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LETTER TO THE EDITOR

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Advancing research on alien species and biological invasions

Mind the gap (between assessing risks and prioritizing management)

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In a recent article, Bertolino et al. (2020) presented a horizon-scanning approach to invasive mammal species in Italy, contributing to ongoing EU and national efforts to assess and manage invasive species. Bertolino et al. 2020 then suggest a list of six priority species for policy-making, combining information from standard impact assessment methods (e.g. Environmental Impact Classification of Alien Taxa, EICAT; Blackburn et al. 2014) with additional considerations about manageability. Moving from risk assessment to management decisions is challenging, and the species prioritization by Bertolino et al. encounters some common pitfalls. Here, we highlight those pitfalls and suggest solutions based on best practices in conservation decision-making. We do not question the assessment of 'Impact' and 'Manageability'. Both are important and we commend Bertolino et al. for providing them. However, we caution against aggregating such different criteria into the 'Impact + Management' prioritization score (the overall ranking by Bertolino et al. 2020), when there is not a clear context or need for it, and the method used is arbitrary.

The first pitfall is that identifying priorities requires a clear decision context (who makes the decision, their objectives, their constraints; Keeney 1982; Game et al. 2013). The criteria used by Bertolino et al. (2020) are clear: impacts on biodiversity, economy, society and human health, and various criteria for manageability. The context for the assessment along these criteria is also clearly defined by the EU and the national requirement to "rank species to be subjected to risk assessment" (Bertolino et al. 2020, p. 32). However, prioritization is necessary only when one must allocate limited resources, typically time and money. If these competing objectives are not defined, assessment cannot be meaningfully converted to prioritization (Kumschick et al. 2020).

To illustrate, imagine a situation where three species must be managed, ranked 1-2-3 from greatest to smallest impacts. If resources were available to manage only one, and they all cost the same, species 1 might be prioritized. If the cost of managing species 1 were equal to the cumulative cost for 2 and 3, one might decide to prevent damage from species 2 and 3, instead of species 1 only. If resources were available for all three, prioritization would be unnecessary regardless of cost. To convert the risk assessment into a decision (prioritization), one must therefore define or assume a decision context, for example imagining the spatial, temporal, social scales where an action is feasible and has certain costs. This must be the case for manageability criteria expressed by Bertolino et al (2020). If the prioritization is to directly guide resource allocation, the context must be clearly defined and truly reflect all situations it might apply to.

On the other hand, management decisions will ultimately be case-specific (e.g. implying "different weights to the various aspects of the invasion process", Bertolino et al. 2020, p. 46). However, in this case prioritizing on a pre-conceived or assumed context is meaningless and possibly misleading, and it would be better to simply list the ranks for each criterion without aggregating them. For example, one may state that the assessment ranked "*S. floridanus* as the most impacting and easiest to manage alien mammal in Italy" (Bertolino et al. 2020, p. 44) and show the assessment criteria (essentially Table 2 in Bertolino et al. 2020, without columns 4–5), avoiding aggregate "prioritization" scores with their assumed value judgments and decision contexts (Game et al. 2013). Decision-makers can use this information to carry out their context-specific prioritization, using multi-criteria decision analysis if needed (Adem Esmail and Geneletti 2018).

Finally, if a clear context is missing but one still wishes to illustrate a "blueprint for similar prioritization initiatives" (Bertolino et al. 2020, p. 48), then this should be based on sound decision-analytic principles and ideally include illustrative examples of how priorities change with different objectives, management costs and resource constraints, such as annual budgets (Joseph et al. 2009; Dodd et al. 2017).

A second pitfall in the aggregation is arbitrariness. EICAT and similar approaches use qualitative scales, e.g. from Minimal Concern to Massive, based on rigorously defined criteria that, while inevitably arbitrary, remain internally consistent. However, this scale is essentially ordinal, not cardinal (i.e. represents order, not quantities): for example, category 4 (Major) is worse than category 2 (Minor), but not necessarily twice as bad. This does not change when converted to a numerical scale (1 to 5) as in Bertolino et al. 2020. Sums and multiplication of such scores still return numerical values that suggest objectivity, but remain fundamentally arbitrary and carry no biological meaning (Wolman 2006).

This problem is repeated by scoring manageability on the same 1–5 scale and multiplying it by the converted impact scores. A species with impact I=1 and manageability M=5 thus scores the same as one with I=5 and M=1, and half the score of a species with I=5 and M=2. But for management, species 1 and 2 might or might not be the same, and might or might not be twice as "priority" as species 3. Because the original scales have no cardinal meaning, such aggregate scores can be uninformative or misleading. In Bertolino et al. (2020), “Spread” and “Overall impacts” range up to 937.5 and 11718.75, respectively. A decision-maker might conclude that an overall impact score of 1000 is half as bad as one of 2000, but there is no logical basis for this interpretation (Game et al. 2013). One should go back to the original verbal scale and ask themselves: what does it mean to multiply “Major impact” *times* “Low manageability”?

To reduce arbitrariness, one might express outcomes using natural scales, like probabilities for chance events (Game et al. 2013). For large-scale assessments, again it is better to resist the temptation to convert constructed scales to numbers and to aggregate scores. If an overall recommendation is needed, it might be equally arbitrary, but more transparent, to rank objectives or impact categories separately, then assign a subjective overall score using the same process, reporting value judgments clearly (Game et al. 2013).

A third pitfall is that uncertainty influences risk attitudes and priorities (Tulloch et al. 2015). For example, the expected impacts of species 1 might range from Minimal to Massive (1–5) and those for species B from Moderate to Major (3–4). Risk-averse managers might prioritize controlling species 1 which has the highest worst-case impacts, whereas risk-neutral managers might prioritize species 2 which has the highest “average” impacts (McCarthy 2014). We recommend making uncertainty explicit in any assessment, following best-practice for horizon-scanning and ICAT assessments (Roy et al. 2018; Volery et al. 2020). Quantifying uncertainty also improves expert performance in elicitations (Speirs-Bridge et al. 2010).

Invasive species are a severe and challenging threat to biodiversity, and rigorous risk assessments help manage them. Principles and methods from decision science can then complement those assessments, clarifying decision problems and reducing arbitrariness, to prioritize efforts and improve outcomes.

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Towards an open, zoomable atlas for invasion science and beyond

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Abstract

Biological invasions are on the rise, and their global impacts on ecosystems, economies and human health are a major challenge. Invasion science is critical to mitigate invader impacts, yet due to the strong increase of data and information in this area, it has become difficult to acquire and maintain an overview of the field. As a result, existing evidence is often not found, knowledge is too rarely transferred to practice, and research is sometimes conducted in pursuit of dead ends. We propose to address these challenges by developing an interactive atlas of invasion science that can be extended to other disciplines in the future. This online portal, which we aim to create in the course of the project described here, will be an evolving knowledge resource and open for anyone to use, including researchers, citizen scientists, practitioners and policy makers. Users will be able to zoom into the major research questions and hypotheses of invasion science, which are connected to the relevant studies published in the field and, if available, the underlying raw data. The portal will apply cutting-edge visualization techniques, artificial intelligence and novel methods for knowledge synthesis.

Keywords

hierarchy-of-hypotheses (HoH) approach, hypothesis networks, interactive knowledge discovery, invasive alien species, invasion biology, Open Knowledge Maps, open science, Wikidata

Introduction

The number of non-native species has been strongly increasing over time worldwide, and there is currently no sign that this trend is going to stop (Seebens et al. 2017). Non-native species (also called alien species) are those species that have been intentionally or unintentionally transported to and introduced in areas outside their natural range (Blackburn et al. 2011; Jeschke et al. 2013). Some of these species establish and spread in their new ranges and/or cause detrimental impacts on ecosystems, economies or human health – these species are called invasive species. Invasion science, the study of non-native (including invasive) species and their environments, is therefore highly relevant to prevent and manage negative consequences for biodiversity, socio-economics and human health (IPBES 2019).

However, due to an exponential increase of data and information in invasion science, it has become difficult to acquire and maintain an overview of the field (Enders et al. 2018, 2019, 2020). This makes research relatively ineffective and inefficient, as existing evidence is often not found, collaboration opportunities are missed, and research is too often conducted in pursuit of dead ends. In addition, there is a slow transfer to practice, as practitioners are often not able to locate experts and knowledge relevant to their problems. This information is scattered across tens of thousands of research papers.

Similar challenges can be observed for many other research fields (Kraker et al. 2021b). The quote by John Naisbitt from the 1980s that we are “drowning in information but starved for knowledge” (p. 24 in Naisbitt 1982) thus seems to be more applicable than ever before (Jeschke et al. 2019; see also Burke 2020). We need novel tools to take full advantage of published scientific findings.

Along these lines, the science philosopher Philip Kitcher wrote in his book “Science in a democratic society”: “Even when informed and well-intentioned scientists try to think broadly about research options, their discussions suffer from the absence of a synthetic vision. Instead of pitting one partial perspective against another, it would be preferable to create a space in which the entire range of our inquiries could be soberly appraised. We would do well to have an institution for the construction and constant revision of an atlas of scientific significance” (p. 127 in Kitcher 2011). We strongly agree such an atlas would be extremely useful, and propose to take significant steps in this direction with the project outlined here.

Existing tools to explore the scientific literature have key drawbacks. Both Clarivate Analytics’ Web of Science and Elsevier’s Scopus are large literature databases behind a paywall, thus only accessible to researchers at institutions with libraries that are both financially able and willing to cover hefty subscription fees. The exact amount of these fees varies according to the size of the subscribing institution. For example, the Texas A&M University Libraries paid in 2019 ca. US$ 212,000 for the Web of Science and ca. US$ 140,000 for Scopus (Tabacaru 2019). While there has been a public debate – and outcry – about high subscription fees for research journals, which cause critical financial challenges for science libraries even in affluent countries, it is often unnoticed that there are other strong paywalls in the scientific universe, such as for literature databases. If researchers do not have access to tools that help them to explore and discover scientific publications, they are not able to really stand “on the shoulders of giants”, but need to reinvent and reinvestigate what others have already done. In addition, even researchers with access to these databases are not allowed to share the re-used data. For example, it is not usually possible to provide data downloaded from these databases along with the articles analyzing the data, leading to the situation that the analyses cannot be reproduced by others.

The freely searchable literature database Google Scholar is probably the tool used by most researchers without access to either the Web of Science or Scopus. Google Scholar is far from an ideal research tool, though. It has largely remained unchanged since its launch in 2004. Its search results are not reproducible by others, which is a problem for scientists, for example when they aim to perform a systematic literature review. Search hits in Google Scholar are created by a black-box algorithm that possibly returns different results depending on where and with which user profile a search was done. Furthermore, Google Scholar returns a list of possibly relevant papers in text form, but such a format does not allow users to grasp, and thus take advantage of, the many papers that are often available for a given scientific topic or search string.

A visual navigation tool would be much more powerful for taking advantage of Big Data (Börner 2014; Vargas-Quesada et al. 2017). The innovative discovery infrastructure Open Knowledge Maps (https://openknowledgemaps.org) provides visual maps when typing in keywords characterizing a scientific topic (Kraker et al. 2019). Open Knowledge Maps is the main driver behind the powerful open source knowledge mapping framework Head Start (Kraker et al. 2020). Head Start provides an interactive, web-based visualization interface and comes with a sophisticated artificial-intelligence backend that is capable of automatically producing knowledge maps from a variety of data, including text, metadata and references (Kraker et al. 2016). Head Start is used in a number of systems and projects, including the H2020 projects OpenUP and TRIPLE, the OpenAIRE Tender Project VIPER (Kraker et al. 2018) and the EOSC Secretariat project CoVis (Kraker et al. 2021b). A challenge for Open Knowledge Maps is that many large literature databases, such as Web of Science, Scopus or Google Scholar, are not open to be used by others, and that they often do not expose semantically enriched data. Open Knowledge Maps is thus restricted in its ability to display and interlink information.

An open database that could possibly be used by Open Knowledge Maps is the open-source linked data system Wikidata (https://www.wikidata.org/wiki/Wikidata:Main_Page; Lemus-Rojas and Odell 2018; Ayers et al. 2019; Waagmeester et al. 2019). Yet although this database has high potential (e.g. Waagmeester et al. 2020; Rutz et al. 2021), it currently does not systematically cover the different scientific disciplines. As preliminary work, we thus included >26,000 publications of the field of invasion science in Wikidata, where they can be explored in a domain-general way through tools like Scholia (https://tools.wmflabs.org/scholia/topic/Q42985020; Nielsen et al. 2017; Rasberry et al. 2019).

Another challenge of existing approaches for exploring scientific publications is that they do not link these to the big research questions, concepts and hypotheses of research fields. The novel hierarchy-of-hypotheses (HoH) approach allows to do so (Jeschke et al. 2012; Heger and Jeschke 2014; Jeschke and Heger 2018; Heger et al. 2021). A first visualization based on the HoH approach where 12 hypotheses in the field of invasion science are connected to >1100 studies is available at https://hi-knowledge.org (Jeschke et al. 2020). We have also explored approaches to create networks of research hypotheses (Enders et al. 2018, 2019, 2020). These approaches can be used to create networks of research questions too, thus making the tools applicable for research disciplines without established major hypotheses.

Objectives and approach

We aim to develop a prototype of a unique interactive atlas of invasion science that can be extended to other disciplines in the future. This interactive knowledge portal will (a) build on the strengths of Open Knowledge Maps in organizing and visualizing scientific knowledge, (b) connect it to Wikidata and (c) be conceptually based on the HoH approach. The portal will also have some similarities to e.g. Google Maps in that it is a zoomable navigation tool. In our case, users will be able to zoom into the field's conceptual structure, its big and smaller research questions, its major hypotheses and more specific operational hypotheses. All of these are connected to the relevant studies published in the field and, if available, the underlying raw data. It will be an openly accessible web portal providing FAIR open data (Wilkinson et al. 2016), all developed under an open source license. As a literature database with search functions, it will complement Google Scholar, where the data cannot be openly reused, and other literature databases such as the Web of Science and Scopus which are extremely expensive and not reusable either (see above). The focal research field is invasion science here, although the web portal will be set up so that it can evolve through time and cover other research fields in the future.

The working title of the proposed knowledge portal is enKORE: EvolviNg KnOwledge Resource. enKORE will be an interactive atlas of up-to-date knowledge that "connects the dots". It will have the following key features:

1. Suitably licensed publications will be made available as full text and connected to the raw data if these are available in an open format. If the raw data or publications are not freely available, key meta-data, such as authors, title and abstract, will be provided together with a link to the journal's website, preferably via persistent identifiers like DOI.
2. An interactive and zoomable visualization of research topics, where major research questions are hierarchically structured into more specific questions and, if applicable, to concepts and hypotheses in the field, which are in turn structured into more specific hypotheses. The publications and raw data will be linked to these questions and hypotheses (Fig. 1). This feature will thus, for example, allow users to easily

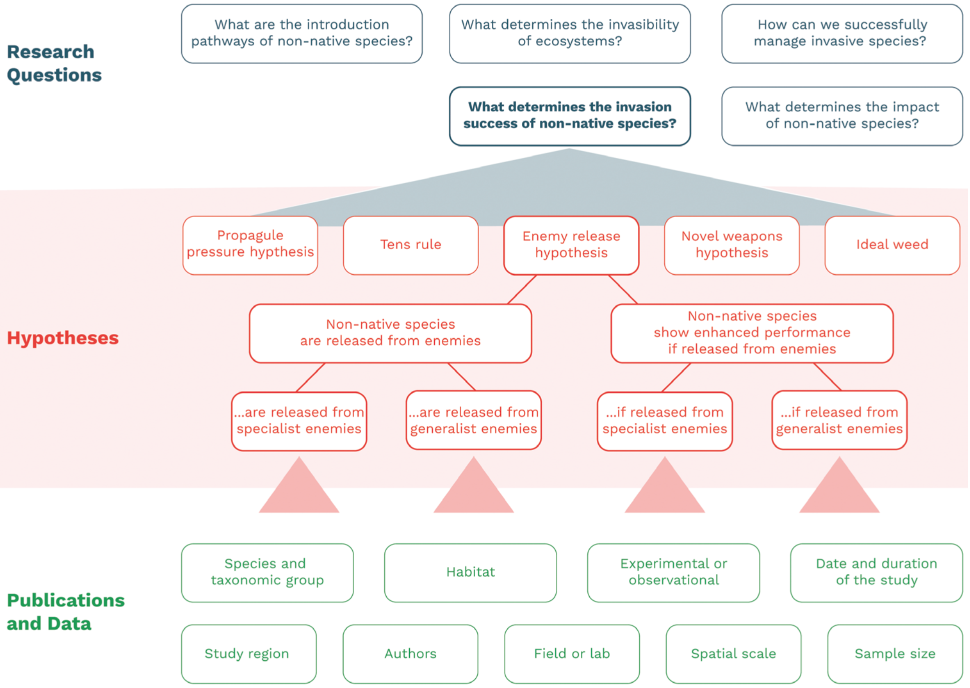


Figure 1. Proposed hierarchical structure of enKORE that will allow for an interactive and zoomable visualization of invasion science. enKORE will allow users to structure research done in biological invasions according to: (a) focal research questions (examples shown on top of the figure), (b) hypotheses addressing (some of) these questions (examples shown for one research question, see Enders et al. 2018, 2019, 2020 for details about these hypotheses) which can be further divided into sub-hypotheses (shown for the enemy release hypothesis, cf. Heger and Jeschke 2014, Jeschke and Heger 2018) and (c) other features of the publications and data. enKORE's hierarchical structure will allow users to zoom from research questions into hypotheses, sub-hypotheses, publications and data, or vice versa to zoom out from publications and data to the hypotheses and research questions these address.

find publications on similar questions and hypotheses by zooming into and out of the conceptual map.

3. Interactive on-demand analyses, allowing users to select studies done in a particular country, region or ecosystem, or focusing on a particular (group of) species. At the moment, such analyses are typically carried out once by researchers summarizing and analyzing the results of studies for a given research question or hypothesis. The results of such analyses are then published as a static paper, but it is not possible to easily repeat the same analyses (i) after some time has passed and the evidence base has changed, or (ii) by changing one or more settings of the analyses, such as additionally including studies following a methodology that the original author did not consider relevant, or studies focusing on animals rather than plants. enKORE will allow for interactive analyses that can be repeated on demand. By including automated processes, it will, for example, be possible to receive notifications about updated analyses.

The web portal will improve shared understanding within and across disciplinary contexts, increase collaboration and enable easier knowledge transfer to education and practice. Our vision is that it will foster theory-building within the discipline, and at the same time allow transfer of knowledge to other parts of society. The approach developed in this project can be easily transferred to other fields, extending its benefits far beyond invasion science, thus harnessing the potential of increased digitization to improve effectiveness and efficiency of global research.

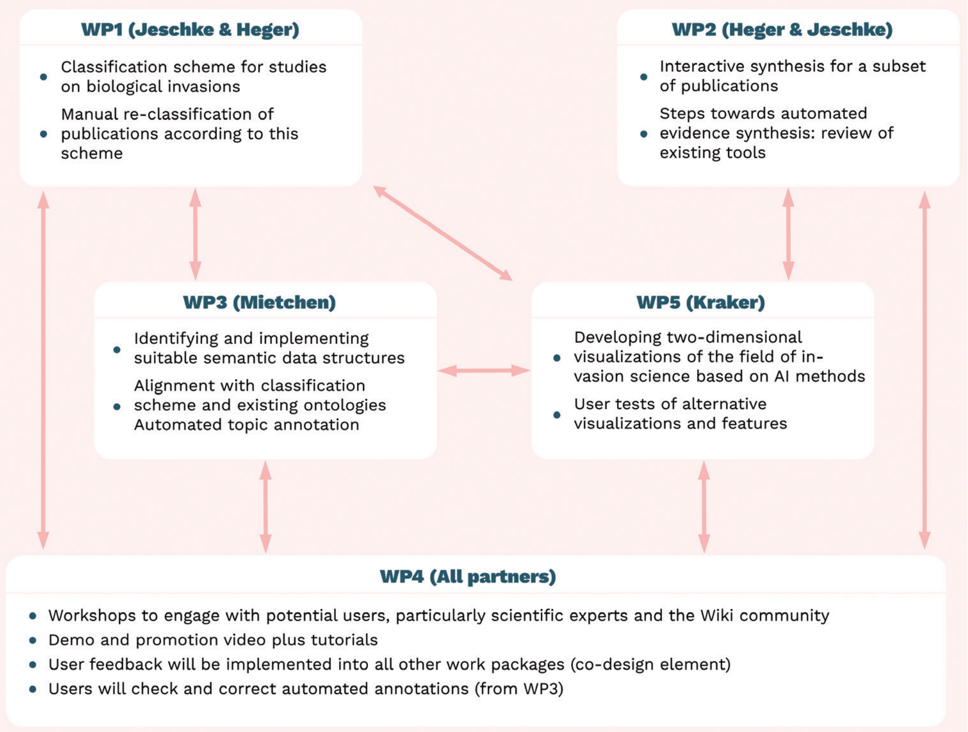
Project structure

These features will be developed in five work packages: (WP1) conceptual classification system integrating research questions and invasion hypotheses; (WP2) interactive evidence synthesis; (WP3) semantic data structures based on WP1 that will automatically ingest the literature into Wikidata; (WP4) engaging with the research and Wiki community; and (WP5) data-driven visualization techniques based on artificial intelligence (Fig. 2).

Work package 1: conceptual classification system integrating research questions and invasion hypotheses

This work package will be based on the hierarchy-of-hypotheses approach and hypothesis networks (see above for references). The website hi-knowledge.org (https://hi-knowledge.org) is a first attempt to combine these two approaches, as it features a zoomable (hierarchically structured) hypothesis network. However, it only includes 12 hypotheses in the field of invasion science, whereas Enders et al. (2018, 2019, 2020) show hypothesis networks with more than 30 invasion hypotheses. In addition, our proposal here is to also include studies that address research questions without reference to established hypotheses.

A core task of WP1 will thus be to create, based on our preliminary work, a conceptual classification system in which all research studies on biological invasions can be integrated. We will construct a hierarchical network of research questions in which major invasion hypotheses (see Ricciardi et al. 2013; Enders et al. 2018, 2019, 2020; Schulz et al. 2019) will be integrated (cf. Fig. 1). This is possible because research hypotheses are based on research questions. For example, several of the hypotheses in Enders et al. (2018, 2019, 2020) relate to the question why some non-native species have a higher invasion success than others; other hypotheses relate to the question why some ecosystems are more vulnerable to biological invasions than others. On the other hand, not all research questions are related to established hypotheses, as for some questions, a major hypothesis does not (yet) exist. This is, for example, the case for observed differences in introduction pathways among non-native species of different taxonomic groups (Saul et al. 2017).



WP1 (Jeschke & Heger)

- Classification scheme for studies on biological invasions
- Manual re-classification of publications according to this scheme

WP2 (Heger & Jeschke)

- Interactive synthesis for a subset of publications
- Steps towards automated evidence synthesis: review of existing tools

WP3 (Mietchen)

- Identifying and implementing suitable semantic data structures
- Alignment with classification scheme and existing ontologies Automated topic annotation

WP5 (Kraker)

- Developing two-dimensional visualizations of the field of invasion science based on AI methods
- User tests of alternative visualizations and features

WP4 (All partners)

- Workshops to engage with potential users, particularly scientific experts and the Wiki community
- Demo and promotion video plus tutorials
- User feedback will be implemented into all other work packages (co-design element)
- Users will check and correct automated annotations (from WP3)

Figure 2. Scheme of the five work packages (WPs) and how they interrelate. Please see main text for details.

The >1100 publications included in Jeschke and Heger (2018) and hi-knowledge.org are so far organized according to hierarchical representations of major hypotheses, but not yet according to research questions. Thus, a second important task of WP1 will be to manually classify these publications according to the newly developed scheme, so that we have a full set of expert-validated links to >1100 publications. This will be done jointly with collaborators and students interested in conceptual work. Such a manual classification is important as a comparison and training opportunity for the algorithm-based classification (WP3).

In addition to research questions and hypotheses, research studies on biological invasions can also be structured according to other factors, such as taxonomic groups (given as scientific names and in several languages), regions in which a study was performed, authors or groups of authors (cf. Lokatis and Jeschke 2018) who performed the studies, the research approach that was applied (experimental vs. observational studies; field vs. enclosure vs. laboratory studies) or the timing of the invasions (Fig. 1). In WP1, we will decide, based on expert and user feedback (see WP4), which features of publications will be included as available information in the future webtool. The aim is to allow future users of enKORE to decide on their own which criteria they want to apply for structuring or filtering the literature.

Work package 2: interactive evidence synthesis

The website hi-knowledge.org (https://hi-knowledge.org) does not only present a hierarchical network of invasion hypotheses, but also shows the level of empirical support for hypotheses according to published literature. In WP2, we will integrate this information into the new web portal enKORE. This will be done for the >1100 publications included in Jeschke and Heger (2018) and hi-knowledge.org. We will develop a possibility to enrich the filtering options developed in WP1 and WP5 such that they take into account the respective levels of evidence. enKORE will thus allow to perform interactive analyses of the level of evidence for specific hypotheses, filtered according to taxonomic group, region, research method and other factors (cf. Fig. 1). In this way, users can assess whether a specific hypothesis has proven useful for the taxonomic group or type of ecosystem they are interested in, or check the robustness of hypotheses across different research approaches (e.g. experimental vs. observational studies, lab vs. field studies).

The information on the level of evidence for or against major hypotheses in invasion science summarized in hi-knowledge.org has been manually extracted from the literature. Integration of additional publications and continuous updates will only be possible with the help of novel approaches including automated methods. A second work step in WP2 will therefore be to review existing approaches, e.g. for the extraction of the respective information from publications, and assess options for a future integration of respective tools in enKORE. Existing contacts with experts working on developing such tools will be very useful in this context, for example the teams behind the Open Research Knowledge Graph (ORKG, Auer et al. 2021) and the Biodiversity Community Integrated Knowledge Library (BiCIKL).

Work package 3: semantic data structures

In WP3, we will build semantic data structures – also known as knowledge graphs – in Wikidata that are based on persistent identifiers for publications, authors, research questions, hypotheses and the relationships between them, focal non-native species, study locations, research methods etc. (Fig. 1). To the extent possible, we will build on existing ontologies and controlled vocabularies (an ontology is a formal representation of the concepts and other key properties of a subject area and how they are related to each other). In a preliminary project carried out in collaboration with Birgitta König-Ries, Ria Stangneth and Alsayed Algergawy from Friedrich Schiller University Jena, Germany, we have already started to build an ontology for the main concepts included in 12 invasion hypotheses featured in https://hi-knowledge.org (Algergawy et al. 2020). We will also work on mechanisms to automatically identify publications in invasion science, to annotate them as to what precise subjects they are about and to classify and categorize them according to their relationship to the identified hypotheses. These classifications will be a first imperfect iteration, and they will need to be reviewed and curated (WP4) by experts in the field. Such experts can themselves be identified through queries to the Wikidata-based knowledge graph set up in this work package, along with relevant datasets, publications, species, study sites, institutions or even conferences or funders and changing trends over time. Since Wikidata uses Semantic Web standards and its data are in the public domain, other knowledge graphs such as ORKG (Auer et al. 2021) will be able to reuse and build on the curation work performed in the framework of enKORE.

Work package 4: engaging with the research and Wiki community

It will be critical that enKORE will be user friendly and that we engage with the research community, citizen scientists, the Wiki community and other stakeholders, such as managers, teachers, policy makers and science journalists. We will do this through workshops and online videos, including a tutorial, in which we explain both the benefits of using enKORE and how it can be used. Wikidata's multilinguality facilitates collaborations of people who do not share a common language, which allows to bring professional researchers together with citizen scientists from around the world, e.g. for specific regions or taxa or from platforms like iNaturalist that are increasingly being integrated with Wikidata. In the future when enKORE will grow beyond invasion science, we will first target related fields in biodiversity science, so that the community will grow in parallel with enKORE's coverage. The enKORE tool itself will, at least initially, only be available in English, but multilinguality will be helpful for future extensions.

For the current project, we aim to organize two large workshops to engage with researchers, the Wiki community and other stakeholders. In these workshops, we will introduce the tools we propose to develop, discover user demands, conduct user tests including options for data curation, and receive feedback. To foster our engagement with user groups, we will additionally develop and distribute a demo and promotion video plus a tutorial in several languages (at least English, German, French, Spanish and Chinese).

This engagement with user groups also serves an additional purpose. As outlined in WP1 above, we have already manually classified more than 1100 publications in the field of invasion science and plan to use this classification to train the algorithm developed in WP3. However, the algorithm will not be perfect and will indeed make classification mistakes. What it will do is provide a rough classification of publications in the field of invasion science. It will be critical that these automated classifications are checked by experts and, if necessary, corrected. We will invite users to provide these corrections online and will use them to further improve the algorithm.

We are confident that researchers will be highly interested in enKORE due to its novel features, particularly because the exact nature of these features will be specified by the users themselves. This co-design element of the project will be possible thanks to the workshops and online channels. In addition, researchers will have an interest that their publications are correctly included in the database, hence invasion scientists will have an incentive to curate their data and improve the algorithm-based classifications where necessary.

Work package 5: data-driven visualization techniques

In WP5, we will develop visualizations and visual search capabilities to enable exploration and discovery of the database developed in WPs 1–3. To create dynamic, two-dimensional representations of the field of invasion science, we will merge machine learning and natural language processing with symbolic reasoning enabled by the semantic data structures (cf. WP 3; for further information about the approach, see Kraker 2015; Kraker et al. 2015, 2016). We will then implement a number of data-driven visualizations to provide these representations in an interactive, web-based format.

The visualizations will be based on design concepts for different types of knowledge maps:

- A visual search within the Wikidata corpus on invasive species that enables topical overviews
- Two variations of the visual search, e.g. a knowledge map for a given hypothesis or a timeline showing the development of research questions over time
- A browse view that allows for hierarchical exploration of the whole corpus

These design concepts will be refined in collaboration with the research and Wiki community as part of the workshops we will organize in WP4. We will carry out two user tests:

1. Different visualizations will be shown at a workshop where we will discuss these in groups with the participants to gather input for the visualizations.
2. A usability test to evaluate the first iteration of the visualizations will be carried out at a second workshop where we will discuss these in groups to gather feedback for the second iteration.

The data-driven visualizations will be implemented in our award-winning knowledge mapping framework Head Start, and will be made available open source during the development phase.

Call for participation, timeline and outlook

This ambitious project aims to take important steps towards an open and interactive atlas of knowledge, in invasion biology and beyond. If you are interested in contributing to it in one way or another, then please do not hesitate to contact us. We invite contributions by interested individuals and organizations with a focus on invasion science or other disciplines. We have started to think more deeply about applications in restoration and urban ecology as well as in freshwater biodiversity research, and also look forward to collaborations in these and other research fields. Strengthening connections to portals with citizen science data (e.g. iNaturalist) will also be very useful, and initiatives like Wikidata's WikiProject Biodiversity can help with this.

The project outlined here is scheduled to run from September 2021 to February 2024. Beyond this time period, it will be important to continue improving the atlas of knowledge, so that it will thrive and its underlying technology remains state of the art. The sustainability of such online tools is critical, hence we are aiming to secure long-term support for the atlas of knowledge. To reach this goal, we will not only apply for future grants: the sustainability of the atlas will also be supported by its integration with Wikidata right from the beginning, as it is part of the Wikipedia ecosystem that has a strong and sustainable community-based funding model centered around small donations from millions of users each year.

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SHORT COMMUNICATION

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Impacts of the invasive round goby (*Neogobius melanostomus*) on benthic invertebrate fauna: a case study from the Baltic Sea

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Abstract

The round goby (*Neogobius melanostomus*) was first observed in the Baltic Sea in 1990 and has since displayed substantial secondary dispersal, establishing numerous dense populations where they may outcompete native fish and negatively impact prey species. There have been multiple round goby diet studies from both the Baltic Sea and the North American Great Lakes where they are similarly invasive. However, studies that quantify their effects on recipient ecosystems and, specifically, their impacts on the benthic invertebrate macrofauna are rare, particularly from European waters. In this study, we conducted the first before-after study of the potential effects of round goby on benthic invertebrate macrofauna taxa in marine-brackish habitats in Europe, focusing of two sites in the Western Baltic Sea, Denmark. Results were in line with those from the Great Lakes, indicating negative impacts on specific molluscan taxa (e.g. Cardiidae bivalves and Neritidae gastropods, which both showed a fall in detected densities of approximately 98% within the Guldborgsund Strait). In contrast, many other groups appeared to be largely unaffected or even show positive trends following invasion. Round goby gut content data were available at one of our study sites from the period immediately after the invasion. These data confirmed that round goby had in fact been preying on the subset of taxa displaying negative trends.

Keywords

Anthropogenic change, infauna, invasion impacts, invasive species, non-indigenous species, predation, predator-prey interactions

The impacts of non-indigenous invasive animals can be closely related to their feeding behaviour, via increased predation pressure and resource competition for native species (Olenin et al. 2017). The round goby, *Neogobius melanostomus* (Pallas, 1814), is native to the Caspian, Black, Azov and Marmara Seas. From there, it was introduced to the Baltic Sea, most likely via ballast water, where it was first observed in the Gulf of Gdansk in 1990 (Kotta et al. 2015). At the same time, the species was also observed in the North American Great Lakes (Kornis et al. 2012). Today, three decades after these first observations, the species has displayed pronounced secondary dispersal in both regions and is now common throughout large parts of the Baltic Sea (Kotta et al. 2015; Puntila et al. 2018) and in three of the four Great Lakes (Corkum et al. 2004; Kornis et al. 2012). They are also found in numerous freshwater systems in Central and Western Europe (Kornis et al. 2012).

Round goby is a bottom-dwelling fish that occurs in a wide range of seabed habitats, from soft substrates (e.g. mud and sand, both with and without vegetation) to hard substrates (e.g. natural boulder reefs or man-made structures like harbour walls and jetties; Young et al. 2010; Kornis et al. 2012). Round gobies possess several invasive characteristics, such as high competitive ability for territory and prey, a broad diet, dispersal ability and broad temperature and salinity tolerances (Kornis et al. 2012; Azour et al. 2015; Behrens et al. 2017; Christensen et al. 2021; Ericsson et al. 2021). As such, the round goby is generally thought to have negative impacts on recipient ecosystems and indigenous taxa.

A handful of studies from freshwater systems in the Great Lakes Region have found evidence that round gobies outcompete indigenous fish species for space and food and may predate on both fish eggs and offspring (e.g. Chotkowski and Marsden 1999; Balshine et al. 2005). Competition with native fish has also been described in European waters (Karlson et al. 2007; Matern et al. 2021), although other studies have not detected effects on other fish species (e.g. Janáč et al. 2016; Piria et al. 2016). In relation to benthic invertebrate macrofauna, studies available from the freshwater Great Lakes system have investigated invertebrate abundances before and after invasion or compared tributaries with and without round goby populations (Lederer et al. 2008; Kipp and Ricciardi 2012; Barrett et al. 2017; Pennuto et al. 2018). These studies often find that round goby invasion has the capacity to alter species compositions and reduce the biomass of certain taxa, for example, in dreissenid bivalves (Lederer et al. 2008) and prosobranch gastropods (Barrett et al. 2017).

In European inlet waters and the marine and brackish habitats of the Baltic Sea, before-after studies of their impacts on the invertebrate macrofauna appear to be nonexistent. In contrast, studies of their diet are quite common (e.g. Polačik et al. 2009; Skabeikis and Lesutienė 2015; Nurkse et al. 2016; Piria et al. 2016; Oesterwind et al. 2017; Schwartzbach et al. 2020), along with a recent valuable experimental field study testing the effects of goby presence on native fauna using caged areas (i.e. goby presence vs. absence, Henseler et al. 2021). The rarity of before-after studies may be due to the difficulties and costs of obtaining site-specific abundance data of benthic fauna communities immediately prior to and after an invasion. This lack of studies is concerning as the limited knowledge of round gobies impacts on Baltic Sea ecosystems and communities has been identified as key a barrier to their management (Ojaveer and Kotta 2015). Therefore, the aim of this study was to test the hypothesis that round goby invasions in the Baltic Sea impact these recipient ecosystems by reducing the abundance of prey taxa.

We focused on two sites in south-eastern Denmark, Guldborgsund and Stege Bugt (see specific locations in Suppl. material 1: Figure S1). The first round goby observation along the main coastline of Denmark was made in Guldborgsund in 2009. By 2010, they were abundant throughout Guldborgsund and, by 2013, had reached an average density of 1.9 individuals per m^2 (Azour et al. 2015). Round gobies were not observed at Stege Bugt until 2011 (Azour et al. 2015), which was likely colonised via secondary dispersal from Guldborgsund. Both are shallow brackish areas where local fishermen continue to catch large quantities of round goby as bycatch (Brauer et al. 2020).

Benthic invertebrate macrofauna data from fixed sampling stations in Guldborgsund and Stege Bugt, collected as part of the Danish national NOVANA marine monitoring programme database (Surface Water Database, ODA: https://odaforalle.au.dk) were mined. All fauna samples were collected in spring using a HAPS core sampler (seabed area: 0.0143 m^2) and multiple samples were taken in each sampling-year (Table 1; Hansen et al. 2017; McLaverty et al. 2020). Species/taxa count data were extracted for the period 2006–2015 from these areas (i.e. ca. four years prior to and four years after invasion), including at least one sampling-year immediately prior to the first goby sighting and at least two sampling-years in a 2–5 year period following their first sighting. In Stege Bugt, invertebrate data were available in spring 2011 (also the year of the first round goby sighting), so for the purposes of this analysis, we considered data from spring 2011 to represent pre-impact abundances. NOVANA data are recorded to species, genus or occasionally higher taxonomic levels, therefore, for our analysis, we defined 20 broader taxonomic groups to aggregate the raw data to order and family levels where possible (see Suppl. material 1: Table S1 for full details of our taxonomic groupings). Species that were rarely detected in samples (in < 5% of cores) and could not be combined into order or family level groupings were excluded from analysis. All groupings were monophyletic, except Littorinimorpha, which we separated, based on morphological differences into two groups: larger periwinkle species (e.g. *Littorina* sp., as 'Littorinimorpha (large)') and several species of much smaller sea snails (e.g. *Hydrobia* sp. and *Rissoa* sp., as 'Littorinimorpha (small)', generally < 5 mm), so that the responses of these morphologically-distinct groupings could be assessed separately.

