiss MS, Johnson VM, Mazzotti FJ, Snow RW (2007) *Python molurus bivittatus* (Burmese Python). Clutch size. Herpetological Review 38: 242–243.
- Brown MT, Cohen MJ, Bardi E, Ingwersen WW (2006) Species diversity in the Florida Everglades, USA: A systems approach to calculating biodiversity. Aquatic Sciences 68(3): 254–277. https://doi.org/10.1007/s00027-006-0854-1
- Burger J, Zappalorti RT, Gochfeld M (1987) Developmental effects of incubation temperature on hatchling pine snakes *Pituophis melanoleucus*. Comparative Biochemistry and Physiology. A. Comparative Physiology 87(3): 727–732. https://doi.org/10.1016/0300-9629(87)90390-2
- Burkett-Cadena ND, Blosser EM, Loggins AA, Valente MC, Long MT, Campbell LP, Reeves LE, Bargielowski I, McCleery RA (2021) Invasive Burmese pythons alter host use and virus infection in the vector of a zoonotic virus. Communications Biology 4(1): 804. https://doi. org/10.1038/s42003-021-02347-z
- Card DC, Perry BW, Adams RH, Schield DR, Young AS, Andrew AL, Jezkova T, Pasquesi GIM, Hales NR, Walsh MR, Rochford MR, Mazzotti FJ, Hart KM, Hunter ME, Castoe TA (2018) Novel ecological and climatic conditions drive rapid adaptation in invasive Florida Burmese pythons. Molecular Ecology 27(23): 4744–4757. https://doi.org/10.1111/ mec.14885
- Currylow AF, Hanley BJ, Holcomb KL, Shields T, Boland S, Boarman WI, Vaughn M (2022a) A decision tool to identify population management strategies for common ravens and other avian predators. Human-Wildlife Interactions 15(3): Article 25. https://doi.org/10.26077/ e056-1a58
- Currylow AF, Falk BG, Yackel Adams AA, Romagosa C, Josimovich JM, Rochford M, Cherkiss M, Nafus MG, Hart K, Mazzotti F, Snow R, Reed RN (2022b) Size distribution and reproductive data of the invasive Burmese python (*Python molurus bivittatus*) in the Greater Everglades Ecosystem, Florida, USA, 1995–2021: U.S. Geological Survey data release. https://doi.org/10.5066/P9CZI2KO
- Currylow AF, McCollister MF, Anderson GE, Josimovich JM, Fitzgerald AL, Romagosa CM, Yackel Adams AA (2022c) Face-off: Novel depredation and nest defense behaviors between an invasive and a native predator in the Greater Everglades Ecosystem, Florida, USA. Ecology and Evolution 12(2): e8639. https://doi.org/10.1002/ece3.8639
- de Vosjoli P, Klingenberg R (2012) Burmese pythons: Plus reticulated pythons and related species. Fox Chapel Publishing, USA.
- Diagne C, Leroy B, Vaissière A-C, Gozlan RE, Roiz D, Jarić I, Salles J-M, Bradshaw CJA, Courchamp F (2021) High and rising economic costs of biological invasions worldwide. Nature 592(7855): 571–576. https://doi.org/10.1038/s41586-021-03405-6

- Dorcas ME, Willson JD, Reed RN, Snow RW, Rochford MR, Miller MA, Meshaka Jr WE, Andreadis PT, Mazzotti FJ, Romagosa CM, Hart KM (2012) Severe mammal declines coincide with proliferation of invasive Burmese pythons in Everglades National Park. Proceedings of the National Academy of Sciences of the United States of America 109(7): 2418–2422. https://doi.org/10.1073/pnas.1115226109
- Easterling I, Bartoszek I (2019) *Python bivittatus* (Burmese python). Maximum male size. Herpetological Review 50: 399–400.
- Emery J-P, Mitchell NJ, Cogger H, Agius J, Andrew P, Arnall S, Detto T, Driscoll DA, Flakus S, Green P, Harlow P, McFadden M, Pink C, Retallick K, Rose K, Sleeth M, Tiernan B, Valentine LE, Woinarski JZ (2021) The lost lizards of Christmas Island: A retrospective assessment of factors driving the collapse of a native reptile community. Conservation Science and Practice 3(2): e358. https://doi.org/10.1111/csp2.358
- Falk BG, Snow RW, Reed RN (2016) Prospects and limitations of citizen science in invasive species management: A case study with Burmese pythons in Everglades National Park. Southeastern Naturalist 15(sp8): 89–102. https://doi.org/10.1656/058.015.sp806
- Fantle-Lepczyk JE, Haubrock PJ, Kramer AM, Cuthbert RN, Turbelin AJ, Crystal-Ornelas R, Diagne C, Courchamp F (2022) Economic costs of biological invasions in the United States. The Science of the Total Environment 806: 151318. https://doi.org/10.1016/j.scitotenv.2021.151318
- Feldman A, Meiri S (2012) Length–mass allometry in snakes. Biological Journal of the Linnean Society. Linnean Society of London 108(1): 161–172. https://doi.org/10.1111/j.1095-8312.2012.02001.x
- Fitch HS (1960) Criteria for determining sex and breeding maturity in snakes. Herpetologica 16: 49–51.
- Fitzgerald A, Josimovich J, Robinson CJ, Reed R, Currylow AF (2021) Identifying negative sentiment polarity in the Judas Technique. Conservation Science and Practice 3(11): e532. https://doi.org/10.1111/csp2.532
- Govindarajulu P, Altwegg R, Anholt BR (2005) Matrix model investigation of invasive species control: Bullfrogs on Vancouver Isand. Ecological Applications 15(6): 2161–2170. https://doi.org/10.1890/05-0486
- Griffith AB, Salguero-Gómez R, Merow C, McMahon S (2016) Demography beyond the population. Journal of Ecology 104(2): 271–280. https://doi.org/10.1111/1365-2745.12547
- Gurevitch J, Fox GA, Wardle GM, Inderjit, Taub D (2011) Emergent insights from the synthesis of conceptual frameworks for biological invasions. Ecology Letters 14(4): 407–418. https://doi.org/10.1111/j.1461-0248.2011.01594.x
- Hanley B, Connelly P, Dennis B (2019a) Another look at the eigenvalues of a population matrix model. PeerJ 7: e8018. https://doi.org/10.7717/peerj.8018
- Hanley BJ, Dhondt AA, Dennis B, Schuler KL (2019b) Using time series data to assess recent population dynamics of bald eagles in the northeast United States. Ecosphere 10(11): e02963. https://doi.org/10.1002/ecs2.2963
- Hanslowe EB, Falk BG, Collier MA, Josimovich JM, Rahill TA, Reed RN (2016) First record of invasive Burmese python oviposition and brooding inside an anthropogenic structure. Southeastern Naturalist 15(sp8): 103–107. https://doi.org/10.1656/058.015.sp809

- Harvey RG, Brien ML, Cherkiss MS, Dorcas M, Rochford M, Snow RW, Mazzotti FJ (2008) Burmese pythons in South Florida: Scientific support for invasive species management; WEC-242; University of Florida, IFAS Extension, 10. https://doi.org/10.32473/edisuw286-2008
- Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success? Biological Invasions 10(4): 483–506. https://doi.org/10.1007/s10530-007-9146-5
- Hoyer IJ, Blosser EM, Acevedo C, Thompson AC, Reeves LE, Burkett-Cadena ND (2017) Mammal decline, linked to invasive Burmese python, shifts host use of vector mosquito towards reservoir hosts of a zoonotic disease. Biology Letters 13(10): 20170353. https:// doi.org/10.1098/rsbl.2017.0353
- Hunter ME, Oyler-McCance SJ, Dorazio RM, Fike JA, Smith BJ, Hunter CT, Reed RN, Hart KM (2015) Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive Burmese pythons. PLoS ONE 10(4): e0121655. https://doi. org/10.1371/journal.pone.0121655
- Hutchison VH, Dowling HG, Vinegar A (1966) Thermoregulation in a brooding female Indian python, *Python molurus bivittatus*. Science 151(3711): 694–696. https://doi.org/10.1126/ science.151.3711.694
- Josimovich JM, Currylow AF (2021) Hatchling growth experiment dataset from invasive Burmese pythons captured in 2015 in southern Florida: U.S. Geological Survey data release. https://doi.org/10.5066/P9WHSSJ6 [accessed April 2, 2022]
- Josimovich JM, Falk BG, Grajal-Puche A, Hanslowe EB, Bartoszek IA, Reed RN, Currylow AF (2021) Clutch may predict growth of hatchling Burmese pythons better than food availability or sex. Biology Open 10(11): bio058739. https://doi.org/10.1242/ bio.058739
- Krysko K, Nifong J, Mazzotti F, Snow R, Enge K (2008) Reproduction of the Burmese python (*Python molurus bivittatus*) in southern Florida. Applied Herpetology 5(1): 93–95. https:// doi.org/10.1163/157075408783489185
- Krysko KL, Hart KM, Smith BJ, Selby TH, Cherkiss MS, Coutu NT, Reichart RM, Nuñez LP, Mazzotti FJ, Snow RW (2012) Record length, mass, and clutch size in the nonindigenous Burmese python, *Python bivittatus* Kuhl 1820 (Squamata: Pythonidae), in Florida. Reptiles & Amphibians : Conservation and Natural History 19(4): 267–270. https://doi. org/10.17161/randa.v19i4.13923
- Lodge TE (2017) The Everglades handbook: understanding the ecosystem. CRC Press, Taylor & Francis Group, Boca Raton, 440.
- McCleery RA, Sovie A, Reed RN, Cunningham MW, Hunter ME, Hart KM (2015) Marsh rabbit mortalities tie pythons to the precipitous decline of mammals in the Everglades. Proceedings. Biological Sciences 282(1805): 20150120. https://doi.org/10.1098/ rspb.2015.0120
- Miller MA, Kinsella JM, Snow RW, Hayes MM, Falk BG, Reed RN, Mazzotti FJ, Guyer C, Romagosa CM (2018) Parasite spillover: Indirect effects of invasive Burmese pythons. Ecology and Evolution 8(2): 830–840. https://doi.org/10.1002/ece3.3557
- Murphy JC, Henderson RW (1997) Tales of giant snakes: A historical natural history of anacondas and pythons. Krieger Publishing Company, Malabar, Florida, 221.

- Nafus MG, Mazzotti FJ, Reed RN (2020) Estimating detection probability for Burmese pythons with few detections and zero recaptures. Journal of Herpetology 54(1): 24–30. https://doi.org/10.1670/18-154
- Parker MR, Currylow AF, Tillman E, Robinson C, Josimovich J, Bukovich I, Nazarian L, Nafus M, Kluever B, Yackel Adams AA (2021) Using enclosed Y-mazes to assess chemosensory behavior in reptiles. Journal of Visualized Experiments 170: e61858. https://doi. org/10.3791/61858
- Pope CH (1961) The giant snakes: the natural history of the boa constrictor, the anaconda, and the largest pythons. Alfred A. Knopf, Inc., New York, 263 pp.
- Reed RN, Rodda GH (2009) Giant constrictors: Biological and management profiles and an establishment risk assessment for nine large species of pythons, anacondas, and the boa constrictor; 2009–1202, 302 pp. https://doi.org/10.3133/ofr20091202
- Reed RN, Hart KM, Rodda GH, Mazzotti FJ, Snow RW, Cherkiss M, Rozar R, Goetz S (2011) A field test of attractant traps for invasive Burmese pythons (*Python molurus bivittatus*) in southern Florida. Wildlife Research 38(2): 114–121. https://doi.org/10.1071/WR10202
- Reed RN, Todd BD, Miano OJ, Canfield M, Fisher RN, McMartin L (2016) Ecology and control of an introduced population of southern watersnakes (*Nerodia fasciata*) in southern California. Herpetologica 72(2): 130–136. https://doi.org/10.1655/HERPETOLOGI-CA-D-14-00061
- Reichert BE, Sovie AR, Udell BJ, Hart KM, Borkhataria RR, Bonneau M, Reed R, McCleery R (2017) Urbanization may limit impacts of an invasive predator on native mammal diversity. Diversity & Distributions 23(4): 355–367. https://doi.org/10.1111/ddi.12531
- Rochford MR, Brien ML, Carrigan J, Snow RW, Mazzotti FJ (2010) Python molurus bivittatus (Burmese python). Clutch size. Herpetological Review 41: 97.
- Rodda GH, Fritts TH (1992) The impact of the introduction of the Colubrid snake *Boiga irregularis* on Guam's lizards. Journal of Herpetology 26(2): 166–174. https://doi. org/10.2307/1564858
- Rogers HS, Buhle ER, HilleRisLambers J, Fricke EC, Miller RH, Tewksbury JJ (2017) Effects of an invasive predator cascade to plants via mutualism disruption. Nature Communications 8(1): 14557. https://doi.org/10.1038/ncomms14557
- Ross RA, Marzec G (1990) The reproductive husbandry of pythons and boas. Institute for Herpetological Research, 270 pp.
- SAS Institute Inc (2021) JMP 16.0.0, SAS Institute Inc., Cary, North Carolina, USA.
- Savidge JA (1987) Extinction of an island forest avifauna by an introduced snake. Ecology 68(3): 660–668. https://doi.org/10.2307/1938471
- Seigel RA, Collins JT, Novak SS (2001) Snakes: ecology and evolutionary biology. The Blackburn Press, Caldwell, New Jersey, 529 pp.
- Shine R, Olsson MM, Moore IT, LeMaster MP, Mason RT (1999) Why do male snakes have longer tails than females? Proceedings. Biological Sciences 266(1434): 2147–2151. https:// doi.org/10.1098/rspb.1999.0901
- Smith BJ, Rochford MR, Matt B, Cherkiss MS, Mazzotti F, Skip S, Hart KM (2015) Largest breeding aggregation of Burmese pythons and implication for potential development of a control tool. IRCF Reptiles & Amphibians 22: 16–19. https://doi.org/10.17161/randa.v22i1.14025

- Smith BJ, Cherkiss MS, Hart KM, Rochford MR, Selby TH, Snow RW, Mazzotti FJ (2016) Betrayal: Radio-tagged Burmese pythons reveal locations of conspecifics in Everglades National Park. Biological Invasions 18(11): 3239–3250. https://doi.org/10.1007/s10530-016-1211-5
- Smith SN, Jones MD, Marshall BM, Waengsothorn S, Gale GA, Strine CT (2021) Native Burmese pythons exhibit site fidelity and preference for aquatic habitats in an agricultural mosaic. Scientific Reports 11(1): 7014. https://doi.org/10.1038/s41598-021-86640-1
- Snow R, Johnson V, Brien M, Cherkiss M, Mazzotti F (2007a) *Python molurus bivittatus*: Nesting. Herpetological Review 38: 93.
- Snow R, Krysko K, Enge K, Oberhofer L, Ludwicka-Bradley A, Wilkins L (2007b) Introduced populations of *Boa constrictor* (Boidae) and *Python molurus bivittatus* (Pythonidae) in southern Florida. In: Henderson RW, Powell R (Eds) Biology of the Boas and Pythons, Eagle Mountain Publishing, Eagle Mountain, Utah, 418–438.
- Snow RW, Wolf AJ, Greeves BW, Cherkiss MS, Hill R, Mazzotti FJ (2010) Thermoregulation by a brooding Burmese python (*Python molurus bivittatus*) in Florida. Southeastern Naturalist 9(2): 403–405. https://doi.org/10.1656/058.009.0215
- Taillie PJ, Hart KM, Sovie AR, McCleery RA (2021) Native mammals lack resilience to invasive generalist predator. Biological Conservation 261: 109290. https://doi.org/10.1016/j. biocon.2021.109290
- Van Mierop LHS, Barnard SM (1976) Observations on the reproduction of *Python molurus bivittatus* (Reptilia, Serpentes, Boidae). Journal of Herpetology 10(4): 333–340. https://doi.org/10.2307/1563071
- Van Mierop LHS, Barnard SM (1978) Further observations on thermoregulation in the brooding female *Python molurus bivittatus* (Serpentes: Boidae). Copeia 1978(4): 615–621. https://doi.org/10.2307/1443687
- Vishnu SN, Ramesh C, Thirumurugan V, Sathish C (2021) Size matters: First record of minimum male size at maturity and mating of free-ranging, endangered Indian Python *Python molurus*. Asian Journal of Conservation Biology 10: 153–158. https://doi.org/10.53562/ ajcb.AQOE1932
- Vitousek PM, D'Antonio CM, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. American Scientist 84: 468–478.
- Wall F (1921) Ophidia taprobanica: or, the snakes of Ceylon. HR Cottle, Government printer, Ceylon. https://doi.org/10.5962/bhl.title.53694
- Wiles G (1987) Current research and future management of Marianas fruit bats (Chiroptera: Pteropodidae) on Guam. Australian Mammalogy 10: 93–95.
- Willson JD, Dorcas ME, Snow RW (2011) Identifying plausible scenarios for the establishment of invasive Burmese pythons (*Python molurus*) in Southern Florida. Biological Invasions 13(7): 1493–1504. https://doi.org/10.1007/s10530-010-9908-3
- Willson JD, Snow RW, Reed RN, Dorcas ME (2014) Python molurus bivittatus (Burmese python). Minimum size at maturity. Herpetological Review 45: 343–344.
- Wolf AJ, Walters TM, Rochford MR, Snow RW, Mazzotti FJ (2016) Incubation temperature and sex ratio of a *Python bivittatus* (Burmese python) clutch hatched in Everglades National Park, Florida. Southeastern Naturalist 15(sp8): 35–39. https://doi.org/10.1656/058.015.sp803

Supplementary material I

Total Burmese python (Python molurus bivittatus)

Authors: Andrea F. Currylow

Data type: figure

- Explanation note: Total Burmese python (*Python molurus bivittatus*) records (n = 4,348) per year in this study by size-stage classes (see Currylow et al manuscript text) and sex from the Greater Everglades Ecosystem, Florida, USA. Total numbers of each sex are listed across the top of the graph section. Size-stage classes are distinguishable by color (adult = burnt orange; sub-adult = purple; juvenile = green) and enumerated in the larger bars.
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Link: https://doi.org/10.3897/neobiota.78.93788.suppl1

Supplementary material 2

Relationship of snout-vent lengths (SVLs) and tail lengths to total lengths

Authors: Andrea F. Currylow

Data type: figure

- Explanation note: Relationship of snout-vent lengths (SVLs) and tail lengths to total lengths (all in cm) for all intact male and female Burmese pythons (*Python molurus bivittatus*) captured 2004–2021 from the Greater Everglades Ecosystem, Florida, USA. Size-stage classes are distinguished by color (adult = burnt orange; sub-adult = purple; juvenile = green) and individual characteristic equations are displayed for each cross variable in the upper left of each graph. Sample sizes for size-age class are listed in the center bottom of each sex group.
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Link: https://doi.org/10.3897/neobiota.78.93788.suppl2

Supplementary material 3

The potential clutch sizes

Authors: Andrea F. Currylow

Data type: figure

- Explanation note: The potential clutch sizes using the number of secondary follicles (pink) or number of oviductal eggs (blue) by snout-vent lengths (SVL in cm) of Burmese pythons (*Python molurus bivittatus*). The shaded areas around the fit lines are 95% confidence of fit buffers. Data were collected from animals across the Greater Everglades Ecosystem, Florida, USA from 2004–2021.
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Link: https://doi.org/10.3897/neobiota.78.93788.suppl3

Supplementary material 4

Percent of total Burmese python (Python molurus bivittatus)

Authors: Andrea F. Currylow

Data type: figure

- Explanation note: Percent of total Burmese python (*Python molurus bivittatus*) captures from southern Florida, USA each month of all years (1995–2021) combined by sex. Total numbers are displayed in the legend and individual month totals by sex are displayed above each bar within that month.
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Link: https://doi.org/10.3897/neobiota.78.93788.suppl4

Supplementary material 5

Total number of adult Burmese python (Python molurus bivittatus)

Authors: Andrea F. Currylow

Data type: figure

- Explanation note: Total number of adult Burmese python (*Python molurus bivittatus*) captures across months for all recorded years (2001–2021) separated by sex (red = females, blue = males). Data were collected from animals across the Greater Everglades Ecosystem, Florida, USA.
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Link: https://doi.org/10.3897/neobiota.78.93788.suppl5

Supplementary material 6

Juvenile Burmese python (Python molurus bivittatus)

Authors: Andrea F. Currylow

Data type: figure

- Explanation note: Juvenile Burmese python (*Python molurus bivittatus*) morphometric data (snout-vent length; SVL \geq 100 cm; weight \geq 200 g) between the months of July through October (window of time when young-of-year (YoY) hatchlings appear on the landscape en masse; see Currylow et al manuscript text) across all years they were encountered (2003–2021) from the Greater Everglades Ecosystem, Florida, USA. During these four months, median sizes for YoY hatchlings were 63.0 cm SVL (interquartile range 58.6–71.5 cm) and 124.6 g (interquartile range = 106.7–194.0 g).
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- Link: https://doi.org/10.3897/neobiota.78.93788.suppl6

RESEARCH ARTICLE



Coming home: Back-introduced invasive genotypes might pose an underestimated risk in the species' native range

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Academic editor: Bruce Osborne | Received 8 August 2022 | Accepted 15 November 2022 | Published 8 December 2022 Citation: Watermann LY, Rotert J, Erfmeier A (2022) Coming home: Back-introduced invasive genotypes might pose an underestimated risk in the species' native range. NeoBiota 78: 159–183. https://doi.org/10.3897/neobiota.78.91394

Abstract

Biological invasions are considered a significant challenge both from an ecological and economical perspective. Compared to the native range, environmental conditions in the invasive range often favor more competitive genotypes. Little attention, however, has so far been paid to the possibility that these invasive and competitive genotypes might also be back-introduced into a species' native range, where they could trigger a problematic increase in abundance or expansion. The frequency with which this occurs in the species' native range might be an underestimated aspect in nature conservation. We transplanted native and invasive individuals of the biennial model species Jacobaea vulgaris into field sites of naturally occurring populations within the species' native range. The aim was to test whether back-introduced invasive origins show decreased performance, e.g., because of the reunion with specialized herbivores or plant-soil-feedbacks or whether they have the potential to trigger problematic population dynamics in the species' native range. We ran an additional greenhouse experiment to specifically address soil-borne effects in the species' native habitats. We found that invasive individuals generally outperformed the native transplants if compared in the field sites. By contrast, there were no origin-dependent differences in the greenhouse experiment. Our findings clearly indicate that testing for origin effects exclusively under controlled conditions might underestimate the potential of invasive genotypes to trigger invasion processes in habitats of the species' native range. Although differences in performance mediated by soil-borne effects were not associated with plant origin, field site susceptibility to J. vulgaris colonization varied largely. Identifying the exact factors driving these differences, offers another focal point to minimize the risk of a detrimental increase in the abundance or expansion of this highly invasive species in its home range.