Gut content data from Guldborgsund (54°43'24.55"N, 11°52'49.70"E) were collected in autumn (November) 2010, in the year immediately following their first arrival in 2009 and immediately preceding the first post-impact sampling at the site. A total of 297 round gobies measuring 7.5–17 cm total length were collected with eel traps set overnight in shallow waters (1–5 m). Gobies were frozen (-20 °C) until processed. The presence/absence and count data for prey detected in gut samples were identified to species where possible. Given the few hours from capture until freezing, there is a risk that soft bodied and very small food items might have been underestimated.

Count data per core sample (aggregated to our taxa groupings) were analysed using general linear mixed effect models for each site ('brms' package v. 2.14.4, Bürkner 2017; negative binomial distribution, log-link function with default non-informative priors, chains = 2 chains, iterations = 6000, warm-up = 2000). A round goby before-after impact fixed effect ('BA') was included, with taxonomic groupings included as a random effect with random slopes (i.e. 'BA|TaxaGroup'). Taxa-specific BA slopes were extracted from posterior distributions with 95% credible intervals to infer positive and negative impacts of goby invasion on each taxon's abundance. Sampling year and core sample ID were also included as random effects to account for non-independence within samples and sampling seasons. Separate models were used for each site (for full model specifications, see Suppl. material 1: Table S2). Despite all sampling occurring in spring, samples were taken in March in 2015, while in previous years, sampling occurred in May, so a sensitivity analysis was conducted to ensure that this difference in timing did not influence our conclusions (see Suppl. material 1: Sensitivity Analyses).

Gut content data from Guldborgsund were summarised as the percentage of total gut samples (n = 297) that each taxon group was detected within (i.e. % occurrence). Further exploratory analysis was also conducted to measure whether a taxon's prevalence in gut contents influenced the BA effect. First, taxa were categorised as present or absent, based on their detection (or not) within gut samples. To test whether the BA effect was more negative in the taxa detected in gut samples than those not detected, we tested for an interaction between BA and taxa presence ('BA*Presence', Guldborgsund data only, using model specifications as above, also see Suppl. material 1: Table S2). To test if there was an overall positive or negative BA impact in each category of taxa, two separate models were used to estimate the BA effect for present and non-present subsets of taxa (Guldborgsund data only).

All credibility intervals below are 95% intervals. Statistically-significant effects are inferred from credibility intervals not overlapping zero. Model performance was assessed by checking diagnostic plots to ensure chains were well mixed and convergence was confirmed (Rhat = 1.00, zero divergent transitions after warm-up). Conditional R^2 values ('R^2_{cond}') were estimated as a measure of the total amount of variance explained by each model (function 'r2_bayes', 'performance' package v. 0.7.0, Lüdecke et al. 2021). Additionally, sensitivity analyses were conducted to check whether our results were sensitive to zero-inflation (see Suppl. material 1: Sensitivity Analyses). All data, models and code are available at the Open Science Framework (https://doi.org/10.17605/OSF.IO/T5R4F)

Taxa-specific BA effects showed non-zero negative responses for Cardiidae bivalves and Neritidae gastropods at both sites, while Bryozoa was the only grouping with positive responses at both sites (Figure 1). Site specific changes at Guldborgsund were negative responses in Littorinimorpha (large) and Littorinimorpha (small) gastropods and positive responses in Capitellidae and Orbiniidae polychaetes (Figure 1a). Site specific changes at Stege Bugt were negative responses in Lymnaeidae gastropods and Chironomidae insects and positive responses in crustacean groups Isopoda and Amphipoda, as well as Spionidae polychaetes (Figure 1b). Overall BA effect estimates across all taxa

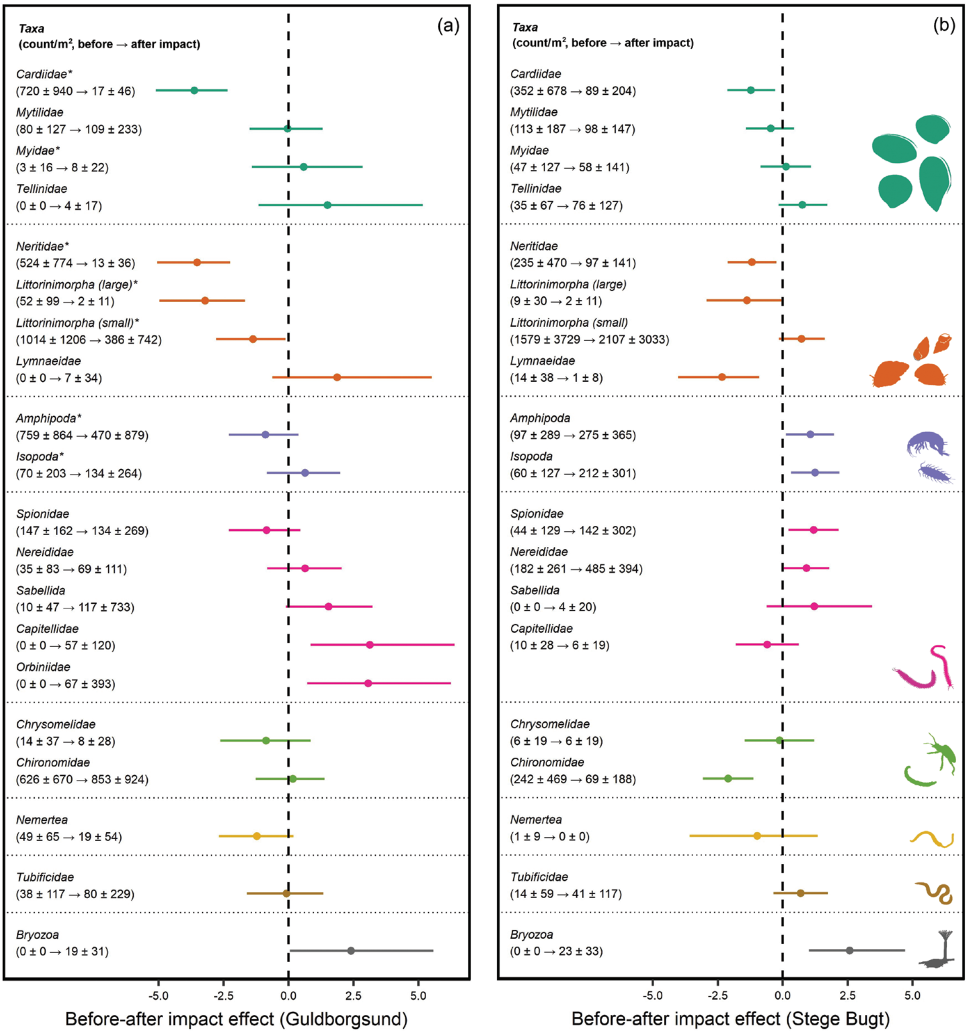

Figure 1. Taxa-specific before-after (BA) effects for (**a**) Guldborgsund and (**b**) Stege Bugt (with 95% credibility intervals). Positive or negative effects (on taxa counts per sample) that do not overlap zero are interpreted as showing a change in abundance following the arrival of round gobies. Mean densities per square meter (± s.d.) in samples before and after invasion are also shown for each taxon group. Taxa groupings are arranged by class/phylum groupings by: (from top to bottom) class Bivalvia, class Gastropoda, class Malacostraca, class Polychaeta, class Insecta, phylum Nemertea, class Clitellata, class Bryozoa. Note: Orbiniidae were not detected at Stege Bugt, so were not included in analysis for that site.

were close to zero on both sites (Gulborgsund: BA: -0.04 [-4.09, 4.05], intercept = -1.12 [-4.78, 2.31], R^2_{cond} = 0.51 [0.46, 0.56]; Stege Bugt: BA: -0.07 [-3.58, 3.54], intercept = -1.12 [-3.85, 1.27], R^2_{cond} = 0.31 [0.22, 0.42]).

Of our twenty taxa groupings, seven were found in gut samples from Guldborgsund (Fig. 2a), of which Littorinimorpha (small) was the most common group de-

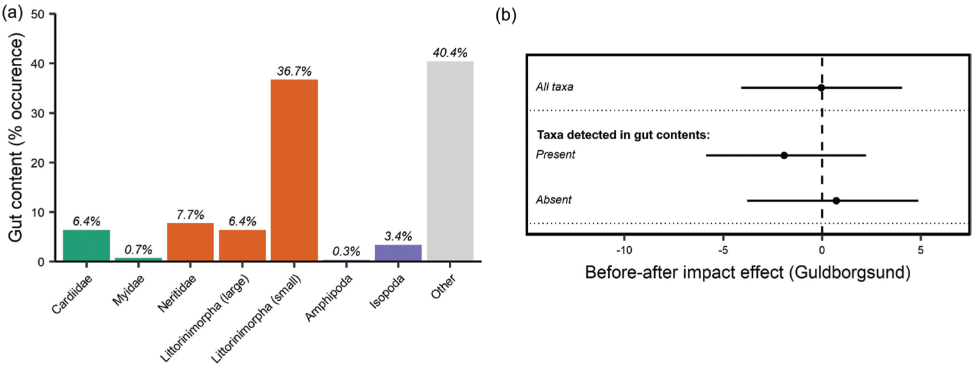

Figure 2. Gut content data for round gobies at Guldborgsund in 2011, including (**a**) the percentage occurrence of taxa groupings in gut content of (n = 297 fish) and (**b**) the overall BA effect estimates for Guldborgsund for all taxa (from the full site model), as well as present and absent subsets of taxa (with 95% credibility intervals). 'Other' taxa found in gut contents were primarily mobile taxa that are poorly detected in HAPS core data (e.g. *Palaemon adspersus*, *Palaemon elegans*) and fish (*Gasterosteus aculeatus*, round goby scales).

tected. Several bentho-pelagic species (e.g. *Palaemon* spp., *Gasterosteus aculeatus*) were detected in the gut content, but were obviously not represented in core samples. The BA effect was influenced by an interaction with prey presence (BA*Presence: -2.66 [-4.63, -0.91], intercept = -2.04 [-5.85, 1.31], R^2_{cond} = 0.52 [0.46, 0.56]), i.e. the BA effect was more negative for taxa found in gut samples than in taxa that were absent from gut samples. The overall BA effect estimate for taxa present in gut contents was negative, but overlapped zero (BA: -1.91 [-5.86, 2.23], intercept = 0.43 [-3.28, 4.08], R^2_{cond} = 0.50 [0.39, 0.58], Figure 2b), while the estimate for taxa absent from gut contents was slightly positive, but also overlapped zero (BA: 0.72 [-3.80, 4.87], intercept = -1.95 [-5.80, 1.95], R^2_{cond} = 0.56 [0.51, 0.61], Figure 2b).

These results represent the first test for the effects of round goby invasion on benthic invertebrate macrofauna in marine/brackish environments. We found that a subset of largely molluscan taxa appear to be negatively impacted by goby invasions. For example, the strongest negative effect at Gulborgsund was in Cardiidae bivalves, where detected densities fell approximately 98% after invasion, while in Stege Bugt, the density of Lymnaeidae gastropods fell approximately 94%. This is generally consistent with the handful of studies available from the Great Lakes Region (i.e. freshwater environments). A study from the upper St. Lawrence River concluded that gastropod richness and median size declined as goby numbers increased, whereas dreissenid bivalves were unaffected and mainly avoided by the round goby (Kipp and Ricciardi 2012). In contrast, in Lake Michigan, dreissenids declined after the invasion of round goby, together with isopods, amphipods, trichopterans and gastropods (Lederer et al. 2008). The negative effect on dreissenids (which are also invasive species of Ponto-Caspian origin) was found to be caused by predation, whereas the effect on the rest of the benthic invertebrate community may have been indirect (i.e. loss of microhabitat and dreissenids pseudo-faeces) (Lederer et al. 2008). Notably, dreissenids do not occur in this area of

Table 1. Overview of NOVANA benthic fauna samples used in the present study.

Sampling site (latitude/longitude)	Pre-impact samples (n, year)	Post-impact samples (n, year)
Guldborgsund (54.70714°N, 11.86273°E)	20 (2007-May)	30 (2011-May); 42 (2013-May); 42 (2015-March)
Stege Bugt (54.99996°N, 12.22708°E)	20 (2009-May): 42 (2011-May)	42 (2013-May): 42 (2015-March)

the Baltic Sea, potentially due to salinity limitations (Werner et al. 2012), but round goby-dreissenid interactions may be more prevalent in lower salinity and freshwater areas of the Baltic catchment. Interestingly, some invertebrates, such as oligochaetes and chironomids, increased in numbers in an invaded bay in Lake Ontario as the gastropods disappeared (Barrett et al. 2017). Increases in abundance were also observed at our sites, particularly in some polychaete groups. This may suggest that the goby can have indirect positive effects on certain taxa: for example, by foraging selectively on certain groups, they may decrease the levels of resource competition for others.

The strong negative effect on gastropods (and to some extent bivalves) seems to be a recurring phenomenon in many of the Great Lakes studies (Kipp and Ricciardi 2012; Pennuto et al. 2018; Barrett et al. 2017). Similarly, previous gut content-based European studies and one field experiment support the notion that round goby show a preference for certain molluscs (e.g. Borza et al. 2009; Oesterwind et al. 2017; Henseler et al. 2021). The present study supports this and, especially for Neritidae and Cardiidae gastropods, strong negative effects were found that were clearly reflected in their observed densities before and after invasion. For example, the average observed density per square metre of both taxa fell by approximately 98% at Guldborgsund, with Stege Bugt showing similar, but more modest decreases of 59% (Neritidae) and 75% (Cardiidae). A strong negative impact on certain gastropods in these areas is a particular concern, as several studies from the Great Lakes Region have highlighted the risk of trophic cascades leading to increased algal biomass as gastropod grazing pressure is reduced (Kipp and Ricciardi 2012; Pennuto et al. 2018; Barrett et al. 2017), potentially signalling a risk of broader changes to ecosystem function and community structure in invaded areas.

As there was a lack of appropriate control sites (i.e. we could not identify a comparable non-impacted site with similar physical parameters, such as depth and salinity and with comparable macrofauna sampling intensity), we therefore lack the ability to directly infer causality between the goby invasion and observed changes. As such, observed trends (negative or positive) should be viewed cautiously. An additional shortcoming of the NOVANA data is the poor detection of mobile taxa, such as decapods (*Palaemon* spp.), which this and other studies in the Baltic have found to be a substantial component of round goby diets (Kornis et al. 2012). Single method monitoring programmes will tend to produce blind spots for certain taxa and limit our ability to measure impacts across the full community.

To mitigate the negative impacts of anthropogenic pressures on our aquatic environments, empirical data are required to plan and prioritise management efforts (Liu et al. 2008). In the Baltic Sea, there is a specific lack of knowledge on the impacts of non-indigenous species on native fauna (Ojaveer and Kotta 2015). Therefore, with this study, we hope to highlight the utility (and some limitations) of environmental monitoring data to assess the impacts of non-indigenous species. In this context, it is important to consider both positive and negative effects of non-indigenous species on ecosystems and our broad analysis approach across a wide range of taxa suggests that, while some groups appear to be severely impacted by this invasion, others may benefit from round goby presence. This also highlights the importance of reporting positive and negative findings (Fanelli 2012). In the anticipation that round goby will continue its secondary dispersal in the western Baltic Sea, we suggest that further multi-year regional monitoring programmes in advance of the invasion front would be valuable. Ideally, ecosystem monitoring would include appropriate control areas allowing before-after-control-impact analysis (as in Conner et al. 2016), which would allow us to better estimate and thus mitigate the impacts of the round goby invasion in northern European waters.

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Supplementary material 1

S1. Sampling Areas (Figure S1); S2. Taxonomic Groupings (Table S1); S3. Model Specifications (Table S2); S4. Sensitivity Analyses (Table S3, Figure S2)

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Data type: document

Explanation note: Map of study locations; Table of taxonomic groupings for analysis; Model specifications for analysis; and, Sensitivity analysis.

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RESEARCH ARTICLE

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A benefit-cost analysis framework for prioritization of control programs for well-established invasive alien species

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Abstract

Invasive alien species (IAS) are identified as a major threat to biodiversity and ecosystem services. While early detection and control programs to avoid establishments of new alien species can be very cost-effective, control costs for well-established species can be enormous. Many of these well-established species constitute severe or high ecological impact and are thus likely to be included in control programs. However, due to limited funds, we need to prioritize which species to control according to the gains in ecological status and human well-being compared to the costs. Benefit-Cost Analysis (BCA) provides such a tool but has been hampered by the difficulties in assessing the overall social benefits on the same monetary scale as the control costs. In order to overcome this obstacle, we combine a non-monetary benefit assessment tool with the ecosystem service framework to create a benefit assessment in line with the welfare economic underpinnings of BCA. Our simplified BCA prioritization tool enables us to conduct rapid and cheap appraisals of large numbers of invasive species that the Norwegian Biodiversity Information Centre has found to cause negative ecological impacts. We demonstrate this application on 30 well-established invasive alien vascular plant species in Norway. Social benefits are calculated and aggregated on a benefit point scale for six impact categories: four types of ecosystem services (supporting, provisioning, regulating and cultural), human health and infrastructure impacts. Total benefit points are then compared to the total control costs of programs aiming at eradicating individual IAS across Norway or in selected vulnerable ecosystems. Although there are uncertainties with regards to IAS population size, benefits assessment and control program effectiveness and costs; our simplified BCA tool identified six species associated with robust low cost-benefit ratios in terms of control costs (in million USD) per benefit point. As a large share of public funds for eradication of IAS is currently spent on control programs for other plant species, we recommend that the environmental authorities at all levels use our BCA prioritization tool to increase the social benefits of their limited IAS control budgets. In order to maximize the net social benefits of IAS control programs, environmental valuation studies of their ecosystem service benefits are needed.

Keywords
benefit points, control measures, ecosystem services, eradication, invasive alien plants, prioritization

Introduction

The consequences of the overall threats and damages caused by invasive alien species (IAS) are growing (Vié et al. 2009; Early et al. 2016; Pyšek et al. 2020). The handling of such species is embedded in the United Nations sustainability goal number 15.8 committing to: "*introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species.... and control or eradicate the priority species*". The damages of IAS have often been correlated with loss of biodiversity (Butchart et al. 2010; Powell et al. 2011; Dueñas et al. 2018; Linders et al. 2019), which in itself can be detrimental for sustainable and resilient ecosystems (Pyšek and Richardson 2010; Vilà et al. 2011; Gallardo et al. 2019) and thus have subsequent effects on supporting, provisioning, regulating and cultural ecosystem services (Vila and Hulme 2017). Even though IAS have been considered a threat to ecosystem services for decades, control programs tend to be implemented late in the invasion process, when such species are well established with large reproducing populations. Invasive species are not evenly spatially distributed and are often highly correlated with infrastructure (Huang et al. 2012; Dodd et al. 2016; Zhou et al. 2020) and trade (Westphal et al. 2008). In fact, nearly all introductions are caused by humans either intentionally, through for example horticulture (Drew et al. 2010), or unintentionally through ballast water, soil/timber import, transportation of goods etc. (Hulme 2009). The introduction pressure of alien species in general is projected to rise with increased globalization (Meyerson and Mooney 2007; Early et al. 2016; Seebens et al. 2018), and with climate change (Bellard et al. 2013, 2018). Many governments have therefore aimed at minimizing current and future threats by IAS through directed control programs. However, such programs have been difficult to implement due to steep economic costs (Rejmánek and Pitcairn 2002) as well as other societal factors (Reaser et al. 2020b). Several tools and recommendations have been proposed to help implement control programs (Genovesi and Carnevali 2011; Hulme et al. 2018; Reaser et al. 2020a, b; Verbrugge et al. 2021).

One of the main obstacles for implementing such control programs is their cost (Invasive Species Specialist Group 2001). Eradicating alien species prior to establishments (i.e., door knocker species) or alien species in an early stage of invasion reduce control costs. This has been coined "Early Detection and Rapid Response" (EDRR) (Westbrooks 2004; Reaser et al. 2020b), and such programs have proved to be highly cost-effective. Although the EDRR approach is an important framework for future invasions, we are still left with the question of how we should best deal with all the IAS that established vast populations decades ago, and which negatively impact ecosystem services. With the large costs needed to eradicate or control an increasing number of well-established invasive species, and the increased competition for governmental funds in the age of the ongoing pandemic and climate change, it is more important than ever to document the social benefits in relation to the costs of IAS control programs. This calls for Benefit-Cost Analysis (BCA) (Boardman et al. 2018). While BCA is routinely used as a decision support tool to evaluate and prioritize governmental projects and programs in the transportation, environment and energy sectors in many countries (including the European Union and the USA) (OECD 2018), BCA has only recently been suggested for use in management of IAS (Hanley and Roberts 2019).

In Norway the recent "Action strategy against alien invasive species 2020–2025" (Ministry of Climate and Environment 2020) calls for assessments of benefits and costs of control programs. However, a main obstacle for large scale use of BCA, which requires monetary estimates for both benefits and costs, is the lack of economic valuation of the mostly non-market benefits of eradicating each of the nearly 1500 alien species found in Norway. There are few non-market environmental valuation studies of effects of IAS on biodiversity and ecosystem services we can transfer/generalize from, and non-monetary assessments tools including impact score systems such as GISS (Generic Impact Scoring System; Nentwig et al. 2016) cannot be used directly as they are not consistent with the welfare economic theory underpinning BCA. This paper aims at closing this gap by combining a GISS-inspired benefit point system with the ecosystem service framework (Millennium Ecosystem Assessment 2005) into a non-monetary benefit assessment based on the contribution to human well-being underlying BCA.

We demonstrate the use of this simplified BCA tool for well-established IAS by applying it to vascular plants in Norway. Although alien species can be found in many organism groups, vascular plants is by far the largest group of IAS in Norway; see Figure 1. Vascular plants are also predominantly found among the ecological impact categories (93% and 82% respectively) of alien species and impose high and severe ecological impact. Thus, we apply the BCA tool to 30 invasive vascular plants which impose severe to high impact on ecosystems. Our approach is carried out as a follow-up of the ecological impact assessment performed by the Norwegian Biodiversity Information Centre (NBIC), and the ecological input builds on the data included within the expert assessments embedded in the NBIC databases. Our approach therefore is a supplement, not a competitor to such ecological impact assessments.

Methods and data

Benefit Cost Analysis (BCA) and the Ecosystem Service (ES) framework

In BCA we consider all the costs and benefits to society as a whole: the social cost and the social benefits. Thus, BCA is often termed Social BCA or Social CBA (Boardman

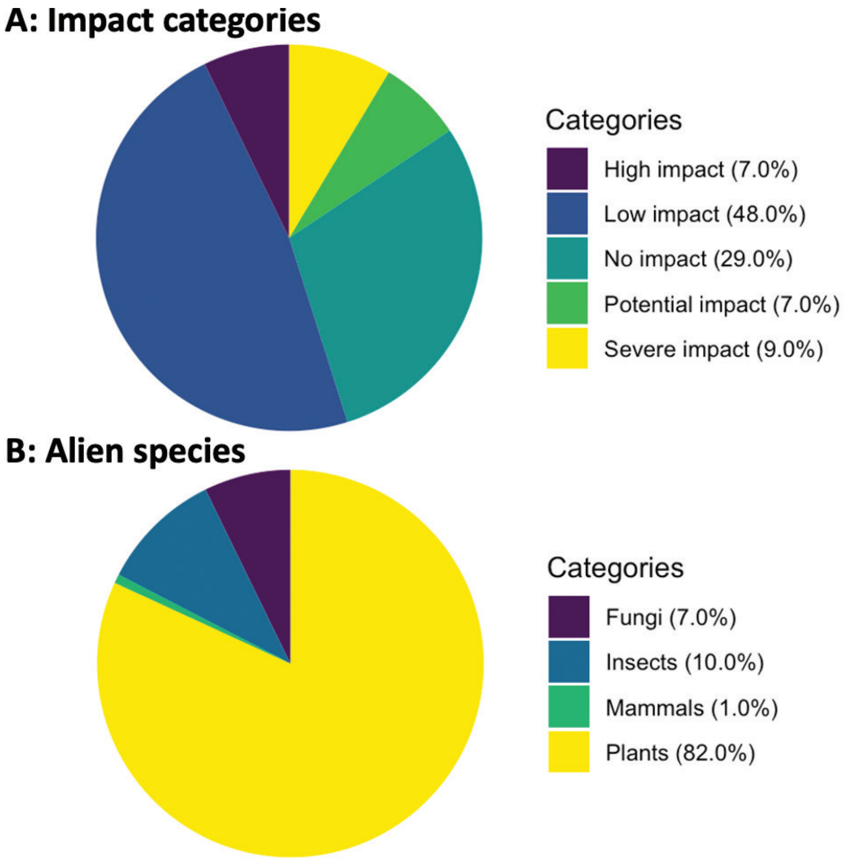

Figure 1. Invasive alien species (IAS) in Norway; distributed on different ecological impact category (A) defined as the assumed ecological impact by an alien species evaluated by the NBIC, and organism groups (B). Source: Norwegian Biodiversity Information Centre; NBIC (www.biodiversity.no).

et al. 2018). BCA is a policy assessment method that quantifies, in monetary terms, the value of all consequences of a policy to all members of a society and is rooted in economic welfare theory. The two main principles used to monetize social costs and benefits are the opportunity costs and individuals' willingness-to-pay, respectively. Valuation of market goods like labor and pesticides are based on (corrected) market prices, e.g., the market price for labor under full employment is the gross wages plus social costs of employment, whereas it is lower and equal to the opportunity cost when there is unemployment. Non-market goods like water quality and biodiversity are assessed using environmental valuation techniques applying the same principles (e.g., recreational value of angling and bathing) and non-use values (e.g., existence and bequest values of attaining good ecological status in lakes, which also accrue to people with no actual use of the lake). Hanley and Roberts (2019) state in their review of the potential for BCA to prioritize invasive species control actions that the economic benefits are not limited to those associated with market-valued goods such as crops but should include increased exposure to disease and disruption to ecosystem service supply and impacts on biodiversity (Hanley and Roberts 2019).

The main challenge in applying BCA to evaluate and prioritize control programs for IAS is to value the social benefits of avoided damages to biodiversity, ecosystem services, and public health in order to directly compare social benefits to costs on the same monetary scale. Although environmental valuation techniques are now well developed (see e.g., Johnston et al. 2017), the number of studies valuing the social benefits of eradicating IAS is too small[*] to be used for benefit transfer to value all or large groups of IAS (Johnston et al. 2021). Therefore, we aim at developing and testing a non-monetary valuation method for assessing the social benefits which utilizes current knowledge of the ecological impacts of IAS and is consistent with economic welfare theory underpinning BCA. A framework for assessment of these benefits which is consistent with economic welfare theory is the ecosystem service (ES) framework. Ecosystem services are defined as outputs, conditions, or processes of natural systems that directly or indirectly benefit humans or enhance social welfare (Millennium Ecosystem Assessment 2005).

Calculating social benefits

Attempts to construct such prioritization tools have been made, for instance by adapting the Project Prioritization Protocol (PPP; Joseph et al. 2009) to IAS and vascular plants (Dodd et al. 2017), and through the Generic Impact Scoring System (GISS; Nentwig et al. 2018). The GISS represents a semi-quantitative impact score system attributing points on a scale from 0 to 5 for twelve different characteristics of alien species. The main criteria are ecological and social effects, and there are six sub-criteria for each main criterion (Nentwig et al. 2018). Each of these sub-criteria are rated by experts and the points are then aggregated, resulting in a list of 149 species which Nentwig et al. (2018) present as a "worst alien species list". An advantage of the GISS is the ranking of all the alien species based on total points across all criteria. It should be noted, however, that this ranking implies assessments of the importance of the different sub-criteria within the two groups, and weighting of ecological versus social criteria. Note that assigning no weight to each sub-criterion when aggregating is also an implicit weighting as each sub-criterion is then given the same weight. We use the system with separate sub-criteria reflecting ecological and other social aspects and a system with points for each sub-criterion for each species as an inspiration for developing our framework. Here, we more explicitly use the ecosystem services framework when assessing each IAS with respect to the criteria for each species, to ensure that the assessment is in line with the welfare theoretical foundation of BCA.

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[*] Only 20 of the 5140 non-market valuation studies worldwide found in the Environmental Valuation Reference Inventory (EVRI) database (www.evri.ca; accessed June2021) value the damages from IAS.

In our framework, the benefits of controlling the species are assessed based on the ecosystem services that are affected by the respective species, as well as whether the species impacts human health or infrastructure. The ecosystem services assessed are supporting (ecological impact and effects on endangered nature), provisioning (food, fiber/materials), regulating (pollination, water regulation, erosion) and cultural services which are related to the use values (recreation, aesthetic beauty) and non-use values (natural heritage). Although not all categories were found to be affected by the alien invasive species considered in our analysis, they can be relevant for other taxa, and are thus kept in the presentation of this methodology. For each benefit category, we assessed the benefits of controlling the species on a scale from 0 to 4. The scale used for each ecosystem service and the source of this assessment are shown in Table 1.

Calculating social costs

The first step in the cost calculations was to estimate the costs per decare (1000 m^2) to carry out relevant control measures applicable for each IAS. One obvious part of the cost is the direct cost of carrying out the measure, for example costs of labor for weeding or other mechanical removal of the IAS and costs of inputs like pesticides. Other direct social costs include administrative costs, i.e., cost of surveillance after the control measures are carried out. The social costs of collecting taxes should in principle be added to the control costs that are publicly funded but are not included here. These social costs are in Norway assumed to add 20% to the control costs according to guidelines for Benefit Cost Analyses given by the Norwegian Ministry of Finance (Norwegian Ministry of Finance 2014). In some cases, the control measures themselves may have negative effects (costs to society) like environmental damage from using pesticides as a control measure, or in terms of lost benefits to those who value the IAS. The latter may be the case for horticulture species like *Rhododendron* (Dehnen-Schmutz and Williamson 2006) or other pretty and/or historical IAS. These costs are harder to estimate in monetary terms and make it more challenging to compare costs across control measures. Here, we have excluded such indirect costs and potential benefits of IAS, and calculated the direct control cost only, which in most cases dominate total control costs. The species included in our analysis are not known to have substantial benefits, and therefore omitting potential benefits is not thought to be important for our results. In other cases, the IAS are known to have potentially large benefits as well as negative effects on ecosystem services, human health and infrastructure, and in these cases the benefits on for example provisioning services such as pollination should also be included. It is possible within our methodology to include such benefits, either as monetary values, which would reduce the net costs to society, or as unpriced effects.

In some cases, there are several control measures available to eradicate an invasive alien species. The control measures include several forms of mechanical removal, use of pesticides, a mix of the two, as well as covering the ground and hot water treatment. Different measures usually have different costs. In cases with several alternative control measures, we have evaluated all of them and included them in the cost ranges for the

Table 1. Benefit assessment of controlling invasive alien species (IAS) in terms of avoided damages to ecosystem services (ES), human health and infrastructure. Description of the benefit point scale (0–4) and data source used for each benefit category are provided within the table. Two categories were found to have no effect based on the 30 IAS included in this study: the provisioning service "fibers/materials" and regulating services in general. Source: Modified from Magnussen et al. (2019).

	Benefit point scale					
Benefit category	**0**	**1**	**2**	**3**	**4**	**Source**
1. Supporting ES: 1.1 Ecological impact	No known ecological impact (NK)	Low ecological impact (LO)	Potential high ecological impact (PH)	High ecological impact (HI)	Severe ecological impact (SE)	NBIC[#] Alien Species list
1.2 Effect of IAS species on threatened ecosystems	Intact (LC)	Near threatened (NT)	Vulnerable (VU)	Endangered (EN)	Critical (CR)	NBIC[#] Alien Species list/ Norwegian Red list for ecosystems
2. Provisioning ES 2.1 Food	No effects	Small effects, i.e., some reduction in area/ production	Somewhat larger effects, i.e., large reduction in area/production/ grazing area	Large effects, i.e., large reduction in area/production/ poisonous for grazing/livestock, etc.	Very large effects, i.e., very large reduction in area/production/ Deadly for livestock etc.	Expert assessment*
2.2 Fiber/ materials	No effects of species in our analysis Benefit point scale not developed					
3. Regulating ES	No effects of species in our analysis Benefit point scale not developed					
4. Cultural ES 4.1 Recreation, aesthetic values	No effects	Minor changes in landscape. Small plants, low visibility, not restricting recreational activities	Aesthetic disturbance of landscape, not restricting recreational activities	Aesthetic disturbance of landscapes, restricting activities in areas but not growing in typical recreation areas	Aesthetic disturbance of Landscape, Restricting recreational activities	Expert assessment*
5. Human health	No effects	Discomfort / Indirect effects		Poisonous to humans	Deadly to humans	NBIC[#] Alien Species list, Expert assessment*
6. Infrastructure	No effects	Indirect effects (obstruct vision/ signs along roadside)			Severe damages to buildings, roads and other infrastructure	Expert assessment*

*The expert assessments are made by researchers at the Norwegian Institute for Nature Research (NINA)

[#] NBIC = Norwegian Biodiversity Information Centre (www.biodiversity.no)

IAS removal. However, all control measures are not applicable for all areas, for instance the use of pesticides might be precluded in nature conservation areas. Hence, it must be made clear which method is most cost efficient in different types of areas. The time it takes for the measures to effectively remove the IAS also varies across measures and species, and so does the need for follow-up measures after the initial treatment. We have made assessment of these aspects for each IAS and control measure. However, it should be noted that there is large uncertainty in these assessments of social costs because of limited systematic experience with different control measures for many of the IAS considered here. In accordance with standard procedure in BCA, we calculated the present value of the control costs for all affected parties, that is the aggregated social costs of all measures carried out over the time period needed for the eradication measures to be 100% effective. Typically, a huge initial effort with corresponding high costs is necessary for the first year or two to eradicate the IAS, followed by a much lower level of annual costs for a varying number of years.

The next step was to calculate the costs for different types of areas and the total area that needs control measures to eradicate the IAS. Based on the calculations described above, we chose the control measures that provided the lowest cost per decare in different types of areas where the IAS should be eradicated. We calculated the total cost of eradication stepwise, first across Norway, then in selected parts of Norway and/or in selected vulnerable ecosystems. Here, we utilized information on the effect of control measures, the total area where the IAS is found, and expert assessments of the density of IAS in different ecosystems; as recorded in the "Alien Species List" and "Alien species observations" in NBIC, respectively. For some IAS, scarce information on the prevalence in different ecosystems precluded estimation of the size of the areas in need of control measures. This prevented the estimation of total costs for these IAS, and they were therefore not included in our analysis.

BCA prioritization tool

In Table 2, we compare benefits and costs for control measures aimed at a hypothetical alien vascular plant species, with the aim to eradicate the species in a geographically limited area. To reach the goal, control measures will be carried out in an area of 2000 decares within an endangered ecosystem. We use the calculated costs per decare for the most cost-effective control measure which is applicable to the IAS and area in question. Then, we calculate the total cost of eradicating this IAS for the specified area; and assess and assign benefit points within each benefit category. Finally, we compare total control costs to total benefit by constructing different cost-benefit ratios.

Table 2 shows the costs of a specific control program for species A, the number of benefit points from the avoided damage to ecosystem services, and the size of the area for eradication. The last three rows in Table 2 shows three different ways of comparing the monetary costs and the non-monetary benefits. The simplest way is to summarize benefit points and total costs without comparing them in terms of cost-benefit or benefit-cost-ratios. This results in an illustration of control costs, benefits provided, and the overall importance of the benefits (expressed as the total number of benefit points). This procedure may work well in assessing different control measures for each individual IAS. However, it would be very demanding to rank 30 or more species and 1–2 control goals per species only by visual inspection of total benefit points compared to costs. Therefore, we have constructed three alternative composite measures comparing total costs to benefits (see the last three rows of Table 2). These are: (i) total cost per benefit point, (ii) control costs divided by the number of benefit categories with 4 benefit points (if one does not want to aggregate all benefit points, or do not agree with the scale for benefit points for each benefit), and (iii) including the size of the area of control in the costs per benefit point. Although the latter might be a good idea, it is far from obvious how this should be done.

Note that the method where benefits points are aggregated implies that we implicitly assign the same weight to all benefit categories. This means that avoided damage

Table 2. Illustration of IAS BCA tool. Control costs of eradication programs and benefit points (BP) for avoided ecosystem services (ES) damages (according to the methodology in Table 1) for a specified control program for the hypothetical invasive alien species (IAS) «A».