Keywords

cryptic invasions, enemy release, local adaptation, plant-soil-feedback (PSF), ragwort, re-introduction, transplant experiment

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Introduction

Invasive alien plant species pose a significant threat to biodiversity (Gaertner et al. 2009; McGeoch et al. 2010; Vilà et al. 2011) and particularly for endangered native species (Pimentel et al. 2005). Given that invasive species might benefit from ongoing climate change (Liu et al. 2017) and increasing human activity (Sardain et al. 2019), the situation might even exacerbate in the future as attempts for predicting invasive species as well as vulnerable habitats have so far been proven to be difficult (de Andrade et al. 2019; Miller et al. 2020). This might particularly apply to "cryptic invasions", a special case of these biological invasions (Morais and Reichard 2018) often completely overlooked when addressing threats to biodiversity. Recognition of invasion processes might fail because invasive plant species are misidentified (i.e., interspecific cryptic invasion) or because the invasion is triggered by the addition of a non-native lineage/ genotype (i.e., intraspecific cryptic invasion). Cryptic back introduction, whereby genetic material from an invasive population is translocated back to the species' native range, might pose a particularly high risk (Guo 2005). However, while interspecific cryptic invasions are increasingly being noticed (e.g. Alves et al. 2021; Goodman et al. 2021; Mezhzherin et al. 2022), intraspecific cryptic invasion might be the hidden cause of a sudden increase in abundance or rapid range expansion in a native species that is regularly attributed to changes in the environmental conditions instead (Morais and Reichard 2018). Subsequently, an observed range expansion of a native species might go undetected as a biological invasion.

Given the increasing human mobility around the globe that allows plant species to overcome the first barrier in the invasion process (Blackburn et al. 2011), as well as the intensified global floristic homogenization (Yang et al. 2021), it seems reasonable to assume that, once established, invasive populations might also be transported back to the species' native range. In fact, the lizard *Anolis sagrei* originating from Cayman Brac and Little Cayman, became invasive on Grand Caymen and was later back-introduced to Cayman Brac (Kolbe et al. 2017). In a similar vein, cultivars of *Ilex aquifolium* were found to promote the native range expansion of wild populations (Skou et al. 2012). Those cryptic invasions, similar to biological invasions by native-alien populations, might be underestimated in their impact (Nelufule et al. 2022). In a study regarding assisted migration, Mueller and Hellmann (2008) found that intracontinental invasions, though rare, are usually as detrimental as intercontinental invasions. Brandes et al. (2019) were able to suggest a cryptic intracontinental invasion to have taken place in the native range of scotch broom by alien genotypes. However, uncovering cryptic invasions, in particular, is difficult as conspicuous signs are usually absent.

In general, range expansions in any population, native or invasive, are associated with adaptations that facilitate high reproduction rates (Fronhofer and Altermatt 2015) and allow counteracting formerly limiting environmental factors (Colautti and Barrett 2013). In a novel range, populations may encounter biotic and abiotic conditions that differ from the species' native range (e.g. Mitchell et al. 2006) and that thus represent selective forces to adapt to. Introduced populations may ultimately exhibit fundamental niche shifts, for example in regard to climatic conditions in a species' novel range (Broennimann et al. 2007; Early and Sax 2014). If these adapted novel genotypes are back-introduced to the species' native range they might show considerably different performance back in the species' native habitat than in the novel one.

For example, species naturalized outside their native range often experience a release from natural enemies during the invasion process. Once exempt from the necessity to defend against specialist herbivores occurring in the plant species' native range, resources can be used for other purposes (Enemy Release Hypothesis) (Keane 2002). In a broader understanding, differences in the biotic environment with its trophic interactions, i.e. pathogens, herbivores (specialists as well as generalists) and/or predators, are part of a possible enemy release. A release from those constraints may then be transformed into a stronger competitive force through resource redistribution (Erfmeier 2013). Consequently, selection then favors genotypes that invest less in now obsolete defenses and more into other traits supporting fitness. Ultimately, invasive genotypes are often subject to selection towards a higher competitive ability compared to their native counterparts (Evolution of Increased Competitive Ability hypothesis) (Blossey and Nötzold 1995; Broennimann et al. 2007). These processes can cause a noticeable increase in both the distribution and local abundance of invasive species. When those invasive genotypes are subsequently back-introduced, the loss of defenses against specialized herbivores and/or pathogenic soil biota could prove to be a maladaptation but could also facilitate subsequent changes in native populations. In the past, common garden experiments in a species' native range often found invasive populations to show inferior performance when compared to their native counterparts (Maron et al. 2004; Hock et al. 2019; Pal et al. 2020).

Besides the often-addressed aboveground factors, such as herbivory, plant-soil-feedbacks (PSFs) have gained more and more attention and call for a belowground focus. In recent years, PSFs are of increasing interest as part of the environmental factors contributing to the success or failure of the invasive range expansion process. While the aforementioned effects of enemy release mostly refer to aboveground herbivores, invasive populations might similarly be freed from enemies in the soils (Beckmann et al. 2016), lose beneficial mutualists (Zenni et al. 2017) or they can encounter new detrimental (pathogens) or beneficial (mutualists) interactions (Reinhart and Callaway 2006). For *Centaurea solstitialis*, e.g., the release from soil pathogens may have been a crucial factor determining its success as a highly invasive species (Montesinos and Callaway 2020). Furthermore, allelopathy exerted by naturalized alien plant species on native plant species was found to have a greater negative effect than allelochemicals from other co-evolved native plant species (Zhang et al. 2020). This indicates that belowground processes may certainly contribute to invasive population dynamics and range expansion.

Accordingly, both above- and belowground agents, such as aboveground herbivory and plant-soil feedbacks, should be considered jointly when trying to judge the success or failure of (exotic) populations. Yet, most studies usually have an either exclusively aboveground or belowground perspective. However, trophic interactions may affect all plant organizational components. Furthermore, common gardens are typically not established as in-site experiments within naturally occurring populations of a species under consideration. This is understandable for reasons of nature conservation. However, abstaining from this kind of test means disregarding the role of PSFs. They can be exerted by the species itself or by co-occurring species and should be addressed in the complex context of the whole set of environmental factors. Consequently, conducting controlled experiments in-site is the best way to adequately assess the risk of both classical biological invasions and cryptic invasions.

The biennial model species *Jacobaea vulgaris* Gaertn. (ragwort), native to Eurasia, is a successful invader on at least two continents and several studies have already provided evidence for genotypic differentiation between native and invasive plant origins. Invasive *J. vulgaris* individuals were shown to grow larger both in a greenhouse (Joshi and Vrieling 2005) and in a common garden experiment (Stastny et al. 2005), thereby providing evidence for an evolutionary shift, that is at least partly connected to herbivore composition differences between the species' ranges. Another common garden experiment, however, found no differences in biomass between origins (Rapo et al. 2010). Here, the authors concluded that the absence of origin-dependent differences might be due to the high-competition setting of their experiment, thus further emphasizing the necessity to study the performance in real-life settings.

In the last two decades, J. vulgaris also exhibited a severe increase in abundance in Northern Germany as part of the species' native range which made it a target species for management efforts in the species' native range as well (Möhler et al. 2021; Schwarz et al. 2021). Therefore, although invasive J. vulgaris genotypes might be expected to underperform in the species' native range, it is a good candidate to assess the risk for cryptic invasions through back-introduction that might counteract management efforts in the species' native range. Additionally, there is evidence for PSFs exerted by *J. vulgaris* affecting the performance of other species (Kos et al. 2015a, b) but also for PSF effects on the species itself (van de Voorde et al. 2011, 2012a). In particular, autotoxicity, as one agent transmitting PSFs in this species, has been proposed as a factor driving the decline in abundance in later successional stages (van de Voorde et al. 2012b; Möhler et al. 2018). Hence, when examining the invasive potential of J. vulgaris, common garden experiments including the entirety of environmental conditions should be carried out, i.e., ideally within natural populations that might exert those PSFs. Specifically, when aiming to elucidate the potential for cryptic invasions by back-introduction of invasive genotypes, experiments should be carried out within natural native populations to receive realistic outcomes of such a scenario.

We carried out a transplant experiment in field sites of naturally occurring ragwort populations in the species' native range. We aimed to test whether *J. vulgaris* individuals of invasive origins do, in fact, underperform in field sites in its native range. Alternatively, if they grow better than native plants, they thus have a potential to contribute to intraspecific cryptic invasions once back-introduced. To address how differences in observed performance might be related to environmental factors, we assessed soil abiotic information (soil moisture, CN and pH) just as biotic community information (*J. vulgaris* population density, species richness/α-diversity and vegetation height as proxy for productivity).

We additionally considered relative light availability, and bare soil proportion within every plot. This set-up allowed us to test (I) whether invasive genotypes show maladaptation to the species' native habitat. If maladaptation does not prove true, *J. vulgaris* would then be a potential candidate for problematic outcomes of back-introduction events. We also tested (II) what environmental factors might contribute to the observed patterns. Furthermore, in an additional greenhouse experiment using the same populations as in the field trial, we studied (III) the extent as to which, in particular, negative soilmediated impacts display genetic divergence between origins, i.e., are more expressed in individuals originating from the invasive than from the native range.

Materials and methods

Study species

Jacobaea vulgaris Gaertn. (Asteraceae, formerly *Senecio jacobaea*) is a predominantly biennial herbaceous plant species regularly observed with annual or perennial lifecycles (Wardle 1987). Flowering in the second year is positively influenced by size of the first-year rosette (van der Meijden and van der Waals-Kooi 1979) and nutrient availability (Prins et al. 1990). Each flowering individual may produce up to 30,000 achenes (Harper and Wood 1957). *Jacobaea vulgaris* is native to Eurasia (Harper and Wood 1957). After several introductions to various places, *J. vulgaris* today, is considered invasive in North America (Isaacson 1973) and Australia (Schmidl 1972).

Seed collection and preparation

Seed collection was carried out in the summer of 2018 in the Pacific Northwest (invasive range) and Central Europe (native range) at the same time. For species identification, we referred to "Rothmaler - Exkursionsflora von Deutschland" (Jäger 2017) and local plant identification literature. We sampled 22 invasive and 24 native populations, spanning a maximum distance of 524 km and 742 km, respectively. For each population, achenes (hereinafter seeds) of 20 maternal plants were collected, if available. The seeds were stored separately by seed family (i.e., seeds from the same maternal plant) in paper bags at room temperature in the lab of the Institute for Ecosystem Research (Kiel University) until use.

Six populations each by range of origin (invasive – native) were chosen according to seed quality and availability to be included in this experiment. In addition, we intentionally included populations varying in size and density in order to cover a broad range of variation within ranges. The sites in the native range served both as donor populations for seed sourcing and target sites for (re-)transplantation. For the selection of these six native populations, we thus additionally had to acquire permission from local authorities, landowners, and the tenant farmers for conducting a transplant experiment on their sites. All field sites for this experiment are owned by the Stiftung Naturschutz Schleswig-Holstein (for population information, s. Suppl. material 1: fig. S1). From each population, seeds from seven randomly selected seed families were sown in potting soil (TKS 2 pot Medium Coarse, Floragard Vertriebs-GmbH, Oldenburg, Germany) on germination trays in April of 2019. The seeds were covered by 1 cm of soil layer to prevent them from drying out. The germination trays were placed in a greenhouse cabinet with ambient temperature and a photoperiod of 12:12 (night/day) hours and watered daily in the following days. After four weeks, five seed families with the highest germination success within each population were chosen to be included in the experiment and seedlings were thinned to allow optimal growth. Once established, the germination trays were placed outside to allow acclimatization of the separated individuals to outdoor conditions.

Experimental set-up: Field experiment

The field experiment was designed to estimate performance of invasive individuals compared to native individuals in the species' original native range. The location of the six native populations used for seed material sampling also served as transplantation sites. In each of these six sites, we established five experimental plots. Plot locations were assigned randomly within site with coordinates marking the southwest corner of each plot. Starting from this corner, an area of 0.9 m × 1.2 m was established, where transplants were arranged in 4×5 rows (all plants were 0.3 m apart) leading to a total of 20 planting positions. One individual from each of the six invasive populations and two individuals from each of the six native populations (and therefore also originating from the experimental sites (= at their population home)) were randomly assigned to the planting positions leaving out the southwest and northwest spots. Thus, a total of 18 individuals were planted per plot. Each two individuals from the six native populations were replicates from the same seed family. For each of those replicates, one individual was a priori randomly chosen for the present experiment while the second one was assigned to remain into the summer of 2020 as part of an additional experiment (s. Suppl. material 1: fig. S2 for schematic overview). Therefore, 12 of the 18 individuals per plot (6 native and 6 invasive) originally belonged to the experiment described here. In case of mortality, however, the native replicate individual served as a substitute in the present experiment (except for the assessment of survival). All remaining individuals were removed before flowering in summer 2020 to ensure prevention of seed set and dispersal.

Planting was carried out starting June 15th 2019 (approx. 2 months after sowing). The vegetation in the plots was cut to approx. 0.3 m to reduce heterogeneity during early establishment of the experimental plants. Subsequently, experimental plants were brought out with the adhering potting soil and labelled for recognition. After planting, each experimental plot was watered with 10 l water right away and two additional times after one week to assure establishment and survival of the planted individuals.

Next to each of the five experimental plots, a $2 \text{ m} \times 2 \text{ m}$ monitoring plot was established following the diagonal extension 5 m apart in a northeastern direction. The monitoring plots served for recording vegetation composition and structure, including information on overall vegetation height (as a measure for productivity), coverage using a modified Londo scale (Londo 1976) and percentage of bare soil. Soil samples were taken next to the monitoring plot for soil C:N determination (with a EURO Elemental Analyzer) and determination of pH in the laboratory at the Institute for Ecosystem Research, Kiel University. In the monitoring plots, *Jacobaea* abundance (i.e., naturally occurring individuals) was additionally determined to infer local population density. Soil moisture was measured at the four corners of the experimental plot using Time Domain Reflectometry with ML3 Theta-soil moisture sensor (Delta-T devices Ltd, Cambridge, United Kingdom). Mean relative light intensity was determined from four evenly distributed measurements taken within each experimental plot (LI-1500 Light Sensor Logger, Li-COR Biosciences, Lincoln, United States).

The monitoring of the field experiment ran from June 28th until September 28th. After 6 weeks and 14 weeks of experimental runtime, we determined specific leaf area (SLA) and leaf dry matter content (LDMC) of transplants. For this, the third fully developed leaf from the top was taken from each plant and stored in a moisturized plastic bag in a cooler box for transportation to the lab. Fresh leaves were scanned (Expression 11000XL, EPSON Deutschland GmbH, Meerbusch, Germany) and leaf area was determined using WinFolia (WinFolia Pro 2015, Regent Instruments Inc., Quebec, Canada). Leaf fresh weight was determined using a precision scale (Sartorius 1702MP8, Sartorius AG, Göttingen, Germany). All leaves were dried at 65 °C for 48 hours afterwards for subsequent dry weight determination. At the end of the experimental runtime, we determined the transplants' expansion in two directions (to calculate rosette size), the number of healthy leaves, and length of the longest leaf. Herbivory was assessed as a binary trait and considered present when parts of the leaf were missing or by the appearance of characteristic "bullet-holes" caused by Longitarsus jacobaeae. For biomass determination, all invasive individuals and half of the native individuals were dug up (i.e. 12 individuals per plot). Dry weight was separately determined for aboveground and belowground biomass after drying in a drying oven at 65 °C for 48 hours. We additionally calculated the root:shoot ratio as a measure for resource distribution strategy.

Experimental set-up: Greenhouse experiment

The greenhouse experiment was set up analogous to the field experiment and ran from August 21st (approx. 4 months after sowing of seeds) to November 13th. To decouple the influence of soil biota effects from other environmental factors varying with the field sites, soil samples were taken from all six native field sites used in the field experiment. These soil samples served as an inoculum for soil-biota treatments to all native and invasive individuals. For this, soil material was sampled about 0.5 m south of the southwestern corner of each experimental plot. After careful sod removal, a volume of 1 l soil was taken per plot, sieved through a 2 mm mesh and collected in a sterilized bucket. Separate soil samples from all plots were pooled and merged by site and served as the site-specific donor substrate. Soil sampling equipment was sterilized between sites to avoid cross-contamination.

All 12 population origins incorporated in the field experiment were also used in the greenhouse with three seed families randomly chosen out of the five used in the field. For each seed family, each one individual was grown with soil addition from one of the six field sites or only using standard substrate (control). Standard substrate consisted of 60% fine sand provided by the Botanical Garden of Kiel University and 40% unfertilized potting soil (F.E. Typ Nullerde, HAWITA Gruppe GmbH, Vechta, Germany) constituting an environment especially low in nutrients. This led to a total of seven different treatments for each seed family, thus resulting in a total of 252 individuals in the greenhouse experiment. All individuals were transferred to 1.5 l planting pots filled with 1.26 l standard substrate supplemented either by 0.14 l of soil collected from one of the six field sites (9:1 standard substrate:field soil) or an additional 0.14 l standard substrate for the control group. The standard substrate was processed by an autoclave (Webeco Dampf-Sterilisator, Matachana Germany GmbH, Selmsdorf, Germany) to reduce already present soil biota to a minimum. Each pot additionally received 3 g slow-release fertilizer (2.14 g/l) (Basacote Plus 6M 16 + 8 + 12 (+ 2 + 5), Compo Expert GmbH, Münster, Germany) corresponding to low levels of nutrient availability as per the manufacturer's specifications. Planting pots were put on saucers and distributed in the greenhouse. Their position on benches was randomized every week. Predatory mites and sticky traps were installed at the beginning of the experiment to reduce infestation risk with insects. After one month, an insecticide was used on all plants (Spruzit Schädlingsfrei, W. Neudorff GmbH KG, Emmerthal, Germany), and milk and neem oil were applied to all individuals to prevent the spread of mildew. Throughout the experiment all plants were watered with 75 ml of tap water every 1-3 days as needed. Excess water from the saucers was emptied after every watering.

After the experimental runtime, monitoring and biomass harvest were carried out analogous to the field experiment. SLA and LDMC were assessed after 4 weeks and 12 weeks, respectively.

Statistical analysis

Statistical analyses were performed with R (Version 4.1.1) (R Core Team 2019). For the field experiment, a linear mixed effects model was fitted using lme4 (Version 1.1.27) (Bates et al. 2015) and ImerTest (Version 3.1.3) (Kuznetsova et al. 2017). Response variables were transformed if necessary (s. Suppl. material 1: table S1 for transformations used). We included origin (native/invasive) as a fixed effect and the random intercept for plot nested in site and seed family nested in population. We performed a correlation test (function rcorr from the package Hmisc (Harrel 2021), type = "pearson") with the environmental variables. Mean moisture was significantly correlated with maximum plant height, C:N ratio as well as cover of bare soil, whereas relative light was significantly correlated with maximum plant height, α -diversity as well as cover of bare soil, respectively. Soil pH was significantly correlated with α-diversity. Subsequently we excluded mean moisture, pH and relative light intensity as covariates in model fitting. We included number of leaves one week after planting, population density of *J. vulgaris* individuals as well as α-diversity (i.e. number of species), C:N ratio, maximum vegetation height and percentage of bare soil in the monitoring plot as covariates in the full model. The best model was subsequently selected using the step

function (lmerTest) and retained covariates were additionally tested for their interaction with the fixed effect origin in the final model. For survival and herbivory, we fitted generalized linear mixed effects models using the binomial-family and the same structure as for the other variables; however, model selection was done manually.

Similarly, for the greenhouse experiment, we fitted a lmer with origin and provenance of the soil (site) as fixed effects. As a covariate we included either the respective response variables' value at the beginning of the experiment or the initial number of leaves if no starting value was available (for biomass variables, SLA, LDMC). We added the random intercept for seed family nested in the population. For the number of leaves, we fitted a glmer with the poisson family. Differences between the treatments were examined using the Tukey post-hoc test in emmeans (Version 1.7.0) (Lenth 2019). All plots were created using ggplot2 (Version 3.3.5) (Wickham 2016). Predicted values for response variables used in plots were obtained using effects (Version 4.1.4) (Fox and Weisberg 2018, 2019).

Data availability

The datasets generated during and/or analyzed during the current study as well as the code used for analysis are available from the corresponding author upon reasonable request.

Results

Field experiment

After 14 weeks of experimental runtime, individuals originating from the invasive range had developed larger rosettes (Table 1, Suppl. material 1: table S2) and showed a significantly higher aboveground biomass than those from the native range (Fig. 1a). There was no difference in the belowground biomass (Fig. 1b), which led to a smaller root:shoot ratio in invasive origins (Fig. 1c).

Irrespective of origin, C:N ratio and maximum height of the vegetation in the monitoring plot displayed a significantly negative relationship with *J. vulgaris* belowground and total biomass as well as with length of the longest leaf (not shown, Table 1). The higher the maximum vegetation, the higher was also the number of individuals with signs of herbivory (Table 1) and this pattern was more pronounced in native than in invasive individuals (Fig. 1d, Table 1). There was no difference in survival depending on origin (Table 1).

For functional leaf traits, no significant origin effect could be detected (Table 2, Suppl. material 1: table S3). Specific leaf area (SLA) after 6 weeks of experimental runtime, however, was negatively correlated with the number of species in the monitoring plot (α -diversity) for individuals originating from the species' invasive range (Fig. 2a). After 14 weeks, SLA was higher with increasing height of the surrounding vegetation, irrespective of plant origin (Fig. 2b). In addition, after 14 weeks, there was an interaction effect of LDMC with increasing α -diversity displaying an increase in LDMC in invasive individuals but no such pattern in native origins (Fig. 2c).

Table 1. Field experiment – performance traits. Results from the ANOVA for the linear-mixed effects and generalized-mixed effects model (Herbivory and Survival) in the field experiment for performance traits. Significant effects (p<0.05) are printed in bold.