Species "A"		**Qualitative description/comments**
IAS control program goal	Eradicate species A from 2000 decares of endangered ecosystems	
Costs		
Cost per decare	150 USD per decare	
Total cost	150 USD per decare x 2000 decares = 300 000 USD	
Benefit types	**Benefit points (BP)**	
Avoided negative ecological impact (non-use value)	4	The species is in impact category SE (Severe negative impact)
Avoided damage to endangered ecosystems (non-market /non-use value)	4	The species is eradicated in areas with CR (critically endangered) ecosystems
Avoided damage to food production (market / use value)	0	No effect
Avoided damage to fibers and other non-food provisioning ES (market / use value)	0	No effect
Avoided damage to regulating ES (non-market/ non-use value)	0	No effect
Avoided damage to cultural ES: recreation and aesthetic services (non-market/ use value)	4	The species is eradicated in areas where it is a nuisance to recreational activities and landscape aesthetics.
Avoided damage to human health (market and non-market/	0	No effect
Avoided damage to human infrastructure (market / use value)	0	No effect
Total benefit points (BP) of avoided damage	12	
Total cost per BP	300 000 USD/12 BP = 25 000 USD/BP	
Total cost per (number of ES with full score i.e., 4 BP)	300 000 USD/3 BP = 100 0000 USD	
Total cost per (BP * controlled area)	300 000 USD/ (12 BP x 20 000 decare) = 1.25 USD per BP x decare controlled	

to infrastructure in a certain geographic area is weighted equally to non-use values e.g., avoiding damages to endangered ecosystems in the same area. Alternatively, one could aggregate the different benefit categories by assigning different weights to different categories, for example by weighting effects on the non-use values of damaged endangered ecosystems to be ten times higher compared to the effects on infrastructure. However, we have no information for assigning a specific set of such weights, and thus do not do this here. According to the principles of social BCA, the weights of these benefit categories should ideally reflect the preferences of the people whose wellbeing/utility is affected by the IAS. However, these weights can only be derived in monetary terms by applying revealed and stated preference techniques to derive people's preferences and willingness-to-pay and thereby avoid the damages caused by IAS on e.g., ecosystem services. As both revealed and stated preference techniques elicit the preferences of people in local communities, their preferences will be included, but participatory approaches and social impact assessments could also be used to elicit people' preferences (Frank et al. 2015; Moon et al. 2015; Crowley et al. 2017). One could involve experts and/or communities in this weighting process, which could influence the results. It is also worth noting that the total number of benefit points for each species, will depend on how many and which benefit categories are included and assessed in the framework. The system is open and transparent, and thus makes it possible to test how sensitive the results are to the number and definition of each benefit category and the benefit points scale used.

Data collection

We have collected data from several sources. We have used observational citizen science data from the Norwegian species map service hosted by the Norwegian Biodiversity Information Centre (NBIC; www.biodiversity.no). The databases are based on a citizen science approach where data points can be added by either experts or lay people. However, it is partly quality controlled and curated by experts. Such citizen science data have proven to be of great value in biodiversity monitoring (Pocock et al. 2018). However, systematic variation in taxonomic coverage and geographic areas covered have been noted in such programs (Chandler et al. 2017). Still, data collected in this way has also been demonstrated to be high-quality non-biased data containing similar properties to data collected by scientists (Lewandowski and Specht 2015; Petrovan et al. 2020). The 30 IAS included in this study are conspicuous and easily identified, and therefore probably neither wrongly identified nor overlooked. An uncertainty-range was previously estimated for each of the IAS species within the NBIC database, based on expert judgment (Sandvik et al. 2020). Each data point within the NBIC database represents either an individual species or, more likely for IAS, a population. Three experts on the respective 30 species estimated individually the size of the area one location of the different species normally covers. After agreeing on the spatial size, the area was summed up across Norway based on the number of points per species respectively. These area estimates are uncertain due to the lack of systematic mapping of all IAS across the country. Also, some points may be invalid due to eradication or death. Thus, the size of the total area needed to be controlled contains uncertainty. However, we think our approach produces sufficiently large total control areas for eradication measures to be effective. For assessing ecological impact, we have used the evaluations from the Norwegian Biodiversity Information Centre (2018) where an expert panel evaluated 1473 alien species using the methodology described in Sandvik et al. (2012, 2019). Data for estimating control costs and eradication efficiency were based on previous cost estimates derived from municipality data and county officials (Blaalid et al. 2018). Benefit points were assessed partly based on information from NBIC and partly on expert judgments; see Table 2 for details.

Results and discussion

Estimation of total costs for control measures

We have estimated (i) costs per decare and (ii) total costs for eradication of each of the selected 30 vascular plant species (see list in Suppl. material 1: Table S1). Due to lack of data, cost/benefit ratios for two of the 30 species within this study, *Eutrochium purpureum* and *Solidago canadensis* were not calculated (N=28). Eradication costs and effects are calculated based on previous cost estimates gathered from eradication projects and expert assessments on plant biology and traits, carried out by a group of three individual plant biologist. These costs are uncertain due to two factors: i) lack of documented effects of IAS control programs across space and time, and ii) uncertainties whether the number of observations reflects the population size and distribution of each of the species. The latter is the main contributor to the overall uncertainty. Our results show that there are large variations in eradication costs for the 30 IAS included here. Note that the vascular plants chosen is not a random sample, but comprises species identified to constitute severe to high ecological impact by the Norwegian Biodiversity Information Centre. Figure 2 shows that nine plants (all herbs) have relatively low eradication costs of less than 10 million USD, nine plants (mixture of herbs and woody plants) have medium eradication costs (10–100 million USD) and ten plants (mixture of herbs and woody plants) have high eradication costs (more than 100 million USD). In the latter group, four species, have extreme eradication costs (>1 billion USD), in which three of them are *Reynoutria* spp. These plants are known for their extreme high decare control costs, as they have very deep root systems and aggressive vegetative reestablishment. This makes eradication both difficult and expensive. It is important to note that the species included in this study share characteristics such as: (i) they are mostly assessed as having severe negative ecological impact (SE-species), (ii) they are relatively well-established, often through their use in horticulture; and (iii) most of them have additional negative effects on vulnerable (red-listed) ecosystems and/or species. Some species, such as *Lupinus* spp. have also been planted deliberately due to potential positive effects. *Lupinus polyphyllus* was considered a highly attractive choice for vegetation cover alongside new roads, and was often planted to prevent erosion, and for its ornamental value as it is both colorful and willing to germinate. Currently, this species has over 50,000 records within the map services of NBIC, making nationwide eradication highly unlikely. This is reflected in the calculated eradication costs of more than 20 million USD given that it is only eradicated in areas where it is a threat to red-listed ecosystems. Notably, its close relatives, *L. perennis* and *L. nootkatensis* have far lower associated eradication costs; less than one million USD. This can potentially be ascribed to their smaller number of records. It is important to note that we here assume no conflicts during the control process such as private landowners opposing eradications, and that all populations of each IAS are identified and successfully removed. Thus, we assume the control measures to be 100% effective. Despite the relatively low number (30) of vascular plant species assessed here, a general pattern in total eradication costs seems to emerge: Species with (i) large distributions or (ii) woody plant species are associated with higher eradication costs.

There are substantial differences in the upper estimates for costs for the different species, which is largely due to differences in the estimated area of distribution, but also differences in the control costs per decare. The knotweed (*Reynoutria* spp.) and fly honeysuckle (*Lonicera caerulea*) stand out, as the upper estimate of costs for measures

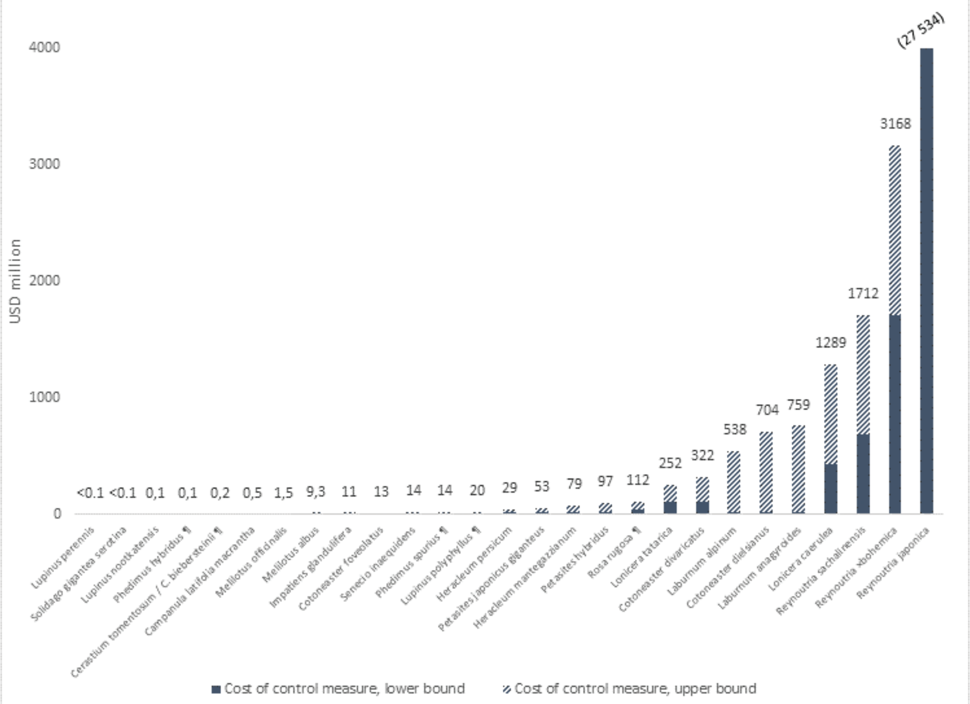

Figure 2. Total social costs (i.e., present value in million 2019-USD), upper and lower bound, of control measures for each invasive alien species (IAS), ranked in terms of increasing costs from left to right. For most species, the control costs would eradicate the IAS in all of Norway, but for five IAS (denoted ¶) the control costs are estimated for eradication in vulnerable ecosystems only, as this is considered to be the most realistic control program for these five species. The number in parenthesis implies the number is far above the scale of the y-axis. Converting Norwegian Kroner (NOK) to US dollars (USD), we have for simplicity used the approximate Purchase Power Parity corrected exchange rate of 1 USD = 0.1 NOK. (https://data.oecd.org/conversion/purchasing-power-parities-ppp.htm).

is considerably higher (more than USD 1,200 million) compared to the remaining vascular plant species. For all but five species we have estimated the costs of total eradication of species populations within Norway.

Assessment of benefits for control measures

The benefits of eradicating each of the 28 species were calculated using the benefit point scale for affected ecosystem services shown in Table 1. The total benefit point score for each species, and the ecosystem services that is affected, is shown in Figure 3. All species are identified to have severe or high ecological effect (NCBI) and are thus given similar score within the supporting ecosystem service category (i.e., category 1 in Table 1). The variation in benefit points for other ecosystem service categories are larger, but overall, most species also score benefit points within "Endangered ecosystems" and "Recreational/aesthetic impacts". Both the *Laburnum* spp. and *Heracleum* spp. are given higher scores within the "Human health" category as they have documented harmful effects in addition to high scores within all categories, which gives them overall higher benefit points compared to the remaining species. The *Reynoutria* spp. has been given points in the "Infrastructure" category, as they obstruct vision and signs along roads in Norway (Blaalid, pers. obs.).

Assessment of benefits and costs for control programs

Comparing costs and benefits of the control programs is not straightforward as costs are measured in monetary terms while the benefits are measured in "benefit points". The data reveals that some species with high total costs have few benefit points, whereas other species have low costs but many benefit points. Some species can be controlled for a relatively low cost, while the benefits are likely to be quite large, as in the case of wild perennial lupine (*Lupinus perennis*) and narrow-leaved ragwort (*Senecio inaequidens*). It is less obvious that control measures should be undertaken for species such as *Reynoutria* spp. and *Lonicera* spp. as they have relatively high costs compared to the expected benefits. However, public and private developers as well as the public administrations at the municipal and county level will have to adhere to national laws and regulations for the handling of soils where alien species are present, for example in connection with road construction projects, even when costs are very high (Ministry of Climate and Environment 2015). Figure 4 depicts the comparison between costs and benefits of a control program in terms of costs per benefit point for each IAS, with the upper panel showing those with control costs (in million USD) per benefit point below 10, and the lower panel the IAS with the cost/benefit ratios above 10. This way of combining costs and benefits enable us to rank the IAS in terms of which species will be cheapest to control relative to a non-monetary measure of benefits. Uncertainties in both control costs and the benefit measure may affect the order of ranked species; however, with such a large span in costs, it will potentially affect closely ranked species. Testing it further across taxonomic groups of IAS will give a better resolution of this BCA tools robustness. Our results show that the species to the left in the upper panel of Figure 4, with low costs relative to the benefits including *Lupinus perennis, Lupinus nootkatensis, Solidago gigantea serotina, Phedimus spurius, Cerastium* spp. and *Senecio inaequidens*, should be given priority when allocating funds to control measures for the invasive alien vascular plant species considered here.

Notably, our BCA tool does not include the costs of preventing potential re-invasions after eradication. De facto re-invasions, defined as another event compared to un-successful eradications, are less likely given that the species have been successfully removed from the country, and may thus be subjected to a new BCA analyses with updated parameters. However, in cases where re-introductions are expected (e.g., cases where IAS species are removed from a given area) costs of preventing re-invasions are important to account for and should be assessed in future studies.

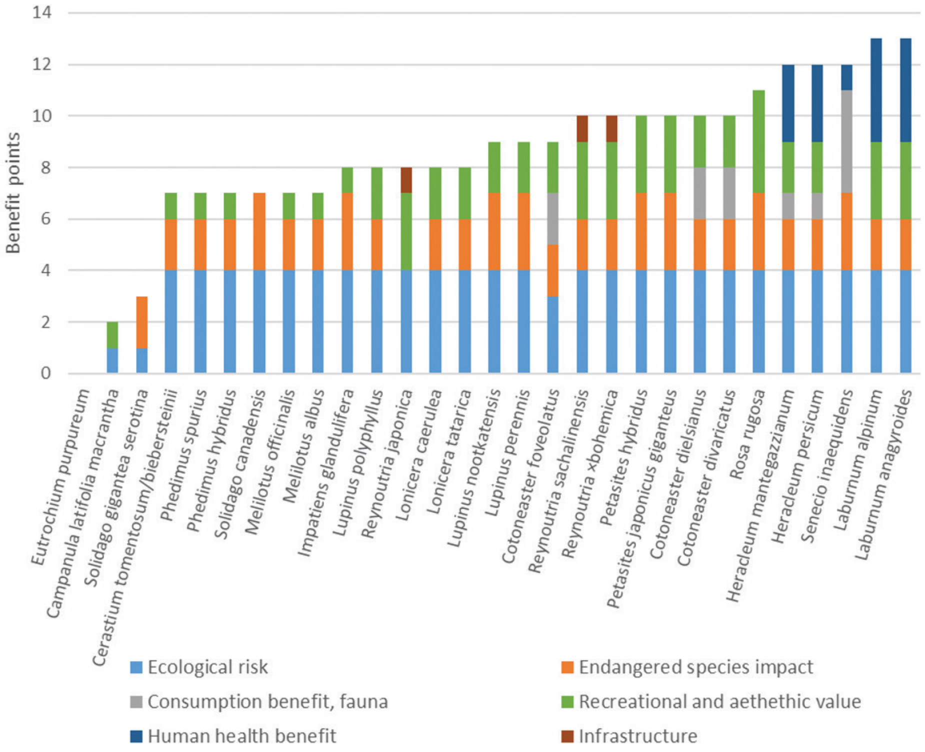

Figure 3. Benefit points (BP) of control measures for invasive plant species in Norway; total BP and for each benefit category.

Conclusions and recommendations

BCAs of eradicating/controlling different IAS provide vital information to policy makers and practitioners that can be used to rank IAS, and through this maximize the social benefits for a given control cost budget. However, the large number of IAS prohibits complete BCAs of different control programs for each individual IAS as it is difficult and time consuming to assess all social costs and benefits. The costs of controlling the different IAS are difficult to assess; not only due to uncertain costs of the different control measures themselves, but also due to incomplete information about the effectiveness of the control measures and which measures, or combinations of measures would suffice to control the IAS in question. In addition, there are uncertainties regarding the total distribution of IAS species in question.

Previous studies have identified lack of legal frameworks (Smith et al. 2014), lack of knowledge and variation in knowledge interpretation (García-Llorente et al. 2008), and lack of public support (Vane and Runhaar 2016) as main obstacles for implementation of control measures for IAS. Most of the 30 invasive alien vascular plant species considered here are species known to be included in control programs by municipalities and

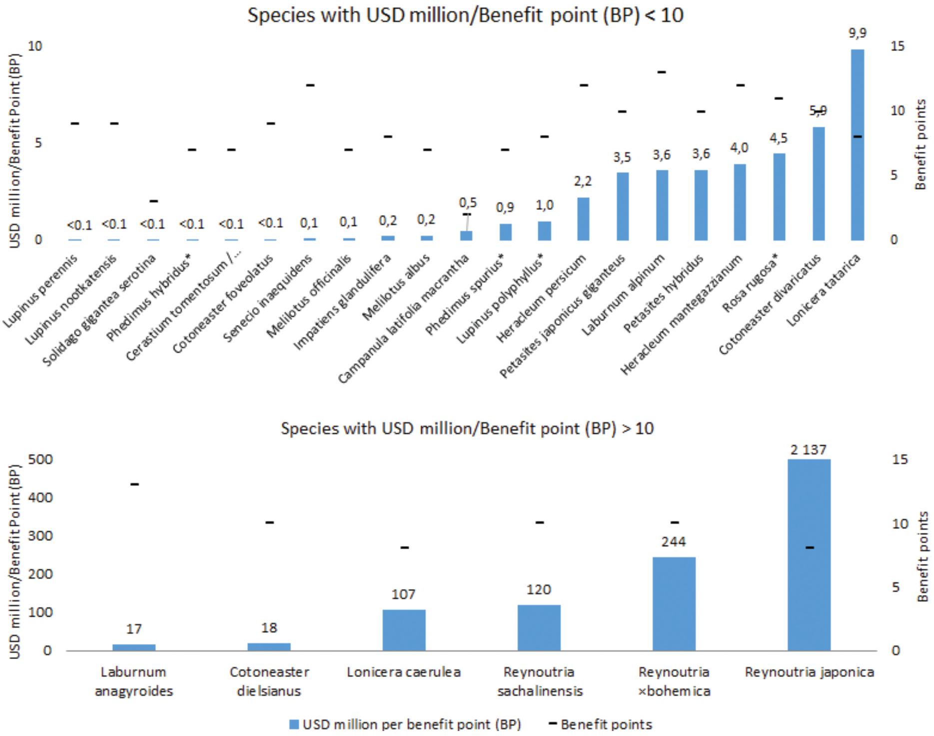

Figure 4. Control cost measures per benefit point (BP) for each invasive alien species (IAS) Average total cost of control measure in million USD divided by total BP. The upper panel and lower panel show the results for IAS with total cost (in in million USD.)/BP being larger and smaller than 10 respectively. N=28, as control costs were too uncertain to estimate this cost/benefit ratios for two IAS, *Eutrochium purpureum* and *Solidago canadensis.*

environmental authorities at the county level (Blaalid et al. 2018). Their status at the national alien species list in Norway states they have severe to high ecological impact. Therefore, they are all potentially candidates for eradication or control programs. However, our results clearly demonstrate that this is not feasible due to the huge economic costs. Currently, 10–20 million USD are used annually for control programs in Norway (Magnussen et al. 2014), and Figure 2 shows this would cover the costs of eradicating only a few of the 30 species at the national level in the next few years (see Figure 2).

Notably, our results indicate that the current IAS control budgets in Norway provide relatively small benefits per dollar, as over 50% of the resources are allocated to *Reynoutria* spp. and *Heracleum* spp. (Blaalid et al. 2018). We demonstrate how using our simplified BCA prioritization tool could aid environmental authorities at local, regional and national levels in spending their limited budgets to obtain higher benefits, potentially eradicating species within an area, compared to just preventing further spread. While BCAs have been performed for one or a few IAS in specific case studies (see e.g., Roberts et al. 2018), and theoretical models for economic optimal prioritization of larger numbers of IAS have been developed (Courtois et al. 2018), there is , to our knowledge, no applied BCA tool to prioritize among the large number of species of alien invasive vascular plants as considered here. The prioritization tool we develop serves as an attempt to develop a simple BCA tool tailored to assess control of large numbers of individual IAS at relatively low costs. We estimate the average cost of applying the BCA tool to an individual IAS to be about 1,500–2,500 USD. Further, as opposed to the PPP and GISS assessment methods it uses the ecosystem service framework, which is a well-established and acknowledged way of assessing impacts on human welfare from changes in the ecosystems, and thus adhere to the welfare economic underpinnings of BCA. We should be careful in directly transferring and generalizing the results from this study across countries and other groups of IAS as there are large variations in awarded benefit points for a single species due to the variation in impact at the regional level. For example, we suspect that *Reynoutria* spp. would be awarded higher benefit points within the category "infrastructure" in Great Britain, as this plant is here known to destroy asphalt roads through erosions and even disrupt housing foundation (Fennell et al. 2018), illustrating that benefit points associated with individual plants may alter with space and time. Control costs can also vary across countries, e.g., labor costs in mechanical removal of plants in Norway may tend to be higher compared to many European countries and the US.

One of the main challenges for control programs of IAS is the funding scheme and the lack of interagency budgeting (Reaser et al. 2020a). Although we should aim at reaching an understanding and collaboration between organizations dealing with IAS control programs, the use of the BCA approach may overcome such obstacles as the benefit points can be compared to the cost at the national or regional level to establish an overall priority list. The simplified BCA prioritization tool described here, relies on both ecological and economic knowledge, and contributions from several disciplines are needed to reach sound prioritizing and management of invasive alien species. Benefits of eradicating alien species have often been estimated in terms of e.g., crop losses valued at market prices, without due consideration to non-market ecosystem impacts. This would easily underestimate the social benefits of invasive species control measures. More environmental valuation studies of avoiding damages to ecosystem services, will provide estimates of people's preferences which is the ideal way to aggregate benefits of IAS control in BCAs. Meanwhile, our simplified BCA tool with no need for monetary assessment of benefits provides a cost-effective tool for constructing a priority list determining which IAS to control among a large number. It still provides a ranking of IAS which at least qualitatively will maximize net social benefits of IAS management. The tool developed here can also be coupled with other impact assessment systems and can thus be versatile and adjustable. The tool has so far been tested on vascular plants only, mainly because the prioritization between the many alien plant species is high on the agenda of the environmental authorities. However, this tool could also quite easily be developed and adapted to other organism groups, as both costs of mitigation and benefits in terms of ecosystem services are general tools and not applicable to plants only.

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Supplementary material 1

Table S1

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Data type: table

Explanation note: List over all species included in the analysis with additional risk categories.

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RESEARCH ARTICLE

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Diversity and distribution of cytochrome oxidase I (COI) haplotypes of the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera, Pentatomidae), along the eastern front of its invasive range in Eurasia

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Abstract

The arrival, establishment and pest status of *Halyomorpha halys* in Europe and non-native countries in Asia have been well-documented, with thorough characterisation of the genetic diversity and occurrence of cytochrome oxidase I (COI) haplotypes in Switzerland, France, Hungary, Italy and Greece. However, a number of gaps exist in terms of the characterisation of the haplotype diversity and occurrence of *H. halys* along the invasion front that covers eastern Europe, western and central Asia. To contribute towards filling this gap, the COI haplotype diversity and distribution were investigated for *H. halys* collected in Serbia, Ukraine, Russia, Georgia and Kazakhstan. A total of 646 specimens were analysed and five haplotypes were found (H1, H3, H8, H33 and H80). Haplotype H1 was present in all five countries investigated and was the only haplotype detected amongst > 500 specimens collected from Ukraine, Russia and Georgia. H1 (82%) was the dominant haplotype found in Kazakhstan, alongside H3 (18%). In contrast to the low or no diversity observed in these four countries, Serbia had higher haplotype diversity and was represented by five haplotypes. Although H3 was dominant (47%) in Serbia, H1 was also prevalent (40%); the remaining haplotypes (H8, H33 and H80) were minor contributors (1–11%) to the haplotype composition. The results are discussed in context with other known populations in neighbouring countries and patterns of haplotype diversity indicate the movement of successful invasive populations in Europe to generate secondary invasions along the eastern front of the invasion in Eurasia. Possible scenarios regarding the spread of particular haplotypes in these regions are discussed, along with suggestions for future research to fill existing gaps.

Keywords
Agricultural pest, DNA barcoding, haplotype, Heteroptera, invasive alien species, mtDNA, range expansion, secondary invasion

Introduction

Halyomorpha halys Stål (Hemiptera: Pentatomidae) is native to East Asia [China (including Taiwan), Japan, Korea, Myanmar and Vietnam; Wang and Liu 2005], but has become invasive in a number of locations in North America, South America and Eurasia (Hoebeke and Carter 2003; Leskey et al. 2012; Haye et al. 2015; Gapon 2016; Faúndez and Rider 2017; Hamilton et al. 2018). In many of these locations, this highly polyphagous insect has become a serious economic pest of agricultural crops, including field, fruit, vegetable and nut crops (Pansa et al. 2013; Rice et al. 2014; Haye et al. 2015; Maistrello et al. 2017; Bosco et al. 2018; Hamilton et al. 2018; Musolin et al. 2018). The global spread of *H. halys* has largely been facilitated through travel, transportation and commercial trade, as the pest gains entry into new locations as a stowaway inside containers, vehicles, packaging and luggage (Hamilton et al. 2018). This poses a significant risk of spread when invasive species establish in a region with a significant transportation hub (Bacon et al. 2012) and, in fact, Turbelin et al. (2017) state that the unintentional ingress of invasive alien species is largely due to trade and ignorant possessions, including stowaways, as well as assisted transport in motor vehicles, trains, boats and planes.

The arrival and establishment of *H. halys* in Europe and non-native areas of Asia has been well-documented (see timeline in Table 1), with the first invasive populations established in Liechtenstein (2004) and Switzerland (2007). Populations expanded rapidly throughout several Swiss Cantons (Haye et al. 2014) and were found in neighbouring Germany and France within five years (Table 1). Prior to 2011, populations of *H. halys* were only known from western European countries; however, the discovery of the pest in Greece in 2011 (Milonas and Partsinevelos 2014) and Italy in 2012 (Maistrello et al. 2014) signalled the arrival of the pest in southern Europe as well (Table 1). The first establishment in eastern Europe was documented in 2013, when *H. halys* was found in Sochi, Russia (Mityushev 2016), with subsequent spread into western Asia in 2015 as the pest moved within the Caucasus region into Georgia (Gapon 2016; Musolin et al. 2018; Table 1), where populations increased rapidly from 2015 to 2016

Table 1. Timeline of the establishment of *Halyomorpha halys* in invaded areas of Eurasia, including western Europe, southern Europe, eastern Europe, western Asia, and central Asia.

Year	Country	Geographical region*	Reference
2004	Liechtenstein	western Europe	Arnold 2009
2007	Switzerland	western Europe	Wermelinger et al. 2008
2011	Belgium	western Europe	Claerebout et al. 2018
	Germany	western Europe	Heckmann 2012
	Greece	southern Europe	Milonas and Partsinevelos 2014
2012	France	western Europe	Callot and Brua 2013
	Italy	southern Europe	Maistrello et al. 2014
2013	Russia	eastern Europe	Mityushev 2016
2014	Hungary	eastern Europe	Vétek et al. 2014
2015	Austria	western Europe	Rabitsch and Friebe 2015
	Serbia	southern Europe	Šeat 2015
	Romania	eastern Europe	Macavei et al. 2015
	Georgia and Abkhazia	western Asia	Gapon 2016; Musolin et al. 2018
2016	Bulgaria	eastern Europe	Simov 2016
	Kazakhstan	central Asia	Temreshev et al. 2018
	Slovakia	eastern Europe	Hemala and Kment 2017
	Spain	southern Europe	Diolo et al. 2016
	Ukraine	eastern Europe	Uzhevskaya 2017
2017	Azerbaijan	western Asia	Nuriyeva 2018, 2020
	Croatia	southern Europe	Šapina and Jelaska 2018
	Slovenia	southern Europe	Rot et al. 2018
	Turkey	western Asia	Güncan and Gümüş 2019
2018	Albania	southern Europe	Claerebout et al. 2018
	Bosnia and Herzegovina	southern Europe	Zovko et al. 2019
	Czech Republic	eastern Europe	Kment and Březíková 2018
	Malta	southern Europe	Tassini and Mifsud 2019
	Poland	eastern Europe	Claerebout et al. 2018; Bury 2021
	Portugal	southern Europe	Grosso-Silva et al. 2020
2019	Moldova	eastern Europe	Derjanschi and Chimişliu 2019
	North Macedonia	southern Europe	Konjević 2020

* Geographic regions as defined by the United Nations online publication “Standard country codes for statistical use”; https://unstats.un.org/unsd/methodology/m49/

(Bosco et al. 2018). In 2016, *H. halys* was found in Kazakhstan (Esenbekova 2017; Temreshev et al. 2018), thereby indicating the first population in central Asia. The documented establishment in each country within each region in Europe and non-native areas in Asia is shown in Table 1. Southern and eastern Europe have seen a great deal of expansion of *H. halys* populations since they were first documented in these regions. From 2015 to 2019, an additional nine southern European countries (Serbia, Spain, Croatia, Slovenia, Bosnia and Herzegovina, Albania, Malta, Portugal and North Macedonia; Table 1) have documented the establishment of *H. halys*. Similarly, following the initial establishment in Russia, eight eastern European countries have documented *H. halys* populations from 2014 to 2019 (Hungary, Romania, Bulgaria, Slovakia, Ukraine, Czech Republic, Poland and Moldova; Table 1). The explosion of new populations in recent years suggests the continued movement and spread of *H. halys* in non-native areas of Eurasia. Already, observations in the Caucasus region suggest the occurrence of 2 or 3 generations per year, allowing substantial numbers to build during a single growing season and likely resulting in the severe agricultural damage observed in Russia and Georgia (Musolin et al. 2018, 2019). Similarly, the population density of *H. halys* in agricultural areas in Greece and Turkey is increasingly high and severe agricultural damage is likely or imminent (Ak et al. 2019; Damos et al. 2020). As this pest continues its outward expansion from the originally-established populations in Europe, knowledge of which populations are present and spreading may improve our understanding of the movement of *H. halys*, particularly in locations that experience high levels of economic damage in southern and eastern Europe and western Asia.

Mitochondrial DNA sequence data have frequently been used to trace the origin and spread of invasive insect species (Grapputo et al. 2005; Corin et al. 2007; Auger-Rozenberg et al. 2012; Chapman et al. 2015). The mitochondrial Cytochrome Oxidase I (COI) gene has shown utility in species identification and separation of genetic lineages (Bucklin et al. 2011; Stephens et al. 2011), in particular as it relates to reconstructing routes of invasion (Auger-Rozenberg et al. 2012; Chapman et al. 2015). Although the Cytochrome Oxidase II (COII) gene of *H. halys* has also been sequenced (Xu et al. 2014; Cesari et al. 2015, 2018; Yan et al. 2021), the COI gene has been used more extensively in the characterisation of the invasion history, diversity and identity of *H. halys* haplotypes in both native and invaded regions (Gariepy et al. 2014, 2015; Cesari et al. 2015, 2018; Zhu et al. 2016; Morrison et al. 2017; Valentin et al. 2017; Lee et al. 2018; Horwood et al. 2019; Schuler et al. 2020; Yan et al. 2021). Based on the COI haplotype analysis of *H. halys*, it has been suggested that multiple invasion events took place in the initial / early stages of invasion in Europe (2007–2012), with the population in Switzerland (primarily haplotype H3 and H8) resulting from the establishment of individuals that arrived directly from China; the population in Italy (primarily H1) resulting from the establishment of individuals from the invasive population in the USA; and the population in Greece (predominantly haplotype H33) resulting from a separate establishment of *H. halys* from China (Cesari et al. 2015; Gariepy et al. 2015; Valentin et al. 2017). The continued and consistent detection of one or more of these same haplotypes in Europe following the initial invasion (e.g. H1, H3, H8 and H33; Cesari et al. 2015, 2018; Gariepy et al. 2015; Morrison et al. 2017; Šapina and Jelaska 2018; Schuler et al. 2020; Yan et al. 2021), largely supports the occurrence of a bridgehead effect, wherein particularly successful invasive populations have given rise to secondary invasions in other locations (Lombaert et al. 2010; Lawson Handley et al. 2011). However, in Italy and Greece, subsequent introductions directly from Asia may have also taken place between 2013 and 2019, as a combined total of 20 additional haplotypes (including 14 previously undescribed haplotypes) have been reported in more recent studies in these two countries (Morrison et al. 2017; Cesari et al. 2018; Schuler et al. 2020). To date, none of these haplotypes is known from other invaded countries, indicating that spread through the bridgehead effect has not occurred. In addition, the majority of the new haplotypes have not yet been described from Asia and their origin remains unknown.

Halyomorpha halys COI haplotypes have been reported from Austria, Croatia, France, Georgia, Greece, Hungary, Italy, Romania, Serbia, Slovenia, Switzerland and

Turkey (Gariepy et al. 2014, 2015; Cesari et al. 2015, 2018; Morrison et al. 2017; Šapina and Jelaska 2018; Schuler et al. 2020; Yan et al. 2021). In some cases, the reports are based on a few specimens from a single location within a given country, based on their availability for study (e.g. Croatia, Serbia and Turkey). Although they may not provide a thorough account of the haplotype composition in a given country, these reports are valuable, as they provide information on the presence of a given haplotype and can continue to be built upon as additional samples become available to generate a more complete picture of the haplotype composition. *Halyomorpha halys* haplotype composition in more recently invaded areas of southern and eastern Europe, as well as western and central Asia, remain relatively undescribed. Identification of the haplotypes that are present in these areas may provide insight into the origin and spread of *H. halys* in these regions, particularly as it relates to secondary invasions from other countries in the invaded range. To address this, the DNA barcode region of the COI gene was analysed from samples collected from Georgia (including Abkhazia), Kazakhstan, Russia, Serbia and Ukraine and compared to the haplotype framework generated in previous studies (Gariepy et al. 2014, 2015; Cesari et al. 2015, 2018; Zhu et al. 2016; Morrison et al. 2017; Schuler et al. 2020; Yan et al. 2021). The present study builds upon a recent haplotype report from Georgia and Serbia (Yan et al. 2021), but with more extensive sampling and assessment of the haplotype composition in these two countries and characterises haplotype composition from Russia, Ukraine and Kazakhstan, which have not previously been assessed. Based on the characterisation of haplotypes from these areas and comparison with global records of *H. halys* haplotypes, we speculate on the pattern of diversity and spread and discuss the possible pathways of entry into these regions.

Methods

Acquisition of material

Halyomorpha halys adults were field-collected (by sweep net, hand-picking from vegetation or in pheromone traps) between 2016 and 2019 from locations with recently-established populations in Georgia (including Abkhazia; 2016–2018; n = 293), Kazakhstan (2017; n = 11), Russia (2016, 2018, 2019; n = 202), Serbia (2018; n = 129) and Ukraine (2017; n = 11) (Fig. 1). Note that Abkhazia is a disputed territory within the Caucasus; however, for the purpose of this study, we considered Abkhazia as located within Georgia, based solely on the geographic continuity of the agricultural landscape in this area. As such, throughout this manuscript, samples from Georgia and the disputed territory of Abkhazia will collectively be referred to as samples from Georgia. Complete specimen and collection data are publicly available at www.boldsystems.org (Project *Halyomorpha halys* in eastern Europe and Eurasia, EEUR) and are summarized in Suppl. material 1: Table S1. Individual insects were stored in 95% ethanol for subsequent molecular analysis.

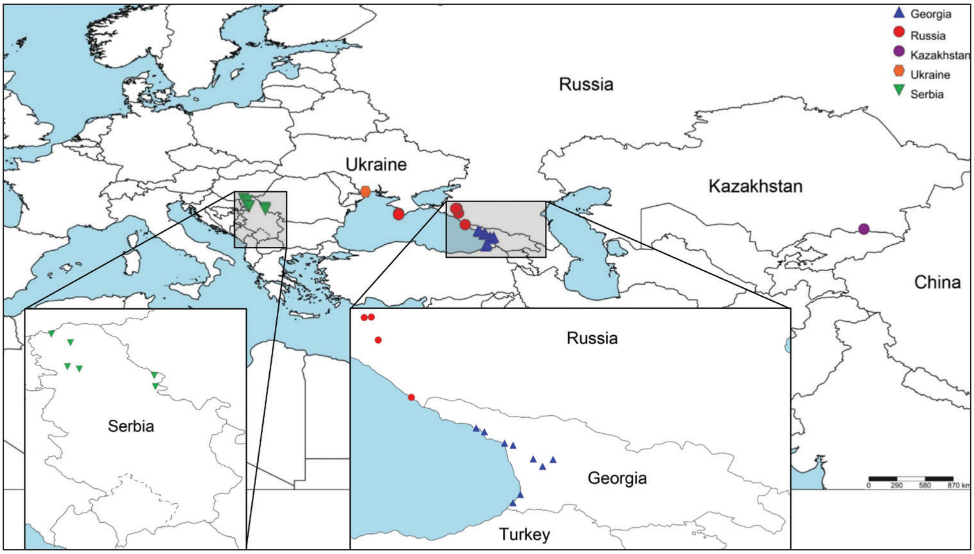

Figure 1. Map of *Halyomorpha halys* collection locations in Serbia, Ukraine, Russia, Georgia and Kazakhstan.

DNA extraction, amplification and sequencing

As described by Gariepy et al. (2014), a single leg was carefully removed from each insect using flame-sterilised forceps and placed in an individual 200 µl well of a 96-well microplate, along with 2 µl of proteinase K (20 mg/ml) and 100 µl of 5% Chelex 100 Molecular Grade Resin (Bio-Rad Laboratories, Hercules, CA, USA). A negative extraction control containing the Chelex and Proteinase K solutions, but no insect tissue, was included in each microplate. Sealed microplates were incubated overnight at 55 °C, followed by 10 min at 99 °C. Samples were centrifuged at 5800 g for 5 min to pellet the Chelex solution and 50 µl of supernatant (containing DNA) was transferred to wells in a new plate, taking care not to transfer the Chelex residue along with the sample. Microplates containing the extracted DNA were stored at –20 °C until further analysis.