	Rosette Expansion [cm ²]					Number of Leaves [count]						
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV
Origin	1	287.15	7.1117	0.008	147.09 \pm	194.14 \pm	1	9.602	4.6268	0.058	$7.29~\pm$	$8.63~\pm$
					216.97	251.87					5.82	6.76
Week 1**	1	297.41	5.1658	0.024			1	227.745	22.79	<0.001		
C:N ratio	NA	NA	NA	NA			NA	NA	NA	NA		
Max. vegetation height	NA	NA	NA	NA			NA	NA	NA	NA		
Origin × C:N ratio	NA	NA	NA	NA			NA	NA	NA	NA		
Origin × Max.	NA	NA	NA	NA			NA	NA	NA	NA		
vegetation height												
	Length of longest Leaf [cm]						g]					
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV
Origin	1	286.431	0.5499	0.458	12.07 ± 6.48	13.94 ± 8.23	1	290.39	7.6368	0.006	0.60 ± 1.95	1.01 ± 2.82
Week 1**	1	298.693	10.7502	0.001			1	303.44	10.4424	0.001		
C:N ratio	1	7.334	6.8443	0.033			NA	NA	NA	NA		
Max. vegetation height	1	16.219	5.289	0.035			NA	NA	NA	NA		
Origin × C:N ratio	1	285.911	0.0208	0.885			NA	NA	NA	NA		
Origin × Max.	1	286.344	0.3022	0.583			NA	NA	NA	NA		
vegetation height												
	Belowground Biomass [g]							Total Bior	nass [g]			
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV
Origin	1	281.277	0.2909	0.590	0.51 ±	0.53 ±	1	290.009	0.0228	0.880	1.11 ±	1.54 ±
					0.57	0.52					2.47	3.31
Number of leaves	1	315.251	26.8191	< 0.001			1	289.342	18.0332	< 0.001		
C:N ratio	1	27.973	5.6967	0.024			1	29.04	4.3788	0.045		
Max. vegetation height	1	29.692	4.8105	0.036			1	30.241	5.9063	0.021		
Origin × C:N ratio	1	278.043	0.7901	0.374			1	278.227	0.1853	0.667		
Origin × Max.	1	279.297	0.0001	0.993			1	279.264	0.0392	0.843		
vegetation height												
		Root:Sho	oot Ratio									
	NumDf	DenDF	F	Р	NAT	INV	-					
Origin	1	54.908	11.7481	0.001	2.31 ±	1.76 ±						
-					2.27	1.70						
Number of leaves	1	290.887	0.4528	0.502								
J. vulgaris density	NA	NA	NA	NA								
Max. vegetation height	NA	NA	NA	NA								
Origin × J. vulgaris	NA	NA	NA	NA								
density												
Origin × Max.	NA	NA	NA	NA								
vegetation height												
	H	erbivory [probabili	ty]		Survival [probability]						
	Esti	mate	Z	Р	NAT	INV		Estimate	z	Р	NAT	INV
Origin		14.4039	2.317	0.021	0.13 ± 0.34	0.11 ± 0.32		0.1614	0.403	0.687	0.86 ± 0.35	0.84 ± 0.36
Number of leaves		NA	NA	NA				NA	NA	NA		
J. vulgaris density		-0.0381	-1.487	0.137				NA	NA	NA		
Max. vegetation height		0.1737	2.821	0.005				NA	NA	NA		
Origin × J. vulgaris		-0.0382	-1.013	0.311				NA	NA	NA		
density												
Origin × Max.		-0.1296	-2.27	0.023				NA	NA	NA		

J. vulgaris density = density of naturally occurring individuals in experimental plot; Number of Leaves was counted at initial monitoring one week after planting; †Week 1 refers to the values of the response variable at the start of the experiment, e.g. for Rosette Expansion (week 14) this is the Rosette Expansion at week 1. NAT = mean and standard deviation for native individuals. INV = mean and standard deviation for invasive individuals.



Figure 1. Origin effects (field experiment). Response of performance (**a**–**c**) traits in relation to origin of the seeds for each individual. Data shown are predicted values from the model \pm SE. Native individuals (left, blue) originated from the field sites where the experimental plots were located. Invasive individuals (right, orange) originate from the Pacific Northwest. Herbivory (**d**) was assessed as a binary trait (presence/absence) only. N = 319 (**a**–**c**) and n = 322 (**d**). For depiction of raw data s. Suppl. material 1: fig. S3.

	SLA (week 6)					LDMC (week 6)							
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV	
Origin	1	156.816	1.6254	0.204	$21.84~\pm$	$20.37~\pm$	1	43.488	3.1219	0.084	$0.13 \pm$	$0.13 \pm$	
					6.16	4.17					0.03	0.02	
Number of leaves	1	173.993	3.6489	0.058			1	170.423	0.8594	0.355			
α-Diversity	1	7.592	12.0547	0.009			NA	NA	NA	NA			
C:N ratio	1	5.373	5.8519	0.056			NA	NA	NA	NA			
Origin $\times \alpha$ -Diversity	1	139.819	6.3232	0.013			NA	NA	NA	NA			
Origin × C:N ratio	1	155.856	0.4553	0.500			NA	NA	NA	NA			
		SLA (w	eek 14)		LDMC (week 14)								
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV	
Origin	1	284.181	1.4873	0.224	$27.38~\pm$	$27.29~\pm$	1	292.377	0.1939	0.660	$0.11 \pm$	$0.09 \pm$	
					7.54	7.49					0.02	0.02	
Number of leaves	1	299.233	1.469	0.226			1	255.374	0.7518	0.387			
Max. vegetation height	1	31.503	5.1708	0.029			NA	NA	NA	NA			
Origin * Max.	1	276.967	1.435	0.232			NA	NA	NA	NA			
vegetation height													
α-Diversity	NA	NA	NA	NA			1	27.654	2.552	0.122			
C:N ratio	NA	NA	NA	NA			1	16.52	3.7026	0.072			
Origin $\times \alpha$ -Diversity	NA	NA	NA	NA			1	276.883	6.7717	0.010			
Origin × C:N ratio	NA	NA	NA	NA			1	280.836	0.0623	0.803			

Table 2. Field experiment – functional traits. Results from the ANOVA for the linear-mixed effects in the field experiment for functional leaf traits. Significant effects (p<0.05) are printed in bold.

Number of Leaves was counted at initial monitoring one week after planting; SLA = specific leaf area, LDMC = leaf dry matter content.



Figure 2. Origin effects × covariate (field experiment). Effects of origin in interaction with α -diversity (**a**, **c**) and maximum vegetation height of surrounding vegetation within the experimental plot (**b**). Data shown are predicted values from the model with upper and lower range. O = origin, α -Div = α -diversity, mvh = maximum vegetation height (of surrounding vegetation), SLA = specific leaf area, LDMC = leaf dry matter content. Week 6 and Week 14 indicates that the leaves for analysis were harvested after 6 weeks of experimental runtime or at the final harvest of the plants after the entire experimental runtime, respectively. N = 193 (**a**), 311 (**b**), 319 (**c**). SLA after 6 weeks was only taken for plants that had a sufficient number of healthy leaves and therefore constitutes a reduced subset. For depiction of raw data s. Suppl. material 1: fig. S4.

Greenhouse experiment

In the greenhouse experiment, there was no difference in biomass depending on the origin of the individuals (Table 3, Suppl. material 1: table S4). Provenance of the soil (treatment) did not affect the aboveground biomass (Fig. 3a) and root:shoot ratio (Fig. 3d) but belowground biomass (Fig. 3b) and total biomass differed significantly between the treatments (Fig. 3c). Number of leaves was the only trait exhibiting significant soil provenance × population origin interaction effects, but post-hoc test revealed no significant difference for origins within any given site.

For the functional traits SLA and LDMC, no difference depending on the origin of the individuals could be detected (Table 4, Suppl. material 1: table S5). However, soil provenance incurred differences in SLA after four (Fig. 4a) and 12 weeks

	Ro	sette Expa	nsion [cr	n²]			Number of Leaves							
	NumDf	DenDF	F	Р	NAT	INV		Chisq	Df	Pr	NAT	INV		
Origin	1	10.302	0.3555	0.563	515.6 ±	525.75 ±		0.343	1	0.558	$26.35 \pm$	$25.19 \pm$		
					198.34	185.82					8.19	8.42		
Treatment	6	224.597	2.6195	<0.001				19.756	6	0.003				
Initial†	1	224.9	3.93	0.090				19.2468	1	<0.001				
${\rm Origin} \times {\rm Treatment}$	6	224.529	0.9115	0.487				24.9001	6	<0.001				
	Leng	gth of long	gest Leaf	[cm]										
	NumDf	DenDF	F	Р	NAT	INV	-							
Origin	1	10.007	3.462	0.092	17.83 ±	$18.66 \pm$								
					3.80	3.83								
Treatment	6	200.665	3.9741	<0.001										
Initial†	1	220.839	16.663	<0.001										
${\rm Origin} \times {\rm Treatment}$	6	200.924	1.3928	0.219										
	Abo	oveground	Biomass	[g]		Belowground Biomass [g]								
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV		
Origin	1	10.211	2.915	0.160	$4.80 \pm$	5.39 ±	1	223	0.0646	0.800	8.77 ±	$2.66 \pm$		
					2.15	2.66					5.39	8.77		
Treatment	6	225.441	2.0788	0.057			6	223	3.7241	0.002				
Number of leaves	1	224.85	2.4574	0.118			1	223	0.0208	0.886				
$Origin \times Treatment$	6	225.216	1.1429	0.338			6	223	0.4702	0.930				
		Total Bio	mass [g]				Root:Shoot ratio							
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV		
Origin	1	10.65	1.8419	0.203	12.24 ±	$14.24~\pm$	1	36.516	0.3564	0.554	1.77 ±	1.65 ±		
					8.20	8.78					1.25	1.26		
Treatment	6	226.95	3.7727	0.001			6	187.414	2.0053	0.067				
Number of leaves	1	171.56	0.1229	0.726			1	190.777	0.0396	0.842				
$Origin \times Treatment$	6	226.55	0.9688	0.447			6	186.814	0.2409	0.962				

Table 3. Greenhouse experiment – performance traits. Results from the ANOVA for the linear-mixed effects in the greenhouse experiment for performance traits. Significant effects (p<0.05) are printed in bold. Treatment refers to the provenance of the added soil. All soils originate from field sites within the species' native range.

Number of leaves was counted at initial monitoring. Initial[†] refers to the values of the response variable at the initial monitoring, e.g. for Rosette Expansion (week 12) this is the Rosette Expansion at the initial monitoring, Treatment refers to the provenance of the soil used.

Table 4. Greenhouse experiment – functional traits. Results from the ANOVA for the linear-mixed effects in the field experiment for functional leaf traits. Significant effects (p<0.05) are printed in bold.

		SLA (v	week 4)		LDMC (week 4)										
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV			
Origin	1	10.142	0.9933	0.342	23.99 ± 4.46	22.73 ± 3.55	1	9.911	0.1945	0.669	$0.12 \pm$	$0.12 \pm$			
											0.00	0.02			
Treatment	6	202.852	2.807	0.012			6	203.173	3.3796	0.003					
Number of	1	235.607	3.1885	0.075			1	205.374	10.3484	0.002					
leaves															
Origin ×	6	202.57	0.4798	0.823			6	202.779	1.0921	0.368					
Treatment															
		SLA (w	reek 12)			LDMC (week 12)									
	NumDf	DenDF	F	Р	NAT	INV	NumDf	DenDF	F	Р	NAT	INV			
Origin	1	10.214	1.6375	0.229	21.22 ± 3.33	19.95 ± 3.35	1	9.894	0.0999	0.759	$0.20 \pm$	$0.20 \pm$			
											0.03	0.04			
Treatment	6	200.084	3.7331	0.002			6	200.154	1.2052	0.305					
Number of	1	232.749	8.0692	0.005			1	219.386	1.3717	0.243					
leaves															
Origin ×	6	199.909	0.2384	0.963			6	199.852	0.177	0.983					
Treatment															

SLA = Specific leaf area, LDMC = leaf dry matter content.



Figure 3. Treatment effects on performance traits (greenhouse experiment). Effects of soil provenance in the greenhouse experiment. Data shown are predicted values from the model \pm SE. Different colors represent different soil origins (only soil from native sites were included in this experiment). Different letter combinations in the panels indicate significant differences according to the Tukey post-hoc test. ALB = Albersdorf, ARB = Arpsdorf, BUN = Bünsdorf/Wittensee, Con = Control (no soil added from any field site), PRE = Preetz, ROT = Rotenhahn/Eidertal, VOL = Vollstedter See. For location information see Suppl. material 1: fig. S1. N(**a**) = 250, n(**b**) = 238, n(**c**) = 251, n(**d**) = 230. For depiction of raw data s. Suppl. material 1: fig. S5.

(Fig. 4b), and LDMC differed significantly depending on soil provenance after four weeks (Fig. 4c). No differences in LDMC were observed after 12 weeks of experimental runtime (Fig. 4d).

Discussion

Since its initial appearance in the Pacific Northwest about a century ago, there was, theoretically, ample time for adaptive evolutionary adjustment to occur in *Jacobaea vulgaris* populations. Local adaptation is supposed to lead to fitness advantages of invasive populations adjusted to environmental conditions in the novel range, including an absence of specialist herbivores (Kawecki and Ebert 2004). However, it would be expected that such a shift would not pay off anymore when back-introduced to the species' native range (Eurasia) and rather provide evidence of maladaptation. Contrary to this gen-



Figure 4. Treatment effects on functional traits (greenhouse experiment). Effects of soil provenance in the greenhouse experiment. Data shown are predicted values from the model \pm SE. Different colors represent different soil origins (only soil from native sites were included in this experiment). Different letter combinations in the panels indicate significant differences according to the Tukey post-hoc test. ALB = Albersdorf, ARB = Arpsdorf, BUN = Bünsdorf/Wittensee, Con = Control (no soil added from any field site), PRE = Preetz, ROT = Rotenhahn/Eidertal, VOL = Vollstedter See. For location information see Suppl. material 1: fig. S1. N(**a**) = 215, n(**b**) = 248, n(**c**) = 251, n(**d**) = 249. For depiction of raw data s. Suppl. material 1: fig. S6.

eral assumption, in the present study, invasive origins outperformed the native origins when (re-)transplanted to native field sites in Northern Germany. Our findings are, in fact, more in line with considerations associated with the potential risk of cryptic invasions triggered by back-introductions of invasive genotypes and multiple factors might have contributed to our observation (hypothesis I).

Origin effects and maladaptation

There is some evidence that specialist herbivores prefer invasive individuals of *J. vulgaris* over native ones (Lin et al. 2015). In the experimental area of Northern Germany, these specialist herbivores are naturally present. However, the specialist *Tyria jacobaeae* usually prefers larger individuals (van der Meijden 1976) while additionally, theory and evidence suggest that generalist herbivores prefer native origins (Keane 2002; Joshi and Vrieling 2005). The fact that our transplants were relatively unattractive to specialist herbivores while previous studies predict higher attractiveness of native origins to generalists, might have contributed to the observed increased performance in invasive origins and increased occurrence of herbivory in native origins. For moths and

butterflies, specialist species were found to prefer lower vegetation compared to generalists (Pöyry et al. 2006), which in combination with the significant herbivory × maximum vegetation height interaction in our experiment, further corroborates this assumption.

In contrast, the absence of any origin-dependent differences in the greenhouse experiment was unexpected given previous studies with *J. vulgaris* showing higher performance of invasive origins (Joshi and Vrieling 2005). Different factors could explain this outcome. First of all, greenhouse experiments, by definition, involve herbivory to be kept at a minimum. This design therefore neither favors native (more specialists) nor invasive (more generalists) individuals of *J. vulgaris* in a greenhouse. Secondly, the greenhouse experiment was set up later in the year while relying on the same growing material. Accordingly, the experimental individuals for the greenhouse experiment were older than the transplants. Seasonal timing might thus have additionally affected plant development differently.

In the field experiment, the invasive individuals might also have benefitted from atypically high temperature and decreased precipitation during the experimental runtime (Broennimann et al. 2007; Early and Sax 2014). During the summer of 2019, the average temperature in Germany was 2.9 °C higher and precipitation on average was 27.7% lower than predicted according to the international climatological reference period (Deutscher Wetterdienst 2020). For the populations included in this experiment, the invasive range is both warmer (native: 15.6 ± 1.38 C; invasive: 16.21 ± 1.18 °C) and drier (native: 232.5 ± 6.75 mm; invasive: 125.5 ± 34.49 mm) during the warmest quarter on average (Data from BioClim, accessed Nov. 10^{th} 2020; Fick and Hijmans 2017), so the present finding might be indicative of a possible climatic niche shift in invasive origins (Broennimann et al. 2007). However, previous studies with *J. vulgaris* showed that, inter alia, competitive ability and regrowth (Lin et al. 2015), growth and regrowth (Lin et al. 2018) as well as growth, photosynthetic rate, and LMR (Lin et al. 2019) differ between native and invasive origins of *J. vulgaris*, but these differences were explicitly not driven by climatic conditions.

The role of environmental conditions and soil-borne effects

In the present study, the provenance of soil (treatment) differently impacted *J. vulgaris* performance (e.g., aboveground biomass), thereby confirming that soil-borne biotic effects contribute to differentiation among populations, as expected (hypothesis III). Strong negative feedbacks on *J. vulgaris* populations themselves have previously been shown in native populations (van de Voorde et al. 2011, 2012a). They furthermore increased with population density (van de Voorde et al. 2012a) and over time in an interspecific competition setting (Bezemer et al. 2018). Additionally, other species were found to exert PSFs on *J. vulgaris* (Bezemer et al. 2006; van de Voorde et al. 2011; Wubs and Bezemer 2018), showing that PSFs are a factor not to be neglected when studying this species' performance and population dynamics.

Knowledge about soil provenance × plant origin interactions in general is lacking for this model species to date and we found no signs for enemy release on the belowground level as shown for *Centaurea maculosa* performance (Callaway et al. 2004). Our findings are more in line with a study on *Verbascum thapsus* that found signs of coevolution between plants and soil microbes on the between-population level but not between ranges of origin (Dieskau et al. 2020). To ensure that the patterns observed in our experiment are not co-affected by differences in soil properties, soil washes as applied by Dieskau et al. (Dieskau et al. 2020) should preferably be used in future experiments to address pure biotic effects most precisely.

In the present experiment, maximum vegetation height (strongly linked to light availability with r = -0.62, p<0.001 with Pearson's rank) was a relevant environmental factor for both origins, with the typical responses of increasing specific leaf area with decreasing light availability (Cornelissen 1992). LDMC varied more in the further course of the experiment, whereas variation in SLA was higher earlier in the experiment with different responses to increasing α -diversity depending on the range origin of the transplants. Given that the invasive individuals allocated more biomass by the end of the experiment, this pattern points towards a reaction to different environmental conditions rather than a sign of stress. Higher phenotypic variation was not only shown to pay off for invasive species compared to native congeners (Funk 2008), but may also apply within a species when comparing native and invasive origins (Caño et al. 2008; Hock et al. 2019). Under these considerations, our results suggest that high phenotypic plasticity might be one important factor contributing to the invasion success of *J. vulgaris* as well.

In summary, we cannot conclude explicitly which factors are the main drivers of increased performance of invasive transplants in the species' native range (hypothesis II). It is, therefore, also difficult to accurately predict the long-term consequences of back-introduction of propagules or individuals of invasive origin into the species' native range. However, genetic admixture might accelerate geographic expansion and invasion (Qiao et al. 2019), thus re-introduction of these invasive genotypes could therefore be problematic. Re-introduction might return originally common but then modified ("adapted") genotypes that have undergone selection in the invasive range. At the same time, it could also add genotypes from other parts of the species' native range, especially in J. vulgaris. In our experiment, we cannot determine whether the invasive transplants are re-introduced in the strict sense or originally came from other parts of the species' native range, especially as invasive populations of *J. vulgaris* were found to probably be admixed before spreading within the invasive range (Doorduin 2012). In Northern Germany, J. vulgaris is assumed to form large panmictic metapopulations (Jung et al. 2020). However, for other species, intraspecific hybridization was previously shown to promote invasion processes (Kolbe et al. 2004; Geiger et al. 2011) and these findings could also apply to cryptic invasions. Therefore, introducing genetic material from the invasive range irrespective of its history, may evoke concerns among nature conservationists for the species' native range.

Conclusion

Invasive genotypes of *J. vulgaris* seem to exhibit higher levels of phenotypic variation, giving them more leeway when confronted with changing environmental conditions. This appears to be especially applicable under favorable environmental conditions as

found in the field experiment, but our findings do not preclude that invasive genotypes might also show superior performance under certain more stressful conditions. It is probable that invasive genotypes, in the future, might do even better in the species' native range, as the environmental conditions might converge to the environmental conditions of the invasive regions.

With regard to a possible cryptic invasion of *J. vulgaris* in the native range, the present study suggests two main messages: Primarily, (back-) introduction of propagules from the invasive ranges of *J. vulgaris* should be prevented as much as possible. Secondly, it might be beneficial to invest more in further identifying the characteristics that decrease the susceptibility of a field site for *J. vulgaris* in general. For *J. vulgaris*, in particular, highly controlled greenhouse experiments under realistic conditions should aim to validate the observed patterns in generative (second-year) flowering plants and assess fitness traits.

However, it is likely that cryptic invasions will occur more often than identified so far. To date, invasion research mostly focuses on the unidirectional introduction into the novel range, and little is known about the possibility of back-introduction. Furthermore, knowledge about the frequency with which back-introductions happen is so far lacking. Generally, pathways of biological invasions are complex and vary in their relative importance over time (Hulme 2009; Essl et al. 2015); climate change may further increase human movement and subsequently the spread of non-native biota (Robinson et al. 2020). Future approaches elucidating the risk of cryptic invasions should apply more controlled back-introduction experiments as real-world tests with further candidate species that have shown to be successful invaders in the past.

Acknowledgements

We thank P. Music and F. Meyer for helping to set up the field experiment and subsequent support in monitoring as well as H. Bülow for plant care in the greenhouse. For technical help, especially in soil sterilization, we want to thank I. Meyer and C. Plieth for providing the necessary equipment. We are grateful to the Stiftung Naturschutz Schleswig-Holstein and A. Huckauf, in particular, for providing permission and access to their field sites.

The study was financially supported by the Evangelisches Studienwerk Villigst e.V. with a Ph.D. scholarship awarded to LYW and with a Quick and Tiny grant from Kiel University awarded to JR and LYW. We acknowledge financial support by Land Schleswig-Holstein within the funding programme Open Access Publikationsfonds.