PCRs were performed in a 25 µl volume containing 0.125 µl of Taq Platinum, 2.5 µl of 10× PCR buffer, 1.25 µl of 50 mM $MgCl_2$, 0.125 µl of 10 µM dNTPs (Invitrogen, Carlsbad, CA, USA), 0.25 µl of 10 µM forward and reverse primer (respectively), 19.5 µl ddH_20 and 1 µl of template DNA. A 658-bp sequence of the mitochondrial gene Cytochrome C oxidase subunit I (COI) was amplified by PCR using primers LCO1490 and HCO2198 (Folmer et al. 1994). Thermocycling conditions included initial denaturation at 94 °C for 1 min, followed by five cycles of 94 °C for 30 s, annealing at 45 °C for 40 s, extension at 72 °C for 1 min, followed by another 35 cycles of 94 °C for 30 s, 51 °C for 40 s and 72 °C for 1 min and a final extension period of 5 min at 72 °C.

PCR products were visualised with a QIAxcel Advanced automated capillary electrophoresis system (Qiagen, Hilden, Germany) using the DNA screening cartridge and method AL320. Results were scored with QIAXCEL SCREENGEL Software (version 1.2.0) and only those samples of the expected fragment size with a signal strength exceeding 0.1 relative fluorescent units were scored as positive.

Samples, scored as positive, were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA), following the manufacturer's instructions. Purified PCR products were bidirectionally sequenced on an ABI 3730 DNA Analyser at the Robarts Research Institute (London Regional Genomics Centre, ON, Canada). Forward and reverse sequences were assembled and edited using CODONCODE ALIGNER, version 9.0.1 (Codon-Code Corporation, Centreville, MA, USA). Sequence data and trace files were uploaded to the Barcode of Life Datasystems (BOLD; www.boldsystems.org) in the Project *Halyomorpha halys* in eastern Europe and Eurasia (EEUR).

Haplotype and nucleotide diversity measures

Samples were grouped, based on their country of collection (Georgia, Kazakhstan, Russia, Serbia and Ukraine) and standard measures of diversity were calculated for each group using DnaSP v.5.10.01 (Librado and Rozas 2009), including number of haplotypes, haplotype diversity (h, the probability that two randomly-selected haplotypes are different; Nei 1987) and nucleotide diversity (π, the average number of nucleotide differences per site between two randomly-selected DNA sequences; Nei and Li 1979).

Frequency and distribution of haplotypes of *Halyomorpha halys*

Samples were grouped, based on their country of collection (Serbia, Ukraine, Russia, Georgia and Kazakhstan) and the proportion of each haplotype within each group (i.e. country) was calculated in order to obtain a representation of the haplotype composition.

Additionally, based on current data and previous publications (e.g. Gariepy et al. 2014, 2015; Cesari et al. 2015, 2018; Šapina and Jelaska et al. 2018; Schuler et al. 2020; Yan et al. 2021), the number of COI haplotypes and the identity of dominant haplotypes from invaded European and central Asian countries were tallied and used to generate an overview of the trends and dominant haplotypes in these areas.

Results

Haplotype and nucleotide diversity measures

Genetic diversity measures for *H. halys* collected in Georgia, Kazakhstan, Serbia, Russia and Ukraine are shown in Table 2. For all samples combined, there were five haplotypes with a total of nine polymorphic sites. Overall haplotype and nucleotide diversity was 0.223 ± 0.021 (mean ± SD) and 0.00052 ± 0.00007 (mean ± SD), respectively. Haplotype and nucleotide diversity was zero in samples collected from Russia, Georgia

Table 2. Genetic diversity measures for *Halyomorpha halys*.

Country	Number of specimens	Number of Polymorphic sites	Number of haplotypes	Haplotype diversity (*h*) (mean ± SD)	Nucleotide diversity (π)(mean ± SD)
Kazakhstan	11	1	2	0.327 ± 0.153	0.0005 ± 0.00023
Georgia	293	0	1	0	0
Russia	202	0	1	0	0
Serbia	129	9	5	0.620 ± 0.022	0.00163 ± 0.00019
Ukraine	11	0	1	0	0
All	**646**	**9**	**5**	**0.223 ± 0.021**	**0.00052 ± 0.00007**

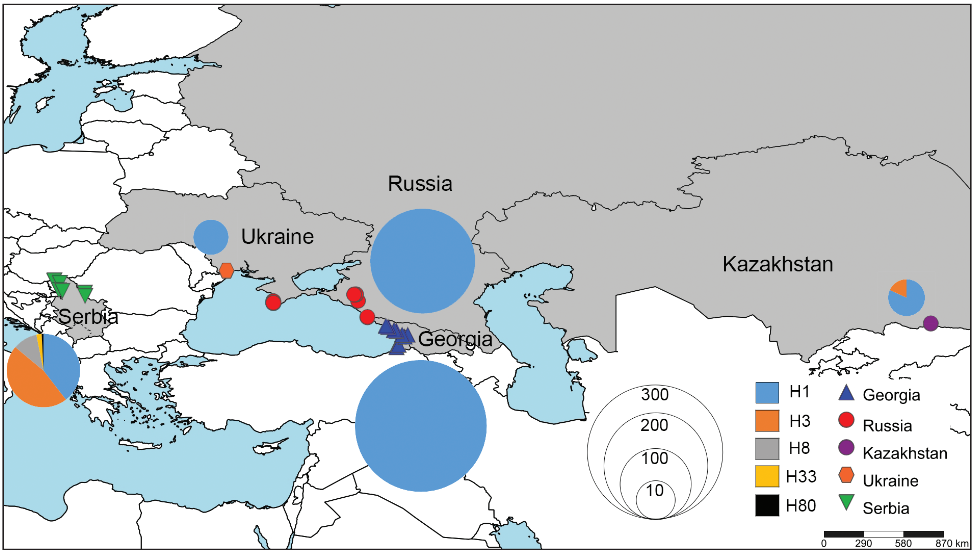

Figure 2. Map of *Halyomorpha halys* collection locations, with the COI haplotype frequency shown in pie charts sized proportionally to the sample size from each country.

and Ukraine, as only a single haplotype was observed. Serbia had the most diverse population observed in the present study, with five haplotypes recorded and showed the highest haplotype and nucleotide diversity (Table 2).

Frequency and distribution of haplotypes of *Halyomorpha halys*

A total of 646 samples were analysed and a 658-bp fragment of the DNA barcoding region of the COI gene was generated (Genbank Accession numbers MZ871818 - MZ872463). Collectively, five COI haplotypes (H1, H3, H8, H33 and H80) were identified. The majority of the samples were identified as haplotype H1 (87.6%), followed by H3 (9.6%), H8 (2.1%), H33 (0.5%) and H80 (0.2%).

The proportion of haplotypes from each country is shown Fig. 2 and Table 3. Haplotype H1 was recorded in all five countries and was either the dominant haplotype or the only haplotype in four of the five countries: Georgia (100%), Kazakhstan (82%), Russia (100%) and Ukraine (100%) (Table 3). In Serbia, H1 was the second

Table 3. Proportion (%) of each COI haplotype from *Halyomorpha halys* collected in Eastern Europe and Eurasia.

Haplotype	Georgia (n = 293)	Kazakhstan (n = 11)	Serbia (n = 129)	Russia (n = 202)	Ukraine (n = 11)
H1	100	82	39.5	100	100
H3	0	18	46.5	0	0
H8	0	0	10.9	0	0
H33	0	0	2.3	0	0
H80	0	0	0.8	0	0

Figure 3. Trends in the number of cytochrome oxidase I (COI) haplotypes in the invasive range of *Halyomorpha halys* in Eurasia.

most common haplotype, but nonetheless, represented 39.5% of the haplotype composition, making it a major contributor to the haplotype composition. H3 was the dominant haplotype from Serbia, representing 46.5% of the haplotype composition and was also recorded from Kazakhstan (18% of the haplotype composition). Three additional haplotypes were recorded from Serbia: H8 (10.9%), H33 (2.3%) and H80 (0.8%) (Fig. 2 and Table 3). The known global distribution of these haplotypes in their native and invasive ranges is presented in Table 4.

An overview of the number of haplotypes and the dominant haplotypes in non-native countries in Eurasia is presented in Table 5. The haplotype composition in the majority of countries in this invaded region consists of a single haplotype or relatively few haplotypes (≤ 4) (Fig. 3). Only four countries demonstrate a moderate (5–7 haplotypes: Switzerland and Serbia) to high (> 8 haplotypes: Italy and Greece) number of reported haplotypes (Fig. 3). All countries with *H. halys* COI haplotype data in Eurasia are dominated or co-dominated by H1, with the exception of Switzerland and France, which are dominated by H3 (Table 5, Fig. 4).

Discussion

Haplotype and nucleotide diversity

Invasive species typically have reduced genetic variation due to the occurrence of genetic bottlenecks upon colonisation of new locations (Fauvergue et al. 2012). Nonetheless,

Table 4. Overview of *Halyomorpha halys* haplotypes found in Eastern Europe and Central Asia, and their known global distribution in the native Asian range and in the invaded ranges. Countries in **bold** lettering indicate new records for a given haplotype.

Haplotype	Known distribution within native and invasive ranges		Original haplotype and/or location descriptions
H1	Native	China	Gariepy et al. 2014
	Invasive	USA, Canada	Gariepy et al. 2014
		Switzerland, France, Greece, Hungary	Gariepy et al. 2015
		Italy	Cesari et al. 2015
		Croatia	Šapina and Jelaska 2018
		Romania	Cesari et al. 2018
		Austria, Serbia, Slovenia, Georgia, Turkey, Chile	Yan et al. 2021
		Kazakhstan, **Russia**, **Ukraine**	Present study
H3	Native	China	Gariepy et al. 2014
	Invasive	Switzerland	Gariepy et al. 2014
		France, Hungary, Greece	Gariepy et al. 2015
		Italy	Cesari et al. 2015
		Austria, Serbia, Slovenia, Chile	Yan et al. 2021
		Kazakhstan	Present study
H8	Native	Unknown	-
	Invasive	Switzerland, France	Gariepy et al. 2014
		Italy	Cesari et al. 2015
		Austria	Yan et al. 2021
		Serbia	Present study
H33	Native	China	Valentin et al. 2017
	Invasive	Greece	Gariepy et al. 2015
		Italy	Cesari et al. 2018
		Serbia	Present study
H80	Native	China	Zhu et al. 2016
	Invasive	Italy	Cesari et al. 2018
		Serbia	Present study

some species have become very successful colonisers despite strong founder effects (Sax and Brown 2000), particularly when multiple introductions from different locations contribute to enhanced diversity (Miller et al. 2005; Dlugosch and Parker 2008; Lawson Handley et al. 2011). Previous studies in Europe have suggested establishment of *H. halys* from multiple sources, including the direct establishment of Asian populations, as well secondary invasions via previously-established populations through the bridgehead effect (Gariepy et al. 2015; Valentin et al. 2017; Schuler et al. 2020). A low genetic diversity in invasive *H. halys* has been observed in European countries where only a single haplotype is present (e.g. Romania; Cesari et al. 2018; Yan et al. 2021) or where one haplotype is dominant amongst a small number of haplotypes (e.g. Switzerland, Hungary and France; Gariepy et al. 2015). In these countries, *H. halys* populations have established either directly from Asia (e.g. from China to Switzerland) or by secondary invasion from neighbouring European countries (e.g. from Switzerland to France), with haplotype diversity (*h*) ranging from 0 to 0.27 and nucleotide diversity (π) ranging from 0 to 0.0008 (Gariepy et al. 2014, 2015; Cesari et al. 2018; Yan et al. 2021).

Figure 4. Trends in the distribution of dominant cytochrome oxidase I (COI) haplotypes in the invasive range of *Halyomorpha halys* in Eurasia.

In contrast to other invaded countries in Europe, *H. halys* populations in Italy and Greece are substantially more diverse (h = 0.702–0.724; π = 0.0036–0.0054; Gariepy et al. 2015; Cesari et al. 2018), with establishments that have originated from multiple source locations, including directly from China, Japan and Korea and/or via the bridgehead effect from established populations in the USA (Cesari et al. 2015, 2018; Gariepy et al. 2015; Morrison et al. 2017; Valentin et al. 2017; Schuler et al. 2020).

In the present study, the overall haplotype (h = 0.223) and nucleotide diversity (π = 0.00052) of the COI barcode region was relatively low and is consistent with the values mentioned above from previous studies in most European countries (excluding Italy and Greece). However, diversity spanned a broad range, with no diversity in samples from Russia, Ukraine and Georgia (where a single haplotype was found) to haplotype and nucleotide diversity values of 0.62 and 0.00163 (respectively) from samples collected in Serbia (where a total of five haplotypes were found). Yan et al. (2021) found similar results in terms of a lack of haplotype diversity in their samples collected in Georgia, where a single COI haplotype was found. Our larger number of samples from several additional areas in Georgia provides a more thorough assessment of the populations in this region and confirms the observations by Yan et al. (2021) in that a single haplotype is (currently) present in this country. The population from Kazakhstan yielded diversity values similar to those reported in European countries with < 5 haplotypes (Fig. 3); however, it is based on a small sample size (n = 11) collected from a single site and, therefore, may not accurately represent the diversity in that country. Goodall-Copestake et al. (2012) recommend sample sizes ≥ 25 for accurate comparisons of population-level COI diversity; nonetheless, our limited samples from Kazakhstan provide a baseline dataset from a previously-unrepresented country that can be built upon with further sampling. This may also be the case with our samples from Ukraine, as the sample size was small and originated from a single region (Odessa). However, given the fact that current and previous studies in neighbouring countries (Romania, Hungary and Russia) have also shown little to no haplotype diversity, it is not surprising that similar results were found in Ukraine in the present study, despite the small sample size. Nonetheless, more thorough sampling in additional,

Table 5. Summary of the number of samples analyzed, the number of haplotypes detected, and the proportion of the dominant haplotypes (H1, H3, H33) in the overall haplotype composition in invaded countries in Eurasia.

Country	Reference	Number of samples	Number of haplotypes	Proportion (%)			
				H1	H3	H33	Other
Austria	Yan et al. 2021	16	4	25	50	0	12.5
Croatia	Šapina and Jelaska 2018	2	1	100	0	0	0
France	Gariepy et al. 2015	139	3	0.6	98	0	1.4
Georgia	Present study	293	1	100	0	0	0
Greece	Gariepy et al. 2015	57	7	32	7	40	≤14
	Morrison et al. 2017	195	11	32	4.1	46.2	0.5–8.2
	Cesari et al. 2018	10	3	20	0	60	20
Hungary	Gariepy et al. 2015	84	2	99	1	0	0
	Morrison et al. 2017	194	2	99.5	0	0	0.5
	Yan et al. 2021	92	3	93	5	0	2
Kazakhstan	Present study	11	2	82	18	0	0
Italy	Cesari et al. 2015	42	3	76	21	0	3
	Morrison et al. 2017*	187	11	60	22.5	0	0.5–3
	Cesari et al. 2018	212	13	50	12	0	0.5–15
	Schuler et al. 2020	162	15	53	15	1	0.5–10
Romania	Cesari et al. 2018	8	1	100	0	0	0
	Yan et al. 2021	23	1	100	0	0	0
Russia	Present study	202	1	100	0	0	0
Serbia	Present study	129	5	39.5	46.5	2.3	0.8–11
Slovenia	Yan et al. 2021	16	3	69	25	0	6
Switzerland	Gariepy et al. 2015	225	4	0.9	85	0	0.4–14
	Morrison et al. 2017	110	4	1.8	89.5	0	2.6–6
Turkey	Yan et al. 2021	11	1	100	0	0	0
Ukraine	Present study	11	1	100	0	0	0

*Original data were presented separately from three regions within Italy (Emilia-Romagna, Veneto, and Piedmont); the values reported here were estimated by combining the values for each region. See Morrison et al. (2017) for detailed breakdown of haplotype frequencies in different regions of Italy.

geographically-diverse locations would help to confirm this observation and/or record changes in diversity over time as the invasion progresses.

The most diverse population in the present study was recorded from Serbia. Although Yan et al. (2021) found lower values for haplotype and nucleotide diversity from their Serbian samples, their values were based on nine specimens collected from a single site, which likely underestimated the actual diversity in Serbia (as per Goodall-Copestake et al. 2012). This is supported by the fact that Yan et al. (2021) recorded only two COI haplotypes, whereas the present study recorded five haplotypes and consisted of a much larger sample size from several locations (Fig. 1; Table 5). The diversity measures that we recorded from Serbia (h = 0.62; π = 0.00163) are just slightly lower than those reported in Italy and Greece (h = 0.702–0.724; π = 0.0036–0.0054; Gariepy et al. 2015; Cesari et al. 2018), where several haplotypes from multiple source populations (including China, Japan, Korea and USA) are known to occur (Gariepy et al. 2015; Valentin et al. 2017; Cesari et al. 2018). As will be discussed more thoroughly below, we suspect that *H. halys* populations in Serbia are derived from multiple source populations from more than one of the surrounding European countries, resulting in diversity levels more similar to those found in countries with multiple sources of invasion (e.g. Italy and Greece).

Frequency and distribution of COI haplotypes and potential sources of *Halyomorpha halys*

Based on the present study and the collective dataset available in literature for *H. halys* (see Table 5), the occurrence of COI haplotypes is fairly uniform across most of eastern Europe and central Asia (Figs 3 and 4). In most countries, only a single haplotype (H1) is present (e.g. Croatia, Romania, Ukraine, Turkey, Russia and Georgia) or is dominant alongside a minor contributing haplotype (typically H3; Kazakhstan and Hungary) (Figs 3 and 4). The exception in the present study is Serbia, where a total of five haplotypes were detected, including two dominant haplotypes (H1 and H3) and three additional haplotypes (H8, H33 and H80), two of which were minor contributors (< 5%; H33 and H80). A similar exception in previous studies was observed in Greece, where two dominant haplotypes are also known (H1 and H33; Fig. 4 and Table 5), along with several minor contributing haplotypes (H3, H13, H22, H30, H31, H32, H158, H159 and H160; Gariepy et al. 2015; Morrison et al. 2017).

Greece and Italy are known hotspots of invasive *H. halys* haplotypes, with 11 (Gariepy et al. 2015; Morrison et al. 2017) and 20 COI haplotypes (Morrison et al. 2017; Cesari et al. 2018; Schuler et al. 2020), respectively. Further, within the invaded range, many of these haplotypes are unique to these two countries (i.e. are not found elsewhere in Europe). For example, in the native and invasive ranges of *H. halys*, H80 was previously only known from Shandong Province in China (Zhu et al. 2016) and from northern Italy (Cesari et al. 2018; Schuler et al. 2020). However, in the present study we found this haplotype at very low levels in Serbia. Given the relatively low occurrence of this haplotype in Italy (Cesari et al. 2018), it is difficult to speculate whether the source of H80 is the result of movement and spread of H80 from Italy or whether it is a separate establishment originating from China. Similarly, H33 was previously only known from Greece (Gariepy et al. 2015; Morrison et al. 2017), but was recently detected for the first time in Italy by Schuler et al. (2020). Although H33 is known from China (Shanxi, Shaanxi and Anhui Provinces; Zhu et al. 2016; Valentin et al. 2017; Cesari et al. 2018), Schuler et al. (2020) suggest that the movement and spread of this haplotype from the already-established population in Greece is more likely the source of H33 in Italy, especially given the prevalence and persistence of this haplotype in Greece (Gariepy et al. 2015; Morrison et al. 2017). The detection of H33 in Serbia in the present study also suggests movement and spread of H33 through secondary invasion from Greece; however, it is unclear whether it is due to passive dispersal or associated with commercial trade and travel (Konjević 2020). As the occurrence of *H. halys* haplotypes in most of the countries that share a border with Serbia and Greece has not yet been investigated, it would be important to determine the occurrence of this (and other) haplotype(s) in such locations where *H. halys* is also known to occur (Table 1), in particular Bulgaria, North Macedonia, Albania, Montenegro and Bosnia and Herzegovina. This may provide insight as to whether the distribution of H33 is widespread or continuous across the Balkan countries or whether it is primarily in urban centres associated with commercial trade and travel. All of our samples were collected in north and north-eastern Serbia (Fig. 1), which is bordered by countries where a single haplotype (H1) is known or dominant (e.g. Croatia, Hungary and Romania; Fig. 4) and where H33 and H80 have not been reported. The movement of H33 and H80 into Serbia is, therefore, unlikely from this direction – neither through natural dispersal nor from commercial trade or travel. As such, sampling in the southern portion of Serbia would provide a more thorough account of the haplotype distribution across the entire country and provide insight on the movement and spread of H33 and H80, particularly from countries along the southern border of Serbia. In contrast, the movement of haplotype H1 likely occurred from the spread of this haplotype from neighbouring countries to the west (Italy), north (Hungary) and/or north-east (Romania), where H1 is dominant. *Halyomorpha halys* populations in Serbia were first observed in areas near or along the Serbian-Romanian border (Šeat 2015) and in close proximity to the railway line that connects Bucharest, Romania with Belgrade, Serbia (Musolin et al. 2018). Thus, the trapping and interception records support the secondary invasion of H1 from neighbouring eastern European countries (as opposed to separate establishment events from China), possibly associated with railway travel or movement of commodities on railway cars, as suggested by Musolin et al. (2018). In terms of H3, which was dominant in Serbia, the prevalence of this haplotype in western Europe (in particular Switzerland, France and Austria), as well as the known presence (albeit at low levels) in Hungary, could indicate movement and spread from this direction. Similarly, H8 is the second most common haplotype in Switzerland and is also present (at lower levels) in France and Italy (Gariepy et al. 2014, 2015; Cesari et al. 2018; Schuler et al. 2020), suggesting the direction of movement of H8 is likely from western Europe to Serbia. In neighbouring Croatia, only H1 has been reported by Šapina and Jelaska (2018), but this is only based on two specimens; further analysis of samples in Croatia would help clarify the occurrence and diversity of additional haplotypes, in particular H3 and H8, which would be interesting in terms of evaluating the spread of haplotypes from this direction. Serbia is located directly at the centre of the invasive range of *H. halys*, surrounded by countries with different haplotype compositions. Although we cannot exclude the possibility of separate invasion(s) from China, the likely scenario (based on location, haplotype and trapping data) is that *H. halys* is entering Serbia from more than one direction simultaneously (through natural dispersal, via commercial/horticultural trade and/or travel). Although largely speculative, H1 may have initially arrived in Serbia from neighbouring countries to the east (possibly with additional invasions from other directions, given that surrounding countries all have a high proportion of H1), with H3 and H8 arriving from the western European countries and H33 and H80 from Greece and Italy.

The first established population of *H. halys* in the area of eastern Europe and central Asia occurred in Sochi City, Russia in 2013–2014 and at the time the pest was not present in neighbouring countries (see Table 1). The fact that this establishment was geographically disconnected from the rest of the invaded range in Europe suggests that secondary invasion from natural dispersal of the pest is unlikely (Musolin et al. 2018). Although the source of *H. halys* could be the result of a separate introduction from Asia, a secondary invasion via the bridgehead effect is more likely, based on the timing and location of the arrival of *H. halys* and the events surrounding its establishment. Musolin et al. (2018) suggest that the pest was accidentally introduced from Italy or Greece with infested plant material that was used in massive landscaping efforts associated with the 2014 Winter Olympic Games hosted in Sochi; plants from northern Italy were regularly imported in 2012–2013 due to a similarity between the climates of the two regions and low availability of local stock to meet the landscaping demands leading up to the Olympic Games. This coincides with the occurrence of high population levels of *H. halys* in northern Italy and reports of economic damage to crops (Pansa et al. 2013; Maistrello et al. 2014). The *H. halys* population in Italy is predominantly H1 in most regions, in particular Emilia Romagna and Lombardy (Cesari et al. 2015; Morrison et al. 2017; Cesari et al. 2018; Schuler et al. 2020). This information, combined with the fact that the present study demonstrated that H1 is the only haplotype in Russia (based on > 200 specimens), lends support to the theory proposed by Musolin et al. (2018) that *H. halys* in Sochi may have originated from locations in Italy where H1 is dominant. From the focal point of Sochi City in Russia, *H. halys* may have dispersed to other regions in the Caucasus. However, populations of H1 in some of these locations (e.g. Sevastopol), as well as neighbouring locations in Ukraine (Odessa) and Georgia (Abkhazia, Adjara and Samegrelo), could be due to the spread of *H. halys* from other locations associated with the movement of commercial goods, as all of these cities are important seaports within the region. A similar concern in Australia was also flagged as a threat, when a significant number of live *H. halys* were intercepted in shipments arriving from Italy; haplotype H1 and H23 were both identified from shipments originating from Italy (Horwood et al. 2019), demonstrating how easily secondary invasion could occur, even over long distances.

In Kazakhstan, *H. halys* was first reported in 2016 in Almaty and establishment was confirmed when populations continued to expand in the area in 2017 and 2018 (Esenbekova 2017; Temreshev et al. 2018). Although the present study consisted of a limited number of samples from Almaty, Kazakhstan (n = 11), two haplotypes (H1 and H3) were detected, with H1 being dominant. Almaty shares a border with Xinjiang Province in China; however, *H. halys* is not known to occur in Xinjiang or Qinghai (Yu and Zhang 2007), indicating that the source of this pest in Kazakhstan is not from natural dispersal near or along the Kazakhstan-China border. Similarly, Almaty is far removed from the distribution of *H. halys* in the Caucasus region, suggesting that natural dispersal from this region is not responsible for the occurrence of the pest in Kazakhstan. However, Almaty is the major commercial centre of Kazakhstan and, as such, we speculate that commercial trade (with China, Russia and/or other European countries) would be the source of H1 and H3 in this country. Kazakhstan has a relatively low number of invasive alien species currently recorded; however, the recent rise in international trade and oil, gas and mining development in Kazakhstan, Turkmenistan, and Uzbekistan will also likely result in an increase of invasive species in these countries (Turbelin et al. 2017). Given Kazakhstan's geographic location (i.e. directly between the invasive range of *H. halys* in Europe / Russia and the native range in China), its importance as the hub of international trade in central Asia and its position as a major transportation hub linking China to Russia and western Europe by air, rail, road and sea (Selmier 2020), we speculate that *H. halys* invasion from Europe, Russia and China are all very likely. Another invasive hemipteran, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae), was recently discovered in Kazakhstan (Barclay and Nikolaeva 2018) and is similarly far-removed from other known established populations. Its arrival is likely due to passive transportation of adults as stowaways in cargo or through nursery trade (Barclay and Nikolaeva 2018), which is likely the same pathway of entry for *H. halys* in this region. The collection and COI haplotype analysis of additional *H. halys* samples from this area would provide a more thorough documentation of the haplotype diversity in Kazakhstan. However, given its separation from the centre of other *H. halys* invasions in Eurasia and without interception records to corroborate potential pathways of entry, additional COI haplotype analysis will only tell us which countries have a similar haplotype composition and is unlikely to clarify whether the pathway of entry is directly from the native range in Asia or whether it is the result of a secondary invasion via Europe, Russia or some combination thereof (but see future directions below).

Future directions

The present study focused solely on the COI gene, as this gene has shown reliability in terms of revealing geographic patterning (O'Loughlin et al. 2008; Valade et al. 2009) and has been widely utilised in haplotype studies on *H. halys* and has the most comprehensive, publicly-available global haplotype network available for this species. However, more in-depth multilocus analysis, based on microsatellite DNA or high-resolution genomic data (e.g. single nucleotide polymorphisms, SNPs; restriction site associated DNA sequencing, RADseq), may reveal additional patterns regarding invasion pathways (Garnas et al. 2016; Sunde et al. 2020). As techniques for generating high-resolution genomic data become more mainstream in ecological studies (see Andrews et al. 2016), future research that makes use of these newer techniques to investigate the global diversity of *H. halys* may provide a more fine-tuned interpretation of patterns of dispersal, in particular in terms of dissecting pathways of entry from the area of pest origin versus movement and spread of already-established populations to new locations.

Conclusions

The results, presented here, provide haplotype coverage of previously uninvestigated or under-investigated regions along the easternmost front of the invasion of *H. halys* in Eurasia. The evaluation of *H. halys* haplotypes along the eastern front of the invasion in Eurasia demonstrates the continued spread and successful establishment of haplotype H1 in newly-invaded areas, where it is often the only haplotype. Secondary invasions within Europe are likely responsible for the movement and spread of additional haplotypes (e.g. H3, H33 and H80) that are moving beyond their first detection and establishment points. In addition, countries at the centre of the invaded range, such as Serbia, are proving to be more diverse due to multiple invasion events from neighbouring countries with differing haplotype compositions.

Several gaps remain to be filled to generate a more complete picture of the haplotype composition across this region. For example, additional collection and haplotype analysis of *H. halys* is necessary in countries where the pest is known to occur, but where large-scale haplotype analysis has not yet been done. Further, in some locations, more thorough collections are warranted in order to more accurately estimate the haplotype composition and diversity across the entire range of the pest (e.g. Kazakhstan, Ukraine and Croatia). Despite the presence of multiple haplotypes in the invasive Eurasian range, H1 is clearly dominant (Fig. 4) and associated with the majority of recent invasions along the eastern front of the spread of *H. halys* in these areas. A number of examples exist in literature where the range expansion of an invasive insect species is primarily associated with one mitochondrial haplotype (e.g. Grapputo et al. 2005; Dittrich-Schröder et al. 2018; Brookes et al. 2020; Machado et al. 2020), with some populations contributing disproportionally to global spread (Garnas et al. 2016). The same pattern is observed in the spread of *H. halys*; haplotype H1 is now known from a total of 18 countries outside of its native range (Table 4) and is the dominant haplotype in the majority of these countries (Table 5). Interestingly, reports of serious agricultural damage are known primarily from those countries where H1 is prevalent (e.g. Georgia, Greece, Italy, Russia and Turkey; Maistrello et al. 2017; Bosco et al. 2018; Musolin et al. 2018; Ak et al. 2019; Damos et al. 2020). This prevalence of H1 across Eurasia could be linked to high levels of traffic and commercial trade between these countries (i.e. global connectivity of non-contiguous areas; Garnas et al. 2016) facilitating the spread of an already-established, dominant haplotype through the bridgehead effect (Lombaert et al. 2010). In addition, climatic factors in some regions may permit more rapid population growth (and increased agricultural impacts) due to multiple generations of any given successfully-established haplotype (Musolin et al. 2019; Stoeckli et al. 2020). However, variation in performance traits in invasive insect species can be an important predictor of their success in establishment and spread across a broad geographic range (Thompson et al. 2021). Future research investigating the different *H. halys* haplotypes would be of interest to determine whether there is any validity to the observation that H1 appears to be a more successful invader and to determine whether this success is linked to intraspecific variation in biological traits, such as phenology, thermal performance, flight capacity, overwintering survival or fecundity.

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Supplementary material 1

Table S1. Collection information and GPS coordinates

Authors: Tara Gariepy, Dmitry Musolin, Aleksandra Konjevic, Natalia Karpun, Vilena Zakharchenko, Elena Zhuravleva, Luciana Tavella, Allison Bruin, Tim Haye

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DISCUSSION PAPER

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Advancing research on alien species and biological invasions

Some reflections on current invasion science and perspectives for an exciting future

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Abstract

Species spreading beyond their native ranges are important study objects in ecology and environmental sciences and research on biological invasions is thriving. Along with an increase in the number of publications, the research field is experiencing an increase in the diversity of methods applied and questions asked. This development has facilitated an upsurge in information on invasions, but it also creates conceptual and practical challenges. To provide more transparency on which kind of research is actually done in the field, the distinction between invasion science, encompassing the full spectrum of studies on biological invasions and the sub-field of invasion biology, studying patterns and mechanisms of species invasions with a focus on biological research questions, can be useful. Although covering a smaller range of topics, invasion biology today still is the driving force in invasion science and we discuss challenges stemming from its embeddedness in the social context. Invasion biology consists of the building blocks 'theory', 'case studies' and 'application', where theory takes the form of conceptual frameworks, major hypotheses and statistical generalisations. Referencing recent work in philosophy of science, we argue that invasion biology, like other biological or ecological disciplines, does not rely on the development of an all-encompassing theory in order to be efficient. We suggest, however, that theory development is nonetheless necessary and propose improvements. Recent advances in data visualisation, machine learning and semantic modelling are providing opportunities for enhancing knowledge management and presentation and we suggest that invasion science should use these to transform its ways of publishing, archiving and visualising research. Along with a stronger focus on studies going beyond purely biological questions, this would facilitate the efficient prevention and management of biological invasions.

Keywords
biological invasions; evidence-based management; grand unified theory; invasion science; open science; philosophy of science; social-ecological systems; theory development

Introduction

During biological invasions, organisms spread and establish outside their native range. These processes are investigated in a vibrant and still-growing research field, with the number of papers published in specific outlets, as well as in general ecological journals having increased exponentially during the past decades (Vaz et al. 2017; Cassey et al. 2018). Reasons for studying biological invasions are manifold. Invasive species are amongst the five most significant global drivers of biodiversity loss (IPBES 2019). Applied research is thus needed to deliver guidance for how to prevent further invasions and how to manage invasive species, where necessary. At the same time, biological invasions are highly interesting study objects, because they represent 'natural experiments', allowing the study of how species respond to novel biotic interactions and environmental conditions. Research in this field is challenging for many reasons, including the high complexity of factors influencing the process, amongst them ecological and evolutionary as well as social-economic feedbacks (Heger et al. 2013; Courchamp et al. 2016).

Several times, it has been questioned whether 'invasion biology' should be addressed as a discipline at all, the main argument being that the process of invasion does not fundamentally differ from other ecological processes as, for example, colonisation (e.g. Davis 2009; Valéry et al. 2013). In line with Blondel et al. (2014), we suggest that the ongoing increase in publications on biological invasions clearly demonstrates that a broad community of researchers disagrees with this argument and, actually, the field still grows and makes substantial progress (see, for example, Hui and Richardson 2017; Vaz et al. 2017; Wilson et al. 2020). In addition, the topic of biological invasions is central to biodiversity conservation (IPBES 2019) and has become an integral part of international policy (e.g. European Union 2014), underlining the societal need for a scientific discipline dedicated to it.

Due to the diversity of reasons for studying the phenomenon, as well as the high complexity of influencing factors, biological invasions are investigated in a multitude of different ways. This has substantially increased our knowledge about invasive species and their impacts, while the expansion of the field increasingly creates conceptual and practical challenges. For example, it is nearly impossible to keep track of all case studies that are published on the patterns and processes of biological invasions and improved efforts are needed to ensure that individual results become integrated into the body of theory (Jeschke and Heger 2018a). Given the breadth of topics and approaches and the rate at which new publications accumulate, gaining an overview of the field or even on the state of knowledge in some more specific sub-field is becoming difficult.

In light of these challenges, we suggest three topics that, from our point of view, need further consideration. First, we discuss the delineation of the field, recalling the previously-proposed distinction between 'invasion science' and 'invasion biology'. Second, we will discuss the structure of invasion biology and ask whether, given the increasing breadth and diversity of the field, there is a need for a unified theoretical framework. We will draw from recent publications in philosophy of science and argue that invasion biology may be a well-functioning discipline without one grand unifying theory, but that more integration, nevertheless, is desirable. Third, we will suggest future steps that could be taken to reach such integration, given the ongoing rapid technological advances and the current changes in the processes involved in scientific publication.

Invasion biology and invasion science

Research at the interface between nature and society

As one of many problem-orientated disciplines, invasion biology, just like ecology in general, is located at the intersection between nature and society (Fig. 1) and thus encompasses basic as well as applied research. In a review of 500 studies published in 2008, a large proportion (74%) covered basic ecological questions and had a focus on community ecology, biogeography, population biology, evolutionary biology or molecular ecology (Richardson 2011). Such studies aim at a mechanistic understanding of patterns and processes and can be classified as generating 'systems knowledge' (Richardson 2011, with reference to Kueffer and Hirsch Hadorn 2008). The high percentage of remaining studies in the dataset, however, focuses on the phenomenon of biological invasions from an applied perspective. In this sample, 14% of the studies aimed at clarifying conflicts of interest and values and perception of people ('target knowledge', Kueffer and Hirsch Hadorn 2008; Richardson 2011). These were, for example, studies on risk assessment or from the fields of environmental ethics or resource economics. The remaining publications in the dataset aimed at finding appropriate actions for management ('transformation knowledge'), stemming, for example, from restoration ecology.

Studies creating target knowledge and transformation knowledge are clearly outside the realm of ecology as a natural science. Consequently, Richardson (2011) suggested distinguishing between 'invasion ecology' as the "study of causes and consequences of the introduction of organisms to areas outside their native range" and 'invasion science', describing the "full spectrum of fields of enquiry that address issues pertaining to alien species and biological invasions" (Richardson et al. 2011). The term 'invasion ecology' is often used interchangeably with 'invasion biology'. We regard ecology as a sub-discipline of biology and, therefore, prefer 'invasion biology' as the broader term, explicitly including, for example, evolutionary and genetic topics as well.

In the following, we will argue that the distinction between invasion biology and invasion science can still be helpful today, as it stresses the difference between studies focusing on biological research questions and other fields of enquiry. It can, thus, contribute to more transparency concerning which kind of research is actually done in the field and thus has the potential to enhance the diversity of research approaches.

Invasion science: biological invasions as processes affecting and effected by social-ecological systems

For a long time in ecology and biological conservation, humans have been treated as apart from natural processes (Mace 2014; Inkpen 2017). During the past decades, however, this mindset has largely changed. Today, ecology is no longer focused on studying only systems ‘untouched’ by humans, but instead, sub-disciplines are thriving that explicitly focus on ecosystems influenced by humans, like urban ecology, global change ecology or indeed invasion biology. Conservation today focuses on ‘people and nature’ (Mace 2014) and relational approaches to environmental ethics are gaining momentum as well (Chan et al. 2016; Eser 2016; Klain et al. 2017; Himes and Muraca 2018). Consequently, an increasing number of authors call for more explicit consideration of the effects of society on patterns and processes in nature and the creation of closer links between ecological and social sciences (e.g. Díaz et al. 2015; Ellis 2015; Perring et al. 2015). In the Anthropocene, human activities affect every ecosystem and it is argued that, in order to understand current ecological patterns and processes, the environment has to be viewed and studied as coupled social-economic and ecological systems (Ostrom 2009; Collins et al. 2011).