References

Alves DFR, Barros-Alves S de P, Dolabella SS, de Almeida AC, Martinez PA (2021) Invasive shrimp *Cinetorhynchus erythrostictus* (Decapoda: Caridea) misidentified in the marine aquarium trade: Niche overlap with a native congeneric species. Estuarine, Coastal and Shelf Science 258: 107411. https://doi.org/10.1016/j.ecss.2021.107411

- Deutscher Wetterdienst (2020) Monthly description summer 2019. Deutscher Wetterdienst (DWD). https://www.dwd.de/EN/ourservices/klimakartendeutschland/klimakartendeutschland_monatsbericht.html
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67(1): 1–48. https://doi.org/10.18637/jss.v067.i01
- Beckmann M, Bruelheide H, Erfmeier A (2016) Reduced tolerance to simulated herbivory on clonal organs in alien genotypes: A multi-species experiment with native and introduced origins. Biological Invasions 18(2): 549–563. https://doi.org/10.1007/s10530-015-1027-8
- Bezemer TM, Harvey JA, Kowalchuk GA, Korpershoek H, van der Putten WH (2006) Interplay between *Senecio jacobaea* and plant, soil and aboveground insect community composition. Ecology 87(8): 2002–2013. https://doi.org/10.1890/0012-9658(2006)87[2002:IBSJAP] 2.0.CO;2
- Bezemer TM, Jing J, Bakx-Schotman JMT, Bijleveld E-J (2018) Plant competition alters the temporal dynamics of plant-soil feedbacks. Journal of Ecology 106: 2287–2300. https:// doi.org/10.1111/1365-2745.12999
- Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26(7): 333–339. https://doi.org/10.1016/j.tree.2011.03.023
- Blossey B, Nötzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology 83(5): 887–889. https://doi. org/10.2307/2261425
- Brandes U, Furevik BB, Nielsen LR, Kjær ED, Rosef L, Fjellheim S (2019) Introduction history and population genetics of intracontinental scotch broom (*Cytisus scoparius*) invasion. Diversity & Distributions 25(11): 1773–1786. https://doi.org/10.1111/ddi.12979
- Broennimann O, Treier UA, Müller-Schärer H, Thuiller W, Peterson AT, Guisan A (2007) Evidence of climatic niche shift during biological invasion. Ecology Letters 10(8): 701– 709. https://doi.org/10.1111/j.1461-0248.2007.01060.x
- Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427(6976): 731–733. https://doi.org/10.1038/nature02322
- Caño L, Escarré J, Fleck I, Blanco-Moreno JM, Sans FX (2008) Increased fitness and plasticity of an invasive species in its introduced range: a study using *Senecio pterophorus*: Increased plasticity of an invasive species. Journal of Ecology 96(3): 468–476. https://doi. org/10.1111/j.1365-2745.2008.01363.x
- Colautti RI, Barrett SCH (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342(6156): 364–366. https://doi.org/10.1126/science.1242121
- Cornelissen J (1992) Seasonal and year to year variation in performance of *Gordonia acuminata* seedlings in different light environments. Canadian Journal of Botany 70(12): 2405–2414. https://doi.org/10.1139/b92-299
- de Andrade AFA, Velazco SJE, De Marco Jr P (2019) Niche mismatches can impair our ability to predict potential invasions. Biological Invasions 21(10): 3135–3150. https://doi. org/10.1007/s10530-019-02037-2
- Dieskau J, Bruelheide H, Gutknecht J, Erfmeier A (2020) Biogeographic differences in plantsoil biota relationships contribute to the exotic range expansion of *Verbascum thapsus*. Ecology and Evolution 10(23): 13057–13070. https://doi.org/10.1002/ece3.6894

- Doorduin L (2012) Rapid evolution or preadaptation in invasive *Jacobaea vulgaris*. Universiteit Leiden.
- Early R, Sax DF (2014) Climatic niche shifts between species' native and naturalized ranges raise concern for ecological forecasts during invasions and climate change. Global Ecology and Biogeography 23(12): 1356–1365. https://doi.org/10.1111/geb.12208
- Erfmeier A (2013) Constraints and release at different scales The role of adaptation in biological invasions. Basic and Applied Ecology 14(4): 281–288. https://doi.org/10.1016/j. baae.2013.04.004
- Essl F, Bacher S, Blackburn TM, Booy O, Brundu G, Brunel S, Cardoso A-C, Eschen R, Gallardo B, Galil B, García-Berthou E, Genovesi P, Groom Q, Harrower C, Hulme PE, Katsanevakis S, Kenis M, Kühn I, Kumschick S, Martinou AF, Nentwig W, O'Flynn C, Pagad S, Pergl J, Pyšek P, Rabitsch W, Richardson DM, Roques A, Roy HE, Scalera R, Schindler S, Seebens H, Vanderhoeven S, Vilà M, Wilson JRU, Zenetos A, Jeschke JM (2015) Crossing frontiers in tackling pathways of biological invasions. Bioscience 65(8): 769–782. https://doi.org/10.1093/biosci/biv082
- Fick SE, Hijmans RJ (2017) WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37(12): 4302–4315. https://doi. org/10.1002/joc.5086
- Fox J, Weisberg S (2018) Visualizing fit and lack of fit in complex regression models with predictor effect plots and partial residuals. Journal of Statistical Software 87(9): 1–27. https:// doi.org/10.18637/jss.v087.i09
- Fox J, Weisberg S (2019) An R Companion to Applied Regression. 3rd edn. Thousand Oaks, CA. http://tinyurl.com/carbook
- Fronhofer EA, Altermatt F (2015) Eco-evolutionary feedbacks during experimental range expansions. Nature Communications 6(1): 6844. https://doi.org/10.1038/ncomms7844
- Funk JL (2008) Differences in plasticity between invasive and native plants from a low resource environment. Journal of Ecology 96(6): 1162–1173. https://doi.org/10.1111/j.1365-2745.2008.01435.x
- Gaertner M, Den Breeyen A, Hui C, Richardson DM (2009) Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: A meta-analysis. Progress in Physical Geography 33(3): 319–338. https://doi.org/10.1177/0309133309341607
- Geiger JH, Pratt PD, Wheeler GSA, Williams D (2011) Hybrid vigor for the invasive exotic brazilian peppertree (*Schinus terebinthifolius* Raddi., Anacardiaceae) in Florida. International Journal of Plant Sciences 172: 655–663. https://doi.org/10.1086/659457
- Goodman CM, Jongsma GFM, Hill JE, Stanley EL, Tuckett QM, Blackburn DC, Romagosa CM (2021) A case of mistaken identity: Genetic and anatomical evidence reveals the cryptic invasion of *Xenopus tropicalis* in Central Florida. Journal of Herpetology 55(1): 62–69. https://doi.org/10.1670/20-083
- Guo Q (2005) Possible cryptic invasion through "Back Introduction"? Frontiers in Ecology and the Environment 3(9): 470–471. https://doi.org/10.2307/3868629
- Harper JL, Wood WA (1957) Senecio jacobaea L. Journal of Ecology 45(2): 617–637. https:// doi.org/10.2307/2256946

- Harrel Jr FE (2021) Hmisc: Harrell Miscellaneous. R package version 4.5–0. https://CRAN.R-project.org/package=Hmisc
- Hock M, Hofmann RW, Müller C, Erfmeier A (2019) Exotic plant species are locally adapted but not to high ultraviolet-B radiation: A reciprocal multispecies experiment. Ecology 100(5): e02665. https://doi.org/10.1002/ecy.2665
- Hulme PE (2009) Trade, transport and trouble: Managing invasive species pathways in an era of globalization. Journal of Applied Ecology 46(1): 10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x
- Isaacson DL (1973) Population dynamics of the cinnabar moth, *Tyria jacobaeae* (Lepidoptera: Arctiidae). Oregon State University. Master thesis.
- Jäger EJ (2017) Gefäßpflanzen: Grundband Rothmaler Exkursionsflora von Deutschland. 21. Auflage. Springer Spektrum, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-49708-1
- Joshi J, Vrieling K (2005) The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores: Evolutionary change in invasive ragwort. Ecology Letters 8(7): 704–714. https://doi.org/10.1111/j.1461-0248.2005.00769.x
- Jung S, Lauter J, Hartung NM, These A, Hamscher G, Wissemann V (2020) Genetic and chemical diversity of the toxic herb *Jacobaea vulgaris* Gaertn. (syn. *Senecio jacobaea* L.) in Northern Germany. Phytochemistry 172: 112235. https://doi.org/10.1016/j.phytochem.2019.112235
- Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecology Letters 7(12): 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x
- Keane R (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17(4): 164–170. https://doi.org/10.1016/S0169-5347(02)02499-0
- Kolbe JJ, Glor RE, Rodríguez Schettino L, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431(7005): 177–181. https://doi.org/10.1038/nature02807
- Kolbe JJ, Wegener JE, Stuart YE, Milstead U, Boronow KE, Harrison AS, Losos JB (2017) An incipient invasion of brown anole lizards (*Anolis sagrei*) into their own native range in the Cayman Islands: A case of cryptic back-introduction. Biological Invasions 19(7): 1989–1998. https://doi.org/10.1007/s10530-017-1432-2
- Kos M, Tuijl MAB, de Roo J, Mulder PPJ, Bezemer TM (2015a) Plant-soil feedback effects on plant quality and performance of an aboveground herbivore interact with fertilisation. Oikos 124(5): 658–667. https://doi.org/10.1111/oik.01828
- Kos M, Tuijl MAB, de Roo J, Mulder PPJ, Bezemer TM (2015b) Species-specific plant-soil feedback effects on above-ground plant-insect interactions. Journal of Ecology 103: 904– 914. https://doi.org/10.1111/1365-2745.12402
- Kuznetsova A, Brockhoff PB, Christensen RHB (2017) ImerTest Package: Tests in linear mixed effects models. Journal of Statistical Software 82(13): 1–26. https://doi.org/10.18637/jss. v082.i13
- Lenth R (2019) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package Version 1.4.3.01.

- Lin T, Klinkhamer PGL, Vrieling K (2015) Parallel evolution in an invasive plant: effect of herbivores on competitive ability and regrowth of *Jacobaea vulgaris*. Ecology Letters 18: 668–676. https://doi.org/10.1111/ele.12445
- Lin T, Klinkhamer PGL, Vrieling K (2018) Evolutionary changes in growth, regrowth and carbohydrate storage in an invasive plant. Scientific Reports 8(1): 14917. https://doi.org/10.1038/s41598-018-33218-z
- Lin T, Klinkhamer PGL, Pons TL, Mulder PPJ, Vrieling K (2019) Evolution of increased photosynthetic capacity and its underlying traits in invasive *Jacobaea vulgaris*. Frontiers in Plant Science 10: 1016. https://doi.org/10.3389/fpls.2019.01016
- Liu Y, Oduor AMO, Zhang Z, Manea A, Tooth IM, Leishman MR, Xu X, van Kleunen M (2017) Do invasive alien plants benefit more from global environmental change than native plants? Global Change Biology 23(8): 3363–3370. https://doi.org/10.1111/gcb.13579
- Londo G (1976) The decimal scale for releves of permanent quadrats. Vegetatio 33(1): 61–64. https://doi.org/10.1007/BF00055300
- Maron JL, Vilà M, Bommarco R, Elmendorf S, Beardsley P (2004) Rapid evolution of an invasive plant. Ecological Monographs 74(2): 261–280. https://doi.org/10.1890/03-4027
- McGeoch MA, Butchart SHM, Spear D, Marais E, Kleynhans EJ, Symes A, Chanson J, Hoffmann M (2010) Global indicators of biological invasion: species numbers, biodiversity impact and policy responses: Invasive alien species indicator: 2010 Biodiversity Target. Diversity & Distributions 16(1): 95–108. https://doi.org/10.1111/j.1472-4642.2009.00633.x
- Mezhzherin SV, Tsyba AA, Kryvokhyzha D (2022) Cryptic expansion of hybrid polyploid spined loaches *Cobitis* in the rivers of Eastern Europe. Hydrobiologia 849(7): 1689–1700. https://doi.org/10.1007/s10750-022-04813-z
- Miller KM, McGill BJ, Weed AS, Seirup CE, Comiskey JA, Matthews ER, Perles S, Schmit JP (2020) Long-term trends indicate that invasive plants are pervasive and increasing in eastern national parks. Ecological Applications 00(2): e02239. https://doi.org/10.1002/eap.2239
- Mitchell CE, Agrawal AA, Bever JD, Gilbert GS, Hufbauer RA, Klironomos JN, Maron JL, Morris WF, Parker IM, Power AG, Seabloom EW, Torchin ME, Vázquez DP (2006) Biotic interactions and plant invasions. Ecology Letters 9(6): 726–740. https://doi.org/10.1111/ j.1461-0248.2006.00908.x
- Möhler H, Diekötter T, Herrmann JD, Donath TW (2018) Allelopathic vs. autotoxic potential of a grassland weed—Evidence from a seed germination experiment. Plant Ecology & Diversity 11(4): 539–549. https://doi.org/10.1080/17550874.2018.1541487
- Möhler H, Diekötter T, Donath TW (2021) Regulation of *Jacobaea vulgaris* by varied cutting and restoration measures. Ecology [preprint]. https://doi.org/10.1101/2021.02.22.432250
- Montesinos D, Callaway RM (2020) Soil origin corresponds with variation in growth of an invasive *Centaurea*, but not of non-invasive congeners. Ecology 101(10): e03141. https:// doi.org/10.1002/ecy.3141
- Morais P, Reichard M (2018) Cryptic invasions: A review. The Science of the Total Environment 613–614: 1438–1448. https://doi.org/10.1016/j.scitotenv.2017.06.133
- Mueller JM, Hellmann JJ (2008) An assessment of invasion risk from assisted migration. Conservation Biology 22(3): 562–567. https://doi.org/10.1111/j.1523-1739.2008.00952.x
- Nelufule T, Robertson MP, Wilson JRU, Faulkner KT (2022) Native-alien populations—An apparent oxymoron that requires specific conservation attention. NeoBiota 74: 57–74. https://doi.org/10.3897/neobiota.74.81671
- Pal RW, Maron JL, Nagy DU, Waller LP, Tosto A, Liao H, Callaway RM (2020) What happens in Europe stays in Europe: Apparent evolution by an invader does not help at home. Ecology 101(8): e03072. https://doi.org/10.1002/ecy.3072
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52(3): 273–288. https://doi.org/10.1016/j.ecolecon.2004.10.002
- Pöyry J, Luoto M, Paukkunen J, Pykälä J, Raatikainen K, Kuussaari M (2006) Different responses of plants and herbivore insects to a gradient of vegetation height: An indicator of the vertebrate grazing intensity and successional age. Oikos 115(3): 401–412. https://doi. org/10.1111/j.2006.0030-1299.15126.x
- Prins AH, Vrieling K, Klinkhamer PGL, de Jong TJ (1990) Flowering behaviour of *Senecio jacobaea*: Effects of nutrient availability and size-dependent vernalization. Oikos 59(2): 248–252. https://doi.org/10.2307/3545541
- Qiao H, Liu W, Zhang Y, Zhang Y, Li QQ (2019) Genetic admixture accelerates invasion via provisioning rapid adaptive evolution. Molecular Ecology 28(17): 4012–4027. https://doi.org/10.1111/mec.15192
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/
- Rapo C, Müller-Schärer H, Vrieling K, Schaffner U (2010) Is there rapid evolutionary response in introduced populations of tansy ragwort, *Jacobaea vulgaris*, when exposed to biological control? Evolutionary Ecology 24(5): 1081–1099. https://doi.org/10.1007/s10682-010-9367-4
- Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. The New Phytologist 170(3): 445–457. https://doi.org/10.1111/j.1469-8137.2006.01715.x
- Robinson TB, Martin N, Loureiro TG, Matikinca P, Robertson MP (2020) Double trouble: The implications of climate change for biological invasions. NeoBiota 62: 463–487. https://doi.org/10.3897/neobiota.62.55729
- Sardain A, Sardain E, Leung B (2019) Global forecasts of shipping traffic and biological invasions to 2050. Nature Sustainability 2(4): 274–282. https://doi.org/10.1038/s41893-019-0245-y
- Schmidl L (1972) Biology and control of ragwort, *Senecio jacobaea* L., in Victoria, Australia. Weed Research 12(1): 37–45. https://doi.org/10.1111/j.1365-3180.1972.tb01185.x
- Schwarz K, Donath TW, Kaltenegger E, Diekötter T (2021) Environmental preferences of Longitarsus jacobaeae, a biocontrol agent of Jacobaea vulgaris, in northern Germany. Biological Control 164: 104740. https://doi.org/10.1016/j.biocontrol.2021.104740
- Skou A-MT, Toneatto F, Kollmann J (2012) Are plant populations in expanding ranges made up of escaped cultivars? The case of Ilex aquifolium in Denmark. Plant Ecology 213(7): 1131–1144. https://doi.org/10.1007/s11258-012-0071-z
- Stastny M, Schaffner U, Elle E (2005) Do vigour of introduced populations and escape from specialist herbivores contribute to invasiveness? Journal of Ecology 93(1): 27–37. https:// doi.org/10.1111/j.1365-2745.2004.00962.x

- van de Voorde TFJ, van der Putten WH, Martijn Bezemer T (2011) Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession: Intraand interspecific plant-soil interactions. Journal of Ecology 99(4): 945–953. https://doi. org/10.1111/j.1365-2745.2011.01815.x
- van de Voorde TFJ, van der Putten WH, Bezemer TM (2012a) The importance of plantsoil interactions, soil nutrients, and plant life history traits for the temporal dynamics of *Jacobaea vulgaris* in a chronosequence of old-fields. Oikos 121(8): 1251–1262. https://doi. org/10.1111/j.1600-0706.2011.19964.x
- van de Voorde TFJ, Ruijten M, van der Putten WH, Bezemer TM (2012b) Can the negative plant–soil feedback of *Jacobaea vulgaris* be explained by autotoxicity? Basic and Applied Ecology 13(6): 533–541. https://doi.org/10.1016/j.baae.2012.08.012
- van der Meijden E (1976) Changes in the distribution pattern of *Tyria jacobaeae* during the larval period. Netherlands Journal of Zoology 26(1): 136–161. https://doi. org/10.1163/002829676X00109
- van der Meijden E, van der Waals-Kooi RE (1979) The population ecology of *Senecio jacobaea* in a sand dune system: I. Reproductive strategy and the biennial habit. Journal of Ecology 67(1): 131–153. https://doi.org/10.2307/2259341
- Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems: Ecological impacts of invasive alien plants. Ecology Letters 14(7): 702–708. https://doi.org/10.1111/j.1461-0248.2011.01628.x
- Wardle DA (1987) The ecology of ragwort (Senecio jacobae L.) a review. New Zealand Journal of Ecology 10: 67–76.
- Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://doi.org/10.1007/978-3-319-24277-4
- Wubs ERJ, Bezemer TM (2018) Temporal carry-over effects in sequential plant-soil feedbacks. Oikos 127(2): 220–229. https://doi.org/10.1111/oik.04526
- Yang Q, Weigelt P, Fristoe TS, Zhang Z, Kreft H, Stein A, Seebens H, Dawson W, Essl F, König C, Lenzner B, Pergl J, Pouteau R, Pyšek P, Winter M, Ebel AL, Fuentes N, Giehl ELH, Kartesz J, Krestov P, Kukk T, Nishino M, Kupriyanov A, Villaseñor JL, Wieringa JJ, Zeddam A, Zykova E, van Kleunen M (2021) The global loss of floristic uniqueness. Nature Communications 12(1): 7290. https://doi.org/10.1038/s41467-021-27603-y
- Zenni RD, Dickie IA, Wingfield MJ, Hirsch H, Crous CJ, Meyerson LA, Burgess TI, Zimmermann TG, Klock MM, Siemann E, Erfmeier A, Aragon R, Montti L, Le Roux JJ (2017) Evolutionary dynamics of tree invasions: Complementing the unified framework for biological invasions. AoB Plants: plw085. https://doi.org/10.1093/aobpla/plw085
- Zhang Z, Liu Y, Yuan L, Weber E, van Kleunen M (2020) Effect of allelopathy on plant performance: a meta-analysis. Ecology Letters 24: 348–362. https://doi.org/10.1111/ele.13627

Supplementary material I

Supplementary information

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Data type: images, tables (word document)

- Explanation note: Population information. Schematic overview of one experimental plot with planting scheme. Transformations of variables. Mean values for performance traits (field experiment). Mean values for functional leaf traits (field experiment). Mean values for functional leaf traits (greenhouse experiment). Mean values for functional leaf traits (greenhouse experiment). Origin effects (field experiment). Treatment effects on performance traits (greenhouse experiment). Treatment effects on functional traits (greenhouse experiment).
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Link: https://doi.org/10.3897/neobiota.78.91394.suppl1

RESEARCH ARTICLE



Phenology and morphology of the invasive legume Lupinus polyphyllus along a latitudinal gradient in Europe

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Academic editor: Sven Jelaska | Received 29 June 2022 | Accepted 11 September 2022 | Published 9 December 2022

Citation: Ludewig K, Klinger YP, Donath TW, Bärmann L, Eichberg C, Thomsen JG, Görzen E, Hansen W, Hasselquist EM, Helminger T, Kaiskog F, Karlsson E, Kirchner T, Knudsen C, Lenzewski N, Lindmo S, Milberg P, Pruchniewicz D, Richter E, Sandner TM, Sarneel JM, Schmiede R, Schneider S, Schwarz K, Tjäder Å, Tokarska-Guzik B, Walczak C, Weber O, Żołnierz L, Eckstein RL (2022) Phenology and morphology of the invasive legume *Lupinus polyphyllus* along a latitudinal gradient in Europe. NeoBiota 78: 185–206. https://doi.org/10.3897/neobiota.78.89673

Abstract

Plant phenology, i. e. the timing of life cycle events, is related to individual fitness and species distribution ranges. Temperature is one of the most important drivers of plant phenology together with day length. The adaptation of their phenology may be important for the success of invasive plant species. The present study aims at understanding how the performance and the phenology of the invasive legume Lupinus polyphyllus vary with latitude. We sampled data across a >2000 km latitudinal gradient from Central to Northern Europe. We quantified variation in phenology of flowering and fruiting of L. polyphyllus using >1600 digital photos of inflorescences from 220 individual plants observed weekly at 22 sites. The day of the year at which different phenological phases were reached, increased 1.3-1.8 days per degree latitude, whereas the growing degree days (gdd) required for these phenological phases decreased 5-16 gdd per degree latitude. However, this difference disappeared, when the day length of each day included in the calculation of gdd was considered. The day of the year of the earliest and the latest climatic zone to reach any of the three studied phenological phases differed by 23-30 days and temperature requirements to reach these stages differed between 62 and 236 gdd. Probably, the invasion of this species will further increase in the northern part of Europe over the next decades due to climate warming. For invasive species control, our results suggest that in countries with a large latitudinal extent, the mowing date should shift by ca. one week per 500 km at sites with similar elevations.

Keywords

Flowering phenology, invasive plant, latitudinal gradient, legume, Lupinus polyphyllus, photoperiod

Introduction

Plant phenology is the timing of seasonal events, such as budburst, greening, flowering, and fruit ripening (Lieth 1974). Phenology influences the fitness of individual plants (Volis 2007; Anderson et al. 2012), controls species distribution ranges (Chuine and Beaubien 2001) and may have cascading effects on communities and ecosystems (Fargione and Tilman 2005; McKinney et al. 2012). Temperature is one of the most important drivers of plant phenology (Lieth 1974; Diekmann 1996) together with day length (e.g., Adole et al. 2019). In a long-term dataset, for example, between-year differences in average temperatures often corresponded to variation in the dates of first flowering (Fitter et al. 1995; Fitter and Fitter 2002). Furthermore, phenology studies have presented consistent evidence for the effects of climate change on organisms due to temperature shifts (Walther et al. 2002; Parmesan and Yohe 2003; Menzel et al. 2006; Cleland et al. 2007). The potential for adaptation of phenology may also be key to understanding the success of invasive plant species (Wolkovich and Cleland 2011), which are predicted to benefit from ongoing global change (Hellmann et al. 2008; Willis et al. 2010). A large proportion of introduced terrestrial plants have shown evidence of climatic niche shifts (Atwater et al. 2018). Analyses of long-term data on first flowering dates showed that non-native species were better able to track inter-annual variation in temperature and tended to flower earlier than native plants, owing to higher climate sensitivity (Wolkovich et al. 2013). The invader plasticity model, which links phenology to plant invasions (Richards et al. 2006; Wolkovich and Cleland 2011) states that species with flexible phenology may be more successful invaders outside their native ranges, because their phenology will covary with weather conditions (Matesanz et al. 2010). Furthermore, a species with flexible phenology due to large phenotypic plasticity (Anderson et al. 2012) has the potential to disperse successfully along latitudinal gradients in the non-native distribution range.