Biological invasions are providing prime examples for the multiple ways in which ecological processes and human activities are influencing each other (McNeely 2001; Kueffer 2017). Social-economic activities are strongly affecting invasion processes in many ways and only since this crucial fact has been taken into account (see, for example, Hulme 2009) has it become possible to develop efficient measures for preventing and managing invasions, for example, by tackling major introduction pathways. In an encompassing literature review, Vaz et al. (2017) demonstrate that publications on biological invasions formerly used to report purely ecological research, but since the 1990s and 2000s, social and socio-ecological research on biological invasions has gained importance.

Connections to social sciences are, for example, sought with the aim to enhance the process of evaluating invasive species (e.g. Bacher et al. 2018; Shackleton et al. 2018). Interdisciplinary teams are formed, for example, to study the spread of acacias, taking into account not only ecological, but also historical, political, ethical and aesthetic aspects (Carruthers et al. 2011).

Moreover, several authors meanwhile made concrete suggestions for addressing biological invasions as processes happening within social-ecological systems. Drawing from methods developed in complexity science, Hui and Richardson (2017) explore how invasion science could profit from treating invasion syndromes as complex adaptive systems – as “dynamic systems comprising multiple interacting parts that can adaptively and collectively respond to perturbations” (p. 268). Here, human beings and their agency are considered part of a network and this method would allow taking into account the complex interactions and feedback loops tying together invading species, invaded ecosystems and social-economic systems. In a similar way, Sinclair et al. (2020) suggest subdividing the invasion process into three ‘coupled human and natural systems’ (CHANS), each describing a specific feedback loop interlinking the fate of invading organisms with human activities during specific sections of the invasion process.

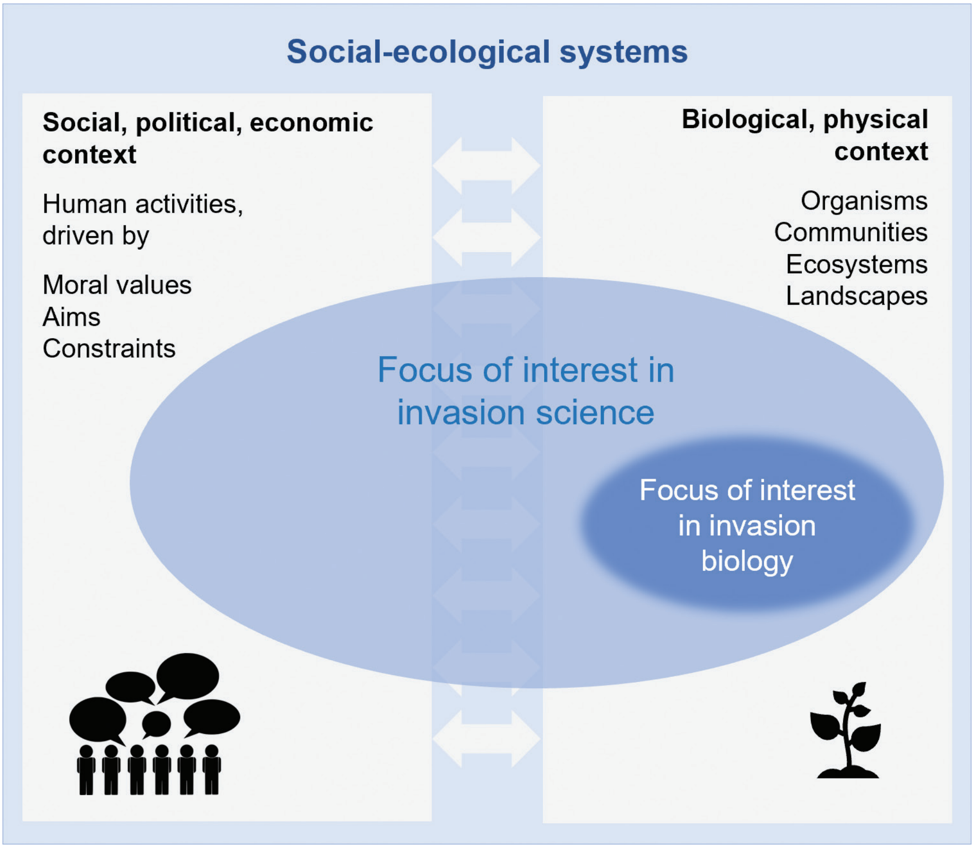

Figure 1. Research on biological invasions is located at the intersection between natural sciences (biological and physical context) and the social sciences and humanities (social, political and economic context). The concept of 'social-ecological systems' (outer light blue box) emphasises that both realms are closely connected, with human activities affecting organisms, communities, ecosystems and landscapes and vice versa. Invasion biology addresses biological questions about patterns and mechanisms of invasions and, thus, has a focal interest in the biological and physical context. The broader field of invasion science contains research analysing patterns and mechanisms of invasions from a social-economic point of view, effects of invaders on people's values and perspectives and many other, non-biological aspects of species invasions.

To give a more concrete example, Ferreira-Rodríguez et al. (2019) applied the interdisciplinary and integrative social-ecological systems framework developed by Ostrom (2009) to analyse the introduction and dispersal of Asian clam *Corbicula fluminea* in Spain. This framework uses a combination of methods from natural sciences (i.e. sampling water bodies) and the humanities (semi-structured interviews) and considers social, ecological, economic and governance subsystems. This way, the authors are able to demonstrate that the distribution of the Asian clam is statistically related, not only to ecological factors as, for example, water temperature, but also to socio-economic variables like education level, the industrial productivity index and the number of NGOs in the region. These findings may help to adjust management and policy actions.

Approaches like these, leaving the realm of pure ecological or biological research, are promising and might be the best choice, especially for finding ways to prevent and manage invasions. However, with their literature review, Vaz et al. (2017) found that, out of more than 9,000 publications addressing biological invasions since the 1950s, 92.4% focused on purely ecological questions. A potential reason for this observation is that research crossing disciplinary boundaries is challenging and there is a lack of regular interaction of the respective peer groups. This is an observation not only made by social-ecological scientists (Ostrom 2009), but also by philosophers: Millgram (2015) argues, for example, that we are living in an age of 'hyperspecialization' and everyone outside of their own field of expertise tends to be a logical alien. Specifically, we are not familiar with the standards and procedures of neighbouring fields and guidance is usually missing on how to apply methods we are not trained to use, how to interpret data that take different forms than we are used to and how to assess results derived with these methods and data (see also Jeschke et al. 2019b). Therefore, a current challenge of invasion science is to increase efforts in overcoming these boundaries and to develop into a truly interdisciplinary field.

Invasion biology: natural science embedded in a societal context

The usefulness and necessity of interdisciplinary studies does not preclude the need for studies focusing on biological research questions (Collins et al. 2011). Basic ecological and evolutionary mechanisms underlying the establishment and spread of species need to be better understood to allow accurate predictions and more efficient management, including the importance of species interactions in hindering establishment or the effects of novel interactions on trait evolution; this is the core of invasion biology (Fig. 1). Richardson et al. (2011) originally defined invasion science as the "full spectrum of fields of enquiry that address issues pertaining to alien species and biological invasions" and invasion biology as the "study of causes and consequences of the introduction of organisms to areas outside their native range". Many significant causes and consequences, however, are closely linked to the societal, political and economic context. We suggest that instead, invasion biology could be defined as the study of patterns and mechanisms of species invasions with a focus on biological research questions. Invasion science is the overarching research area that includes invasion biology and, additionally, amongst others, the study of species invasions as social-ecological phenomena, focusing on social, political and economic processes and their interactions with biological invasions. The broad discipline of invasion science can and should involve the integration of knowledge and methods developed in non-biological disciplines (Fig. 1).

Invasion biology in this sense is studying organisms, communities, ecosystems, landscapes and biomes, typically with a focus on ecological and evolutionary questions. It aims, for example, at explaining how invaders change species interaction networks or at predicting which species compositions increase the probability for invasion. Human activities are important here because their effects on the biophysical context are nearly ubiquitous and, thus, are inseparable parts of the study objects. For answering a biological question (for example, about the interaction of two species), however, it is not necessary to study human activities themselves nor their causes (see also Gounand et al. 2018). With its focus on biological research questions, invasion biology usually does not need to directly incorporate knowledge and methods from the social sciences or humanities.

Nevertheless, invasion biology still is embedded in a social context (Fig. 2) – it is affected by and has effects on society. For example, the social context of a study (e.g. country, lab, knowledge of the principal investigator) influences the research focus, the choice of the study system and methods, as well as the focus of analyses, communication of results and decisions to take management action (inward blue arrows in Fig. 2) (see, for example, Schurz 2014, p. 41–44). Conversely, the outcomes of scientific studies affect opinions and decisions, within invasion biology as well as in society at large (outward blue arrows in Fig. 2).

This embeddedness of invasion biology in a societal context leads to complex relationships between facts and values (Justus 2013). Biological research on invasions is often linked to societal values and goals (Backstrom et al. 2018). This can be problematic, as a common conception of good scientific practice posits that science should be performed objectively. Scientific research should only describe the facts as observed, while deriving value judgements (i.e. a situation is good or bad) or normative claims (i.e. an action is right or wrong) is outside the realm of scientific practice. It is known from philosophy of science that, during the planning of a research project, as well as during the subsequent phase of utilising the results, it cannot be avoided that value assumptions stemming from society have an effect. In invasion biology, for example, researchers prefer studying those species with a strong impact over those that have less impact (Pyšek et al. 2008). Such societal influences create biases that need to be accounted for; but the respective studies themselves can nevertheless represent sound and solid science. During the phases of generating and testing hypotheses and gathering data (i.e. the context of justification), care has, thus, to be taken to avoid that fundamental value assumptions influence the process (Schurz 2014). Otherwise, a statistical negative correlation between the number of native and alien species could, for example, be misinterpreted, leading to false conclusions about underlying causes of observed patterns.

The influence of implicit values on research in invasion biology has been discussed within the discipline (e.g. Larson 2005; Colautti and Richardson 2009). Still, the challenge persists and, for instance, a recent literature survey showed that invasion biology uses militaristic language more frequently than research on other topics in ecology and conservation biology (Janovsky and Larson 2019). Given this observation, it is comforting that, in philosophy of science, there are alternative opinions as well, suggesting that, especially in disciplines driven by ethically relevant questions, values and facts are so closely intertwined that a proper separation is not possible (see Justus 2013 for a review of this discussion). However, we believe there is no question that, in invasion biology, the ideal of objectivity should be pursued during data gathering, analysis and interpretation.

The relationship of invasion science and invasion biology as sketched in Fig. 1 suggests that, given the much broader coverage of invasion science, the majority of

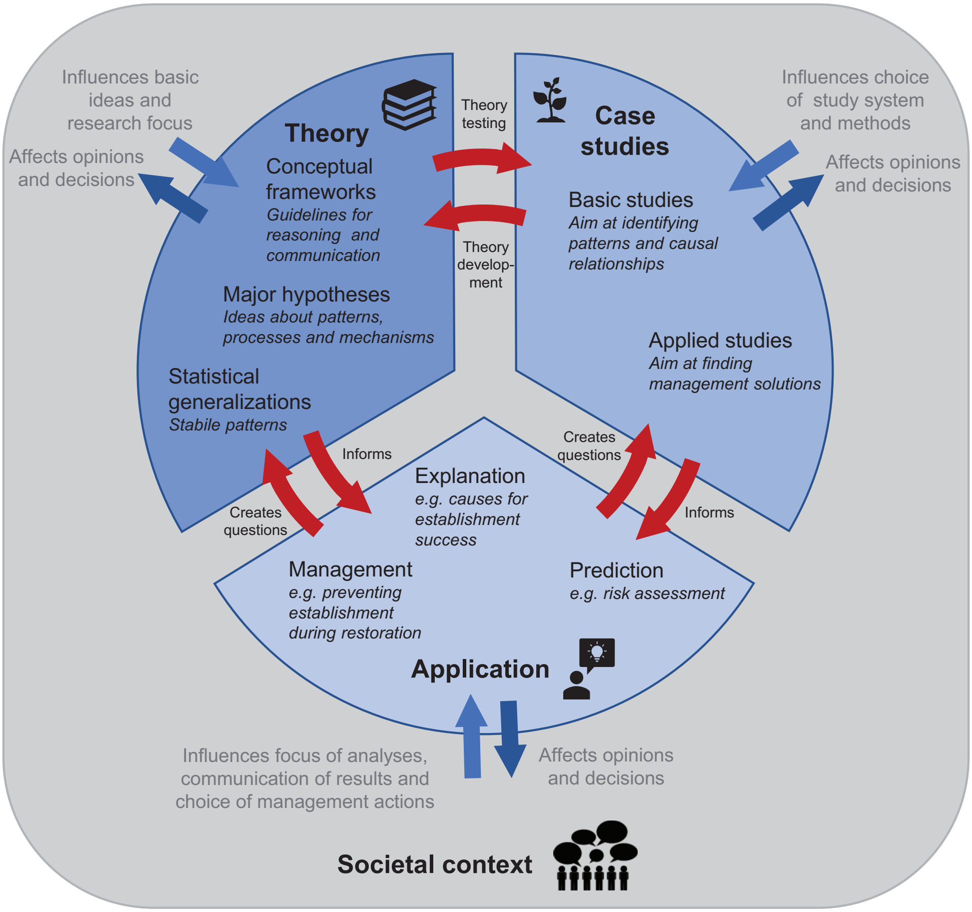

Figure 2. Invasion biology, with its building blocks theory, cases studies and application, is embedded in a societal context. The red arrows show how theory, case studies and application affect each other; the blue arrows depict effects of society on invasion biology and vice versa. The lighter colour of the block 'application' indicates that also non-biological questions beyond invasion biology, as defined above, are addressed here – these are part of invasion science (see Fig. 1).

studies in this field should lie outside of the narrow range of topics covered by invasion biology. The results of Vaz et al. (2017), however, demonstrate the opposite. We agree with Vaz et al. (2017), Hui and Richardson (2017) and others that invasion science can profit from focusing research much more on questions outside of invasion biology. Embracing approaches like network theory (see also Frost et al. 2019) or complex adaptive system modelling and framing invasions from a social-ecological perspective, could strongly aid explanation, prediction and management of invasions. However, we also think that it is useful to keep in mind that there is and, probably, always will be, a sub-field in invasion science that focuses on basic biological research questions and that the broad field of invasion science can profit from such studies as well. Given that invasion biology still represents the core of invasion science, we will now take a closer look at this field.

The role of theory in invasion biology

The structure of invasion biology

A major building block of invasion biology is theory, consisting of conceptual frameworks, statistical generalisations and major hypotheses (Fig. 2). Two other important building blocks are case studies and applications. Being an empirical natural science, the discipline relies on observations and experiments; case studies, therefore, are indispensable elements, delivering a broad and solid basis for knowledge gain. Most publications in invasion biology report on such case studies, i.e. evidence collected in field surveys, common gardens, greenhouses or lab facilities or based on mathematical models (which often are also part of theory). They can either have the aim to identify patterns and causal relationships, thus contributing to answering basic questions or they can aim at identifying management solutions.

According to a classic idea of scientific progress, the main purpose of cases studies is to test specific elements of theory. Indeed, many studies in invasion biology do so, i.e. they test ideas that are grounded in theory (Fig. 2). A prime example are studies that test specific hypotheses in invasion biology (see Jeschke and Heger 2018a), as, for example, the enemy release hypothesis. However, case studies do not regularly test single well-defined hypotheses or other elements of theory. This is a fact that has been noticed by philosophers of science for other parts of biology as well. For example, Elliott (2019) observed that research often focuses on addressing problems (for instance, species X invades a community containing endangered species) rather than testing theory (see also Love 2008) and each research problem can invoke a range of research questions. This conception of science seems to be well in line with the practices of invasion biology and philosophical studies indicate that research directed at addressing problems is not scientifically inferior to research testing hypotheses or other elements of theory.

The knowledge gained in case studies and through theory development can be applied in various ways. With respect to invasion biology, application can mean to use the knowledge for preventing and managing species invasion. In addition to such practical application, new knowledge can be used for prediction and explanation. Explanation is often an implicit part of case studies. An empirical project typically starts with a question or hypothesis, conducts an experiment or survey, analyses the data and then uses the results to explain the observed patterns in the light of theory. If multiple case studies are synthesised, the aim usually is to find explanations that are more broadly applicable; and ideally, these can be used to derive predictions by extrapolating or transferring the insight to other situations.

The abovementioned building blocks (theory, case studies, application) can be linked in various ways (red arrows in Fig. 2): theory and case studies can deliver the knowledge base for application and the three forms of application (explanation, prediction, management) can deliver questions that generate the motivation to perform case studies and develop theory. Theory creates research questions and elements of theory can be empirically tested in case studies. On the other hand, the insight gained from case studies can be used to develop theory.

Is there a need for a grand unified theory of invasion biology?

Theory in invasion biology can take the form of conceptual frameworks, statistical generalisations and major hypotheses (Fig. 2). A recent special issue in this journal provides an overview of conceptual frameworks that are being used in invasion science (Wilson et al. 2020). The 24 contributions demonstrate the usefulness of these elements of theory for research, policy and management. Other work has demonstrated the richness of major hypotheses formulated in invasion biology and has made efforts to show the level of empirical support and their connectedness (Jeschke and Heger 2018a; Enders et al. 2020; Jeschke et al. 2020). These efforts underline that the discipline contains and is based on a well-developed body of theory. It may be asked, however, whether this theory is sufficiently well integrated. The term 'theory' is often used to describe a concise, unified, general framework, analogously to the 'grand unified theory' in particle physics that provides a strong knowledge base in a research field. The question is whether invasion biology has, or will ever have, such a kind of theoretical basis.

In the late 20th century, ecology picked up physics as a role model (Trepl 1987) and philosophy of science commonly praised this discipline as the prime example of how to conduct scientific research. Consequently, the claim was that every proper branch of science should strive for developing a grand unified theory. However, it became increasingly obvious that not all scientific disciplines can be compared to physics and that the development of a grand unified theory may not be a common goal. In philosophy of science, an argument is gaining momentum which posits that, in the so-called special sciences, such as biology, the high complexity of the study objects and high context-dependency of processes make the search for universal laws and a unified theory difficult or even impossible (Reutlinger et al. 2019) and that a discipline can very well produce fruitful results without having a unified theory (Love 2014).

Invasion biology seems to be such a discipline. It does not have one concise unified theoretical framework, but is still based on a substantial body of theory (see, for example, Catford et al. 2009; Enders et al. 2020; Wilson et al. 2020). The absence of a unified theory that can comprehensively explain the phenomenon of invasion and guide research has been regarded as a deficiency of the field by invasion biologists, as well as critics of the discipline (see, for example, Richardson et al. 2008) and there have been calls for developing a "broadly applicable conceptual framework grounded in basic principles of ecology and evolutionary biology" (Gurevitch et al. 2011, p. 407). From recent philosophical studies, we conclude, however, that the search for a unified theory, for 'basic principles' or for an extensive explanatory framework is probably not the most efficient way forward for invasion biology (Love 2014; Elliott-Graves 2016). Theory here, as well as in ecology in general, can rather be viewed as an "ever-changing, context-dependent, collective construct" (Travassos-Britto et al. 2021b) and, as such, is suited well to guide research and build knowledge. Striving for extensive synthesis, by contrast, carries the danger of over-generalisation and of sacrificing too many of the details that are required for truly enhancing explanation, prediction and management (Elliott-Graves 2016).

Even if we conclude that the search for a unified general theory is not a useful aim for invasion biology, this does not mean that integration and synthesis is useless. We suggest the opposite: invasion biology needs more integration and synthesis. The aim, however, should not be to strive for a single general framework or (mathematical) theory that explains everything, but to explore novel ways for integration that allow for plurality and consider the context-dependency of invasions.

The development and harmonisation of conceptual frameworks seems to be a useful way forward. Frameworks have the aim to organise knowledge and can function as guidelines for research and communication. Notably, most of the established frameworks in invasion biology have a focus on classification and description, often in a management context. For example, of the 24 papers included in the already-mentioned special issue (Wilson et al. 2020), only five discuss frameworks with regards to causes and mechanisms of invasions (Hulme et al. 2020; Liebhold et al. 2020; Pyšek et al. 2020; Robinson et al. 2020; Sinclair et al. 2020). This seems to demonstrate that the focus of theory development in the field currently is on producing knowledge useful for application. We suggest that, in order to improve the mechanistic understanding of biological invasions, it is important to foster the development and harmonisation of frameworks addressing causes and mechanisms of invasions as well.

In addition, we believe that invasion biology could profit from a more explicit consideration of how knowledge is generated and from systematically analysing its conceptual basis (see suggestions in Travassos-Britto et al. 2021a; Travassos-Britto et al. 2021b for ecology). Further philosophical analyses of the research practices in invasion biology could help to identify weaknesses in current methods and strategies and could, thus, facilitate methodological improvement. There is a rising interest of philosophers of science in ecology and also invasion biology (e.g. papers cited here and Elliott-Graves 2016; Bausman 2019; Elliott-Graves 2020; Justus 2021) and we should seize this opportunity to build sustainable collaboration, based on an interdisciplinary research agenda, involving invasion biologists and philosophers.

The future of invasion science: opportunities abound

Evidence-based management

A multitude of different methods, ranging from field surveys and experiments to molecular studies and mathematical models are used to address various basic and applied questions in invasion biology. The majority of studies in invasion biology focus on terrestrial plants (Pyšek et al. 2008; Jeschke and Heger 2018b), but even within this group, research approaches are quite diverse. This diversity is necessary to address the entire range of invasion cases and processes involved. It creates the challenge, however, how this wealth of information can be efficiently used for improving theoretical foundations and practical applications.

In ecology, there have been several initiatives to synthesise evidence from empirical studies to allow for efficient, evidence-based conservation (www.conservationevidence.com) and environmental management (www.environmentalevidence.org; see also www.eklipse-mechanism.eu and Nesshöver et al. (2016)). Studies contributing to these initiatives provide guidance for policy decisions and local management and favoured tools are, for example, systematic reviews and statistical meta-analyses, following specific protocols. Species invasions are one out of many topics addressed in these initiatives, but are currently the focus of relatively few synthesis studies; for example, only six systematic reviews out of more than one hundred at www.environmentalevidence.org (search date: 17 May 2021). Systematic reviews and meta-analyses are regularly undertaken in invasion biology, but such studies rarely aim at evidence-based management. A notable exception is the ongoing IPBES assessment on invasive alien species. It is possible that a limited awareness of evidence-based conservation portals in invasion biology or a lack of awareness of meta-analytical methods in researchers interested in application are reasons for this shortcoming. Evidence-based management has much potential for invasion biology and we urgently suggest a more regular use of the available tools and platforms. The management of invasive species is a dynamic research field, as exemplified by the successful bi-annual 'Conference on Ecology and Management of Alien Plant Invasions' (EMAPI) (Pyšek et al. 2019). Making evidence-based management a prominent approach in this field would, for sure, increase the chances for efficient prevention and mitigation.

Enhancing research in the broader field of invasion science

Evidence-based invasion management would become an even more promising approach if human-environment interactions were a regular research topic in invasion science. Modelling invasion syndromes as adaptive cycles or as complex networks including humans as actors has a strong potential to enhance predictability in invasion science (Hui and Richardson 2017). The development and implementation of efficient management, on the other hand, could profit from close cooperation with diverse stakeholders right from the onset (including the design) of a study. This aim could be reached by establishing long-term and reciprocal interactions of invasion scientists and diverse stakeholders (Vaz et al. 2017).

A significant increase in interdisciplinary research is needed, as invasion biological studies with a focus on biological questions will not suffice for facing the diverse challenges posed by biological invasions. Vaz et al. (2017), therefore, suggest the formation of "research teams comprising a balanced pool of social scientists (including scholars from the humanities) and ecologists (and other natural scientists)". We agree with this prospect.

Efficient theory development

A more philosophical, general problem is how to utilise empirical results for theory development. As indicated above, case studies are not necessarily linked to a specific element of theory, but even if they are, their interpretation is not always straightforward. Is a single negative test result sufficient to discard an entire major hypothesis? According to an interpretation of the 'hypothetico-deductive method' based on Popper (1935), which is still rather prominent in ecology (e.g. Farji-Brener and Amador-Vargas 2014), discarding the hypothesis would, indeed, be the best option. Actual practice in ecology, as well as contemporary opinions in philosophy of science, however, do not follow such a strict approach of naïve falsificationism (Andersen and Hepburn 2016). It is a standard requirement for every scientific study that results are carefully discussed, considering results of studies performed in other systems or with different methods. A single negative result will, therefore, usually not be used as an argument to discard a major hypothesis in its entirety. Additionally, it is a standard problem for invasion biologists (and ecologists in general) that empirical studies deliver mixed results. Systematic reviews and statistical meta-analyses are used to deal with these challenges; they require, however, a minimum amount of methodological homogeneity that is not always given in a focal set of empirical studies and can have other challenges (de Vrieze 2018; Heger and Jeschke 2018b).

In addition to methodological heterogeneity, a challenge for synthesising the results of single cases studies is the high complexity of potentially relevant factors driving observed patterns. In the past, a general strategy to deal with the high complexity of interacting factors has been to focus on single factors. Explanation, prediction and management, however, will certainly profit from including more complexity. Respective suggestions have been repeatedly made in invasion biology (e.g. Heger 2001; Pyšek et al. 2020). We suggest that research at the interface of invasion biology, ecology and philosophy of science is needed to improve and implement these ideas and to develop further novel, innovative approaches for efficient theory development that considers complexity (e.g. Heger and Jeschke 2018a; Heger et al. 2021; Schurz 2021). Methods and tools are needed that explicitly consider what has been called 'causal heterogeneity' in philosophy of science, i.e. the fact that, in invasions, ecological entities can have different ways of causing invasions, depending on the situation (Elliott-Graves 2016). A promising way forward could be to defer the search for general patterns and mechanisms that can be found across systems and situations and, instead, focus on how the results of case studies could be used to delineate classes of cases in which there is causal homogeneity and where similar mechanisms apply. This approach could offer a way to balance the need for integration and synthesis with the necessity to account for complexity. Novoa et al. (2020) recently suggested the systematic identification of invasion syndromes, which they define as "a combination of pathways, alien species traits and characteristics of the recipient ecosystem which collectively result in predictable dynamics and impacts and that can be managed effectively using specific policy and management actions". Such a search for recurring patterns could indeed foster the establishment of effective management priorities.

It could be highly rewarding to additionally develop methods that allow for the identification of recurring causal patterns, thus fostering improved possibilities for mechanistic explanations. Parreño et al. (2021), for example, recently suggested a novel meta-analytical method for identifying persistent causal relationships. They took information on the statistical analyses used in a set of biodiversity-productivity studies to infer (backwards) which causal relationships the respective studies have hypothesised. Thus, they identified commonly addressed hypothetical causal relationships, i.e. recurring patterns of hypotheses about causes. They concluded that, so far, data were still too sparse to allow for conclusions on actual recurring causal patterns on biodiversity-productivity relationships; but this method is a promising way forward.

Theory development could also be enhanced by fostering closer connection amongst fragmented elements of theory. For example, it has been suggested to demonstrate links and overlaps of established invasion frameworks by arranging them in a hierarchical way, thus creating a 'hierarchy of invasion frameworks' (Wilson et al. 2020). Ideally, the resulting structure would not only be published as a figure in a publication, but also as an interactive online tool, thus utilising advances in computer sciences and related fields (cf. https://hi-knowledge.org/). Novel developments in various research areas, including network theory (Hui and Richardson 2019), statistics and computer science (e.g. open access data aggregation, machine learning, semantic modelling), are being increasingly utilised in ecology (Algergawy et al. 2020; Heberling et al. 2021). We suggest, however, that much more potential lies in these advances and even more effort should be made to harness these developments for invasion science.

Enhanced knowledge management and presentation

Technological advances in computer science in addition provide innovative tools for visualising knowledge (Börner 2014; Kraker et al. 2016). For obtaining a first overview of a research field, the traditional approach is to search for textbooks summarising the state of knowledge or to use search engines like Google Scholar, Web of Science or Scopus. Textbooks, however, are outdated quickly and often do not perfectly match the specific interests of researchers. The existing scientific search engines deliver up-to-date information and can be adjusted to users' specific needs; to process their output, however, is a challenging and time-consuming effort. In addition, the more professional services are usually behind a paywall, hindering research for those without access to these services.

An openly-accessible, searchable knowledge base for invasion biology that provides search outputs in an intuitively structured way would, therefore, be a major achievement (Jeschke et al. 2021). Ideally, this tool would allow customised searches and interactive displays of search results, with direct links to the respective publications and underlying data. There is a growing number of databases in the field of invasion science, for example, the Global Invasive Species Database (http://www.iucngisd.org/gisd/), the Global Naturalized Alien Flora database (https://glonaf.org/), the Global Alien Species First Record Database (https://dataportal.senckenberg.de/dataset/global-alien-species-first-record-database) or the European Alien Species Information Network (https://easin.jrc.ec.europa.eu/easin) (see also Essl et al. 2015). These services provide valuable data on specific alien species and are a very good basis for comparative analyses. We suggest that, in addition, online tools are needed that provide an overview of the field and deliver theoretical background information; they should also provide information about which major research questions and hypotheses have been empirically addressed for which taxonomic groups and realms and to what degree hypotheses have received empirical support. A tool that could deliver such kind of information is not necessarily an idealistic vision that will never be realised. First suggestions, making use of advances in data visualisation, machine learning and semantic modelling, are already being developed (Jeschke et al. 2021).

In addition to the recent technological advances providing the respective technical possibilities, the ongoing shift in scientific publication practices could also turn out to be facilitative for developing such tools. Calls for openly-accessible data and publications are gaining momentum (Wilkinson et al. 2016; Jeschke et al. 2019a) and services like pre-print servers and public data archives are added to the traditional portfolio of scientific work output. The traditional way of publishing results as a journal paper are increasingly supplemented by other approaches (see Auer 2019). Instead of sifting through high numbers of PDFs for finding those studies that match a certain research question, invasion scientists in the future should be able to utilise powerful tools like knowledge graphs, in which smartly developed algorithms collate the available information in visually appealing and easily understandable ways.

In conclusion, we believe that exciting developments are under way and we hope that our contribution stimulates efforts to seize these upcoming opportunities. Respective projects would require teaming up with experts from other disciplines, but the results would certainly make up for the effort such a crossing of disciplinary boundaries demands.

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COMMENTARY

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Advancing research on alien species and biological invasions

Neonatives and translocated species: different terms are needed for different species categories in conservation policies

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Pervasive human-induced environmental changes are increasingly causing species to move, with profound implications for their conservation and survival (e.g. Chen et al. 2011; Dawson et al. 2011). In a recently published piece on "Global policy for assisted colonization of species", Brodie et al. (2021) call for assisted colonisation (also called managed relocation) to be embraced as a viable management option in post-2020 global conservation policies. They suggest that species, deliberately introduced beyond their historic native range and species that expand their ranges on their own in response to climate changes, should be treated identically for the purposes of policy. They also suggest expanding the use of the term "neonative" – which we previously proposed for range-expanding species that track environmental changes without human assistance (Essl et al. 2019) – so that this term also applies to species targeted for assisted colonisation.

Table 1. Ten key features and associated differences between range-expanding species tracking environmental changes (= neonatives) and species moved purposefully to regions outside their native range in response to (anticipated) environmental changes (= assisted colonisation or managed relocation). The main conservation and challenges associated with the features are shown.

No	Key features	Neonatives	Conservation challenges and implications	Assisted colonisation	Conservation challenges and implications
1	Number of individuals typically involved	(Very) large	No specific conservation challenges	(Very) small	Ensuring that founder populations do not go through genetic bottlenecks
2	Number of species involved	Often large to very large	Monitoring the effects of range-expanding species on resident biota	One or few	Selection of priority species (incl. the potential that translocated species might become invasive), monitoring the effects of translocated species on resident biota
3	Characteristics of species involved	Wide range of species, particularly mobile species and generalists	Applying management measures to ensure survival of less mobile species and of specialists	Charismatic, large, conspicuous species	Identification of alternative conservation options for the vast majority of biota that cannot be realistically translocated
4	Range expansion is reactive or proactive to environmental changes	Always reactive, i.e. species are responding to environmental change that has already occurred	No specific conservation challenges	Reactive or proactive (in anticipation of expected environmental change)	Taking uncertainty of future environmental changes into account
5	Source regions of individuals involved in range expansion	Leading range edge	No specific conservation challenges	Anywhere, often current centres of occurrence	Ensuring that suitable ecotypes of the translocated species are chosen
6	Form and distance of range expansion	Wave-like range expansion from current leading edge to adjacent regions that have become suitable	Improving landscape permeability	Jump dispersal, places of translocations are often distant and disjunct to the native range	Identifying suitable places of release with high likelihood of establishment and low risks of negative impacts
7	Velocity of range expansion	Variable, depending on characteristics of the species, the landscape (e.g. permeability) and the velocity of environmental change	Improving landscape permeability	Abrupt, depending on human activity (i.e. introduction of individuals or propagules to the site of release)	Apply an exhaustive ex ante risk-assessment prior to species translocation
8	Degree of ecological novelty associated with the range-expanding species	Typically low, but with exceptions (e.g. if range-expanding species have novel traits)	Monitoring the impacts on resident biota and potentially managing if negative impacts are observed	Variable, but often high as distances to native range are often large	Monitoring the impacts on resident biota, potentially managing if negative impacts are observed
9	Direct resources involved	Low to non-existing	Typically no resources are directly needed, but potentially for monitoring, or management (e.g. increasing landscape permeability)	Medium to high	The planning, execution and monitoring of translocations requires (substantial) resources
10	Connectivity of native range and newly colonised region	High, newly colonised regions are usually adjacent to (leading edge of) native range	No specific conservation challenge	Low, places of translocations are usually distant from the native range and separated by unsuitable regions in between	Identifying suitable places of release with a high likelihood of establishment and low risks of negative impacts

We recognise the need to proactively consider the opportunities and risks of species translocations as a key tool in policies and management in the Anthropocene. However, we agree with Ricciardi and Simberloff (2021) and consider it crucial to treat distinct phenomena and different categories of species of conservation concern differently in policies. Very careful attention must be given to the precise definition of core concepts and terminology. Human-induced translocations differ from range-expanding species (i.e. 'neonatives' as we defined them in Essl et al. 2019) in key aspects (Table 1), which makes lumping these two categories of species highly problematical with regard to fundamental features that relate to policy. These aspects include dispersal potential, the rate and direction of range expansions, the number and characteristics of species involved and the associated risks and uncertainties. Whereas species targeted for assisted colonisation are currently a limited number of charismatic taxa (Hällfors et al. 2017), range-expansions by "neonatives" (as in our definition) involve a wide range of biota (Essl et al. 2019), some of them with a great potential to spread. Additionally, the risks and benefits associated with the two phenomena differ (IUCN 2013; Ricciardi and Simberloff 2014). Finally, although human decisions on whether or not to move species are pivotal in assisted colonisation (Richardson et al. 2009), this is not the case for species undergoing range expansions independently of direct human action. For the latter, measures to preserve or restore connectivity are most relevant (e.g. Wessely et al. 2017). Consequently, these profoundly different key characteristics of range-expanding species tracking environmental change vs. those subject to assisted colonisation result in very different conservation challenges (Table 1).

We call upon conservation bodies, such as the IUCN and the Convention of Biological Diversity (CBD), to evaluate the full range of conservation opportunities and risks created by species on the move. These efforts should recognise the profoundly different nature of translocated species and those undergoing range changes due to global change, but without direct human assistance. We are convinced that only such a nuanced approach will lead to appropriate conservation action to ensure species survival in the Anthropocene. We argue that species selected for assisted colonisation are a distinct category that should be subject to exactly the same classification as all other species. As they are introduced purposefully outside their natural range, they should be considered as aliens. The protocols for evaluating associated risks are well established (Richardson et al. 2009; Karasov-Olson et al. 2021). However, given that translocated species also differ in some important characteristics from other alien species, it may be warranted to classify these species in a distinct (sub)category.

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RESEARCH ARTICLE

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Advancing research on alien species and biological invasions

A global assessment of the potential distribution of naturalized and planted populations of the ornamental alien tree *Schinus molle*

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Abstract

The Peruvian Peppertree (*Schinus molle* L.) is an evergreen tree native to semiarid environments of Peru and Bolivia in South America. This tree has been introduced and widely planted for ornamental and forestry purposes in several semiarid regions of the world because its seedlings are easily established and have a high survival rate; it also grows quickly, and it is tolerant of dry climates. We compared the global and regional niches of naturalized and planted populations of *S. molle* in order to examine the invasive stages and potential distribution of this species in four regions of the world. This work provides a novel approach for understanding the invasion dynamics of *S. molle* in these areas and elucidates the ecological processes that bring about such invasions. Most naturalized and planted populations were found to be in equilibrium with the environment. In its native range as well as in Australia and South Africa the models of the coverage area of habitat suitability for natural populations were the highest, whereas the coverage area of planted populations was lower. For planted populations in Australia and South Africa, a large percentage of predicted presences fell within sink populations. The invasion stages of *S. molle* vary across regions in its adventive range; this result may be attributable to residence time as well as climatic and anthropic factors that have contributed to the spread of populations.

Keywords

Global niche, niche conservatism, plant invasions, regional niche, stage of invasion, tree invasions

Introduction

Climate change has contributed to shifts or modifications of some tree species´ geographic distributions in recent decades (Hoffman and Sgrò 2011; Urban et al. 2016). Further range shifts (reductions and expansions) of many more species are expected in the coming decades, leading to a major reorganization of ecological communities and, potentially, biodiversity loss (Early and Sax 2011; Radchuk et al. 2019). Invasive species are a major component of global change and threaten native species and ecosystem integrity (Pyšek et al. 2020). Biological invasions have impacted ecosystems in many ways, including through hybridization with native species, effects on ecosystem processes, population dynamics, and by modifying of community structure and composition (Vilà et al. 2011; Vilà and Hulme 2017). Trees are increasingly important as invasive species in many regions of the world and have major impacts in such areas (Richardson et al. 2014; Rundel et al. 2014).