Latitudinal gradients provide the opportunity to study the effects of climate on plants in natural experiments (De Frenne et al. 2013). While biomass, height and seed mass of plants usually decrease with decreasing temperatures along latitudinal gradients (De Frenne et al. 2013), northern populations often require fewer accumulated growing degree days than southern populations to reach the same phenological state (Langvall and Ottosson Löfvenius 2021). At the same time, northern populations experience longer day lengths during summer in the northern hemisphere. Whether the day length can compensate for the overall lower temperatures was rarely studied, but Adole et al. (2019), from a study in the southern hemisphere, conclude that the day length (also called daytime or photoperiod) is an important factor in driving vegetation phenology. However, there is little information on species-specific patterns of flowering and fruiting phenology in relation to temperature and day length of invasive species across long geographic gradients.

Lupinus polyphyllus Lindl. (Fabaceae) is a perennial herbaceous hemicryptophyte originating from western North America that was introduced in Central Europe as an ornamental plant in the 19th century. From Central Europe and Scandinavia, the species spread very successfully to almost all parts of Europe, now ranging from the Pyrenees in the West to the Ural (and beyond) in the East (Eckstein et al., unpublished data). From North to South, *L. polyphyllus* is currently covering the full range of Europe, except for Mediterranean zones such as the Iberian Peninsula and Italy (GBIF 2022). This species typically flowers relatively early and invades different habitats ranging from road verges to forest understories and different types of mountain meadows (Wissman et al. 2015; Ramula 2017; Klinger et al. 2019). Dominant stands of *L. polyphyllus* have significantly negative effects on local plant diversity (Valtonen et al. 2006; Hejda et al. 2009; Ramula and Pihlaja 2012), plant community composition (Hansen et al. 2021), and arthropod abundance (Valtonen et al. 2006). In contrast to other invasive neophytes in Europe, *L. polyphyllus* can, in particular, colonise nutrient-poor habitats of high nature conservation value (Hejda et al. 2009).

Our overall aim was to understand how the timing, temperature dependence of flowering and fruiting, and performance (canopy height, potential seed production and seed release height) of *L. polyphyllus* change along the latitudinal gradient from Central to Northern Europe. We tested differences between populations that were assigned to different climatic zones and quantified variation in phenology in relation to latitude. To our knowledge, the present study is the first attempt to quantify variation in phenology of an invasive plant across a large latitudinal gradient in the field. This information may help to develop more effective management schemes for this invasive plant. Generally, the species is managed by cutting at fixed calendar dates (Ramula 2017; Klinger et al. 2020). A phenology-based management, accounting for the timing of flowering and seed ripening may be key for optimal control of invasive species (Taylor et al. 2020). A strong positive correlation between the time of cutting and the germinability of seeds was found for L. polyphyllus in mountain meadows in Central Germany (Klinger et al. 2020). This suggests a tradeoff between an early cut for avoiding ripe seeds and a late cut for avoiding a second flowering and therefore the need for another cut. However, plant phenology is rarely used for determining the timing of management practices and, further, it is poorly understood how to account for phenology in management (Ansquer et al. 2009; Taylor et al. 2020).

We wanted to test the following hypotheses:

1. Morphology is affected by latitude: canopy height, seed release height and the length of the inflorescence (as a proxy of potential seed production) decrease with decreasing temperatures; all three variables are significantly smaller in northern populations or populations in colder environments than in more southern populations in climatic zones with higher annual temperatures.

2. Reproduction is delayed with latitude: flowering and seed ripening are delayed in northern populations or populations in colder environments and there will be significant differences between populations of different climatic zones.

3. The number of accumulated growing degree days and accumulated growing degree day length (hours) required to reach flowering and seed ripening is negatively related to increasing latitude; accumulated growing degree days and growing degree day length (hours) to reach flowering and seed ripening vary significantly between climatic zones.

Methods

Study area

We analysed *Lupinus polyphyllus* populations along a latitudinal gradient ranging > 2000 km from Luxembourg, in Central Europe, to Umeå, in northern Sweden $(49^{\circ}38' - 63^{\circ}49', Fig. 1)$. Twenty-two sites (Table 1) were included in the study with a minimum distance of 130 m (JAWO1 to JAWO2) and a maximum distance of 2196 km (LUXE to UMEA) between pairs of sites. We used the environmental stratification of Europe (Metzger et al. 2005; Metzger 2018) to assign each site to



Figure 1. Position of the 22 study sites (for site abbreviations, see Table 1). The background map depicts climatic zones according to the environmental stratification of Europe (Metzger et al. 2005; Metzger 2018). ATC – atlantic central, ATN – atlantic north; ALS – alpine south; CON – continental; NEM – nemoral; BOR – boreal; ALN – alpine north. For certain analyses, sites in BOR and ALN were pooled.

a climatic zone. The most south-westerly sites (LUXE, TRIE) are assigned to the **AT**lantic **C**entral climatic zone (ATC), most of the Central European sites (GIES, ERZG, JAWO1, JAWO2, WROC, ODEN) belong to the **CON**tinental climatic zone (CON), while four sites belong to the **AT**lantic **N**orth climatic zone (ATN: MARB, HAMB, KIEL, SCHL). Two sites at low latitudes (50–51°N) but at the highest elevations of the data set (SWHA: 508 m a.s.l., RHON: 767 m a.s.l.) are characterised by exceptionally cold climate for their latitude and thus belong to the **AL**pine **S**outh climatic zone (ALS). All sites in southern Sweden are part of the **NEM**oral climatic zone (NEM: LINK, OREB, KRIS, KARL), whereas the northern sites are assigned to the **BOR**eal climatic zone (BOR: RATT, RESE, UMEA). To avoid statistical problems due to climatic zones represented by a single site, we also included the TRON site to the BOR group although it has a more Atlantic climate and belongs to the alpine north zone (ALN) according to Metzger et al. (2005). However, TRON is most similar to the other sites in the boreal climatic zone with respect to mean annual temperatures, day length, and latitude (Table 1).

Table 1. Characteristics of the 22 study sites along the latitudinal gradient. For abbreviation of climatic zones, see Figure 1. Mean annual temperature (MAT) and mean annual sum of precipitation (MAP) for the study sites were derived from the nearest weather station. Distance (km) indicates the distance between the weather station and the respective study site.

Study	Full site	Coordinates	Elevation	Climatic	Weather station	Distance	MAT	MAP
site	name	(Latitude, Longitude)	(m a.s.l.)	zone		(km)	(°C)	(mm)
UMEA	Umeå	63.82961°N, 20.33164°E	38	BOR	Umeå Flygplats ¹	4.3	2.6	644
TRON	Trondheim	63.41364°N, 10.40789°E	41	ATN	Trondheim-Voll Plu ²	2.3	4.7	925
RESE	Resele	63.34757°N, 17.00437°E	55	BOR	Forse ¹	22.4	2.5	536
RATT	Rättvik	60.87959°N, 15.12866°E	209	BOR	Leksand 1	18.3	4.1	591
KARL	Karlstad	59.40300°N, 13.62328°E	78	NEM	Karlstad Flygplats ¹	17.0	5.7	635
KRIS	Kristinehamn	59.33775°N, 14.19258°E	139	NEM	Kristinehamn ¹	6.1	5.8	659
OREB	Örebro	59.26483°N, 15.33968°E	26	NEM	Örebro Flygplats ¹	17.3	5.8	586
LINK	Linköping	58.17554°N, 15.71404°E	99	NEM	Malexander A ¹	30.6	5.9	519
ODEN	Odense	55.36990°N, 10.42298°E	21	CON	Odense Lufthavn ³	5.9	8.1	583
SCHL	Schleswig	54.48772°N, 9.56911°E	10	ATN	Schleswig ⁴	4.0	8.0	926
KIEL	Kiel	54.34886°N, 10.10497°E	24	ATN	Kiel-Holtenau ⁴	3.5	8.4	754
HAMB	Hamburg	53.54843°N, 9.86951°E	9	ATN	Hamburg-Fuhlsbüttel ⁴	12.1	8.6	770
SWHA	Südwest-Harz	51.66625°N, 10.60720°E	508	ALS	Braunlage ⁴	6.3	5.9	1263
WROC	Wrocław	51.04966°N, 17.25088°E	128	CON	Wroclaw-Strachowice ⁵	25.3	8.4	588
ERZG	Erzgebirge	50.93647°N, 13.71082°E	432	CON	Dresden-Klotzsche ⁴	20.2	8.9	667
MARB	Marburg	50.80591°N, 8.80855°E	332	ATN	Cölbe, Kr. Marburg-	5.6	8.9	756
BUIGHT	D1	50 ((2400)) 40 0 (00 (07)	- / -		Biedenkopf	o ((0	100/
RHON	Rhön	50.46310°N, 10.04884°E	767	ALS	Wasserkuppe ⁴	8.4	4.8	1084
GIES	Gießen	50.45559°N, 8.58841°E	303	CON	Giessen-Wettenberg ⁴	25.5	8.2	719
JAWO2	Jaworzno	50.23825°N, 19.22854°E	273	CON	Krakow -Balice ⁵	43.3	7.8	679
JAWO1	Jaworzno	50.23744°N, 19.22739°E	275	CON	Krakow ⁵	43.0	7.8	679
TRIE	Trier	49.81556°N, 6.57417°E	374	ATC	Trier-Petrisberg ⁴	10.7	9.1	784
LUXE	Luxembourg	49.63619°N, 6.17952°E	365	ATC	Luxembourg/Luxembourg ⁴	3.4	8.3	875

Sources for climatic data (1961–1990): 'Swedish Meteorological and Hydrological Institute (SMHI), 'Det Norske Meteorologiske Institutt (DNMI), 'Danmarks Meteorologiske Institut (DMI), 'Deutscher Wetterdienst (DWD), 'Institute of Meteorology and Water Management – National Research Institute (IMGW – PIB).

Study design and data sampling

Each participant in the study selected a population of *L. polyphyllus* along a road verge or in close vicinity to a road, in an open, sunny locality. For each population we documented geographic coordinates, elevation, and climatic parameters from the nearest weather station (Table 1).

Ten randomly selected adult individuals, representative for each population, were marked early in the season in 2019. We focussed on the first developed central inflorescence of each of these marked individuals. Starting when the inflorescences were visible (mid-end April), we made digital photos of the inflorescence of each marked individual against a scale bar, usually a meter stick. We used photos for consistent measurements across sites and stored them for future analysis. The photos were sent to the project coordinator (RLE), who analysed all photos together with a student assistant. Additionally, all participants measured the maximum height from the ground to the top of the basal leaves (**canopy height**) and to the top of the inflorescence (**seed release height**) of the 10 marked individuals per measuring event on site. Usually, the photos and direct measurements were taken once per week until the first pods of the inflorescences were ripe. We obtained between five and twelve observations per site, resulting in 180 site × date combinations. Depending on the site location, observations ranged from 29 April to 4 August, with the majority of observations (160 site × date combinations) made between 6 May and 8 July.

In total, 1633 photos from 22 sites were analysed using the software ImageJ 1.52a (Schneider et al. 2012). We used the scale bar in each photo to calibrate distances before we estimated the following variables (Fig. 2a, b, Table 2) for each plant individual per measuring event:

1. Total length of the inflorescence from the lowermost flower bud, flower or flower scar to the top (A-C),

2. Length of the inflorescence with open flowers from the lowermost flower/ flower scar to the uppermost open flower (A–B); open flowers were defined as flowers with unfolded standard, visible keel, and elongated pedicel (Fig. 2c).

Calculated variables

Using the photo measurements, we calculated the **relative length of the inflorescence with open flowers** (*RLF*) for each plant individual per measuring event (Table 2). *RLF* represents an indicator of progress of flowering phenology that can relatively easily be estimated in the field, e.g., for management purposes. Since *RLF* is bound between zero and one, we used these estimates (available for each individual per measuring event) to perform logistic regressions per site. The logistic regressions for *RLF* against the **day of the year (doy)** of the measuring events were significant (p<0.05). Using the parameters of all significant logistic regressions, we estimated the doy, at which half of the actual length of the inflorescence carried open flowers (doy.flow.half, Fig. 2d).

Variable	Lleage*	Description	Formula/Domortes
variable	Usage	Description	Formula/ Remarks
Canopy height	R	Maximum height from the ground to the top of the basal leaves (cm)	Measured in the field (usually weekly)
Seed release height	R	Maximum height from the ground to the top of the inflorescence (cm)	Measured in the field (usually weekly)
Total length of	R	Length of inflorescence (cm) from the lowermost flower	Determined via photos
inflorescence		bud, flower or flower scar to the top (A-C in Fig. 2)	•
Length of the	А	Length from the lowermost flower/flower scar to the	Determined via photographs; open flowers
inflorescence with		uppermost open flower (A–B in Fig. 2);	were defined as flowers with unfolded
open flowers			standard, visible keel, and elongated pedicel
RLF	А	Relative length of the inflorescence with open flowers at	
		each measuring event (t_x)	
doy	A/E/R	Day of the year	Day number (1 st of January = day 1)
gdd	A/E/R	Number of growing degree days using a base temperature of 5 $^{\circ}\mathrm{C}$	GDD= $(T_{max} - T_{min})/2 - T_{Base}$, if $T_{Mean} > T_{Base}$
gdh	Е	Cumulated day length: growing degree day length (hours), called growing day hours	GDH=GDD*Day length of each day included in calculation of GDD
Flow.half	R	Day of the year (doy.flow.half) or number of growing degree days (gdd.flow.half) when half of the actual length of the inflorescence carried open flowers	Estimated per site via RLF using logistic regressions
First.flow	R	Day of the year (doy.first.flow) or number of growing degree days (gdd.first.flow) when the first flower was formed	Determined via photographs
First.ripe	R	Day of the year (doy.first.ripe) or number of growing degree days (ødd.first.ripe) when the first black pod was formed	Determined via photographs

Table 2. Measured and calculated variables used in the manuscript.

*Usage: A=Auxiliary (used to calculate other variables), E=Explanatory variable, R=Response variable.

Additionally, we recorded the doy of the measuring event at which the first open flower (doy.first.flow, Fig. 2c) and the first black pod (doy.first.ripe, Fig. 2e) were visible in the photographic documentation. This was not possible for the sites ODEN and RESE because the observation period did not include these phenological stages. For site RATT, no pods were black at the time of the second last observation date but all pods were black on the last observation date. However, as the last observation was done one month after the second last, the timing of this phase was clearly overestimated and we omitted this data point.

We obtained temperature measurements from meteorological stations located closest to the field sites (distances between 2.3 and 43.3 km from the studied sites (Table 1). Using the minimum and maximum daily temperatures, we calculated **growing degree days (gdd)** from January 1, using the standard base temperature of 5 °C (Table 2). Temperatures preceding a phenological phase (e.g., day of first flowering) are important drivers of phenology (e.g., Fitter et al. 1995) and the accumulation of gdd with the standard base temperature of 5 °C represents a simple but powerful proxy for predicting the flowering phenology of plants (e.g., Diekmann 1996; De Frenne et al. 2010). We then calculated the accumulated gdd for each observation date at each site from the temperature data of the meteorological stations (Table 2). Finally, we used the same logistic regression approach as above, but using accumulated gdd until the first doy to estimate gdd.flow.half. We also estimated the accumulated gdd until the first



Figure 2. Measurements taken along the inflorescence of *Lupinus polyphyllus* (**a**): A – C: total length of inflorescence; A – B: length of inflorescence with open flowers; an example photo showing the flower development taken at the population KARL, on the 7th of June 2019 by Lutz Eckstein (**b**) and example photos showing the different stages first.flow, on 24^{th} of May (**c**), flow.half, on 31^{st} of May (**d**), and first. ripe, on 5^{th} of July (**e**). White arrows show an open flower and a ripe pod, in (**c**) and (**e**), respectively.

open flower (gdd.first.flow) and the first black pod (gdd.first.ripe) were visible in the photographic documentation.

Since light is an important driver of phenology alongside temperature, and day length increases with latitude, we aimed at incorporating differences in day length in our analyses by creating a variable (**growing degree day length, in accumulated hours: gdh**) that combines growing degree days and day length. For each population and day of the year, the day length was calculated using the 'geosphere' package in R (Hijmans 2017). We then multiplied the respective gdd (with the base temperature of 5 °C) with day length hours of that day. In addition to growing degree days, the combined factor growing day hours was included for all populations. In the literature about phenology studies, day length is rarely accounted for, although it may be an important factor (Adole et al. 2019; Ettinger et al. 2021).

Statistical tests

For all variables, we used mean values from the ten measured individuals per site. We analysed the data in two different ways. Firstly, we calculated linear regressions of doy.first.flow, doy.flow.half, doy.first.ripe, gdd.first.flow, gdd.flow.half, and gdd.first. ripe against latitude (decimal degrees north) to quantify the rate of change in flowering, seed ripening, and seed shedding phenology with latitude. In these analyses, we excluded the two sites of the Alpine south climatic zone (SWHA, RHON), since these populations potentially experience much higher temperature selection due to a cold montane climate as compared to other sites at similar latitudes and would lead to confounding latitude and elevation. Secondly, we did one-way ANOVAs of all six dependent variables with the climatic zones according to Metzger et al. (2005) to test whether climatic zones differed significantly with respect to the timing (using doy) and energy dependence (using gdd and gdh) of phenology. Test assumptions were checked visually and homogeneity of variances tested using Levene's test. In case of violation of variance homogeneity, we did a White-adjusted ANOVA (White 1980) using the R-package car (Fox and Weisberg 2019). When we found significant differences using ANOVA, we performed a pair-wise comparison to determine significant differences among climatic zones using the Tukey HSD test of the R-package agricolae (Mendiburu 2015). We tested the residuals of all statistical models for spatial autocorrelation, using a global Moran's I with the spdep package in R (Bivand and Wong 2018). Only in two of 18 tests, autocorrelation was detected, in both cases for the response variable first.ripe (Appendix 1: Table A1), which had no significant results in the ANOVA tests. Thus, we assume that spatial autocorrelation is not relevant in our analyses. All statistical analyses were done using R version 3.6.2 (R Core Team 2020).

Results

The maximum canopy height varied by a factor of 2.5 between sites (min: HAMB – 34.9 cm; max: JAWO2 – 88.0 cm). Averaged across sites of the same climatic zone (Fig. 3a), it varied by a factor of 1.4, ranging from the Atlantic north zone (ATN) with a mean canopy height of 55.2 cm to the continental zone (CON) with a mean canopy height of 76.9 cm. Overall, we detected a marginally-significant effect of climatic zone on canopy height ($F_{5,16} = 2.59$, p = 0.0674), but canopy height patterns were not consistent with latitudinal direction. We found a significant effect of climatic zone on seed release height ($F_{5,16} = 5.96$, p = 0.0028), but even here there was no consistent pattern with latitude or temperature (Fig. 3b). Seed release height varied by a factor of 1.9 between single sites (min: LINK – 74.5 cm; max: TRON – 141.2 cm). On average, it ranged between 90.3 cm (ALS) and 122.7 cm (BOR) among climatic zones. The length of the inflorescence varied by a factor of about 2 between sites (min: LINK – 24.9 cm; max: JAWO1 – 49.3 cm) and ranged between 36.0 cm (NEM) and 43.1 cm (ATN) among the climatic zones (Fig. 3c); there was neither a significant effect of climatic effect of climatic zones (Fig. 3c); there was neither a significant effect of climatic zone.



Figure 3. a canopy height (cm) **b** seed release height (cm) **c** length of the inflorescence (cm) during the time of biomass maximum for sites grouped according to climatic zones according to Metzger (2018). Climatic zones: ATC: atlantic central, CON: continental, ATN: atlantic north, NEM: nemoral, ALS: alpine south; BOR: boreal (includes the climate zone ALN, i.e. site TRON). Climatic zones are ordered according to decreasing mean annual temperatures. With the exception of ALS, the ordering corresponds to increasing northern latitude. Different letters indicate significant effects of climatic zone (p < 0.05).

The doy at which the inflorescences of *L. polyphyllus* reached first.flow, flow.half, and first.ripe increased with decreasing mean annual temperatures of the climatic zones (Fig. 4). The effects of climatic zone were highly significant for first.flow ($F_{5.16}$ = 22.06, p <



Figure 4. Day of year (doy), at which **a** the first open flower was observed (first.flow) **b** half of the length of the inflorescence bears open flowers (flow.half) **c** the first ripe (black) pods were observed (first.ripe) for each climatic zone (Metzger 2018). Climatic zones are ordered according to decreasing mean annual temperatures. With the exception of ALS, the ordering corresponds to increasing northern latitude. Different letters indicate significant effects of climatic zone (p < 0.05). For abbreviations of climatic zones, see Fig. 3.

0.001), flow.half ($F_{5,16}$ = 21.49, p < 0.001), and first.ripe (White-adjusted ANOVA: $F_{5,13}$ = 12.98, p < 0.001) and there was a monotonic increase of doy with latitude. Lupines in the boreal climatic zone (BOR) needed significantly more days to reach flow.half (doy = 168, 17th of June) than in all other climatic zones (Fig. 4b). Both, the populations in the BOR and in the alpine south climatic zone (ALS) needed significantly more days to reach first.



Figure 5. Growing day hours (gdh), at which **a** the first open flower was observed (first.flow) **b** half of the length of the inflorescence bears open flowers (flow.half) **c** the first ripe (black) pods were observed (first.ripe) for each climatic zone (Metzger 2018). Climatic zones are ordered according to decreasing mean annual temperatures. With the exception of ALS, the ordering corresponds to increasing northern latitude. For abbreviations of climatic zones, see Fig. 3.

flow (on 18th of June and 31st of May, respectively) than in the Atlantic north zone (ATN, 18th of May), in the continental zone (CON, 16th of May) or in the Atlantic central zone (ATC, 1th of May) (Fig. 4a). The same patterns as in first.flow and flow.half were visible for first.ripe (Fig. 4c). The difference in average number of days between the earliest and the latest climatic zone was 30 days for first.flow and ca. 23–24 days for flow.half and first.ripe.