An example of a major invasive species is the Peruvian Peppertree (*Schinus molle* L.), a native tree of the Andes in South America (Ramírez-Albores et al. 2020), which has also been introduced, planted, and naturalized in several regions of the world (Taylor 2005). It is evergreen, fast-growing, drought- and -heat resistant, and has been introduced mainly to cities, towns, villages (in parks, gardens, and sidewalks), and farms as an ornamental tree, as well as along drainage lines, water bodies (rivers, streams, dams), agricultural fields (as shade trees, windbreaks, or hedgerows) and roads as an amenity. The successful introduction of *S. molle* in non-native ranges is attributed to its high drought-and-heat tolerance, its ability to compete for nutrients and light, its fast growth rate, and its prolific seed production (Iponga et al. 2008, 2009; Zahed et al. 2010). For these reasons, it has recently expanded its range considerably (Rouget et al. 2004; Iponga et al. 2008; Ramírez-Albores and Badano 2013; Rejmánek and Richardson 2013). Understanding which factors encourage the progress of the introduction-naturalization-invasion continuum is important when seeking measures to manage the invasive species' effects on native biota (Richardson et al. 2000; Pyšek and Richardson 2006; Richardson et al. 2014).

One way of assessing whether evolutionary changes have occurred in an invasive species is to compare the climatic niche between its native distribution range to that of an introduced distribution range. Such studies assume that the niche of a species is formed by a series of vectors, each representing an environmental condition, the magnitudes of which define the range of conditions within which a species can exist (Soberón and Peterson 2011). Thus, if the magnitudes of the vectors that comprise a niche are similar in the native and introduced ranges, it can be concluded that the requirements of the species have not changed. In other words, this situation indicates that the species retains its niche in the introduced range, and therefore will colonize only sites with similar environmental characteristics to those that exist in their native range (Soberón and Peterson 2011). However, if significant differences are detected in the magnitude of the vectors of the niche between native and introduced ranges it can be concluded that the species is being, or has been, shaped by selective pressures within the introduced range (Soberón and Peterson 2011).

In this sense, Gallien et al. (2012) proposed that invasion processes could be inferred by comparing the outputs of global and regional niche models. The global model can be constructed using all occurrence records for the species climatic spaces worldwide. Meanwhile, the regional model, captures all the abiotic and biotic conditions that the species occupies in its native or invaded range. Knowledge of the species' populating stages during invasion has the potential to provide important insights into the future dynamics and potential threat of an invader (Roura-Pascual et al. 2009; Gallien et al. 2012). For instance, many naturalized species occupy climatic conditions that are more extreme than the conditions found in their native range (Broennimann et al. 2007; Gallagher et al. 2010; Petitpierre et al. 2012; Sax et al. 2013). Although in the realized niche, some invasions and survival in situ beyond conditions in the distribution could be the result of evolutionary change, it is likely that some cases prevail because conditions in the native distribution represent only a subset of the existing fundamental niche of the species (Buswell et al. 2011; Sax et al. 2013). Another reason for this is that human actions substantially change environmental factors, e.g., through disturbance, irrigation, or nutrient addition, thereby totally altering and rendering invalid any correlations between occurrence and environmental factors (González-Moreno et al. 2015; Walker et al. 2017). The latter case seems particularly likely for naturalizations of long-lived species such as trees, where there has typically been limited time for evolutionary change in recently established populations (Sax et al. 2013).

This study focuses on the modeling and comparison of the regional and global climate niches of *S. molle*). The long residence time and large extent of plantings and invasion of *S. molle* across multiple regions make this a good species for such a study. This comparison allowed us to infer the stage of invasion for *S. molle* and to determine which sites are most susceptible to invasion by this species. We hypothesize that there will be a differentiation between models (regional and global models) generated within a climatic niche if this species has responded to local selective pressures in *S. molle* naturalized populations (i.e., populations in natural environments without human subsidization) or planted (i.e., planted populations in urban or rural environments where their occurrence is at least partly attributable to human actions) within its introduced range.

Materials and methods

Study species

Schinus molle is native to semiarid Andean ecosystems of Peru and Bolivia and has been introduced to several regions of the world as an ornamental in human settlements as well as for forestry purposes as hedgerows and windbreaks in rural areas (Ramírez-Albores et al. 2016, 2020). Peppertrees recently began colonizing abandoned agroecosystems (Ramírez-Albores et al. 2016, 2020; Guerra-Coss et al. 2021). In addition to the several countries where populations have already been established (Iponga et al. 2008, 2009; Ramírez-Albores et al. 2016), *S. molle* has the potential to invade other semiarid environments (Iponga et al. 2008; Ramírez-Albores et al. 2020) in tropical and temperate regions (Milton et al. 2007; Guerra-Coss et al. 2021).

Occurrence data

Occurrence records of *S. molle* were obtained from our own fieldwork (in Mexico and South America) and were complemented with global occurrence data from scientific collections (see Suppl. material 1: Table S1), as well as literature and online sources. The references that report of the occurrence of *S. molle* without clear evidence of establishment were not included in the dataset (Table 1). Occurrence data records were grouped into (*i*) "naturalized populations" (i.e., individuals occurring in natural environments where they were not reliant on human nurturing), and (*ii*) "planted populations" (i.e., individuals that occur in urban and rural zones where their occurrence is potentially subsidized by human activities). Because sampling of occurrence data is commonly biased in favor of easily accessible areas, spatial data may not be completely independent. This can affect the performance of species distribution models (SDMs), which could lead to misinterpretations of models (Boria et al. 2014; Regos et al. 2019).

Bioclimatic variables

Environmental parameters were obtained from the WorldClim database (available at https://www.worldclim.org/). We used the altitudinal layer and bioclimatic variables pertaining to temperature and precipitation with a spatial resolution of 2.5 minutes (about 5 km^2). We performed a principal component analysis (PCA) and selected the subset of variables that were most strongly associated with the first two principal axes of ordination (Table 2). Collinearity between bioclimatic variables was reduced by eliminating highly correlated variables (Pearson correlation values ≥ 0.70) (Beaumont et al. 2005). We ran models with combinations of minimally correlated variables. Using these criteria, we selected 11 variables for the SDMs: altitude, mean diurnal range, isothermality, annual mean temperature, mean temperature of wettest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of driest month, precipitation seasonality, precipitation of warmest quarter and precipitation of coldest quarter. ArcGIS 10.2 was used to process the environmental layers (ESRI 2014).

Table 1. Date of first record and number of data records of Peruvian Peppertree (*Schinus molle* L.) in study regions.

	Global	Australia	California	Mexico	South Africa	Native region
Date of first record	–	1860	1650–1750	1540–1550	1880	–
Naturalized populations	905	62	23	128	19	81
Planted populations	1022	219	64	649	189	76

Table 2. Contribution (%) of the bioclimatic variables selected for the global and regional Peruvian peppertree (*Schinus molle* L.) distribution models.

	Model											
Variable	**Global**	**Global**	**Australia**	**Australia**	**California**	**California**	**Mexico**	**Mexico**	**South Africa**	**South Africa**	**Native region**	**Native region**
	Planted	Naturalized	Planted	Naturalized	Planted	Naturalized	Planted	Naturalized	Planted	Naturalized	Planted	Naturalized
Altitude	0.8	0.4	14	2.7	10.1	5.1	26.2	13.1	28.6	7	19.8	40.8
Annual mean temperature	36.9	34.2	10.3	25.9	2.1	6.6	1.6	4.2	15.1	7.4	24.1	20.8
Mean diurnal range	2	0.2	2.9	2.9	6.5	0	1.2	1.2	0	4.1	0.4	0
Isothermality	47.7	58.4	3.5	0.4	4.2	21.4	24.3	19.3	0	2.1	4.4	6.5
Temperature annual range	6.4	0.7	1.9	6.8	11.7	30	6.3	4.3	8	2	5.2	2.6
Mean temperature of wettest quarter	0.3	0.6	3.8	19.3	22.4	1.8	4.5	21.5	7.2	5.7	0	0
Annual precipitation	0.7	1.2	5	6.6	9.1	12.2	13.3	15.5	0	1.2	1.9	3.1
Precipitation of driest month	0.8	1.1	16.3	16.7	6.6	9.7	3.7	1.3	6.7	21.7	1.3	0.8
Precipitation seasonality	0.4	0.1	3.8	4	14.4	1.7	2.1	10.2	0.4	6.4	3.8	2.8
Precipitation of warmest quarter	0.9	0.2	3	4.5	4.7	2.5	7.7	5.7	16.1	33	1.5	0.7
Precipitation of coldest quarter	3.2	2.4	35.4	10.3	8.2	8.9	9.1	4.6	17.8	9.4	37.6	21.8

Regional and global distribution models

The occupied climate space was compared between the native and invaded ranges using direct climate comparisons and PCA before ecological niche modeling; this allowed us to make a quick assessment of the relative positions of populations in climate space, using the 11 selected bioclimatic variables. A kernel function was used by converting the presence points to density values (Broennimann et al. 2012).

We then compared the regional versus the global niche range to assess whether the *S. molle* niche differed. To compare the distribution models, we projected the potential distribution from the regional niche and compared it with the potential distribution projected from the global niche (Medley 2010). To compare the global (where the invader species could spread) and the realized regional niches (where the invader species is already observed) we followed the framework proposed by Gallien et al. (2012). This framework allows us to infer both the stage of invasion for each population in the niche space and the degree of regional range filled by the invading species in geographical space. According to this framework, which was applied analytically by Kumar et al. (2015), if the regional and global niche models predict probabilities higher than 0.5 for the presence of the species, the species is in quasi-equilibrium (i.e., the populations are in a stabilizing stage). In contrast, if both niche models predict probabilities lower than 0.5 for presence of the species, this means that the locations may represent population sinks (i.e., sink populations). If the probability of presence of the species is higher than 0.5 in the global niche but in the regional niche the probability is lower than 0.5, this finding suggests colonization from different sources, including areas already invaded in the regional invaded range (i.e., populations colonizers). In contrast, if the probability of presence of the species is higher than 0.5 in the regional niche, but lower than 0.5 in the global niche, populations may be adapting to new environmental conditions (i.e., locally adapted populations). All analyses were performed in R (R Development Core Team 2019) using functions as ecospat and SDMtools (Broennimann et al. 2012; Di Cola et al. 2017).

We used MaxEnt (v.3.4) to construct the regional and global models of *S. molle.* MaxEnt computes the probability distribution of maximum entropy for the set of climatic variables with the occurrence records of the target species, but this procedure is constrained by the incomplete knowledge of the distribution of the species (Phillips et al. 2006; Graham et al. 2008; Elith et al. 2011). The resulting model is then a geographical projection of habitat suitability for the target species (i.e., probability for finding the species) where values close to 0 indicate sites that do not match with the niche requirements of the species, and values close to 1 indicate sites that fully match their niche requirements. Although other computer programs have also been used to model species climatic niches, several authors have shown that MaxEnt usually performs better when presence-only data are available (Graham et al. 2008; Elith et al. 2011). This produced a model of a suitable habitat for the species based on the climatic variables, expressed as a probability distribution (Phillips et al. 2006). Each niche model was calibrated with a random selection of 75% of the occurrence points used as training data; the remaining 25% of the points were used as test data to validate the models. All models were regularized, modifying the value of the β parameter to avoid over-parametrization (only models with $\beta \cong 1$ were retained), therefore selecting the most conservative models (i.e., those with the best compensation between complexity and predictive capacity; Phillips et al. 2006; Peterson et al. 2011). For each model, we created 100 replicas considering a cross-validation approach in which the occurrence points are repeatedly split into two subsets: one for training and one for testing. Model performance was evaluated using the area under the curve (AUC) and partial ROC test (pROC) (Barve 2008; Peterson et al. 2008). Our product consisted of a projection (continuous map) of the habitat suitability for *S. molle* in the invaded range (Mexico, California, South Africa, and Australia). According to the predicted habitat suitability (Pachauri et al. 2014), four types of potential habitat suitability for *S. molle* were defined as follows: high suitability (>0.60), medium suitability (0.40–0.60), low suitability (0.20–0.40), no suitability (<0.20) (Pachauri et al. 2014). ArcGIS 10.2 (ESRI 2014), which was used to visualize and interpret the output in raster format.

Results

For all models, factors related to temperature were more important than those related to precipitation. The variable that contributed most strongly to the global models was isothermality followed by annual mean temperature (Table 2). For the regional models, the variables fluctuated, with the altitude, isothermality and precipitation of coldest quarter emerging as the most important (Table 2). All of the models had a good performance, with AUC values ranging from 0.758 to 0.973, and pROC values from 1.75 to 1.92: Global $_{\text{planted}}$ (AUC = 0.924 ± 0.001), Global $_{\text{naturalized}}$ (AUC = 0.952 ± 0.002); and Native region $_{\text{planted}}$ (AUC = 0.971 ± 0.03), Native region $_{\text{naturalized}}$ (AUC = 0.952 ± 0.04) (Table 3).

Table 3. Areas of calibration and performance statistics for naturalized and planted populations models of Peruvian peppertree distribution.

Model	Boyce index (β)	Test AUC	pROC
Global $_{\text{planted}}$	0.98	0.924 ± 0.001	1.91 ± 0.002
Global $_{\text{naturalized}}$	0.99	0.952 ± 0.002	1.90 ± 0.003
Australia $_{\text{planted}}$	0.99	0.927 ± 0.017	1.91 ± 0.030
Australia $_{\text{naturalized}}$	0.99	0.949 ± 0.005	1.85 ± 0.001
California $_{\text{planted}}$	0.99	0.932 ± 0.014	1.91 ± 0.030
California $_{\text{naturalized}}$	0.99	0.958 ± 0.014	1.85 ± 0.001
Mexico $_{\text{planted}}$	0.99	0.942 ± 0.005	1.89 ± 0.040
Mexico $_{\text{naturalized}}$	0.99	0.973 ± 0.004	1.90 ± 0.001
South Africa $_{\text{planted}}$	0.97	0.758 ± 0.006	1.75 ± 0.006
South Africa $_{\text{naturalized}}$	0.97	0.830 ± 0.070	1.80 ± 0.003
Native region $_{\text{planted}}$	0.99	0.971 ± 0.030	1.88 ± 0.020
Native region $_{\text{naturalized}}$	0.99	0.952 ± 0.040	1.79 ± 0.001

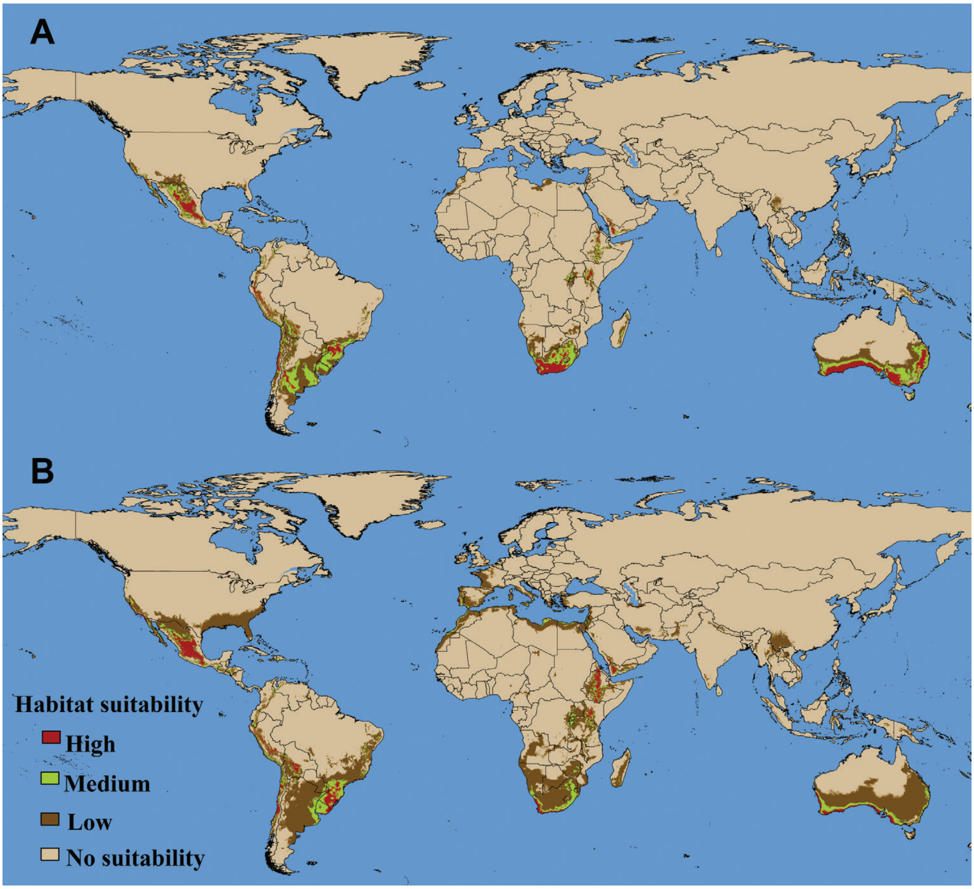

Figure 1. Global distribution model of Peruvian Peppertree (*Schinus molle* L.) with naturalized (**A**) and planted populations (**B**).

In the global models, the highest habitat suitability (> 0.60) occurred in central Mexico, the coastal regions of South Africa, some regions of eastern Africa, and the Andean Plateau of Peru and Bolivia, all of which correspond to arid and semi-arid climates (Figure 1). However, the Global $_{\text{planted}}$ model had a higher coverage, with a low and medium habitat suitability in temperate, such as Mediterranean, and arid climates (Figure 1). This was similar for the regional models of Mexico, California, Australia, and South Africa in which the largest area of suitable habitat was found from the northern to central Mexico, along the entire coast to north-central California, the southern coast and the east part of Queensland, Victoria, and New South Wales in Australia, as well as the Cape coast of South Africa (Figs 2–3, 5–6). The coverage area of habitat suitability of naturalized populations in its native region was the highest, whereas the coverage of planted populations was lower (Figure 4).

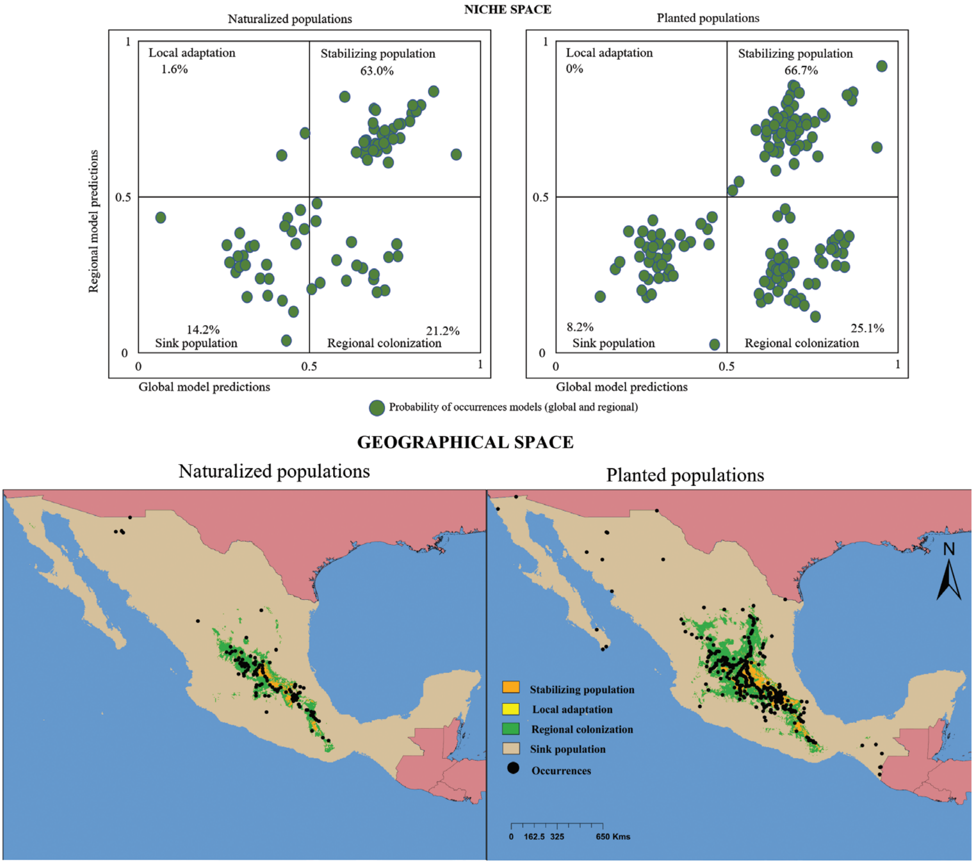

Figure 2. Invasion stages for the Peruvian Peppertree (*Schinus molle* L.) with naturalized and planted populations in Mexico.

In the niche space, the highest proportion of the predicted presences for naturalized and planted populations fell within the regions with stabilized populations (Figures 2–6). For the cases of the planted populations of Australia and South Africa, a substantial proportion of predicted presences fell within sink populations (Figures 5, 6). However, in the niche space, in the range associated with native regions, in both the naturalized and planted populations the highest proportion of predicted presences was within sink populations (Figure 4).

Discussion

The global invasion of *S. molle* suggest source-sink dynamics from the native to the invaded range, and its populations are found at different stages of invasion in Australia, California, Mexico, and South Africa. Although most *S. molle* populations are

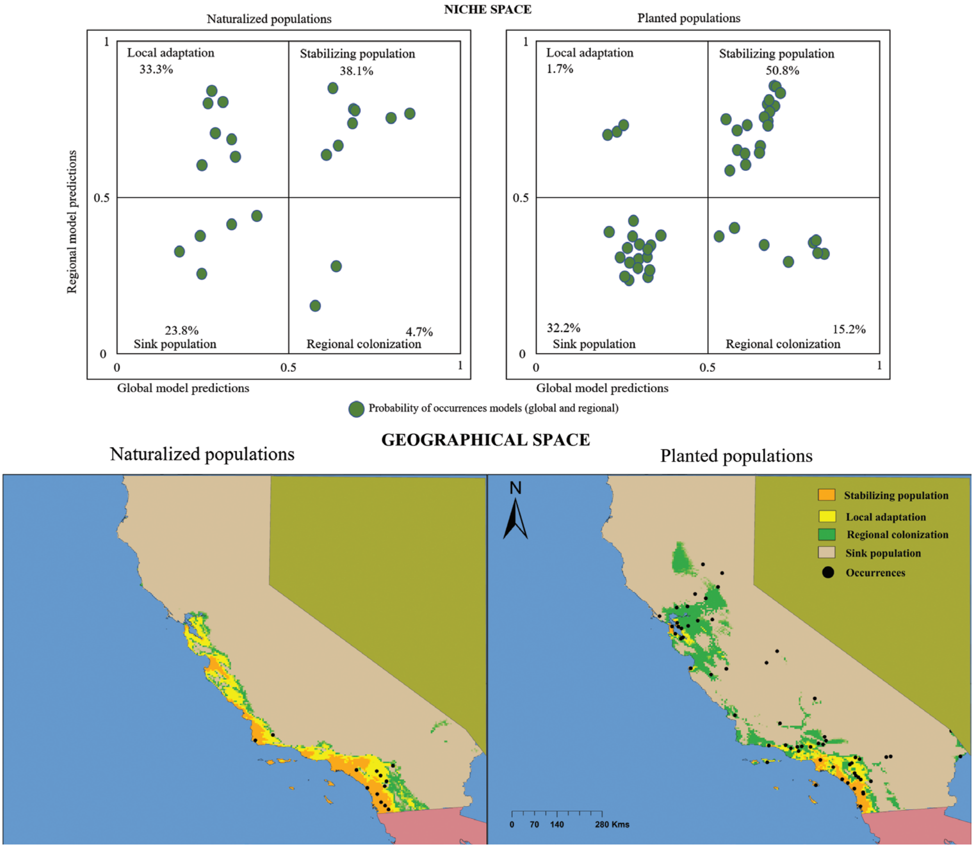

Figure 3. Invasion stages for the Peruvian Peppertree (*Schinus molle* L.) with naturalized and planted populations in California, USA.

stable, some exhibit high extinction risk (and persist as sink populations). Our findings suggest that in Mexico and California, both naturalized and planted populations of *S. molle* are stabilized, whereas only naturalized populations in natural environments of Australia and South Africa are stabilized. Our analysis allowed us to predict the regions that are most susceptible to invasion of the *S. molle* based on its climatic niche requirements. Although the invasion process is complex and different for each species, comparing global and regional climatic niches provides a useful tool that initially addresses these complexities and generates different hypotheses to be tested in future experimental studies (Taucare-Ríos et al. 2016).

In Mexico, Australia, California, and South Africa, both niche models predicted the most suitable habitats in the central part and the Mexican Plateau in Mexico, the Californian coast, the southern coast and the east part of Queensland and New South Wales in Australia, as well as the Cape coast of South Africa. Factors relating to temperature were the most important for defining the potential distribution of this species. In this regard, our results confirm those of earlier studies on *S. molle* (Iponga et al.

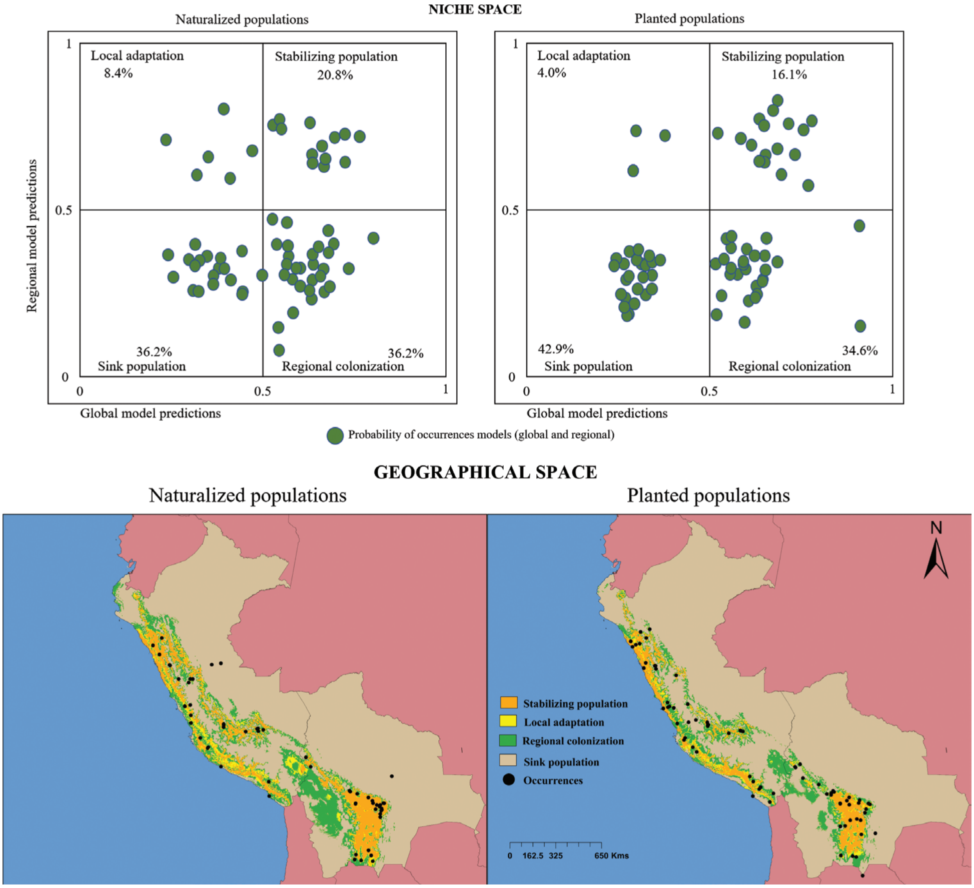

Figure 4. Invasion stages for the Peruvian Peppertree (*Schinus molle* L.) with naturalized and planted populations in South America (native region).

2008; Lemos et al. 2014, 2019; Ramírez-Albores et al. 2020; Guerra-Coss et al. 2021). *Schinus molle* can be established easily in arid and semiarid climates in Mexico, California, Australia, and South Africa, probably because of the similarities in the climate in their native range (e.g., Richardson and Thuiller 2007 [figure 2] for South Africa).

In this context, and similar to other studies (e.g., Taucare-Ríos et al. 2016), our models showed that in regions with longer residence times (i.e., longer time elapsed since the first record), the number of stable populations increases, while the regional colonization remains low. This suggests that the species' current distribution is not limited primarily by abiotic factors, but instead reflects human aid in facilitating the expansion of this highly invasive species beyond its climatic thresholds. When comparing the native niche model with the global niche model, the ecological requirements of *S. molle* are maintained despite the climatic differences; in other words, the populations show niche conservatism in the invaded regions. In the regional models, *S. molle* populations appear to occupy new niches, which may be facilitated by human actions such

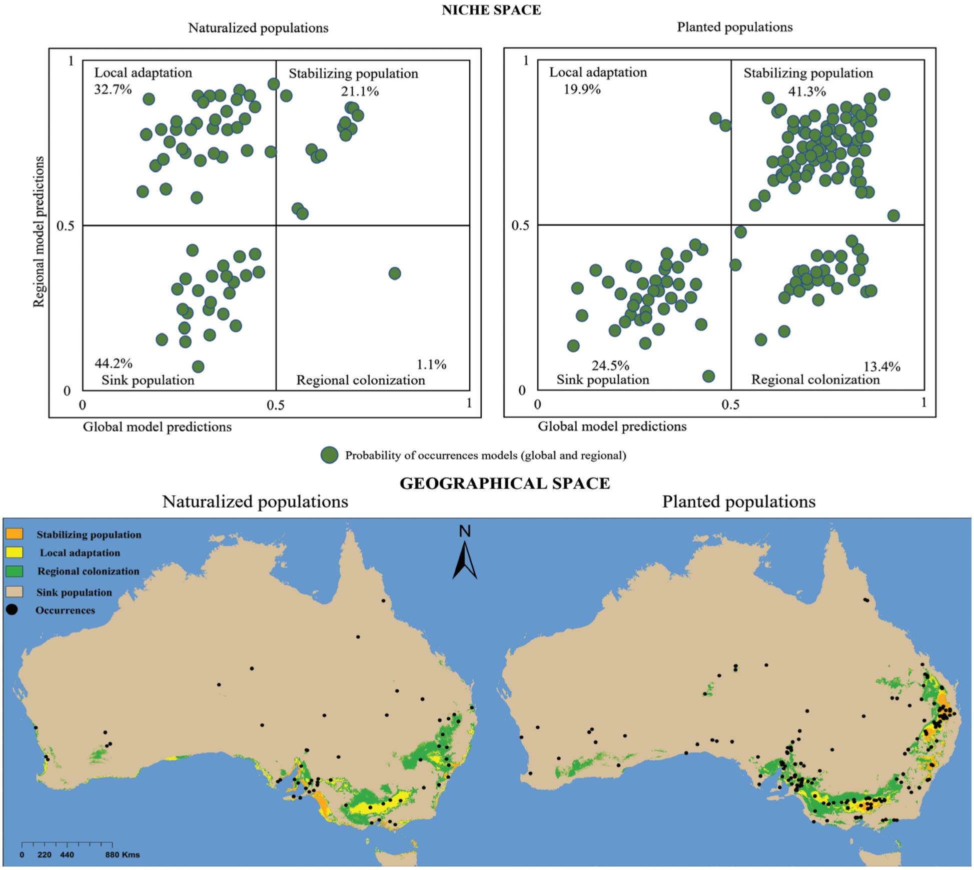

Figure 5. Invasion stages for the Peruvian Peppertree (*Schinus molle* L.) with naturalized and planted populations in Australia.

as irrigation, which generates new environments favorable to the species even though the natural climate is outside the species' niche (Taucare-Ríos et al. 2016, 2018).

Although the range-filling analysis showed that the naturalized and planted populations in these regions may still colonize more suitable habitats, the populations may be in equilibrium with the environment. This partial filling of the native niche in the invaded region has been reported for other invasive plants (Rouget et al. 2004; Petitpierre et al. 2012; Goncalves et al. 2014; Kolanowska and Konowalik 2014; Peña-Gómez et al. 2014) and has been documented in other studies of *S. molle* (Richardson et al. 2010; Ramírez-Albores et al. 2020). This would explain why *S. molle* has only colonized a fraction of the environments that are climatically suitable for the species. It is well known that when humans translocate species across biogeographic barriers, the introduced individuals often constitute a biased sample of the genetic variability of the populations across the entire native range (Chun et al. 2009). Therefore, invasive plants may fail to occupy the full range of climatic conditions that occur in their native niches,

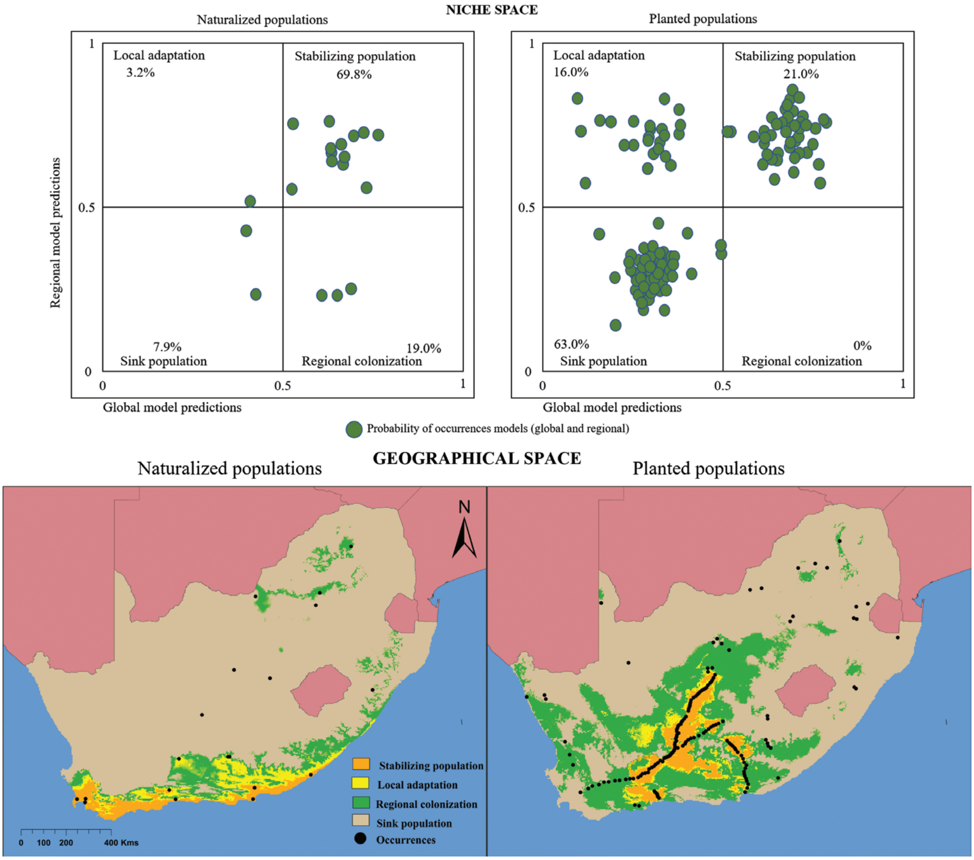

Figure 6. Invasion stages for the Peruvian Peppertree (*Schinus molle* L.) with naturalized and planted populations in South Africa.

even when these conditions are available in the invaded regions (Early and Sax 2014; Alexander 2016). There is no evidence of genetic rescue (i.e., increased genetic variability due to reintroductions) in the global history of introductions of the species. Invasive peppertrees may have experienced a genetic bottleneck because a small, random, and nonrepresentative fraction of the full niche of the species was captured when seeds were harvested in the native range. Testing this hypothesis would require comparing genetic profiles between the native and invasive peppertrees. However, *S. molle* populations in these regions probably experienced genetic bottlenecks, as often happens when alien plants are introduced into new regions (Prentis et al. 2008; Xu et al. 2015; Xia et al. 2020). On the other hand, significant genetic structure and a low levels of population genetic diversity were observed in an analysis of nine populations of natural occurrence in the Brazilian Pampa biome (Lemos et al. 2015). Thus, if multiple introductions are performed in new areas using seeds from different origins, the genetic variation can be increased since different alleles may be sampled across divergent populations.

Nonetheless, the high capacity of colonizing new areas seems to be relatively independent of the level of genetic variation of the introduced plants and of the human interference, like irrigation. Although the Incas planted and irrigated *S. molle* around palaces, temples, and public building (as it was considered a sacred tree; Mendonça-Rocha et al. 2012), irrigation does not seem to be essential for seedling establishment. In a controlled experiment in California, where the species was introduced and naturalized, seedling growth was fast through the summer regardless of the irrigation regime (Howard and Minnich 1989). The high plasticity of this species seems to be efficient in driving its capacity of introduction into and surviving in different environments (Lemos et al. 2015). This capacity has been corroborated if we consider the large number of different sites were *S. molle* was introduced and is currently naturalized, regardless of human intervention. The rate of local adaptation and populations stabilization predicted in our model suggests that planted populations in the natural occurrence range of *S. molle* (Figure 2) is similar to Mexico, California, Australia, and South Africa (Figures 3–6). Thus, local adaptation seems to be independent of human interference and can occur as a natural process for this species.

The Global $_{\text{planted}}$ model predicts large areas of suitable habitat areas in the western and Mediterranean regions of Europe and Africa, the Brazilian Atlantic coast, and the Pampa region of Argentina, Brazil, and Uruguay, showing a high proportion of stable populations and few sink populations compared to the Global $_{\text{naturalized}}$ model. This pattern is similar to that reported by Richardson et al. (2010) and Lemos et al. (2019), who mentioned that the largest areas of suitable habitat for the establishment of *S. molle* are subsidized by humans. In general, sink populations were found in tropical and cold climates as well as deserts which represent stressful and extreme temperatures. Also, in several South American countries, we found populations that had undergone regional colonization in Ecuador, Colombia, Chile, Paraguay, Brazil, and Argentina. However, *S. molle* populations in Mexico suggest that the seeds used in founder populations came from sites that cover less than 10% of the full range of climatic conditions over which this species occurs in its native region (Ramírez-Albores et al. 2016, 2020). Indeed, as far as we know, the *S. molle* was introduced in Mexico only once.

There were some areas for which local adaptation was predicted (see Figures 2–6). We suggest possible explanations for local adaptation in our naturalized niche models as the species' ability to exploit empty niches, or on account of local disturbances have created new habitats (Sax et al. 2013). Our results support the hypothesis that alien species are more successful in human-modified environments (Pyšek and Richardson 2010). Indeed, such environments can result in the creation of vacant niches that can be filled by alien species (Catford and Downes 2010). On the other hand, planted models may have overestimated the potentially suitable areas because not all predicted areas have suitable habitats for *S. molle* (e.g., tropical climates). Furthermore, occurrences in urban areas where the species may be subsidized by human activities may cause the models to overpredict suitability in nonurbanized areas with similar environmental features in other regions. Therefore, we suggest that it is plausible in this case that the geographical range of *S. molle* can be further extended as humans continue to use this species for ornamental and forestry purposes in urban and rural environments, thereby modifying the fundamental niche of *S. molle* (Ingeloff et al. 2017; Qiao et al. 2017). However, this could also be established by natural processes as it occurs in the Brazilian Atlantic coast and the Pampa region (Lemos et al. 2014, 2019).

Conclusions

The invasion stages of *S. molle* vary across regions in its adventive range; this is the result of the complex interplay of stochastic factors and abiotic and biotic mediators. Residence time as well as climatic and anthropic factors have contributed to the success of *S. molle* populations. This study provides a preliminary approach for understanding the process of invasion by this invasive tree, thereby helping to elucidate the dimensions of the "invasion debt" (*sensu* Rouget et al. 2015) that clearly exists for *S. molle* in many areas. Such insights will be crucial for developing strategies for the management of this important invasive tree to avoid or at least reduce its future impacts in recipient ecosystems.

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Supplementary material 1

Table S1

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Data type: Databases consulting

Explanation note: Databases consulted in collating occurrence records of Peruvian Peppertrees (*Schinus molle* L.).

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Farmers' knowledge, perception and management practices of fall armyworm (*Spodoptera frugiperda* Smith) in Manica province, Mozambique

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Abstract

This study aimed to gather information about farmers' knowledge, perception and management practices of the newly introduced insect pest, the fall armyworm *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Manica province, Mozambique. A total of 200 smallholder farmers with experience in maize cultivation were surveyed using a semi-structured questionnaire. The survey was conducted between May and August 2019 in four districts: Macate, Manica, Sussundenga and Vanduzi. Most farmers were unable to morphologically identify fall armyworm (FAW) (from 93.9% in Vanduzi to 98.0% in Manica). Most farmers have experienced FAW damage in their farms (from 92% in Macate to 98.0% in Manica). Maize is mostly planted in October and November (from 44.0% in Sussundenga to 60.0% of farmers in Manica), but the highest infestation period is believed to be between November and February. With the exception of Vanduzi where 65.3% of farmers apply insecticides, most farmers in other districts do not use any method to control FAW (from 60.8% in Macate to 88.0% in Manica and Sussundenga respectively). Among those applying insecticides, from 65.0% in Manica to 75.0% in Vanduzi have confidence in the efficiency of the insecticides being used against FAW. Most farmers reported an increase in the spread of FAW. The lack of financial resources is reported as the main constraint in the fight against FAW. This study is the first of its nature in the province of Manica and provides valuable information that may support extension services and researchers when designing FAW management options for local smallholder farmers.

Keywords
Attack symptoms, constraints, morphological identification, pest management, smallholder farmers

Introduction

The fall armyworm *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) is a polyphagous insect pest originating from the Americas, where it has more than 350 different host plants including both crop and non-crop species (Montezano et al. 2018). Despite its ability to survive in different host plants, fall armyworm (FAW) is known to have a high preference for maize (Molina-Ochoa et al. 2001; Nagoshi et al. 2018). In Africa, FAW was first reported in West and Central Africa in 2016 (Goergen et al. 2016) and rapidly spread to the rest of the continent with devastating consequences on maize production (Feldmann et al. 2019).

The larval stage of FAW consists of six instars. Young larvae usually feed on leaves creating windows and moistened sawdust-like frass near the funnel and upper leaves. During daylight, young larvae hide in the funnel, becoming active during the night. Older larvae stay in the funnel where they are protected from insecticide application and natural enemies, making it difficult to control them (Prasanna et al. 2018). Development rate is affected by diet (Abrahams et al. 2017) and temperature (Early et al. 2018). FAW populations can expand rapidly in tropical areas, where warmer temperatures allow more generations per year (Assefa and Ayalew 2019). At an average temperature of 28 °C, the life cycle of FAW is completed in around 30 days but can be extended in cooler temperatures (Prasanna et al. 2018).

In Mozambique, FAW was confirmed in early 2017 (Cugala et al. 2017). In 2018, FAW was also reported in Asia (Sharanabasappa et al. 2018). The rapid spread of FAW is largely attributed to its migratory potential (Meagher et al. 2004) and high dispersal capacity (Kumela et al. 2018). The problem of FAW in sub-Saharan Africa is exacerbated because its preferred host plant, maize, is a staple food in the region (Midega et al. 2018; Prasanna et al. 2018; Harrison et al. 2019). In Mozambique, for example, 21 to 90% of households depend on maize for daily consumption (MASA 2016). In 2017, it was estimated that by 2018, FAW would have caused an economic loss of around US$ 3 billion in Africa (Abrahams et al. 2017). In the absence of proper control methods, FAW has the potential to cause huge yield losses (Prasanna et al. 2018) as was reported in Mozambique where a year after its detection, around 49 thousand tons of maize were reported to be lost as a direct consequence of FAW attack (FAO 2018).

Farmers have various forms of indigenous knowledge to tackle pest problems, but such knowledge is often neglected (Mendesil et al. 2007). Surveys designed to ascertain farmers' knowledge and practices regarding pest management are important because they can highlight the need for the training of farmers in the identification of pests and the debunking of pest management misconceptions (Arshad et al. 2009). Crop losses due to insect pests may be prevented, or reduced, by deploying effective crop protection measures, which to a large extent depend on farmers' knowledge and behaviour towards pest management (Midega et al. 2016; Kansiime et al. 2019).

For effective deployment of control methods for a given pest, farmers should be able to morphologically identify the target pest and distinguish it from non-target organisms. In cases of economically important and invasive insect pests such as FAW, it is crucial to know how familiar farmers are with the pest, what options they have to control it and what their main constraints are. Understanding these factors is critically important for setting a research agenda, designing extension strategies, and formulating research that meets farmers' demands (Arshad et al. 2009; Mendesil et al. 2016; Kumela et al. 2018). To respond to these concerns, this study was designed to provide baseline information that can be used by extension services and research institutions in determining how the problem of FAW should be addressed at the smallholder farmers' level. This study aimed to assess the knowledge, perception and management practices of FAW among smallholder farmers of the central province of Manica, Mozambique.

Materials and methods

Description of the study area

This study was carried out in the districts of Macate (19°24'50.9"S, 33°30'54.6"E), Manica (18°56'13.2"S, 32°52'33.6"E), Sussundenga (19°24'39.0"S, 33°16'33.0"E) and Vanduzi (18°57'09.4"S, 33°15'51.6"E) in the central province of Manica, Mozambique. According to MASA (2016), the area of the survey belongs to the Agro-Ecological Region (AER) number 4, which is characterized by the large occurrence of ferralsols and litosols with an annual mean temperature around 24 °C and annual mean precipitation ranging between 800 and 1000 mm (Figure 1). In Mozambique, maize is the main food crop and is cultivated in both dry and rainy seasons. The rainy season starts from mid-November to late March. During the dry season, maize is cultivated mainly in areas with irrigation systems or in valleys and river banks. Maize is often grown in small plots (less than 1 ha), in different cropping systems and mainly for family consumption. In general, no fertilizers or pesticides are used for the production of maize by smallholders. It is usually intercropped with roots and tubers (cassava and sweet potato), legumes (cowpea, pigeon pea, groundnut and common beans) and cucurbits (pumpkin, watermelon, melon).

Selection of farmers and questionnaire delivery

The survey was conducted from May to August 2019. Although the main cropping season is between November and March due to the rainy weather, the survey period was intentionally chosen because during the rainy season, some locations would have been inaccessible due to flooding. Furthermore, FAW was officially detected in

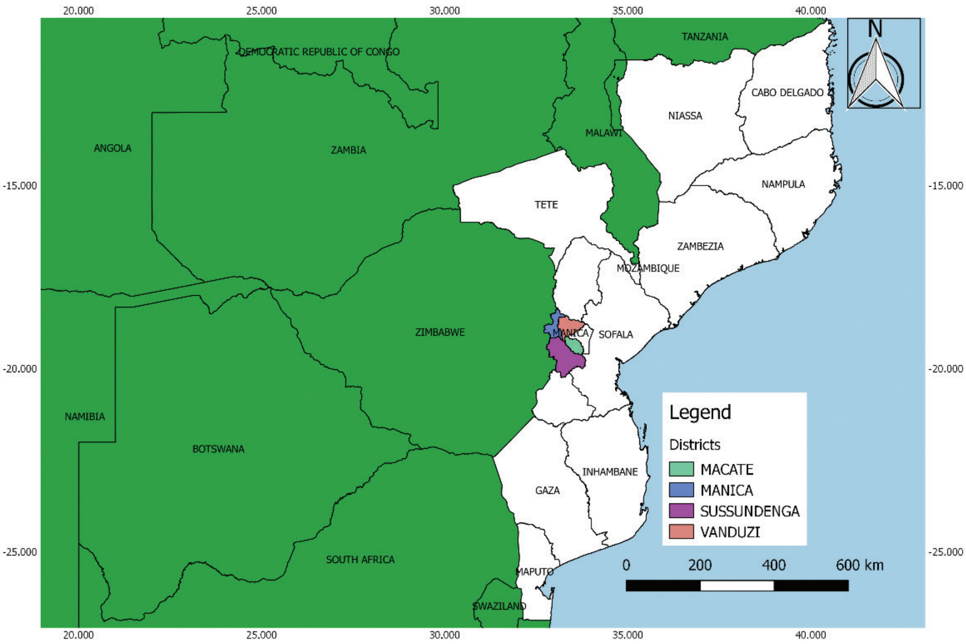

Figure 1. Sampling locations in Mozambique (colored areas within the province of Manica).

Mozambique two years prior to the survey and there was some information about the pest among farmers. Districts were selected based on their potential for maize production combined with the reported occurrence of FAW. Undergraduate finalist students of Agricultural Engineering from Instituto Superior Politécnico de Manica were recruited and trained as enumerators. Enumerators were selected based on their knowledge of the area and the ability to communicate in local languages. A semi-structured questionnaire, written in Portuguese, was used to interview farmers. The questionnaire was pre-tested for its validity and suitability for the survey. Farmers were selected by convenience based on their willingness to be interviewed and on their experience in maize cultivation.

At the beginning of the interviews, farmers were informed of the aim of the study. A leaflet including pictures of FAW and its damage on maize was used to facilitate recognition of the pest by farmers. A total of 200 farmers were interviewed as follows: 50 in Macate, 51 in Manica, 50 in Sussundenga and 49 in Vanduzi. Although the questionnaire was written in Portuguese, interviews were conducted either in Portuguese or in one of the following local languages which are common in the area of study: Chiuté, Chi-Shona and Chi-Ndau. Whenever the interview was conducted in a local language, questions were translated into that language but responses were recorded in Portuguese. In order not to limit the responses from farmers, some of the questions were left "open". Interviews were conducted face-to-face either on the farm or around farmers' homes.

Each interview lasted on average 16 minutes. Information related to farmers' socioeconomic characteristics (age, gender, education level, number of individuals per household, head of the household, monthly income, number of farms and land possession per household), knowledge and perceptions about FAW (morphological identification, recognition of attack symptoms, incidence and spread of the pest), management practices (methods of control, handling of insecticides) and constraints for its control were collected. Whenever farmers were unable to tell their age, they were asked to show their IDs. In cases where no ID was provided, farmers' ages were estimated based on the information provided by other family members.

Data analysis

Data were summarized per district. Descriptive statistics such as means and percentages were calculated through the Statistical Package for Social Sciences (SPSS) version 26. For each question, similar answers were grouped and the percentage of farmers who gave similar responses was determined for each district. Whenever two or more responses were given to the same question, they were again grouped by similarity and the percentage of farmers who gave a similar response was determined for each district. In some cases, the percentage of farmers was determined based on the total number of farmers who gave a particular response.

Results

Socio-economic characteristics

With the exception of the district of Sussundenga, where 64.0% of the interviewed farmers are women, most farmers in the rest of the districts are men (57.0% to 68.0%). The average age for women varied from 37.6 years in Sussundenga to 45.0 years in Macate, while for men, the average age varied from 34.6 years in Sussundenga to 43.4 years in Vanduzi. The average size of households varied from 6.8 individuals in Macate to 7.7 individuals in Manica. Although there is a considerable proportion of illiterate farmers (16.3% to 28.0%), most of them received primary education (54.0% to 69.4%), and have farming as their primary source of income (70.0% to 83.7%). The average monthly income of households ranges from US$ 36.5 in Sussundenga to US$ 82.6 in Macate. Each household has between 2.2 to 3.5 farms with total land possession varying from 3 ha in Macate to 5.2 ha in Sussundenga (Table 1).

Cropping systems, maize varieties and purpose of production

Most farmers have more than 10 years of experience in maize cultivation (from 68.0% in Macate to 90.2% in Manica). While in Manica and Vanduzi most farmers acquire

Table 1. Socio-economic characteristics of farmers per district.

Characteristics of respondents	Number of responses per district (%)			
	Macate (n = 50)	Manica (n = 51)	Sussundenga (n = 50)	Vanduzi (n = 49)
Gender				
Women	40.0	32.0	64.0	42.9
Men	60.0	68.0	36.0	57.1
Age/gender (years)				
Women	45.0	43.2	37.6	44.6
Men	39.6	42.3	34.6	43.4
Head of household				
Women	6.0	12.0	22.0	14.3
Men	94.0.	88.0	78.0	85.7
Number of individuals/household	6.8	7.7	7.0	6.9
Education level				
No education	24.0	22.0	28.0	16.3
Primary education	60.0	54.0	54.0	69.4
Secondary education	14.0	20.0	12.0	10.2
High school	2.0	4.0	4.0	4.1
Tertiary education	0.0	2.0	2.0	0.0
Another occupation				
Yes	20.0	23.5	30.0	16.3
No	80.0	76.5	70.0	83.7
Monthly income/household (US$)*	82.6	69.2	36.5	58.6
Number of farms owned/household	2.2	2.6	3.5	2.4
Land possession/household (ha)	3.0	3.6	5.2	3.0

*1 US$ = 58 MZN.

their seeds from authorized dealers (56.9% and 63.3% respectively), in Macate and Sussundenga the primary source of maize seeds is farmers' grain from the previous cropping season (68.0% and 88.0% respectively). While in Manica and Vanduzi most farmers use hybrid varieties (70.6% and 69.4% respectively), in Macate and Sussundenga, most farmers rely on local maize varieties (74.0% and 78.0% respectively). In Macate and Sussundenga, 72.0% and 74.0% respectively of farmers plant their maize intercropped with other crops. But in Manica and Vanduzi most farmers plant maize as monocrop (51.0% and 57.1% respectively). The majority of farmers in all districts reported that they produce maize for both home consumption and sale (from 63.3% in Vanduzi to 80.0% in Sussundenga) (Table 2).

Identification and recognition of FAW attack symptoms

When farmers were asked about how they first obtained information about FAW, from 69.4% in Vanduzi to 88.0% in Macate, reported that it was through direct observation in their farms. From 94.0% in Macate to 100% of farmers in Vanduzi, reported having seen FAW larvae. Although farmers have seen FAW, most of them are unable to distinguish FAW larvae from other lepidopteran larvae. From 92.0% in Macate to

Table 2. Farmers' experience in maize cultivation, seed provenience and cultural practices per district.

Characteristics of respondents	Number of responses per district (%)			
	Macate (n = 50)	Manica (n = 51)	Sussundenga (n = 50)	Vanduzi (n = 49)
Experience in maize cultivation				
Less than 1 year	2.0	0.0	0.0	0.0
Between 1 and 5 years	6.0	3.9	6.0	14.3
Between 5 and 10 years	24.0	5.9	6.0	8.2
More than 10 years	68.0	90.2	88.0	77.6
Seed provenience				
Authorized dealer	24.0	56.9	30.0	63.3
Own seed (previous season)	68.0	51.0	88.0	36.7
Neighboring farmer	12.0	9.8	0.0	2.0
Extension services/NGO's	6.0	5.9	4.0	4.1
Type of maize variety				
Hybrid	26.0	70.6	22.0	69.4
Local	74.0	29.4	78.0	30.6
Cropping pattern				
Monocrop	30.0	51.0	26.0	57.1
Intercrop	72.0	49.0	74.0	42.9
Purpose of production				
Home consumption	24.0	19.6	18.0	26.5
Sale	0.0	7.8	2.0	10.2
Both	76.0	72.6	80.0	63.3

Table 3. Identification and recognition of FAW attack symptoms by farmers per district.

Characteristics of respondents	Number of responses per district (%)			
	Macate (n = 50)	Manica (n = 51)	Sussundenga (n = 50)	Vanduzi (n = 49)
First source of information about FAW				
Radio	2.0	9.8	4.0	4.1
Extension Services	0.0	3.9	0.0	10.2
Neighboring farmer	4.0	7.8	18.0	6.1
Own observation	88.0	76.5	76.0	69.4
Other	0.0	0.0	0.0	10.2
Has never heard about	6.0	2.0	2.0	0.0
Observation of FAW larvae				
Yes	94.0	98.0	98.0	100
No	6.0	2.0	2.0	0.0
Ability to identify FAW larvae morphologically				
Can identify	4.0	2.0	2.0	6.1
Unable to identify	96.0	98.0	98.0	93.9
Occurrence of FAW damages on own farm				
Yes	92.0	98.0	96.0	98.0
No	8.0	2.0	4.0	2.0
Training in identification and control of FAW				
Trained	6.0	11.8	6.0	28.6
Non-trained	94.0	88.2	94.0	71.4

98.0% of farmers in Manica, have observed FAW damage in their farms. From 71.4% in Vanduzi to 94.0% of farmers in Macate and Sussundenga did not receive any training for the identification and control of FAW (Table 3).

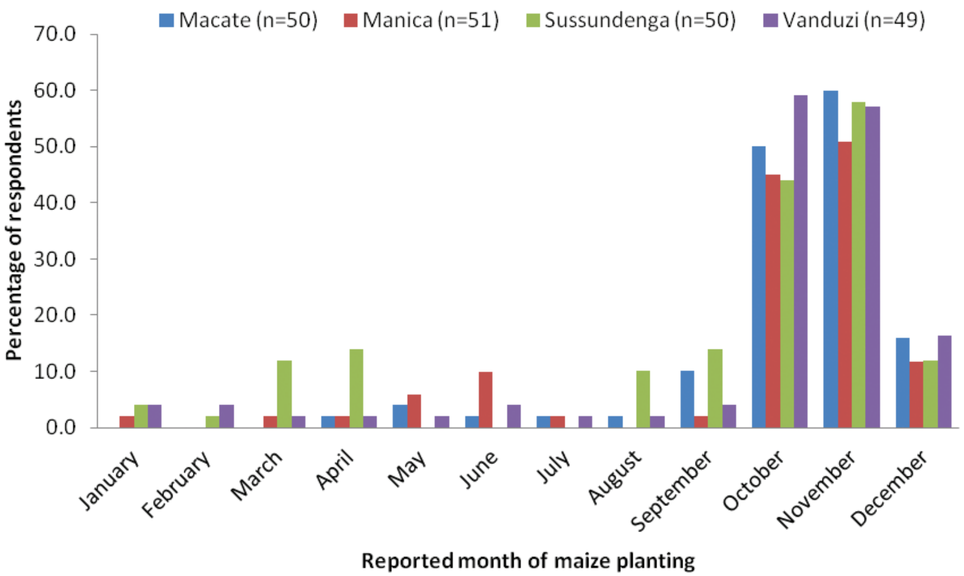

Figure 2. Reported month of maize planting per district.

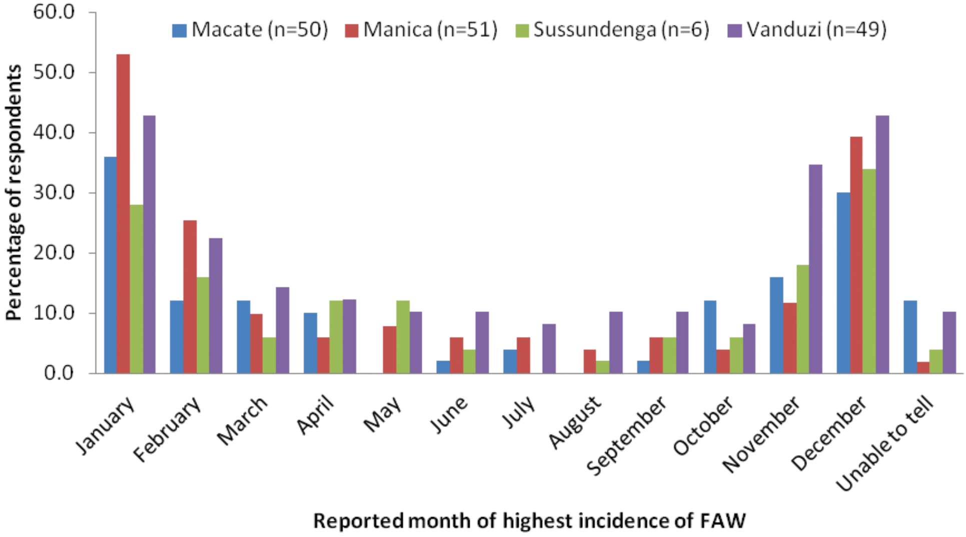

Figure 3. Reported month of the highest incidence of FAW in maize fields per district.

Maize planting and FAW infestation periods

As reported by farmers, maize is mainly planted in October and November, which coincides with the beginning of the rainy season. Nevertheless, the incidence of FAW is reported to be high between November and February as in this period, maize plants are still young (Figures 2 and 3).

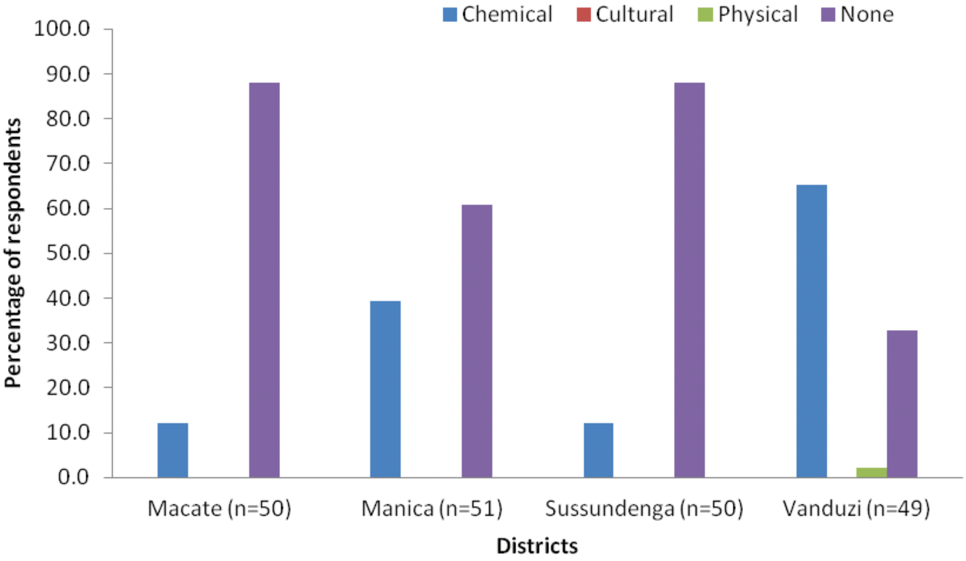

Figure 4. Methods of control of FAW used by farmers per district.

Methods of control of FAW

Except for Vanduzi, where 65.3% of farmers apply chemical insecticides, in other districts, most farmers do not use any method of control of FAW (60.8% in Manica to 88.0% in Macate and Sussundenga) (Figure 4).

Management and application of insecticides

Among those using chemical insecticides to fight FAW, their primary source of insecticides is authorized dealers' shops (from 50.0% in Sussundenga to 90.0% in Manica). But in some cases, insecticides are either acquired through street vendors or donated by extension services. The majority of farmers spray by themselves (66.7% in Sussundenga to 83.3% in Macate). While in Macate and Manica most farmers are partially equipped with protective gear (66.7% and 50.0% respectively), in Sussundenga and Vanduzi at least half of the farmers reported the use of complete sets of protective equipment (suit, rubber boots, gloves, glasses and masks) (50.0% to 59.4% respectively). Between 66.7% and 100% of the farmers reported that they use the recommended dose of insecticides. In Macate 50.0% of farmers using chemical insecticides have the habit of mixing two or more insecticides, but in the remaining districts this practice is not common (16.7% in Sussundenga, 18.8% in Vanduzi and 30.0% in Manica). All farmers reported using backpack sprayers when applying insecticides. The common spraying intervals used by farmers are seven or fourteen days. Between 67.0% and 75.0% of farmers applying insecticides reported that the insecticides used are efficient in the control of FAW. Despite the reported use of insecticides, from 73.5% of farmers

Table 4. Use of insecticides among farmers per district.

Characteristics of respondents	Use of insecticides per district (%)			
	Macate (n = 6)	Manica (n = 20)	Sussundenga (n = 6)	Vanduzi (n = 32)
Source of insecticides				
Authorized dealer	83.3	90.0	50.0	71.9
Street vendor in sealed packaging	0.0	0.0	16.7	3.1
Street vendor in unsealed packaging	0.0	0.0	0.0	3.1
Extension services/NGO's	16.7	10.0	33.3	21.9
Responsible for spraying				
Farmer himself	83.3	75.0	66.7	68.8
Another family member	0.0	20.0	0.0	15.6
Someone hired	16.7	5.0	33.3	15.6
Use of protective equipment				
Fully equipped	33.3	25.0	50.0	59.4
Partially equipped	66.7	50.0	16.7	31.3
Without any equipment	0.0	25.0	33.3	9.4
Dose of application of insecticides				
Recommended	66.7	85.0	100	87.5
Increased	0.0	5.0	0.0	6.3
Reduced	0.0	0.0	0.0	0.0
Unknown	33.3	10.0	0.0	6.3
Mixture of insecticides				
Mix	50.0	30.0	16.7	18.8
No mix	50.0	70.0	83.3	81.3
Application equipment				
Backpack sprayer	100	100	100	100
Spraying interval				
7 days	16.7	50.0	50.0	25.0
14 days	50.0	40.0	50.0	46.9
21 days	0.0	5.0	0.0	9.4
30 days	16.7	5.0	0.0	6.3
Density dependent	16.7	0.0	0.0	12.5
Efficiency of insecticides				
Efficient	66.7	65.0	66.7	75.0
More or less	33.3	30.0	16.7	25.0
Not efficient	0.0	5.0	16.7	0.0
Training in the handling of insecticides				
Trained	6.0	17.7	6.0	26.5
Non-trained	94.0	82.4	94.0	73.5
Application of insecticides based on				
Monitoring	83.3	95.0	83.3	90.6
Calendar	0.0	0.0	0.0	6.3
Recommendation	0.0	5.0	16.7	3.1
Observation of neighboring farmers	16.7	0.0	0.0	0.0

in Vanduzi to 94.0% of farmers in Macate and Sussundenga do not have training in pesticides use and management. Although the number of sprays can go up to 20× per crop cycle, farmers in Sussundenga generally spray once per crop cycle. In Vanduzi the average number of sprays per cycle is 3×. But in Macate and Manica, most farmers spray 4× during the crop cycle. Most farmers reported monitoring as the basis for deciding to apply insecticides (Table 4).

Table 5. Reported incidence, spread and constraints in the control of FAW per district.

Characteristics of respondents	Number of responses per district (%)			
	Macate (n = 50)	Manica (n = 51)	Sussundenga (n = 50)	Vanduzi (n = 49)
Perceived incidence of FAW				
Low	12.0	23.5	8.0	28.6
Average	38.0	29.4	34.0	36.7
High	36.0	45.0	54.0	32.7
Unknown	14.0	2.0	4.0	2.0
Perceived spread of FAW				
Increasing	38.0	58.8	80.0	59.2
Decreasing	38.0	31.4	10.0	34.7
No changes	16.0	7.8	6.0	4.1
Unknown	8.0	2.0	4.0	2.0
Constraints in the control of FAW				
None	18.0	15.7	8.0	28.6
Lack of financial resources	52.0	56.9	74.0	59.2
Inefficiency of insecticides	18.0	3.9	6.0	12.2
Unavailability of insecticides	8.0	7.8	6.0	2.0
Lack of technical assistance	4.0	11.8	2.0	0.0
Other	0.0	3.9	4.0	0.0

Incidence, spread and constraints in the control of FAW

Most farmers believe that the incidence of FAW is average or high. There is a common perception among farmers that FAW is spreading in the region and the lack of financial resources for the acquisition of insecticides and spraying equipment is reported as the main constraint in the control of FAW (Table 5).

Discussion

Traditionally, the head of the household in the area of study is a man. But specific circumstances may compel women to take on this role, such as when they are divorced, widowed or single. The educational background of farmers seems to play a major role in their ability to get alternative/additional jobs. Given that most farmers rely solely on agriculture, it is both a means of subsistence and a source of income. Although in this study no relationship was established between the level of education and knowledge of FAW, Abtew et al. (2016), pointed out the importance of education in farmers' level of knowledge of agricultural pests. Given that the majority of farmers in the present study have primary education or are illiterate, that may well explain their lack of knowledge of the FAW. Although farmers may own 3 ha or more of land, one should note that not all the area is under cultivation. Due to their limited income, farmers prefer to use their stored maize seeds from the previous harvest, as certified seeds are seen as expensive.

Invasive alien species represent a serious challenge in the context of pest management because farmers and local agricultural extension workers rarely know about the presence of a newly arrived and spreading species until disastrous damage occurs (Toepfer et al. 2019). In Mozambique, for example, FAW was initially confused with stem borers by agricultural extension workers (Cugala et al. 2017). A similar scenario was also reported in Nigeria where FAW was also confused with indigenous species of *Spodoptera* (Goergen et al. 2016). Although most farmers in Zambia (91%) (Kansiime et al. 2019) and in Ethiopia (99%) and Kenya (100%) (Kumela et al. 2018) could positively identify FAW larvae through its morphological characteristics, the same could not be observed in the present study, as only a maximum of 6.1% of the farmers could identify FAW larvae. That might be explained by the fact that the majority of farmers (from 71.4% in Vanduzi to 94.0% in Macate and Susundenga) did not receive any training in identification of FAW. It is important to point out that at the time of the study, the extension service workers themselves were under training in identification and control of FAW by the Ministry of Agriculture, FAO and other agriculture related organizations and universities. Because FAW is a new pest, it can be easily confused with other caterpillars, especially those belonging to the same family (FAO and CABI 2019). However, Toepfer et al. (2019) underscored the fact that even if farmers are not trained by extension workers in identification and management of FAW, they will learn, over time, through their own experiences with the new pest.

The infestation of FAW in maize fields is reported to be high between November and February. This can be explained by the fact that in this interval, maize planted from October to December is still in the vegetative stage which is the most preferred by FAW. But a field survey conducted from May to August 2019 and between December 2019 and January 2020 in the same area, found that FAW infestation was higher during the dry season (Caniço et al. 2020).

Despite an official recommendation from the government to use a select range of insecticides composed of 23 different active ingredients belonging to the major groups of pyrethroids, organophosphates carbamates and organoclorades, and highly selective pesticides such as Spinosad, *Beauveria bassiana* and *Bacillus thuringiensis* to fight FAW (Cugala et al. 2017), only about 12.0% in Macate and Sussundenga, 40.0% in Manica and 65.3% in Vanduzi appeared to follow the recommendation. Abate et al. (2000), explained that although local extension services in African countries may encourage the use of pesticides for pest management, most smallholder farmers rely on indigenous approaches when dealing with pests such as crop associations, mechanical control, use of herbal products and, sometimes, in supernatural ways. Concerning the specific case of FAW in Africa, several methods of control of the pest were reported in various countries such as Zambia where farmers use chemical, cultural and biological control (Kansiime et al. 2019), Ethiopia and Kenya where among other methods, farmers use physical and traditional methods (Kumela et al. 2018). In Zimbabwe for example, as reported by Chimweta et al. (2020), most of the farmers applied pesticides recommended by the government to control FAW, although some of them used non-conventional materials such as washing powders, indicating lack of knowledge of the pest and its methods of control.

In this study, in contrast to other countries, chemical control was the only method used by a limited number of smallholder farmers. Because of the importance of the pest and, with the objective of widening available options, African researchers are testing alternative methods of monitoring and control of FAW, such as the push-pull technology tested in Kenya, Tanzania and Uganda with promising results (Midega et al. 2018) and pheromone trap design and lures for monitoring FAW tested in Togo (Meagher Jr et al. 2019). Because insecticides in Africa are mostly used improperly and often traded in dubious markets, it was recommended that the management of FAW should be based on biopesticides such as the fungi *Beauveria bassiana* and *Metarhizium anisopliae* and baculoviruses because they are environmentally safe with a low risk of human intoxication (Feldmann et al. 2019). *Bacillus thuringiensis*, a bacteria-based biopesticide, could also play a role in low-cost methods (Hruska 2019). Furthermore, when biopesticides are combined with good crop management, they can keep pest levels under control (Bateman et al. 2018).

In this study, there was a common belief among farmers from all districts that FAW is rapidly spreading. This behavior of the pest has been predicted in Zimbabwe by Chimweta et al. (2020) and a similar trend was also observed in Ethiopia and Kenya by Kumela et al. (2018). When farmers were asked about their major constraints in the fight against FAW, some reported no constraint while most of them (from 52.0% in Macate to 74.0% in Sussundenga) indicated the lack of financial means to support the acquisition and application of insecticides, combined with the inefficiency of insecticides being used (from 6.0% in Sussundenga to 18.0% in Macate). In Zimbabwe, around 84.1% of the farmers also reported the lack of financial resources as the main constraint, followed by 73.2% who indicated inadequate labor as the main constraint (Chimweta et al. 2020). A similar scenario was reported in Ethiopia, where the major problems affecting FAW management efforts were reported to be lack of adequate knowledge of the pest and its management options, combined with scarcity of financial and material resources (Assefa and Ayalew 2019). Depending on the context, smallholder farmers may have limitations that will define their pest management options (Hruska 2019).

Conclusions

Although farmers are aware of the presence of FAW in maize fields, the majority of them are unable to morphologically distinguish FAW from other caterpillars, which probably affects their ability to control the pest. Despite there being a government list of recommended pesticides to be used in the fight against FAW, a small proportion of farmers apply insecticides while the majority of farmers take no measure against the pest. Nevertheless, most farmers believe that the incidence of FAW in their fields is high and that the pest is spreading to other territories. Given the importance of FAW to food security, educational campaigns addressing the issues of identification and control of the pest should be implemented targeting smallholder farmers. Alternative methods of control of FAW should be investigated as the simple recommendation of insecticides or other methods that are perceived as expensive or hard to implement may not work.

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Supplementary material 1

Questionnaire used to interview farmers

Authors: Albasini Caniço, António Mexia, Luisa Santos

Data type: Pdf. file

Explanation note: Questionnaire used to interview farmers.

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RESEARCH ARTICLE

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Advancing research on alien species and biological invasions

The biology and ecology of the invasive silver-cheeked toadfish (*Lagocephalus sceleratus*), with emphasis on the Eastern Mediterranean

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Abstract

Invasive species pose threats to either human health or inflict ecological and/or economic damage. The silver-cheeked toadfish (*Lagocephalus sceleratus*), a Lessepsian species, is one of the most harmful species in the Mediterranean Sea, because of its potent neurotoxin, impacts on marine biodiversity, and the increased costs and labor they inflict on fishers. Since the catch and consumption of this pufferfish is prohibited by almost all countries bordering the Mediterranean, they have now expanded into the entire Mediterranean and Black Sea. We performed a comprehensive study of *L. sceleratus* covering ecological aspects, growth, reproduction, diet and trophic level based on samples from southwestern coasts of Turkey. The estimated growth parameters were L_∞ = 88.7 cm, K = 0.27 year^{-1}, C = 0.6 and WP = 0.1. Their sex-ratio was M/F = 1:0.69. *Lagocephalus sceleratus* appears to be a batch spawner with discontinuous oocyte recruitment and has different spawning seasons in the Eastern Mediterranean which seem to be based on temperature cues which get shorter in duration as one moves north from the Suez. We also report their first positive ecological trait, that they are controlling some other invasive species through their diets, such as lionfish, Red Sea goatfish, rabbitfish and longspine sea urchins, in addition to controlling themselves through cannibalism, which appears to be density-dependent. They are indeed a top predator in the region with a trophic level of 4.1. We suggest that targeted fishing using improved gear-types to reduce fishing gear damages are initiated, and that finding commercial markets for pufferfish could help to naturally fund ongoing control efforts.