The gdd at which the inflorescences reached first.flow did not differ between the climatic zones ($F_{5,16} = 0.96$, p = 0.468). For the next phenological phase, flow.half, the gdd tended to be affected by the climatic zone ($F_{5,16} = 2.48$, p = 0.076). Finally, the effects of climatic zone on gdd were significant for first.ripe ($F_{5,13} = 5.96$, p = 0.0047). More specific, the gdd at which the inflorescences reached flow.half and first.ripe tended to be lower for the zones NEM, ALS and BOR than for ATC, CON and ATN (data not shown). While these patterns were found for growing degree days (gdd), they disappeared when the day length was taken into account as growing day hours (gdh, Fig. 5). No significant effect of the climatic zones was found on the growing day hours that are needed to reach first.flow ($F_{5,16} = 0.964$, p = 0.468), flow.half ($F_{5,16} = 1.714$, p = 0.189), and first.ripe ($F_{5,13} = 0.729$, p = 0.614) (Fig. 5).

Our regression analyses showed that the day of the year, on which the first open flower was observed (first.flow), half of the inflorescence's length at each site had open flowers (flow.half), and the first ripe pod (first.ripe) was observed increased significantly (p < 0.001) with latitude (Appendix 1: Fig. A1). The day of the year on which first.flow was observed ranged between the 6th of May and the 11th of June across sites; for flow. half between the 23rd of May and the 23rd of June; and for first.ripe between the 17th of June and the 29th of July. The slope of the linear regression was 1.76 days per degree latitude for first.flow (Appendix 1: Fig. A1a, $F_{1,18} = 49.33$, $R^2 = 0.7178$), 1.42 days per degree latitude for flow.half (Appendix 1: Fig. A1b, $F_{1,18} = 35.36$, $R^2 = 0.6439$), and 1.53 days per degree latitude for first.ripe (Appendix 1: Fig. A1c, $F_{1,15} = 27.83$, $R^2 = 0.6264$).

The accumulated growing degree days (gdd) until the inflorescence at each site reaches first.flow, flow.half, and first.ripe, decreased significantly (all p-values < 0.05) with latitude (Appendix 1: Fig. A2a–c). The slope of the linear regression was -5.17 gdd per degree latitude for first.flow (Appendix 1: Fig. A2a, $F_{1,18} = 6.67$, $R^2 = 0.2298$), -8.26 gdd per degree latitude for flow.half (Appendix 1: Fig. A2b, $F_{1,18} = 13.53$, $R^2 = 0.3973$), and -16.14 gdd per degree latitude for first.ripe (Appendix 1: Fig. A2c, $F_{1,15} = 27.61$, $R^2 = 0.6245$). While the growing degree days (gdd) decreased significantly with latitude, this pattern disappeared when the day length was considered (Appendix 1: Fig. A2d–f).

Data accessibility statement

The data supporting the findings of this study are openly available in the repository dryad at https://doi.org/10.5061/dryad.stqjq2c3t (Ludewig et al. 2022).

Discussion

According to our findings, canopy height and the length of the inflorescence of *Lupinus polyphyllus* does not vary significantly among climatic zones. Seed release height shows significant variation among climatic zones but there is no consistent pattern with latitude. Consequently, we found no evidence for our first hypothesis that the latitudinal gradient affects these measures of performance of the invasive *L. polyphyllus*. Plant height and seed mass usually decrease with decreasing temperatures along latitudinal

gradients (De Frenne et al. 2013), though some studies did find no latitudinal variation or even increased performance with latitude (De Frenne et al. 2013). Other factors, including the productivity of the habitat or the height of the competing vegetation, can additionally influence this relationship. Nevertheless, the absence of this relationship and the production of black pods show that although phenology is delayed at more northern latitudes, *L. polyphyllus* is able to produce mature seeds even in Northern Europe.

The day of year (doy) at which the first open flower was found, half of the length of the inflorescence had open flowers and the doy at which the first black, ripe pod was found, increased significantly with northern latitude. Populations in zones with a colder climate reach these phenological phases significantly later than populations in climatic zones with higher annual temperatures. Therefore, and as stated in our second hypothesis, the phenology of flowering and seed ripening is delayed in populations of *L. polyphyllus* in the northern part of the gradient. More specifically, all measured phenological parameters were delayed under colder climate conditions, i.e., at higher latitudes or elevations (boreal and alpine south zone). This is in contrast to studies, in which plant material from latitudinal gradients was collected and grown in common garden experiments (Olsson and Ågren 2002; Kollmann and Bañuelos 2004). For example, Impatiens glandulifera plants grown from seeds flowered faster when the seeds originated from northern populations compared to southern populations (Kollmann and Bañuelos 2004). This finding is not surprising and can be expected when plants at higher latitudes are adapted to flower earlier (relative to the beginning of the vegetation period) and their seeds are then transferred to lower latitudes in a study. Generally, temperature is an important driver for the timing of seasonal events (e.g., Lieth 1974; Diekmann 1996; De Frenne et al. 2013), but also day length plays an important role in our study about *L. polyphyllus*.

While the accumulated growing degree days (gdd) required to reach the different phenological phases decrease with latitude, suggesting that energy requirements for flowering and fruit ripening are lower at higher latitudes, this effect disappeared when day length was considered. This finding shows that longer day lengths may compensate for the fewer growing degree days at northern latitudes. As a result, the energy demands of *L. polyphyllus* to reach the studied phenological phases, measured as growing day hours (gdh), do not differ significantly along the latitudinal gradient. The first finding is in line with Langvall and Ottosson Löfvenius (2021) who showed that the leafing phenology of Betula pubescens and Pinus sylvestris is delayed in northern populations compared to southern populations in Sweden, but that northern populations need fewer growing degree days than southern populations to reach the same phenological phase. However, Langvall and Ottosson Löfvenius (2021) did not include the day length in their study, but identified day length as a possible underlying factor for their results. Overall, we found some evidence for our third hypothesis that the number of accumulated growing degree days required to reach certain flowering and seed ripening phases is negatively related to northern latitude, but this was not the case when day length was accounted for. For future studies on plant phenology across latitudinal gradients, we recommend including day length in the analysis.

Longer day length during summer allows *L. polyphyllus* to fulfil its life cycle relatively quickly in the investigated northern latitudes. Therefore, populations in the northern part of the gradient have probably not changed their climatic niche (Guisan et al. 2014; Atwater et al. 2018) and react plastically to the energy input they get. Similarly, two other species in the same genus show high phenotypic plasticity on small spatial scales. *Lupinus lepidus* responded with differing phenology between established (surviving) and newly colonized populations on lava fields after an eruption of Mount St. Helens (Bishop and Schemske 1998). The second species is the woody *Lupinus arboreus*, which shows small-scale differentiation in traits such as plant size, flowering phenology, or fecundity in subpopulations in dune and grassland habitats < 500 m apart from each other (Kittelson and Maron 2001). This high capability for plastic responses of *Lupinus* species may be advantageous in the light of climate change. We anticipate that the invasion of this species will further expand in the northern part of Europe over the next decades. This will be facilitated by a temperature increase that is predicted to be faster and higher in northern ecosystems (Hewitson et al. 2014). Also, we expect this species to do well in northern latitudes as the shorter growth season is at least partly compensated for by longer days.

For invasive species control, our results suggest that in countries with a large latitudinal extent, the timing of management (e.g. mowing date) should shift by ca. one week every 500 km, at least for sites at lower elevations. For example, in Germany (ca. 900 km south-north extent) or Sweden (ca. 1600 km south-north extent), the southernmost populations should be managed ca. 12 and 22 days earlier, respectively, than the northernmost populations. The variable flow.half may represent a good indicator for the optimal time for management since no viable seeds are present at this stage. In our study year, flow.half was reached in the southernmost populations in Germany and Sweden at the end of May and beginning of June, respectively. With later mowing the possibility of seed shedding increases and the potential to limit the spread of *L. polyphyllus* decreases. The practical planning of phenology-based control of invasive plants (Taylor et al. 2020) would benefit from an open large-scale phenology dataset providing information on the timing of flowering and seed ripening for plant species relevant for nature conservation management.

Acknowledgements

We thank Denis Lafage for help with an app for photo submission and Tobias Knieps and Muriel Fauth for help with analysis of photos, data input and acquisition of climate data. We thank Adrian Wächtershäuser (site GIE) and Katrin Eichberg for help in the field (site TRIE). We thank Nina Sajna and one anonymous referee for valuable comments on the manuscript.

References

Adole T, Dash J, Rodriguez-Galiano V, Atkinson PM (2019) Photoperiod controls vegetation phenology across Africa. Communications Biology 2(1): e391. https://doi.org/10.1038/ s42003-019-0636-7

- Anderson JT, Inouye DW, McKinney AM, Colautti RI, Mitchell-Olds T (2012) Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proceedings of the Royal Society B: Biological Sciences 279(1743): 3843–3852. https://doi.org/10.1098/rspb.2012.1051
- Ansquer P, Al Haj Khaled R, Cruz J-P, Therond O, Duru M (2009) Characterizing and predicting plant phenology in species-rich grasslands. Grass and Forage Science 64(1): 57–70. https://doi.org/10.1111/j.1365-2494.2008.00670.x
- Atwater DZ, Ervine C, Barney JN (2018) Climatic niche shifts are common in introduced plants. Nature Ecology & Evolution 2(1): 34–43. https://doi.org/10.1038/s41559-017-0396-z
- Bishop JG, Schemske DW (1998) Variation in flowering phenology and its consequences for Lupines colonizing mount St. Helens. Ecology 79(2): 534–546. https://doi. org/10.1890/0012-9658(1998)079[0534:VIFPAI]2.0.CO;2
- Bivand R, Wong DWS (2018) Comparing implementations of global and local indicators of spatial association. Test 27(3): 716–748. https://doi.org/10.1007/s11749-018-0599-x
- Chuine I, Beaubien EG (2001) Phenology is a major determinant of tree species range. Ecology Letters 4(5): 500–510. https://doi.org/10.1046/j.1461-0248.2001.00261.x
- Cleland E, Chuine I, Menzel A, Mooney H, Schwartz M (2007) Shifting plant phenology in response to global change. Trends in Ecology & Evolution 22(7): 357–365. https://doi.org/10.1016/j.tree.2007.04.003
- De Frenne P, Graae BJ, Kolb A, Brunet J, Chabrerie O, Cousins SAO, Decocq G, Dhondt R, Diekmann M, Eriksson O, Heinken T, Hermy M, Jógar Ü, Saguez R, Shevtsova A, Stanton S, Zindel R, Zobel M, Verheyen K (2010) Significant effects of temperature on the reproductive output of the forest herb *Anemone nemorosa* L. Forest Ecology and Management 259(4): 809–817. https://doi.org/10.1016/j.foreco.2009.04.038
- De Frenne P, Graae BJ, Rodríguez-Sánchez F, Kolb A, Chabrerie O, Decocq G, Kort H, Schrijver A, Diekmann M, Eriksson O (2013) Latitudinal gradients as natural laboratories to infer species' responses to temperature. Journal of Ecology 101(3): 784–795. https://doi. org/10.1111/1365-2745.12074
- Diekmann M (1996) Relationship between flowering phenology of perennial herbs and meteorological data in deciduous forests of Sweden. Canadian Journal of Botany 74(4): 528–537. https://doi.org/10.1139/b96-067
- Ettinger AK, Buonaiuto DM, Chamberlain CJ, Morales-Castilla I, Wolkovich EM (2021) Spatial and temporal shifts in photoperiod with climate change. The New Phytologist 230(2): 462–474. https://doi.org/10.1111/nph.17172
- Fargione J, Tilman D (2005) Niche differences in phenology and rooting depth promote coexistence with a dominant C_4 bunchgrass. Oecologia 143(4): 598–606. https://doi.org/10.1007/s00442-005-0010-y
- Fitter AH, Fitter RSR (2002) Rapid changes in flowering time in British plants. Science 296(5573): 1689–1691. https://doi.org/10.1126/science.1071617
- Fitter AH, Fitter RSR, Harris ITB, Williamson MH (1995) Relationships between first flowering date and temperature in the flora of a locality in Central England. Functional Ecology 9(1): 55–60. https://doi.org/10.2307/2390090
- Fox J, Weisberg S (2019) An R Companion to Applied Regression, 3rd Edn. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

- GBIF (2022) *Lupinus polyphyllus* Lindl. https://www.gbif.org/species/2964355 [accessed 17th of August 2022]
- Guisan A, Petitpierre B, Broennimann O, Daehler C, Kueffer C (2014) Unifying niche shift studies: Insights from biological invasions. Trends in Ecology & Evolution 29(5): 260– 269. https://doi.org/10.1016/j.tree.2014.02.009
- Hansen W, Wollny J, Otte A, Eckstein RL, Ludewig K (2021) Invasive legume affects species composition and functional diversity of mountain meadow plant communities. Biological Invasions 23: 281–296. https://doi.org/10.1007/s10530-020-02371-w
- Hejda M, Pyšek P, Jarošík V (2009) Impact of invasive plants on the species richness, diversity and composition of invaded communities. Journal of Ecology 97(3): 393–403. https://doi. org/10.1111/j.1365-2745.2009.01480.x
- Hellmann JJ, Byers JE, Bierwagen BG, Dukes JS (2008) Five Potential Consequences of Climate Change for Invasive Species. Conservation Biology 22(3): 534–543. https://doi. org/10.1111/j.1523-1739.2008.00951.x
- Hewitson B, Janetos AC, Carter TR, Giorgi F, Jones RG, Kwon W-T, Mearns LO, Schipper ELF, van Aalst M (2014) Regional context. In: Barros VR, Field CB, Dokken DJ, Mastrandrea MD, Mach KJ, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, White LL (Eds) Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on ClimateChange. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1133–1197.
- Hijmans RJ (2017) Introduction to the "geosphere" package R Package Version 1.5-7. https:// cran.r-project.org/web/packages/geosphere/
- Kittelson PM, Maron JL (2001) Fine-scale genetically based differentiation of life-history traits in the perennial shrub *Lupinus arboreus*. Evolution 55(12): 2429–2438. https://doi. org/10.1111/j.0014-3820.2001.tb00758.x
- Klinger YP, Harvolk-Schöning S, Eckstein RL, Hansen W, Otte A, Ludewig K (2019) Applying landscape structure analysis to assess the spatio-temporal distribution of an invasive legume in the Rhön UNESCO Biosphere Reserve. Biological Invasions 21(8): 2735–2749. https://doi.org/10.1007/s10530-019-02012-x
- Klinger YP, Horlemann D, Eckstein RL, Otte A, Ludewig K (2020) Germination of the invasive legume *Lupinus polyphyllus* depends on cutting date and seed morphology. NeoBiota 60: 79–95. https://doi.org/10.3897/neobiota.60.56117
- Kollmann J, Bañuelos MJB (2004) Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae): Latitudinal trends in growth and phenology of an annual invasive plant. Diversity & Distributions 10(5–6): 377–385. https://doi.org/10.1111/j.1366-9516.2004.00126.x
- Langvall O, Ottosson Löfvenius M (2021) Long-term standardized forest phenology in Sweden: A climate change indicator. International Journal of Biometeorology 65(3): 381–391. https://doi.org/10.1007/s00484-019-01817-8
- Lieth H (1974) Phenology and seasonality modelling. Springer, New York, 203 pp. https://doi. org/10.1007/978-3-642-51863-8
- Ludewig K, Klinger YP, Donath TW, Bärmann L, Eichberg C, Thomsen JG, Görzen E, Hansen W, Hasselquist EM, Helminger T, Kaiskog F, Karlsson E, Kirchner T, Knudsen

C, Lenzewski N, Lindmo S, Milberg P, Pruchniewicz D, Richter E, Sandner TM, Sarneel JM, Schmiede R, Schneider S, Schwarz K, Tjäder Å, Tokarska-Guzik B, Walczak C, Weber O, Żołnierz L, Eckstein RL (2022) Phenology of Lupinus polyphyllus from Central to Northern Europe. Dryad Digital Repository. https://doi.org/10.5061/dryad.stqjq2c3t

- Matesanz S, Gianoli E, Valladares F (2010) Global change and the evolution of phenotypic plasticity in plants: Global change and plasticity. Annals of the New York Academy of Sciences 1206(1): 35–55. https://doi.org/10.1111/j.1749-6632.2010.05704.x
- McKinney AM, CaraDonna PJ, Inouye DW, Barr B, Bertelsen CD, Waser NM (2012) Asynchronous changes in phenology of migrating Broad-tailed Hummingbirds and their early-season nectar resources. Ecology 93(9): 1987–1993. https://doi.org/10.1890/12-0255.1
- Mendiburu FD (2015) agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.2-3. http://CRAN.R-project.org/package=agricolae
- Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kübler K, Bissolli P, Braslavská O, Briede A, Chmielewski FM, Crepinsek Z, Curnel Y, Dahl Å, Defila C, Donnelly A, Filella Y, Jatczak K, Måge F, Mestre A, Nordli Ø, Peñuelas J, Pirinen P, Remišová V, Scheifinger H, Striz M, Susnik A, Van Vliet AJH, Wielgolaski F-E, Zach S, Zust A (2006) European phenological response to climate change matches the warming pattern. Global Change Biology 12(10): 1969–1976. https://doi.org/10.1111/j.1365-2486.2006.01193.x
- Metzger MJ (2018) The Environmental Stratification of Europe, [dataset]. University of Edinburgh. https://doi.org/10.7488/ds/2356
- Metzger MJ, Bunce RGH, Jongman RHG, Mücher CA, Watkins JW (2005) A climatic stratification of the environment of Europe. Global Ecology and Biogeography 14(6): 549–563. https://doi.org/10.1111/j.1466-822X.2005.00190.x
- Olsson K, Ågren J (2002) Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb *Lythrum salicaria*. Journal of Evolutionary Biology 15(6): 983–996. https://doi.org/10.1046/j.1420-9101.2002.00457.x
- Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421(6918): 37–42. https://doi.org/10.1038/nature01286
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/
- Ramula S (2017) The generality of management recommendations across populations of an invasive perennial herb. Biological Invasions 19(9): 2623–2632. https://doi.org/10.1007/ s10530-017-1472-7
- Ramula S, Pihlaja K (2012) Plant communities and the reproductive success of native plants after the invasion of an ornamental herb. Biological Invasions 14(10): 2079–2090. https:// doi.org/10.1007/s10530-012-0215-z
- Richards C, Bossdorf O, Muth NZ, Gurevitch J, Pigluicci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9(8): 981–993. https://doi.org/10.1111/j.1461-0248.2006.00950.x
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9(7): 671–675. https://doi.org/10.1038/nmeth.2089
- Taylor RV, Holthuijzen W, Humphrey A, Posthumus E (2020) Using phenology data to improve control of invasive plant species: A case study on Midway Atoll NWR. Ecological Solutions and Evidence 1(1): e12007. https://doi.org/10.1002/2688-8319.12007

- Valtonen A, Jantunen J, Saarinen K (2006) Flora and Lepidoptera fauna adversely affected by invasive Lupinus polyphyllus along road verges. Biological Conservation 133(3): 389-396. https://doi.org/10.1016/j.biocon.2006.06.015
- Volis S (2007) Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. Evolutionary Ecology 21(3): 381-393. https://doi.org/10.1007/s10682-006-9108-x
- Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416(6879): 389-395. https://doi.org/10.1038/416389a
- White H (1980) A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. Econometrica 48(4): 817–838. https://doi.org/10.2307/1912934
- Willis CG, Ruhfel BR, Primack RB, Miller-Rushing AJ, Losos JB, Davis CC (2010) Favorable climate change response explains non-native species' success in Thoreau's Woods. PLoS ONE 5(1): e8878. https://doi.org/10.1371/journal.pone.0008878
- Wissman J, Norlin K, Lennartsson T (2015) Invasiva arter i infrastruktur. CBM:s skriftserie 98. Centrum för biologisk mångfald, SLU, Uppsala, Sweden, 1–104.
- Wolkovich EM, Cleland EE (2011) The phenology of plant invasions: A community ecology perspective. Frontiers in Ecology and the Environment 9(5): 287-294. https://doi. org/10.1890/100033
- Wolkovich EM, Davies TJ, Schaefer H, Cleland EE, Cook BI, Travers SE, Willis CG, Davis CC (2013) Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. American Journal of Botany 100(7): 1407-1421. https://doi. org/10.3732/ajb.1200478

Appendix I

Dependent	Independent	Figure	Moran's I standard deviate	p-value
Canopy height	Climatic zone	3a	0.08931	0.4644
Seed release height	Climatic zone	3b	-0.12235	0.5487
Length infl.	Climatic zone	3c	-0.11074	0.5441
First.flow(doy)	Climatic zone	4a	-0.21516	0.5852
Flow.half(doy)	Climatic zone	4b	-0.11702	0.5466
First.ripe(doy)	Climatic zone	4c	-0.57733	0.7181
First.flow(gdh)	Climatic zone	5a	-0.93938	0.8262
Flow.half(gdh)	Climatic zone	5b	0.26693	0.3948
First.ripe(gdh)	Climatic zone	5c	2.1708	0.01497
First.flow(doy)	Northern latitude	Ala	0.045778	0.4817
Flow.half(doy)	Northern latitude	A1b	0.34373	0.3655
First.ripe(doy)	Northern latitude	A1c	0.21317	0.4156
First.flow(gdd)	Northern latitude	A2a	0.87796	0.1900
Flow.half(gdd)	Northern latitude	A2b	-0.36804	0.6436
First.ripe(gdd)	Northern latitude	A2c	-0.78436	0.7836
First.flow(gdh)	Northern latitude	A2d	-0.0080847	0.5032
Flow.half(gdh)	Northern latitude	A2e	-0.0091361	0.5036
First.ripe(gdh)	Northern latitude	A2f	2.2439	0.01242



Figure A1. Linear regressions of the day of year (doy) for each site, at which **a** the first open flower was observed (first.flow) **b** half of the inflorescence's length bears open flowers (flow.half) **c** the first ripe (black) pods was observed (first.ripe), against latitude (°N). Only the sites in black were included into the model. White symbols are sites of the high altitude, alpine south climatic zone (RHON, SWHA) that were omitted from this analysis and only shown for comparison. Grey areas depict 95% confidence intervals.



Figure A2. Linear regressions of the accumulated growing degree days (gdd; from January 1, base temperature: 5 °C from weather stations) for each site, until **a** the first open flower was observed (first. flow) **b** half of the inflorescence's length bears open flowers (flow.half) **c** the first ripe (black) pods were observed (first.ripe), against latitude (°N). Furthermore, linear regressions of the accumulated growing day hours (gdh) for each site, until **d** the first open flower was observed (first.flow) **e** half of the inflorescence's length bears open flower sobserved (first.flow) **e** half of the inflorescence's length bears open flower sobserved (first.flow) **e** half of the inflorescence's length bears open flowers (flow.half) **f** the first ripe pod was observed (first.ripe) against latitude (°N). Only the sites in black were included into the model. White symbols are sites of the high altitude, alpine south climatic zone (RHON, SWHA) that were omitted from this analysis and only shown for comparison. Grey areas depict 95% confidence intervals.

SHORT COMMUNICATION



Knowledge needs in economic costs of invasive species facilitated by canalisation

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Academiceditor:S.McDermott|Received18September2022|Accepted28November2022|Published19December2022

Citation: Balzani P, Cuthbert RN, Briski E, Galil B, Castellanos-Galindo GA, Kouba A, Kourantidou M, Leung B, Soto I, Haubrock PJ (2022) Knowledge needs in economic costs of invasive species facilitated by canalisation. NeoBiota 78: 207–223. https://doi.org/10.3897/neobiota.78.95050

Abstract

Canals provide wide-ranging economic benefits, while also serving as corridors for the introduction and spread of aquatic alien species, potentially leading to negative ecological and economic impacts. However, to date, no comprehensive quantifications of the reported economic costs of these species have been done. Here, we used the InvaCost database on the monetary impact of invasive alien species to identify the costs of those facilitated by three major canal systems: the European Inland Canals, Suez Canal, and Panama Canal. While we identified a staggering number of species having spread via these systems, monetary costs have been reported only for a few. A total of \$33.6 million in costs have been reported from species linked to European Inland Canals (the fishhook waterflea *Cercopagis pengoi* and the zebra mussel *Dreissena polymorpha*) and \$8.6 million linked to the Suez Canal (the silver-cheeked toadfish *Lagocephalus sceleratus*, the lionfish *Pterois miles*, and the nomad jellyfish *Rhopilema nomadica*), but no recorded costs were found for species facilitated by the Panama Canal. We thus identified a pervasive lack of information on the monetary costs of invasions facilitated by canals and highlighted the uneven distribution of costs.

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Keywords

aquatic environment, habitat connectivity, inequality, InvaCost, invasive alien species, monetary costs

Introduction

Aquatic invasive alien species (IAS) are a major threat to biodiversity and ecosystem functioning (Ricciardi and Rasmussen 1999; Molnar et al. 2008; Strayer 2010), as well as human health (Galil 2018; Souty-Grosset et al. 2018). New alien species continue to be introduced at increasing rates (Seebens et al. 2017), and the share that becomes invasive brings considerable and increasing economic costs (Diagne et al. 2021a). Recently, an open database which compiled the economic costs of biological invasions (Diagne et al. 2020) has allowed quantification across a variety of geographical regions (e.g. Haubrock et al. 2022a), ecosystems (e.g. Cuthbert et al. 2021) taxa (e.g. Angulo et al. 2022) and languages (Angulo et al. 2021).

Aquatic IAS spread through multiple vectors and pathways, intentionally or unintentionally, through either active or passive transport. For example, they can escape from confinement (Lockwood et al. 2013), be unintentionally translocated as contaminants or parasites of a certain goods item (e.g. food, plants, timber; Lockwood et al. 2013), through hull fouling (Sylvester and MacIsaac 2010; Sylvester et al. 2011) or ballast waters of ships (Briski et al. 2012, 2013). Also, breaching biogeographical barriers allows new or additional species invasions (Gollasch et al. 2006; Kourantidou et al. 2015; Kaiser and Kourantidou 2021) or the further spread of IAS to secondary invaded areas from primary 'stepping stones' (Bertelsmeier and Keller 2018). For example, roads and railways represent important corridors for IAS (Hulme 2009), also increasing their propagule pressure (Woodford et al. 2013).

One of the most important pathways that allow the spread of IAS are canals connecting geographically-isolated aquatic systems (e.g. Asth et al. 2021), such as the trans-isthmian Suez and Panama Canals, and the cross continental North Sea-to-Black Sea Rhine-Main-Danube Canal. These highly trafficked strategic canals connect transport networks of critical economic and socio-political value, mainstays of global trade and globalisation (Amato 2020). They considerably reduce travel time and distance (therefore also CO_2 emissions), as well as operating costs to shippers and consumers, thus increasing commerce and economic growth (e.g. Lloyd 2018; Park et al. 2020; Cordoba 2022). Nonetheless, these economic benefits are counterbalanced by facilitated introduction and spread of IAS in goods, vessels, and in water due to increased connectivity. If established, IAS can have detrimental ecological consequences, as well as negative economic impacts (Bij de Vaate et al. 2002; Leuven et al. 2009; Galil et al. 2017; Turbelin et al. 2021).

This paper aims to quantify the known economic costs associated with IAS considered to have been facilitated by canals, enabling active (i.e. self-moving species) or passive (i.e. hitchhiker species) spread of these species. For this study, we focused on three major canal systems: Suez, Panama, and European Inland Canals (Fig. 1), as



Figure 1. Locations of the main canal systems studied (**a**) the European Inland Canals (**b**), the Panama Canal (**c**), and the Suez Canal (**d**). Red lines represent single canals. As for the European Inland Canals, only the three major canals (the Rhine-Main-Danube Canal, the Volga-Don Canal, and the Volga-Baltic Canal) are represented, for simplicity.

these represent major circumventions of important biogeographical barriers, and for which greater information is available. In particular, we hypothesised that (i) the Suez Canal majorly contributed to IAS economic costs, that (ii) these costs are not evenly distributed among countries of the same canal system, and that (iii) these costs are attributed to different taxa in different canal systems.

Materials and methods

Study systems choice

Canals directly connect distinct biogeographic provinces (as in the case with the Suez and Panama Canals) or contiguous seas in the case of the Kiel and the Corinth Canals, whose biota may intermingle freely. While the latter two can be considered of regional importance (in Germany and Greece, respectively), the former two are globally important. Indeed, the Suez Canal connects the Red Sea with the Mediterranean Sea, while the Panama Canal connects the Atlantic Ocean (Caribbean Sea) with the (eastern) Pacific Ocean, allowing ships to avoid circumnavigating Africa and South America, respectively, and reducing travel by thousands of nautical miles. The Suez Canal, a marine sea-level canal, was officially opened as early as 1869 and was recently doubled by creating a new lane (the 'New Suez Canal', functionally opened in 2016; Bereza et al. 2020), but also by widening and deepening the old canal, to increase its traffic capacity. Following the obstruction of the Suez Canal due to the grounding of a container ship in March 2021 (Ruiz et al. 2022), the Suez Canal Authority accelerated \$10 billion project plans to further extend and enlarge the canal (https://www.maritime-executive. com/article/suez-canal-sets-new-record-for-traffic-volume; https://www.reuters.com/ business/suez-canal-expansion-due-finish-july-2023-sca-chairman-2022-01-16/). The Panama Canal, a freshwater canal ~30 m above sea level, was opened in 1914 and due to the increase in traffic, just like with the Suez Canal, it was recently (2016) expanded and a new set of larger locks was installed, doubling its capacity (Wang 2017). In Europe, the situation is more complicated, with canals connecting multiple water bodies, thus forming a dendritic inland network system of connected major European rivers that ultimately link northern and southern European seas. The major and longest canal-river connections are the Rhine-Main-Danube, Volga-Don and Volga-Baltic Canals, which together with other minor systems form the European Inland Canals connecting the North and Baltic Seas with the Black, Azov and Caspian Seas, crossing all over Europe (Jażdżewski 1980; Bij de Vaate et al. 2002). Among these, the Rhine-Main-Danube Canal, completed in 1992, is the southernmost and longest one and has a particularly high economic importance (Bij de Vaate et al. 2002; Leuven et al. 2009).

Cost data sourcing and filtering

For each canal (Suez, Panama, and European Inland Canals), a detailed list of established IAS reported in the literature to have spread through these pathways, either actively or passively, was compiled by reviewing published papers and datasets (Suppl. material 1: table S1). To analyse the costs of these species, we used data from the InvaCost database, which includes costs from sources written primarily in English (Diagne et al. 2020), but also sources from 21 additional languages (Angulo et al. 2021). InvaCost compiles cost data resulting from systematic searches on the Web of Science, Google Scholar and Google search engine, and opportunistic contacts with experts and stakeholders. Each recorded cost entry was characterised by various descriptors as explained in more detail in Diagne et al. (2020) and in the online database repository (https://doi.org/10.6084/m9.figshare.12668570). InvaCost is a dynamic database that allows new cost entries to be corrected and added as they develop or are reported over time.

The most recent version of InvaCost (4.1 as of January 2022) includes 13,553 cost entries (i.e. rows of data entries with monetary costs) of IAS extracted from published peer-reviewed and grey literature. Although there may be costs that have not been captured (e.g. unpublished or outside the search languages), InvaCost offers the most up-to-date compilation of invasion costs and, therefore, constitutes the best tool available to draw parallels with the current state-of-the-art in cost reporting and associated knowledge gaps. However, considering the dynamic nature of the database, the results are subject to changes in the future as new monetary cost data become available for different species, countries, sectors of the economy, and other factors or as the existing cost data are further refined for accuracy. All costs published in the literature and included in the database were converted to 2017 US\$ values (see Diagne et al. 2020). For this analysis, we filtered cost entries in the InvaCost database by selecting those IAS that were reported to have been facilitated in their invasion as a result of the construction of selected canals (Suez, Panama, and European Inland Canals). Further, we filtered these IAS' costs by the countries involved in these three canal systems. Since costs of aquatic IAS are often under-reported (Cuthbert et al. 2021; Haubrock et al. 2022b), we included not only the countries crossed by the canals, but also those adjacent and those alongside the same water body of the two ends of the canals (i.e. all the countries bordering the North, Baltic, Black, and Mediterranean Seas; Suppl. material 1: table S2), as they could be affected by the further natural spread of the IAS (e.g. Galil et al. 2017).

Global cost descriptions

To describe the costs of IAS facilitated by the canals over time, we used the expandYearlyCosts function of the 'invacost' package (v0.3-4; Leroy et al. 2020) in R version 4.1.1 (R Core Team 2020). This function facilitates consideration of the temporal dimensions of the data, with the estimated costs per year being expanded in line with the length of time over which costs were reported or expected to have occurred as indicated by each respective publication included in the InvaCost database (Diagne et al. 2020) (i.e. the length of time between the Probable_starting_year_adjusted and Probable ending year adjusted columns). For example, the starting and ending years of a cost could reflect the period of which a control measure was implemented against an invasive population, or a period of reported resource damages to a fishery, as per the information in the cost source document (Diagne et al. 2020). To obtain a comparable cumulative total cost for each estimate over the period during which costs were incurred for each invasion, we multiplied each annual estimate by the respective duration (in years). Therefore, the analyses were conducted based on these 'expanded' entries to reflect the likely duration of the costs as reported in each study analysed. This means that costs covering several years (e.g. \$10 million between 2001 and 2010) are divided according to their duration (i.e. \$1 million for each year between 2001 and 2010). Finally, the cumulative costs of the invasion were estimated based on their classification across the following cost descriptors (i.e. columns) included in the database:

i. Method_reliability: indicating the perceived reliability of cost estimates based on the publication type and estimation method. Costs are considered to be of low reliability in those cases where they were derived from grey literature and/or are lacking documented, repeatable or traceable methods. On the other hand, costs are considered of high reliability if they come from peer-reviewed articles, official documents, or grey literature but with a fully documented, repeatable and traceable method (Diagne et al. 2020). While we acknowledge that this binary classification does not capture the widely varying methodologies of underlying studies, it provides a practical, reproducible and objective means of cost assessment and filtering; ii. Implementation: whether the cost estimate was incurred in the invaded area (observed; e.g. a cost directly incurred from investment in managing an invasive species, or an invasion-driven decline in a native fishery that resulted in a realised loss of income) or whether it was extrapolated or predicted over time within or beyond the actual distribution area of the IAS (potential), and thus not empirically incurred (Diagne et al. 2020). We emphasise that costs were compiled in InvaCost based on the information in each cost document (i.e. we did not extrapolate or predict cost estimates independently here, and simply compiled reported costs). For example, potential costs may include estimated reductions in fisheries income because of an invasion (Scheibel et al. 2016), known local costs that are extrapolated to a larger system than the one in which they occur (Oreska and Aldridge 2011), and costs extrapolated over several years based on estimates from a shorter period (Leigh 1998);

iii. Type_of_cost_merged: grouping of costs into categories: (i) damage referring to damages or loss incurred by the invasion (i.e. costs of repairing damage, losses of resources, medical care), (ii) management including expenditure related to control (i.e. surveillance, prevention, management, eradication), (iii) and mixed including mixed cost of damage and control (cases where the reported costs were not clearly distinguishable);

iv. Impacted_sector: the activity, societal or market sector that was affected by the cost. Seven sectors are described in the database: agriculture, authorities-stakeholders (official structures allocating efforts to manage biological invasions), environment, fishery, forestry, health, public and social welfare, and diverse (Diagne et al. 2020).

To analyse the costs of invasive alien species that were facilitated by canals (European Inland Canals, Suez Canal, and Panama Canal), we extracted species lists from several publications (see Suppl. material 1: table S1) and selected neighbouring countries for which invasions are likely to be facilitated by canals (Suppl. material 1: table S2). We then searched the InvaCost database (4.1) for these species in the respective countries and analysed the obtained data following the protocol and criteria described (Diagne et al. 2020; Leroy et al. 2020; Angulo et al. 2021).

Results

A total of 34 established species for the European Inland Canals, 411 for the Suez Canal, and 98 for the Panama Canal were listed to have been facilitated in their introduction and spread by these canals. In the InvaCost database, we identified in total 19 database entries: 8 for European Inland Canals and 11 for Suez. By way of contrast, no recorded costs were available for Panama. After expansion, these entries resulted in 34 annualised cost entries, encompassing 5 species (the fishhook waterflea *Cercopagis pengoi* and the zebra mussel *Dreissena polymorpha* for the European Inland Canals and the silver-cheeked toadfish *Lagocephalus sceleratus*, the lionfish *Pterois miles*, and the nomad jellyfish *Rhopilema nomadica* for the Suez Canal) for a total of \$42.2 million (\$33.6 for European Inland Canals and \$8.6 for Suez). The most surprising result is that costs were recorded for only a few species facilitated by the three canals (9% for European Inland Canals, 0.5% for the Suez Canal, and none for the Panama Canal), and this seems not to depend upon the choice of the countries that could be affected by canal-facilitated invaders, but by the general lack of costs reported for those species. Indeed, only a few cost records associated with the listed species were present in the entire InvaCost database (12% for European Inland Canals, 5% for Panama, and 1% for Suez), even for distant countries.

Fig. 2 summarises the recorded costs for European Inland Canals and Suez Canal. There was a clear difference between the two sites in the taxa associated with the costs. In European Inland Canals, all costs were attributed to invertebrates, specifically almost all to molluscs (*Dreissena polymorpha*, \$33.3 million) and just \$0.3 million to crustaceans (*Cercopagis pengoi*). In the case of the Suez Canal, most costs were attributed to vertebrates (*Lagocephalus sceleratus* and *Pterois miles*, \$8.6 million) with two very high-cost entries recorded and the remainder belonging to Cnidaria (*Rhopilema nomadica*, about \$59,000).

Our analysis also revealed an uneven distribution of the recorded costs. Out of the total 26 countries investigated for the European Inland Canals, only the United Kingdom (\$33.3 million), Finland (about \$146,000), Russia (about \$74,000), and Denmark (about \$58,000) reported costs associated with canal-facilitated invasive species. Similarly, only Turkey (\$5.5 million), Cyprus (\$3.1 millions), and Israel (about \$59,000) reported economic costs associated with the Suez Canal, out of the total 23 countries considered. Despite the low number of recorded costs, most of them were attributed to the high reliability category (\$31.5 million for the European Inland Canals and \$8.2 million for the Suez Canal) rather than the low reliability one (\$2.1 million for the European Inland Canals and about \$459,000 for the Suez Canal).

The total costs were differently attributed to observed and potential costs in the two canal systems. In European Inland Canals, about \$16.4 million of observed costs were recorded against about \$17.2 million of potential costs (though this latter result is mostly due to a single very high potential cost recorded). In contrast, in the Suez Canal, costs were mostly associated with observed entries (\$8.2 million, with two very high costs recorded) rather than potential costs (\$0.4 million). As for the type of costs, the recorded costs for the European Inland Canals were mostly attributed to management (\$31.4 million), followed by damage (\$2.1 million), and mixed (about \$55,000). The recorded costs for the Suez Canal, instead, were mainly associated with damage (\$5.5 million) and management (\$3.1 million). The invasive species associated with the European Inland Canals were recorded to impact multiple sectors: authorities-stakeholders (\$24.4 million), environment (\$1.9 million), and fishery (about \$220,000). Moreover, additional costs were recorded for other sectors (diverse: \$6.9 million). Similarly, the invasive species facilitated by the Suez Canal had recorded impacts on authorities-stakeholders (about \$0.5 million), fishery (\$6.2 million, with two very high reported costs), and public and social welfare (\$1.9 million).



Figure 2. Proportions of monetary costs (outer circle) and cost entries (inner circle) between canals analysed (i.e. European Inland Canals and Suez Canal), according to the cost descriptors studied: species, affected countries, method reliability, implementation, type of costs and impacted sectors.

Discussion

Canals are important corridors for many aquatic IAS, as revealed by the long list of established species that we obtained. The connection of multiple water bodies with distinct ecological communities is well-known to have promoted the spread of numerous invaders (Galil et al. 2008; Leuven et al. 2009; Hulme et al. 2017). These numbers are expected to increase with time, especially after the enlargement of the Suez and the Panama Canals (Galil et al. 2015; Muirhead et al. 2015; Castellanos-Galindo et al. 2020). However, when searching in the InvaCost database for the costs associated with these species, very few entries for very few species (five) were found, even if we opted for an "extensive approach" by including all the countries potentially affected by canal-passing invaders, i.e. not bordering the receiving system directly. This might be unsurprising, as impacts of those species are not well known (hidden below water) or documented in monetary terms, e.g. for the killer shrimp *Dikerogammarus villosus*,

which is widely distributed in Europe (Soto et al. 2022) but only had reported costs from Italy (Tricarico et al. 2010). Also, many of these species could take decades to cause tangible impacts from the moment of their establishment. Moreover, cost data deficiency is common, especially for marine species across many taxa (e.g. Haubrock et al. 2022b; Kouba et al. 2022), countries (e.g. Haubrock et al. 2021a; Renault et al. 2021), and entire regions (e.g. Kourantidou et al. 2021). However, it is very important to stress that this massive lack of data does not mean that there are only a few costs caused by IAS facilitated by the opening of canals, but only that just a few have been recorded or estimated so far. A lack of costs also does not reflect large ecological impacts incurred in these invaded systems, given the challenges for monetisation of environmental effects.

By contrast, the economic benefits arising from commerce through canals such as those examined here can be easily materialised (e.g. Kaluza et al. 2010; Kenawy 2016; Chirosca and Rusu 2021), so that the general perception may be that the benefits far outweigh the drawbacks (Bereza et al. 2020; Cordoba 2022). Indeed, the value from canals includes numerous components that go beyond just income and employment opportunities created locally, but also encompass economic benefits for exporters and consumers of goods at various stages (i.e. from raw materials to consumer goods). Also, some IAS that spread through canals are perceived to have localised benefits, for example for local fisheries (Castellanos-Galindo et al. 2019; van Rijn et al. 2020), without knowledge of their impact. Nevertheless, the results of our analysis show that the data available is insufficient for a trade-off analysis and does not by any means suggest that the benefits of trade facilitated by the canals outweigh the costs of invasions. Since cost-benefit analyses of biological invasions remain difficult, and since beneficiaries are often far removed geographically from the site of environmental damage, this could potentially lead to disparities and social injustices between those parties (countries, stakeholders, economic sectors, and other individuals such as consumers or members of local communities) that benefit from commerce and those that incur the costs of the associated IAS. In turn, this highlights the concepts of environmental accountability, telecoupling and liability from the involved parties at a transnational or even global level (Shafer 2006; Kramarz and Park 2017; Hull and Liu 2018).

Environmental barriers within the respective canal can nevertheless limit the spread of IAS. For example, the Panama Canal is a freshwater canal (mainly composed of Lake Gatun) that marine species need to cross to invade either side. The similar salinity barrier also applies to the Rhine-Main-Danube Canal and the other European Inland Canals, as freshwater conditions in them should prevent the spread of saline species from the Ponto-Caspian region to the North European seas, and the other way around. However, this barrier can halt only stenohaline species actively spreading or fouling the ship hulls, while not impeding biological invasions through ballast waters and sediments (Sylvester and MacIsaac 2010; Briski et al. 2011, 2013). On the other hand, euryoecious species can overcome these barriers. Ponto-Caspian euryhaline taxa have done particularly well in the eastern Baltic Sea because it has low salinity, and many of them have been established in freshwater systems en route from the Ponto-Caspian region to the North and Baltic Seas (Bij de Vaate et al. 2002). In the case of the Suez Canal, the dissolution of the saltbed of the formerly hypersaline 'Bitter Lakes', which served as an effective barrier up to the 1960s, and the accelerated seawater warming in the Mediterranean, boosted by ever more frequent and severe marine heat waves, have likely enhanced the rate of successful invasions (Biton 2020; Galil et al. 2022). While the overwhelming majority of species traversed the Suez Canal northwards (the so-called Lessepsian migrations), a few species, for which monetary impacts are yet unknown, have been considered to traverse it southwards (anti-Lessepsian migrations; Bos et al. 2020; Azzurro et al. 2022). Considering the economic and socio-political importance of canals, and that the commerce through them cannot be easily impeded, we suggest that prevention and mitigation measures should be undertaken or reinforced by the canal authorities, to reduce the ecological and economic impact of IAS.

Some limitations of this study originate from the species and the countries considered. Indeed, in most cases, it can only be presumed that an invader was facilitated by a canal during its spread, especially for species established for a long time, which could have been introduced or spread through other pathways. Other, not easily disentangled, intricacies can also occur. For example, Ponto-Caspian species were sometimes intentionally introduced after canalisation in Europe to stabilise or enrich these new habitats (Arbačiauskas et al. 2010). Also, some IAS further spread from these hubs as secondary, 'stepping stone' invaders (e.g. Gammarus tigrinus, introduced from North America; Rewicz et al. 2019). Moreover, many stowaway species (like those transported via ballast water, or fouling species) have by now become cosmopolitan, being widely and repeatedly translocated. This makes it difficult to attribute them to specific geographic locations, and therefore to follow their spread and their associated costs (e.g. Amphibalanus amphitrite; Wrange et al. 2016). Ultimately, we note that species spreading in the opposite direction, i.e. towards the Ponto-Caspian region or the Red Sea, had no recorded economic costs, likely because movements of alien species from these systems are predominantly unidirectional (e.g. Galil et al. 2015; Cuthbert et al. 2020). However, documented cost flows may also reflect the availability of data, which may be limited due to sources in certain languages not included in InvaCost, inaccessible or very recent literature, or not having been captured in the search terms underlying the database (Diagne et al. 2020; Angulo et al. 2021). As for the countries considered, it should be acknowledged that even those not directly involved through canals can be affected by their facilitation.

Conclusions

Although we tried to be as inclusive as possible, our results underline the paucity of available data. As such, our estimations should be taken with caution, as complex trading relationships and interconnected introduction pathways meant that not all countries invaded as a consequence of canals could be accounted for, i.e. those not immediately bordering the regions linked by canals and those affected by secondary spread (see
fig. 6 in Galil et al. 2021). As the canals considered here are utilised by ships from all over the world, even a very distant country can be affected by hitchhiking species. More focused research is required to elucidate source-sink dynamics for biological invasions and the large-scale effects of pathways and vectors, as well as to quantify the importance of 'stepping stones' for invasion events. In an era of economic uncertainty (Baker et al. 2020), severe economic disparities between those benefiting and those negatively affected will have staggering consequences. Highlighting the magnitude of economic costs and sectors affected due to biological invasions (Cuthbert et al. 2021; Diagne et al. 2021b; Haubrock et al. 2021b) evidences the potential threat to economies and human wellbeing. Here, our results highlight the potential for canals to cause substantial economic costs, in addition to their intended economic benefits, as a result of biological invasions - even for the few species with reported impacts - for which knowledge gaps should be further addressed in future. Thus, we are calling for an increasing effort in (i) identifying the ecological impacts and associated costs of biological invasions in canals and the affected parties, as well as (ii) limiting their staggering increase given the predicted intensification in the use of these infrastructures in the future.

References

- Amato V (2020) The New Routes of Globalization. Containerization, Naval Gigantism and Role of the Suez and Panama Canals. Politics and Knowledge: New Trends in Social Research, 9–19.
- Angulo E, Diagne C, Ballesteros-Mejia L, Adamjy T, Ahmed DA, Akulov E, Banerjee AK, Capinha C, Dia CAKM, Dobigny G, Duboscq-Carra VG, Golivets M, Haubrock PJ, Heringer G, Kirichenko N, Kourantidou M, Liu C, Nuñez MA, Renault D, Roiz D, Taheri A, Verbrugge LNH, Watari Y, Xiong W, Courchamp F (2021) Non-English languages enrich scientific knowledge: The example of economic costs of biological invasions. The Science of the Total Environment 775: 144441. https://doi.org/10.1016/j.scitotenv.2020.144441
- Angulo E, Hoffmann B, Ballesteros-Mejia L, Taheri A, Balzani P, Renault D, Cordonnier M, Bellard C, Diagne C, Ahmed DA, Watari Y, Courchamp F (2022) Economic costs of invasive alien ants worldwide. Biological Invasions 24(7): 2041–2060. https://doi. org/10.1007/s10530-022-02791-w
- Arbačiauskas K, Rakauskas V, Virbickas T (2010) Initial and long-term consequences of attempts to improve fish-food resources in Lithuanian waters by introducing alien peracaridan species: A retrospective overview. Journal of Applied Ichthyology 26: 28–37. https://doi.org/10.1111/j.1439-0426.2010.01492.x
- Asth MDS, Rodrigues RG, Zenni RD (2021) Canals as invasion pathways in tropical dry forest and the need for monitoring and management. Journal of Applied Ecology 58(9): 2004–2014. https://doi.org/10.1111/1365-2664.13950
- Azzurro E, Nourigat M, Cohn F, Ben Souissi J, Bernardi G (2022) Right out of the gate: The genomics of Lessepsian invaders in the vicinity of the Suez Canal. Biological Invasions 24(4): 1117–1130. https://doi.org/10.1007/s10530-021-02704-3

- Baker SR, Bloom N, Davis SJ, Terry SJ (2020) Covid-induced economic uncertainty (No. w26983). National Bureau of Economic Research, 1–17. https://doi.org/10.3386/w26983
- Bereza D, Rosen D, Shenkar N (2020) Current trends in ship movement via the Suez Canal in relation to future legislation and mitigation of marine species introductions. Management of Biological Invasions 11(3): 476–492. https://doi.org/10.3391/mbi.2020.11.3.09
- Bertelsmeier C, Keller L (2018) Bridgehead effects and role of adaptive evolution in invasive populations. Trends in Ecology & Evolution 33(7): 527–534. https://doi.org/10.1016/j. tree.2018.04.014
- Bij de Vaate A, Jazdzewski K, Ketelaars HA, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. Canadian Journal of Fisheries and Aquatic Sciences 59(7): 1159–1174. https://doi.org/10.1139/f02-098
- Biton E (2020) Possible implications of sea level changes for species migration through the Suez Canal. Scientific Reports 10(1): 1–16. https://doi.org/10.1038/s41598-020-78313-2
- Bos AR, Ogwang J, Bariche M, Horoiwa M, Megahed M, Ouf A, Yasuda N (2020) Anti-Lessepsian migration rectified: The Comber *Serranus cabrilla* (L. 1758) existed in the Red Sea prior to the Suez Canal opening. Marine Biology 167(9): 1–10. https://doi. org/10.1007/s00227-020-03748-0
- Briski E, Bailey SA, MacIsaac HJ (2011) Invertebrates and their dormant eggs transported in ballast sediments of ships arriving to the Canadian coasts and the Laurentian Great Lakes. Limnology and Oceanography 56(5): 1929–1939. https://doi.org/10.4319/ lo.2011.56.5.1929
- Briski E, Wiley CJ, Bailey SA (2012) Role of domestic shipping in the introduction or secondary spread of nonindigenous species: Biological invasions within the Laurentian Great Lakes. Journal of Applied Ecology 49(5): 1124–1130. https://doi.org/10.1111/j.1365-2664.2012.02186.x
- Briski E, Bailey SA, Casas-Monroy O, DiBacco C, Kaczmarska I, Lawrence JE, Leichsenring J, Levings C, MacGillivary ML, McKindsey CW, Nasmith LE, Parenteau M, Piercey GE, Rivkin RB, Rochon A, Roy S, Simard N, Sun B, Way C, Weise AM, MacIsaac HJ (2013) Taxon-and vector-specific variation in species richness and abundance during the transport stage of biological invasions. Limnology and Oceanography 58(4): 1361–1372. https://doi.org/10.4319/lo.2013.58.4.1361
- Castellanos-Galindo GA, Robertson DR, Pacheco-Chaves B, Angulo A, Chong-Montenegro C (2019) Atlantic Tarpon in the Tropical Eastern Pacific 80 years after it first crossed the Panama Canal. Reviews in Fish Biology and Fisheries 29(2): 401–416. https://doi.org/10.1007/s11160-019-09565-z
- Castellanos-Galindo GA, Robertson DR, Sharpe DMT, Torchin ME (2020) A new wave of marine fish invasions through the Panama and Suez canals. Nature Ecology & Evolution 4(11): 1444–1446. https://doi.org/10.1038/s41559-020-01301-2
- Chirosca AM, Rusu L (2021) Statistical and economic analysis of the Rhine-Main-Danube Canal, the bridge between the North Sea and the Black Sea. Journal of Danubian Studies and Research 11: 192–201.
- Cordoba GF (2022) The impact of the Panama Canal transfer on the Panamanian economy. Economics Letters 211: 110208. https://doi.org/10.1016/j.econlet.2021.110208

- Cuthbert RN, Kotronaki SG, Dick JT, Briski E (2020) Salinity tolerance and geographical origin predict global alien amphipod invasions. Biology Letters 16(9): 20200354. https://doi.org/10.1098/rsbl.2020.0354
- Cuthbert RN, Pattison Z, Taylor NG, Verbrugge L, Diagne C, Ahmed DA, Leroy B, Angulo E, Briski E, Capinha C, Catfordk JA, Dalu T, Essl F, Gozlan RE, Haubrock PJ, Kourantidou M, Krameru AM, Renault D, Wasserman RJ, Courchamp F (2021) Global economic costs of aquatic invasive alien species. The Science of the Total Environment 775: 145238. https://doi.org/10.1016/j.scitotenv.2021.145238
- Diagne C, Leroy B, Gozlan RE, Vaissière AC, Assailly C, Nuninger L, Roiz D, Jourdain F, Jarić I, Courchamp F (2020) InvaCost, a public database of the economic costs of biological invasions worldwide. Scientific Data 7(1): 1–12. https://doi.org/10.1038/s41597-020-00586-z
- Diagne C, Leroy B, Vaissière AC, Gozlan RE, Roiz D, Jarić I, Salles J-M, Bradshaw CJA, Courchamp F (2021a) High and rising economic costs of biological invasions worldwide. Nature 592(7855): 571–576. https://doi.org/10.1038/s41586-021-03405-6
- Diagne C, Turbelin A, Moodley D, Novoa A, Leroy B, Angulo E, Adamjy T, Dia CAKM, Taheri A, Tambo J, Dobigny G, Courchamp F (2021b) The economic costs of biological invasions in Africa: A growing but neglected threat? NeoBiota 67: 11–51. https://doi. org/10.3897/neobiota.67.59132
- Galil BS (2018) Poisonous and venomous: marine alien species in the Mediterranean Sea and human health. In: Mazza G, Tricarico E (Eds) Invasive species and human health. CABI, 1–15.
- Galil BS, Nehring S, Panov V (2008) Waterways as Invasion Highways Impact of Climate Change and Globalization. In: Nentwig W (Ed.) Biological Invasions. Ecological Studies, vol. 193. Springer, Berlin, Heidelberg, 59–74. https://doi.org/10.1007/978-3-540-36920-2_5
- Galil BS, Boero F, Campbell ML, Carlton JT, Cook E, Fraschetti S, Gollasch S, Hewitt CL, Jelmert A, Macpherson E, Marchini A, McKenzie C, Minchin D, Occhipinti-Ambrogi A, Ojaveer H, Olenin S, Piraino S, Ruiz GM (2015) 'Double trouble': The expansion of the Suez Canal and marine bioinvasions in the Mediterranean Sea. Biological Invasions 17(4): 973–976. https://doi.org/10.1007/s10530-014-0778-y
- Galil BS, Marchini A, Occhipinti-Ambrogi A, Ojaveer H (2017) The enlargement of the Suez Canal-Erythraean introductions and management challenges. Management of Biological Invasions 8(2): 141–152. https://doi.org/10.3391/mbi.2017.8.2.02
- Galil BS, Mienis HK, Hoffman R, Goren M (2021) Non-indigenous species along the Israeli Mediterranean coast: Tally, policy, outlook. Hydrobiologia 848(9): 2011–2029. https://doi.org/10.1007/s10750-020-04420-w
- Galil BS, Mienis HK, Mendelson M, Gayer K, Goren M (2022) Here today, gone tomorrow the Levantine population of the Brown mussel *Perna perna* obliterated by unprecedented heatwave. Aquatic Invasions 17(2): 174–185. https://doi.org/10.3391/ai.2022.17.2.03
- Gollasch S, Galil BS, Cohen AN (2006) Bridging divides: maritime canals as invasion corridors (Vol. 229). Springer, Dordrecht, 306 pp. https://doi.org/10.1007/978-1-4020-5047-3
- Haubrock PJ, Cuthbert RN, Tricarico E, Diagne C, Courchamp F, Gozlan R (2021a) The recorded economic costs of alien invasive species in Italy. NeoBiota 67: 247–266. https://doi.org/10.3897/neobiota.67.57747

- Haubrock PJ, Turbelin AJ, Cuthbert RN, Novoa A, Taylor NG, Angulo E, Ballesteros-Mejia L, Bodey TW, Capinha C, Diagne C, Essl F, Golivets M, Kirichenko N, Kourantidou M, Leroy B, Renault D, Verbrugge L, Courchamp F (2021b) Economic costs of invasive alien species across Europe. NeoBiota 67: 153–190. https://doi.org/10.3897/neobiota.67.58196
- Haubrock PJ, Cuthbert RN, Hudgins EJ, Crystal-Ornelas R, Kourantidou M, Moodley D, Liu C, Turbelin AJ, Leroy B, Courchamp F (2022a) Geographic and taxonomic trends of rising biological invasion costs. The Science of the Total Environment 817: 152948. https://doi.org/10.1016/j.scitotenv.2022.152948
- Haubrock PJ, Bernery C, Cuthbert RN, Liu C, Kourantidou M, Leroy B, Turbelin AJ, Kramer AM, Verbrugge LNH, Diagne C, Courchamp F, Gozlan RE (2022b) Knowledge gaps in economic costs of invasive alien fish worldwide. The Science of the Total Environment 803: 149875. https://doi.org/10.1016/j.scitotenv.2021.149875
- Hull V, Liu J (2018) Telecoupling: A new frontier for global sustainability. Ecology and Society 23(4): 41. https://doi.org/10.5751/ES-10494-230441
- Hulme PE (2009) Trade, transport and trouble: Managing invasive species pathways in an era of globalization. Journal of Applied Ecology 46(1): 10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x
- Hulme PE, Bacher S, Kenis M, Kuhn I, Pergl J, Pyšek P, Roques A, Vilà M (2017) Blurring alien introduction pathways risks losing the focus on invasive species policy. Conservation Letters 10(2): 265–266. https://doi.org/10.1111/conl.12262
- Jażdżewski K (1980) Range extensions of some gammaridean species in European inland waters caused by human activity. Crustaceana (Supplement 6): 84–107.
- Kaiser BA, Kourantidou M (2021) Invasive alien species in changing marine arctic economies and ecosystems. CABI Reviews: Perspectives in Agriculture, Veterinary Science. Nutrition and Natural Resources 16: 1–12. https://doi.org/10.1079/PAVSNNR202116022
- Kaluza P, Kölzsch A, Gastner MT, Blasius B (2010) The complex network of global cargo ship movements. Journal of the Royal Society, Interface 7(48): 1093–1103. https://doi. org/10.1098/rsif.2009.0495
- Kenawy E (2016) The economic impacts of the new Suez Canal. IEMed. Mediterranean Yearbook, 282–288.
- Kouba A, Oficialdegui FJ, Cuthbert RN, Kourantidou M, South J, Tricarico E, Gozlan RE, Courchamp F, Haubrock PJ (2022) Identifying economic costs and knowledge gaps of invasive aquatic crustaceans. The Science of the Total Environment 813: 152325. https://doi.org/10.1016/j.scitotenv.2021.152325
- Kourantidou M, Kaiser BA, Fernandez LM (2015) Towards Arctic Resource Governance of Marine Invasive Species. In: Heininen L, Exner-Pirot H, Plouffe J (Eds) Arctic Yearbook 2015. Northern Research Forum, Akureyri, Iceland, 175–194.
- Kourantidou M, Cuthbert RN, Haubrock PJ, Novoa A, Taylor N, Leroy B, Capinha C, Renault D, Angulo E, Diagne C, Courchamp F (2021) Economic costs of invasive alien species in the Mediterranean basin. NeoBiota 67: 427–458. https://doi.org/10.3897/neobiota.67.58926
- Kramarz T, Park S (2017) Introduction: The politics of environmental accountability. The Review of Policy Research 34(1): 4–9. https://doi.org/10.1111/ropr.12223
- Leigh P (1998) Benefits and costs of the ruffe control program for the Great Lakes fishery. Journal of Great Lakes Research 24(2): 351–360. https://doi.org/10.1016/S0380-1330(98)70826-3

- Leroy B, Kramer AM, Vaissière AC, Courchamp F, Diagne C (2020) Analysing global economic costs of invasive alien species with the invacost R package. bioRxiv, 1–18. https://doi. org/10.1101/2020.12.10.419432
- Leuven RS, van der Velde G, Baijens I, Snijders J, van der Zwart C, Lenders HJ, bij de Vaate A (2009) The river Rhine: A global highway for dispersal of aquatic invasive species. Biological Invasions 11(9): 1989–2008. https://doi.org/10.1007/s10530-009-9491-7
- Lloyd RA (2018) The Panama Canal as a Determinant of fdi in Panama. Review of Integrative Business and Economics Research 7: 87–102.
- Lockwood JL, Hoopes MF, Marchetti MP [Eds] (2013) Invasion ecology, 2nd edn. Wiley-Blackwell / John Wiley and Sons, 444 pp.
- Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Frontiers in Ecology and the Environment 6(9): 485–492. https://doi.org/10.1890/070064
- Muirhead JR, Minton MS, Miller WA, Ruiz GM (2015) Projected effects of the Panama Canal expansion on shipping traffic and biological invasions. Diversity & Distributions 21(1): 75–87. https://doi.org/10.1111/ddi.12260
- Oreska MP, Aldridge DC (2011) Estimating the financial costs of freshwater invasive species in Great Britain: A standardized approach to invasive species costing. Biological Invasions 13(2): 305–319. https://doi.org/10.1007/s10530-010-9807-7
- Park C, Richardson HW, Park J (2020) Widening the Panama Canal and US ports: Historical and economic impact analyses. Maritime Policy & Management 47(3): 419–433. https://doi.org/10.1080/03088839.2020.1721583
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Renault D, Manfrini E, Leroy B, Diagne C, Ballesteros-Mejia L, Angulo E, Courchamp F (2021) Biological invasions in France: Alarming costs and even more alarming knowledge gaps. NeoBiota 67: 191–224. https://doi.org/10.3897/neobiota.67.59134
- Rewicz T, Grabowski M, Tończyk G, Konopacka A, Bącela-Spychalska K (2019) Gammarus tigrinus Sexton, 1939 continues its invasion in the Baltic Sea: First record from Bornholm (Denmark). BioInvasions Records 8(4): 862–870. https://doi.org/10.3391/bir.2019.8.4.14
- Ricciardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. Conservation Biology 13(5): 1220–1222. https://doi.org/10.1046/j.1523-1739.1999.98380.x
- Ruiz GM, Galil BS, Davidson IC, Donelan SC, Miller AW, Minton MS, Muirhead JR, Ojaveer H, Tamburri MN, Carlton JT (2022) Global marine biosecurity and ship layups: Intensifying effects of trade disruptions. Biological Invasions 24(11): 3441–3446. https://doi.org/10.1007/s10530-022-02870-y
- Scheibel NC, Dembkowski DJ, Davis JL, Chipps SR (2016) Impacts of northern pike on stocked rainbow trout in Pactola Reservoir, south Dakota. North American Journal of Fisheries Management 36(2): 230–240. https://doi.org/10.1080/02755947.2015.1116472
- Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jäger H, Kartesz J, Kenis M, Kreft H, Kühn I, Lenzner B, Liebhold A, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K,

Tokarska-Guzik B, van Kleunen M, Walker K, Weigelt P, Yamanaka T, Essl F (2017) No saturation in the accumulation of alien species worldwide. Nature Communications 8(1): 1–9. https://doi.org/10.1038/ncomms14435

- Shafer WE (2006) Social paradigms and attitudes toward environmental accountability. Journal of Business Ethics 65(2): 121–147. https://doi.org/10.1007/s10551-005-4606-2
- Soto I, Cuthbert RN, Ahmed DA, Kouba A, Domisch S, Marquez JR, Beidas A, Amatulli G, Kiesel J, Shen LQ, Florencio M, Lima H, Briski E, Altermatt F, Archambaud-Suard G, Borza P, Csabai Z, Datry T, Floury M, Forcellini M, Fruget J-F, Leitner P, Lizée M-H, Maire A, Ricciardi A, Schäfer RB, Stubbington R, Van der Lee GH, Várbíró G, Verdonschot RCM, Haase P, Haubrock PJ (2022) Tracking a killer shrimp: *Dikerogammarus villosus* invasion dynamics across Europe. Diversity & Distributions 00: 1–16. https://doi.org/10.1111/ ddi.13649
- Souty-Grosset C, Anastácio P, Reynolds J, Tricarico E (2018) Invasive freshwater invertebrates and fishes: Impacts on human health. In: Mazza G, Tricarico E (Eds) Invasive Species and Human Health. CABI, 91–107. https://doi.org/10.1079/9781786390981.0091
- Strayer DL (2010) Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology 55: 152–174. https://doi. org/10.1111/j.1365-2427.2009.02380.x
- Sylvester F, MacIsaac HJ (2010) Is vessel hull fouling an invasion threat to the Great Lakes? Diversity & Distributions 16(1): 132–143. https://doi.org/10.1111/j.1472-4642.2009.00622.x
- Sylvester F, Kalaci O, Leung B, Lacoursière-Roussel A, Murray CC, Choi FM, Bravo MA, Therriault TW, MacIsaac HJ (2011) Hull fouling as an invasion vector: Can simple models explain a complex problem? Journal of Applied Ecology 48(2): 415–423. https://doi. org/10.1111/j.1365-2664.2011.01957.x
- Tricarico E, Mazza G, Orioli G, Rossano C, Scapini F, Gherardi F (2010) The killer shrimp, *Dikerogammarus villosus* (Sowinsky, 1894), is spreading in Italy. Aquatic Invasions 5(2): 211–214. https://doi.org/10.3391/ai.2010.5.2.14
- Turbelin AJ, Diagne C, Hudgins EJ, Moodley D, Kourantidou M, Novoa A, Haubrock PJ, Bernery C, Gozlan RE, Francis RA, Courchamp F (2021) Species on the move: Stowaways and contaminants cause the greatest economic impacts. Biological Invasions, 1–27. https://doi.org/10.21203/rs.3.rs-440305/v1
- van Rijn I, Kiflawi M, Belmaker J (2020) Alien species stabilize local fisheries catch in a highly invaded ecosystem. Canadian Journal of Fisheries and Aquatic Sciences 77(4): 752–761. https://doi.org/10.1139/cjfas-2019-0065
- Wang M (2017) The role of Panama Canal in global shipping. Maritime Business Review 2(3): 247–260. https://doi.org/10.1108/MABR-07-2017-0014
- Woodford DJ, Hui C, Richardson DM, Weyl OL (2013) Propagule pressure drives establishment of introduced freshwater fish: Quantitative evidence from an irrigation network. Ecological Applications 23(8): 1926–1937. https://doi.org/10.1890/12-1262.1
- Wrange AL, Charrier G, Thonig A, Alm Rosenblad M, Blomberg A, Havenhand JN, Jonsson PR, André C (2016) The story of a hitchhiker: Population genetic patterns in the invasive barnacle *Balanus (Amphibalanus) improvisus* Darwin 1854. PLoS ONE 11(1): e0147082. https://doi.org/10.1371/journal.pone.0147082

Supplementary material I

Knowledge needs in economic costs of invasive species facilitated by canalization Authors: Paride Balzani, Ross N. Cuthbert, Elizabeta Briski, Bella Galil, Gustavo Castellanos-Galindo, Antonín Kouba, Melina Kourantidou, Brian Leung, Ismael Soto, Phillip J. Haubrock

Data type: tables (word file)

Explanation note: Sources used for the extraction of species for each canal system. Countries considered for each canal system.

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Link: https://doi.org/10.3897/neobiota.78.95050.suppl1