Keywords
Cannibalism, growth, Invasive Alien Species (IAS), pufferfish, reproduction, Tetraodontidae

Introduction

Global biodiversity is currently being threatened by overfishing, pollution and invasive species (Costello et al. 2010). The Mediterranean Sea is both a biodiversity hotspot and the most invaded sea of the planet, with currently about 800 marine non-indigenous species (Galil et al. 2015; Zenetos et al. 2017; Zenetos and Galanidi 2020), approximately 500 of which are Lessepsian species that invaded from the Red Sea after the creation of the Suez Canal (Por 1978; Galil et al. 2018). Due to the combination of overfishing (resulting in a loss of large predators), globalization (increased marine traffic), and the 2015 widening of the Suez Canal, the fauna of the Eastern Mediterranean is becoming more and more akin to that of the Red Sea. In fact, closer proximity to the Suez Canal correlates to a higher incidence of Lessepsian species (Ulman et al. 2019a). Some fish can swim through the canal unassisted, while their larvae may be stowed-away in the ballast tanks of ships, with sessile species (mostly macro-invertebrates) hitchhiking to new destinations on boat-hulls as part of their biofouling communities (Ulman et al. 2019a, 2019b). Given that the connection between the Red Sea and the Mediterranean is anthropogenic, all biota which arrive via the Suez Canal into the Mediterranean are considered to be non-indigenous species, rather than being the result of natural range expansions (European Environment Agency 2012). Lessepsian migrations may represent the ‘most important biogeographic phenomenon witnessed in the contemporary oceans’ (Por 1978), and also present ongoing opportunities to better understand general biological processes such as species interactions, physiological and ecological adaptations, and evolutionary processes (Ruiz et al. 2000; Bernardi et al. 2010). The Eastern Mediterranean is oligotrophic (Longhurst 2010), which limits the productivity of the subregion, which also suffers from severe overfishing (Demirel et al. 2020; Tsikliras et al. 2021). Because of the ‘fishing down marine food webs’ phenomenon (Pauly et al. 1998), which also occurs in the Mediterranean, including its eastern basin (Stergiou 2005; Keskin and Pauly 2018), high-trophic level, large fish (such as sharks) are now rare, leaving room for new non-indigenous top-level predators to establish themselves.

Non-indigenous species (NIS) are called invasive when they cause either ecological, economical damage, or pose a threat to human health. Marine invasive species can pose major threats to biodiversity by altering community structure and function, and by modifying ecosystem processes, which can have long-lasting ecological and economic consequences (Molnar et al. 2008). Once a marine species establishes itself in a new area, its eradication becomes near impossible, due to the three-dimensional nature of marine ecosystems, and their interconnectedness. Thus, managers commonly decide to wait and hope that the situation naturally resolves itself in time, although when the risks are too great, they may need to implement control measures.

Table 1. Pufferfish species found in the Mediterranean, their native region, first year of introduction, first locality introduced, established status in the Mediterranean, and reported in Turkey (Y= yes, N= no).

Common name	Scientific name	Native region	Year	Locality	Established	In Turkey
Prickly puffer	*Ephippion guttiferum*	E. Atlantic & W. Med.	NA	NA	Y	N
Oceanic puffer	*Lagocephalus lagocephalus*	Subtropical	NA	NA	Y	Y
Diamondback puffer	*Lagocephalus guentheri*	Indo-Pacific	1950[1]	Egypt	Y	Y
Suez puffer	*Lagocephalus suezensis*	W. Indian, Red Sea	1977	Lebanon	Y	Y
Silver-cheeked toadfish	*Lagocephalus sceleratus*	Indo-Pacific	2003	Turkey	Y	Y
Guinean puffer	*Sphoeroides marmoratus*	E. Atlantic	1977	Italy	Y	N
Blunthead puffer	*Sphoeroides pachygaster*	Subtropical	1979	Spain	Y	Y
Bandtail puffer	*Sphoeroides spengleri*	W. Atlantic	2000	Spain	Y	N
Yellowspotted puffer	*Torquigener flavimaculosus*	W. Indian	1987	Israel	Y	Y
Spiny blaasop	*Tylerius spinosissimus*	Indo-Pacific	2004	Greece	Y	Y
Spotbase burrfish	*Cyclichthys spilostylus*	Indo-Pacific	1993	Israel	Y	Y
Spotfin burrfish	*Chilomycterus reticulatus*	Subtropical	2009	Sardinia	N	N
Spotfin porcupinefish	*Diodon hysterix*	Circumtropical	1956, 2016	Italy, Spain	N	N

[1] Formerly misidentified as *Lagocephalus spadiceus*.

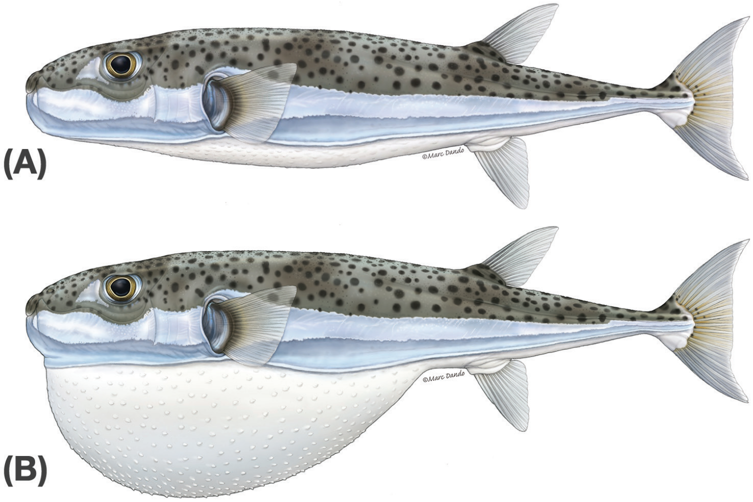

Figure 1. The silver-cheeked toadfish *Lagocephalus sceleratus* (Gmelin, 1789) **A** lateral view **B** 'puffed' lateral view (original drawings by Marc Dando).

There are 197 species of pufferfish globally, 112 of which live in marine environments, 48 in brackish environments and 37 in freshwater (Santharam 2018). In the Mediterranean, there are currently eleven established pufferfish species present, eight of which are found in Turkey; and six of those in Turkey being Lessepsian migrants (Table 1). However, before management can design applicable solutions, at the very least, their biology, and in particular the specific nature of their invasiveness, needs to be assessed. In Turkey, a risk assessment on the invasiveness of five pufferfish species from its southwestern coast, revealed the silver cheeked toadfish *Lagocephalus sceleratus* (Gmelin 1789) (Fig. 1) to be the most invasive based on its diet and life-history traits

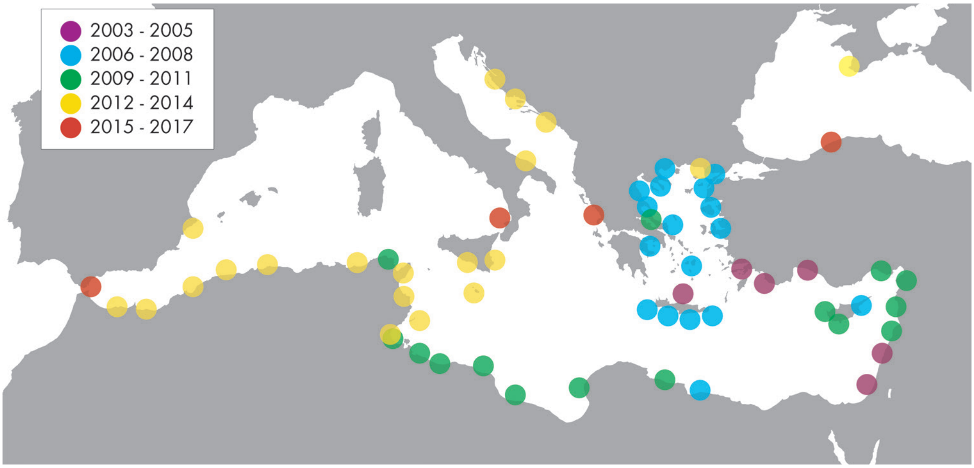

Figure 2. Distribution of *Lagocephalus sceleratus* in the Mediterranean, as documented by records in Bilecenoğlu and Öztürk (2018), Galanidi and Zenetos (2019), Azzurro (2020), and Gücü et al. (2021).

(Filiz et al. 2017). *Lagocephalus sceleratus* causes negative impacts to human health through its high poison content, native ecology from its high abundances and generalist diet, and to the economy from both depredation and fishing gear losses to fishers.

Lagocephalus sceleratus (Fig. 1) is one of the largest and most abundant invasive predatory fish species established in the Eastern Mediterranean. It is a generalist predatory carnivore feeding on crustaceans, fish and cephalopods. Its maximum published weight is 7 kg (Smith and Heemstra 1986; Froese and Pauly 2020), although several Turkish fishers claim to have caught individuals between 10 to 12 kg (pers. comm. to A.U).

An important part of the ecological sucess of *L. sceleratus* is due to their having one of the most advanced forms of teeth in the animal kingdom. The 'first generation teeth' are coated with recurring toothbands which are continously regenerated by stem cells (Thiery et al. 2017). These toothbands fuse to form upper and lower plates, which jointly forms a beak. This strong beak and accompanying plates enable them to crush and slice very tough prey organisms such as decapods and bivalves (Turingan 1994).

The first record of *Lagocephalus sceleratus* in the Mediterranean was from Gökova Bay, southwestern Turkey in 2003 (Filiz and Er 2004; Akyol et al. 2005). *Lagocephalus sceleratus* is normally shy of humans, and thus not readily encountered by snorkelers and divers. This species rapidly and successfully established itself in Turkey and in the waters of Eastern Mediterranean countries such as Egypt, Lebanon, Syria, Cyprus and Greece. Lately, it has now expanded its range to the Black Sea (Bilecenoğlu and Oztürk 2018) and most of the Mediterranean, all the way to the Strait of Gibraltar (Azzurro et al. 2020), implying that it may soon spill into the Atlantic Ocean. Strangely, it has not yet been reported from mainland France, or Corsica, its largest island (Fig. 2). The success of *L. sceleratus* implies a high phenotypic plasticity and an ability to cope with a range of environmental conditions (Golani et al. 2010). For example, the average salinity along the coast of Ukraine in the Black Sea, where *L. sceleratus* has its northernmost occurrence, is only 10–12 psu, much lower than the Mediterranean average of 34 psu. As a result, *L. sceleratus* is found in a variety of benthic habitats, including sandy bottoms, rocky substrates and seagrass meadows (Rousou et al. 2014).

Strong defenses against predation

In *Lagocephalus sceleratus's* 18-year presence in the Mediterranean, only loggerhead turtles (*Caretta caretta*) have been documented preying on adult *L. sceleratus*, while garfish (*Belone belone*), common dolphinfish (*Coryphaena hippurus*), and cannibalism has been documented in juveniles (Ulman et al. 2021). Potential common predators require large throats which can accommodate a puffed up fish, which is the case in loggerhead turtles, and also in predators of *Lagocephalus inermis* from India, which included cobia (*Rachycentron canadum*), and catfish (*Arius* spp.) (Mohamed 2013).

Lagocephalus sceleratus combine two exceptional defense mechanisms which benefit them in evading predators, i.e., the ability to 'puff' themselves up, and their highly toxic tissues. The combination of these two factors contribute, in the Mediterranean, to a scarcity of predators.

Puffing

The peculiar head, buccal cavity and pectoral girdle structures of these fishes facilitate their unique ability to 'puff' themselves up by rhythmic buccal pumping, swallowing and forcing water (or air if they are outside of water) into their stomach. While their 'stomach' can increase its size 50–100-fold depending on the species (Brainerd 1994), it is not a true stomach, having lost its digestive function to allow for puffing. In addition to stretchable skin and a distensible stomach, pufferfish lack pleural ribs and pelvis which would be impediments to 'puffing' (Amores et al. 2004). It has been demonstrated that some predators perished due to the puffing of ingested pufferfish, notably a lemon shark due to blocked gills resulting in asphyxiation (Ulman et al. 2021). Their second major deterrent to potential predators are high levels of tetrodotoxin neurotoxin throughout the body.

Tetrodotoxin

Lagocephalus sceleratus is the second most poisonous Mediterranean pufferfish species after *Torquigener flavimaculosus* Hardy & Randall, 1983 (Ayas 2017; Kosker et al. 2018), and pufferfish are the second most poisonous family after the Synanceiidae family of stonefishes. This is one of the reasons why *L. sceleratus* is viewed as one of the worst invasive species (Streftaris and Zenetos 2006; Otero et al. 2013).

Tetrodotoxin (TTX) is an extremely potent neurotoxin found in *L. sceleratus* and other pufferfish (Amano et al. 2019). TTX inhibits voltage-gated sodium channels, blocking the propagation of nerve impulses (Geffeney and Ruben 2006). Animals that contain tetrodotoxin are resistant to the neurological effects of the toxin themselves

(Kotipoyina et al. 2020). Also, pufferfish containing TTX were found to grow at faster rates, exhibited fewer signs of aggression, and lower stress hormones than pufferfish without TTX (Amano et al. 2019). TTX testing of two very small juvenile *L. sceleratus* (5 cm) found TTX from the muscle of one, and in nearly all tissues from the other, to be above the lethal human 2 mg·kg^{-1} dose (Leonardo et al. 2019), which is a new important finding as juveniles were previously considered to be non-toxic.

Out of the 197 pufferfish species, only 55 (28%) are considered toxic (Santhanam 2018). The high TTX content of *L. sceleratus* preclude this species from being a food fish, even as Japanese *fugu*, which only incorporates pufferfish with TTX values of <2 mg TTX/kg (Noguchi and Ebesu 2001). Generally, the ovaries and male gonads leading up to spawning season have the highest TTX content (Sabrah et al. 2006). High TTX concentrations are also found in the liver, skin, eyes and muscle, but they vary according to season, locality and fish size (Kosker et al. 2016; Rambla-Alegre et al. 2017).

Impacts on fishers and other persons

Lagocephalus sceleratus has strong negative impacts on the livelihoods of small-scale fishers of the Eastern Mediterranean, most of whom are already marginalized due to declining catches and revenues (Ünal et al. 2015). These impacts of *L. sceleratus* are caused by damaging fishing nets, consuming caught fish within the nets (depredation) and eating the bait and hooks from set longlines (Ünal and Göncüoğlu 2017). Thus, losses to fishers occur through fishing gear losses, time losses and losses of catches. Ninety seven percent of surveyed fishers from Turkey's southern Aegean and Mediterranean coasts suffered an average of US$ 183 in fishing gear losses during 2011–2012, which increased to US$ 325 during 2013–2014, and US$ 370 during 2015–2016; note that US$ 370 represents 4.3% of the per capita GDP in Turkey in 2020, equivalent to US$ 8548 in the US (Ünal et al. 2015; Ünal and Göncüoğlu 2017; Öndes et al. 2018). Economic losses due to foregone catches are comparable to fishing gear losses, and were evaluated to be an additional of US$ 353 in 2015–2016 (Ünal and Göncüoğlu 2017). Fishers from southern Turkey are more affected than those in western Turkey due to the higher abundance of *L. sceleratus*, with losses calculated at about US$ 538 per longline vessel in 2016 and US$ 616 per vessels with set nets (Öndes et al. 2018). Gillnets normally used to last between two to five years, but currently, many small-scale fishers are unable to afford replacing their nets in just months after suffering irreparable pufferfish damage; a new two km long gillnet now costs over US$ 2000 in Turkey, which recently increased by 40% due to ongoing currency devaluations.

Interestingly, around Turkey, this species is normally shy of humans and is not commonly encountered while snorkeling or scuba diving. In August 2019, a first human attack by *L. sceleratus* occurred in Kaledran, Turkey where *L. sceleratus* bit a child three times on the left hand, resulting in the amputation of her ring finger (Sümen and Bilecenoglu 2019). In September 2020, a snorkeler was bitten in his calf in Mersin, Turkey (Melih Görkem Bilgin, pers. comm.), and in May 2021, in Antalya, Turkey, there were some snorkelers who had large portions of their fins attacked and eaten by *L. sceleratus*. Aggressive behavior has also been reported by divers in Cyprus (Hasan Deniz Akbora, pers. comm.), and there are growing concerns for safety especially in highly touristic areas of high *L. sceleratus* abundances. For example, during the COVID pandemic, there were several months where locals were restricted from going to the beach, but tourists (very few in number at the time) were permitted, and alarmingly they recorded small aggregations of very large *L. sceleratus* specimens (each between 2–5 kg) in shallow popular beach areas in both Muğla and Antalya provinces, making national news headlines. As Turkey is a popular tourist destination primarily for its beaches and clear waters, an aggressive predatory fish has the potential to negatively impact the tourism sector if interactions with people increase.

Lagocephalus sceleratus poisoning has caused dozens of human fatalities in the Mediterranean region, which is a severe underestimate given that most of these fatalities are not officially recorded (Ben Souissi et al. 2014). In Turkey, from October 2020 to March 2021, five deaths have occurred from consuming *L. sceleratus*.

Despite the multiple negative impacts of *L. sceleratus*, most Mediterranean studies have been dedicated to the high content of tetrodotoxin (TTX) in its tissues, with only a handful investigating its biology (Sabrah 2006; Aydin 2011; Nader et al. 2012; Kalogirou 2013; Farrag 2014; Khalaf et al. 2014; Rousou et al. 2014; Ersönmez et al. 2017; Zengin and Türker 2020). As well, two studies reported on the abundance of *L. sceleratus* in Egypt (Farrag et al. 2015; Elhaweet et al. 2016) and another from Antalya Bay, Turkey (Özbek et al. 2017).

This contribution is an attempt to correct this imbalance. Due to nearly a complete lack of control in the region, its negative impacts to marine biodiversity, human health and fishers' livelihoods continue to worsen. This study presents new data on the species behaviour (eg., spawning, ecology and feeding) based on fishers' knowledge, and from biological studies, presenting new data on their distribution, size, growth, spawning season and reproductive status, reproductive morphology and fecundity, and the taxonomic composition of their prey. This contribution aims to improve current knowledge about this invasive species, to help direct further research needs and management options.

Materials and methods

Study area and sample collection

Pufferfish samples were purchased from small-scale commercial fishers in southwestern Turkey primarily from Datça where they were targeted (36.726°N, 27.685°E) and about 15% of samples were caught as by-catch from Fethiye (36.659°N, 29.126°E), both Muğla Province, Turkey, from June 2019 to November 2020. This stock has not yet been studied and is understood to be a different stock from the neighbouring Antalya province, which has been somewhat studied. This area is very close to Gökova

Bay, where the first Mediterranean *L. sceleratus* occurrence was reported (Filiz and Er 2004; Akyol et al. 2005). The majority of pufferfish were caught by one fisher in Datça, who initially tried using reinforced steel lines with three separate hooks to deter fishing gear losses. However, many of these steel lines were severed by *L. sceleratus* the first day, so the fisher continued both with hook and line, continually replacing lost hooks, and then by trammel net. In the first six months of the study, chicken flesh was used as bait and for the next six months, strips of flesh of adult *L. sceleratus* were used as bait, with similar success (S. Taşkiran, personal observation).

A total of 1013 fish: 456 males, 270 females and 287 juveniles (where juveniles were generally < 25 cm and could not have their sex determined due a lack of gonadal development) were collected for this study from June 2019 to November 2020. Fishers were paid 10 Turkish Lira (≈ US$ 1.20; April 23/2021) per kg for *L. sceleratus* from June 2019 until mid-April 2020, and 20 Turkish Lira per kg from mid-April 2020 onwards. The fish were purchased from approximately 20 fishers from Fethiye and Datça, Muğla province, who all had special permissions to collect them for this study. Permission to collect pufferfish for the specified designated fishers for scientific research purposes was granted from the Turkish Ministry of Agriculture and Forestry and General Directorate of Water Products under Permission #67852565-140.03.03-E.1354602 & #6987137-663.08.

Fisher's knowledge of behaviour

We formally surveyed 45 small-scale fishers face-to-face from the Muğla province (Fethiye to Bodrum) in April 2019 to help understand some of the behavior of this species and to inform them of this study. An initial structured survey consisting of 18 questions pertaining to their contact details, fisher characteristics, vessel and geartypes, average days fished, *L. sceleratus* catches, catch areas, caught depths, average sizes, maximum sizes, fishing gear losses in nets and longline hooks, and interest in catching pufferfish for this study was initially undertaken at the beginning of the study in April 2019. Twelve of those initially interviewed supplied fish afterwards for this study all using trammel nets, after permissions were granted for them to catch pufferfish. Any new information learnt as the study progressed was written down and transferred to the spreadsheets containing the other data. These data were then summarized for each topic. Their responses, aside from the new maximum depth record, should be viewed as anecdotal evidence.

Morphometrics and growth

Information on such basic biological parameters of species, such as growth, reproduction and fecundity are essential in understanding the basic life history traits of a species and are prerequisites needed to develop scientifically sound fisheries management policies. For all 1013 samples, the total length (L) and body weight (W) of fish were measured to the nearest 0.1 cm and the nearest 1 g, respectively, and gonads and livers were weighed to the nearest 0.01 g. The length-weight relationship yields authentic biological information about a species in a particular region and is of great importance in fishery assessments. The parameter of length-weight relationships (LWRs) of the form $W = a \cdot L^b$ were estimated through re-expression of the LWR equations in linearized form, i.e., $log(W) = log(a) + b \cdot log(L)$, where $\boldsymbol{a}$ is a scaling coefficient for the weight at length and $\boldsymbol{b}$ is a shape parameter; note that if $b < 3$, a fish become thinner as it grows, and plumper if $b > 3$.

The growth of water-breathing ectotherms such as fish can be conceived as the net result of two processes with opposing tendencies (Bertalanffy 1938):

$$dW/dt = HW^d - kW \tag{1}$$

where dW/dt is the growth rate, W is body weight (or mass), H and k are the coefficients of anabolism and catabolism, and d is the scaling exponents of anabolism, which depend on oxygen, and hence of the growth of gill surface area (Pauly 1984, 2021). Assuming that $d = 2/3$ and integrating, i.e., re-expressing the differential Equation 1 as a growth curve leads to the von Bertalanffy growth function (VBGF), which is commonly used to describe the growth of fish and which has the form:

$$L_t = L_\infty\,(1 - e^{-K(t-t0)}) \tag{2}$$

where L_t is the length at age t, L_∞ is the asymptotic length, i.e. the mean length the individuals of a given population would reach if they grew indefinitely, K is rate, or dimension time^{-1} (here: year^{-1}) at which L_∞ is approached, and t_0 is the age at $L = 0$.

The mutual compatibility of the growth parameters L_∞ and K can be evaluated by $\text{Ø}' = \log(K)+2\log(L_\infty)$ which should be roughly similar between populations of the same species (Longhurst and Pauly 1987; Pauly 1998).

Here, a seasonally oscillating variant of the von Bertalanffy growth function (VBGF) was used to estimate growth parameters from the length-frequency data available for *L. sceleratus*; this version of the VBGF has the form:

$$L_t = L_\infty\{1 - e^{-[K(t - t0) + S(t) - S(t0)]}\} \tag{3}$$

where $S(t) = (CK/2\pi) \cdot sin(2\pi(t - t_s))$, $S(t_0) = (CK/2\pi) \cdot sin(2\pi(t_0 - t_s)$, and L_∞, K and t_0 are defined as above; see Pauly (1991) for a first application to a pufferfish.

Equation (3) involves two parameters more than the standard VBGF: C and t_s. Of these, the former is easiest to visualize, as it expresses the amplitude of the growth oscillations. When $C = 0$, Equation (3) reverts to Equation (2). When $C = 0.5$, the seasonal growth oscillations are such that growth rate increases by 50% at the peak of the 'growth season' (i.e., in 'summer'), and, briefly, declines by 50% in 'winter'. When $C = 1$, growth increases by 100%, doubling during 'summer', and becoming zero in the depth of 'winter'. The other new parameter, t_s expresses the time elapsed between $t = 0$ and the start of a sinusoid growth oscillation. However, visualization is facilitated if we define $t_s + 0.5 = WP$ ('Winter Point'), which expresses, as a fraction of the year, the period when growth is slowest. *WP* is often close to 0.1 (i.e., early February) in the Northern Hemisphere and 0.6 (early August) in the Southern Hemisphere.

The parameters of Equation 3 were estimated through the ELEFAN method, which fits growth to the peaks of length-frequency (L/F) samples arranged in time (represented by black, positive histograms, and deemed to represent age classes) while avoiding the trough between peaks (represented by white, negative histograms). Peaks and troughs are identified by a simple high-pass filter, i.e., a running average which leads to definition of peaks as those parts of a length-frequency distribution that are above the corresponding running average and conversely for the troughs separating peaks. Then, hundreds of growth curves, each with a different set of growth parameters, are traced, and the growth curve (i.e., parameter set) is retained which has the highest score in linking the peaks of L/F distributions, whose 'point' values are positive, while avoiding troughs, whose point values are negative (Pauly 1991, 1998). The software used here to implement the ELEFAN method was FiSAT, documented in Gayanilo et al. (2005).

Reproduction

Variations in fish gonadal morphology explain important behavioral and ecological adaptations during reproduction. Particularly knowledge about the reproductive period is considered a major life-history trait and evaluating the changes in gonadal development, liver size and body weight can help to understand energy trade-offs in the development of reproductive strategies, notably in the inverse relationship between the gonadosomatic index (GSI) and the hepato-somatic index (HSI), while condition factor (CF) shows the relative health of the fish.

To estimate fecundity, the gonads were removed, weighed and preserved in formalin. To identify the reproductive season, temporal changes in the gonadosomatic index were assessed using the relation: $GSI = 100 \cdot \times [G_W/(TW - G_W)]$ where G_W is the gonad weight and TW is the total weight. Also, the hepato-somatic index analyses was computed as an indicator of reserves in the liver, i.e., $HSI = 100 \cdot \times [H_W/(TW - H_W)]$ where H_W and TW represent liver weight and total weight, respectively. Understanding changes in liver reserves, helps to better understand how energy is transferred from storage to reproduction. Finally, the overall plumpness of individuals was determined from their condition factor $CF = 100 \cdot W/L^3$.

The size at first maturity (and spawning) was estimated by plotting the fraction of mature individual females and males against their lengths, and fitting a logistic curve. Mean length at first maturity (L_m) was the length at which, in a given population, 50% of individuals were mature. This was evaluated separately for fish sampled during the main spawning season (i.e., in June) and outside, to test if *L. sceleratus* reach maturity at smaller sizes within than outside the spawning season.

We also used the lengths of first maturity and maximum lengths in several population of *L. sceleratus* to indirectly estimate their ratios of metabolic rate at first maturity

(Q_m) to maintenance rates (Q_{maint}). These ratios were then used to test whether their mean value is compatible with earlier estimate ranging from 1.22 to 1.53 and suggesting that it is a declining relative oxygen supply which triggers maturation and spawning (Pauly 2021); see 'Gill-Oxygen Limitation Theory' in Suppl. material 1: Appendix 1.

Knowledge on fecundity is used to calculate the reproductive potential of a stock and is another important factor for effective fish stock management. Ovary samples were collected in May and June 2020, to capture the peak GSI values. The oocyte size–frequency method (Murua et al. 2003) was applied to females with migratory nucleus or early hydrated oocytes to assess the fecundity. Murua et al. (2003) explained that if highly advanced oocytes (≥500 mm) were used for batch fecundity estimation, the results become typically similar to the hydrated-oocyte method. Given these considerations, three subsamples, weighing between 20–40 mg, were taken from the anterior, middle and posterior parts of the ovaries. The relationships between number of eggs per batch, length, and ovary free weight were determined by (log)linear regression. The diameters of the oocytes were measured using the Zeiss Labscope App (version 1.3.1) for iPad.

Examination of oocyte development is evaluated to help identify reproductive strategies of species such as ovary organization, fecundity type and spawning patterns (Murua et al. 2003). In order to examine spawning strategy of this species, histological analyses were performed on 70 ovaries. Tissues were removed from the center of each ovary, fixed in 10% formalin solution, dehydrated in an increasing series of ethanol and embedded in paraffin. Tissue sections of 5 μm were stained with Mayer's hematoxylin and eosin and examined with an Olympus BX51 light microscope equipped with an Olympus DP72 digital camera (Roberts et al. 2012). The diameters of oocytes were validated by a second person using Leica image analysis software.

Prey

Knowledge on predator-prey interactions for species are essential to understanding their role in the ecosystem, impacts on biodiversity, and are essential in building accurate ecosystem models for a region. Two complementary studies were conducted on the diet of *L. sceleratus*. The prey/diet preferences were examined by a visual taxonomic examination of stomach content for 563 samples from Fethiye and Datça, Muğla province in Turkey. Food items were removed from the esophagus, stomach and intestine and identified to the lowest taxonomic level possible; fishing hooks and pieces of fishing net were also accounted for, as were sand and algae. The prey taxa were then grouped into three main categories: crustaceans, fish and cephalopods, and also identified as either indigenous or non-indigenous taxa where possible. A t-test was performed on the ratios of the three prey groups for juvenile (< 45 cm) and adult fish (> 45 cm) to determine if they target different taxonomic groups as they grow.

To better understand the role of *L. sceleratus* in the ecosystem, and to estimate their trophic level (TL), their mean fractional level of their prey for 34 stomachs, where the contribution of prey items in numbers (%N), weight (%W) and frequency of occurrence (%F) was recorded. These values were then used for calculating the Index of Relative Importance (IRI) of prey item (IRI = %F × (%N + %W)), which was then re-expressed using %IRI = (IRI/ SIRI) × 100 (Cortes 1997). SIRI is the percentage which a discrete prey taxon contributes to the sum of all IRI values in the prey spectrum. Based on the dietary composition (expressed as W%), the mean fractional trophic level (TP) of the *L. sceleratus* was estimated using the method of Pauly et al. (2000), as implemented in their TrophLab software and the equation: $TL_i = \Sigma j\, TL_j \times DC_{ij}$ where TL_j is the fractional trophic levels of prey *j*, and DC_{ij} represents the fraction of *j* in the diet of *i*. Trophic levels range from 1 for primary producers to 5 for apex predators such as marine mammals and sharks. Stomach fullness was evaluated using a 5-point scale, where 0 = empty, 1 = food residues, 2 = less than half full, 3 = more than half full, and 4 = full (Gaykov and Bokhanov 2008).

Results

Fisher's knowledge of behavior

The fishers who informed this study consisted of 12 using trammel nets, 12 using longlines, 21 using both trammel nets and longlines, and five occasionally using rods. The fishers who provided fish for this study used trammel nets, with three sometimes using fishing rods.

According to these fishers, when *L. sceleratus* first appeared along the southwestern Turkish coast, it was found mostly in rocky areas from depths of about 10 m, and never deeper than 100 m. However, over time *L. sceleratus* were increasingly found in deeper locations to a maximum of 220 m depth (recorded in April 2021 from Fethiye Bay). In June, i.e., during their spawning season, they aggregate in the shallows of bays, between 5–10 m depths; however, a few individuals have also been caught at the surface. Based on the accounts of 45 fishers in Muğla Province, Turkey, *L. sceleratus* regularly consumes bait from rods and longlines, severing many of the hooks and even steel lines in the process. Some fishers reported hook losses from 50–90% of their longlines in extreme cases, but the majority of long-line fishers claimed an average of about 10–20% of hooks lost. Hooks were found in 8% of *L. sceleratus* stomachs; nearly all samples were collected by net. *Lagocephalus sceleratus* uses its fused parrot-like teeth to bite holes in set trammel nets and consume the fish caught in the nets, as evidenced by nine pieces of fishing net between 3–20 cm in diameter in their stomachs, weighing up to 10 g. All fishers in the region are regularly affected by this and try to cast their nets away from *L. sceleratus* hotspots to minimize damages. One fisher from Fethiye (Mehmet Taniş, pers. comm.) explained that on several occasions, *L. sceleratus* bit through his trammel nets, and consumed the stingrays caught inside, leaving only the needle tail portion behind as evidence.

As one fisher, S. Taşkıran, was the main fisher in Datça that targeted *L. sceleratus* for this study, and thus has the most experience with this species, his observations are

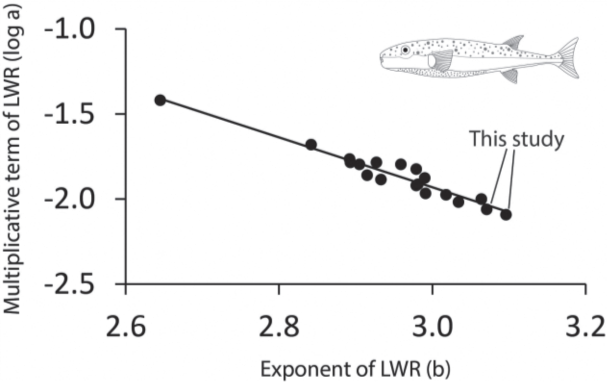

Figure 3. Illustrating the relationship between the multiplicative term (*a*) and the exponent (*b*) of length-weight relationship in *Lagocephalus sceleratus*. (Based on the results of 13 studies from data in Suppl. material 1: Table S3 which based their measurements on TL).

separately noted here. He estimated that in June 2020, there were approximately 10 tonnes of *L. sceleratus* spawning in InçiBurnu Bay near Datça. At this locality, during their spawning period, the fish were inactive at night, and actively fed at dusk and dawn. In July and August, they fed very little, but in September onwards for a few months, they again fed very aggressively.

Morphometrics and growth

In total, 1013 fish were examined, and of those, 456 were male, 270 were female and 287 were juveniles generally below 25 cm whose sex could not be determined. The overall sex-ratio was calculated as M:F = 1.0:0.69. Total length ranged from 13 to 77.2 cm. The mean lengths of females and males were not statistically different ($p > 0.05$, $p = 0.71$) but males were more abundant throughout the entire year. Suppl. material 1: Table S1 compares sex ratios from different localities.

Suppl. material 1: Fig. S2 illustrates the LWRs that we obtained; the slopes (*b*) of the LWRs for females, males, and unidentified individuals were compared and were found to be statistically different ($p < 0.05$, $p = 0.001$; see also Suppl. material 1: Table S2). Notably, females were plumper than males of the same length. Figure 3 compares the LWR results of this study compared to other published studies from Suppl. material 1: Table S2.

The close inverse relationship of log(*a*) vs *b* in Fig. 3 implies that the LWRs in Suppl. material 1: Table S3 (i.e., including those in Figure S2) are all mutually compatible, and predict similar weight for a given length. Pauly (2019), p. 94–95 shows that the different locations of LWRs along gradients log(*a*) vs *b* such as illustrated in Fig. 3 are largely due to different sampling periods of the L-W data pairs used to establish each LWR; such gradients are also well documented in FishBase (Froese and Pauly 2020), for example for the well-studied Atlantic cod (*Gadus morhua*).

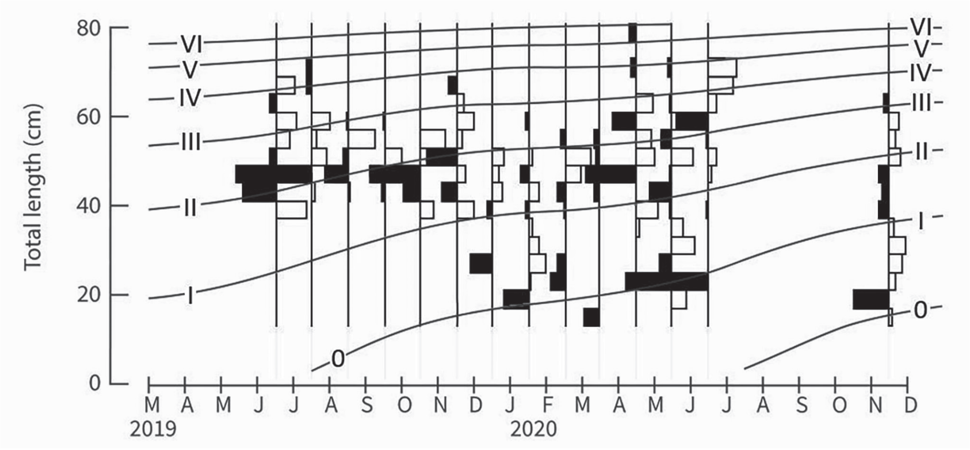

Figure 4. Seasonally oscillating growth curve fitted using ELEFAN to 14 length-frequency samples of *Lagocephalus sceleratus* (n = 1013) collected from June 2019 to November 2020; the estimated growth parameters were L_∞ = 88.7 cm (TL), K = 0.27 year^{-1}, C = 0.6 and WP = 0.1. The Roman numerals (& 0) refer to the 7 cohorts (= sequences of peak, i.e., black histograms) that were identified by ELEFAN (Based on data in Suppl. material 1: Table S4).

The best fit to the length-frequency data that we gathered (see Fig. 4, Suppl. material 1: Table S4) was obtained for the growth parameter L_∞ = 88.7, K = 0.32 year^{-1}, C = 0.6 and WP = 0.1. The estimates for C and WP imply that the seasonal changes in water temperature in the sampling area impact the growth of *L. sceleratus*, which the estimate of WP implies is most reduced in early February 2020. The growth parameters that we estimated are compatible with those estimated by other authors from other parts of the Mediterranean Sea (see values of Ø' in Suppl. material 1: Table S5).

Reproduction

The ovarian organization of *L. sceleratus* appears to be based on synchronous development of groups of oocytes. Two concurrent populations of oocytes were found during spawning, i.e., larger oocytes and a more heterogeneous group of smaller oocytes (Fig. 5). Post-ovulatory follicles were not observed in our samples, but atresia (the degeneration of ovary follicles which do not ovulate) occurred in both the previtellogenetic (before formation of the yolk) and vitellogenetic (yolk formation process in the oocyte) phases (Fig. 5). Overall, the spawning pattern thus appears to be batch spawning with discontinuous oocyte recruitment.

Oocyte diameter during vitellogenesis were found to range between 0.42–0.58 mm, with an average oocyte size of 0.50 mm for the migratory nucleus stages. Oocyte counts were performed on 23 female ovaries from the peak reproductive period. Average fecundity was calculated as 134,000 oocytes for females of 55 cm and

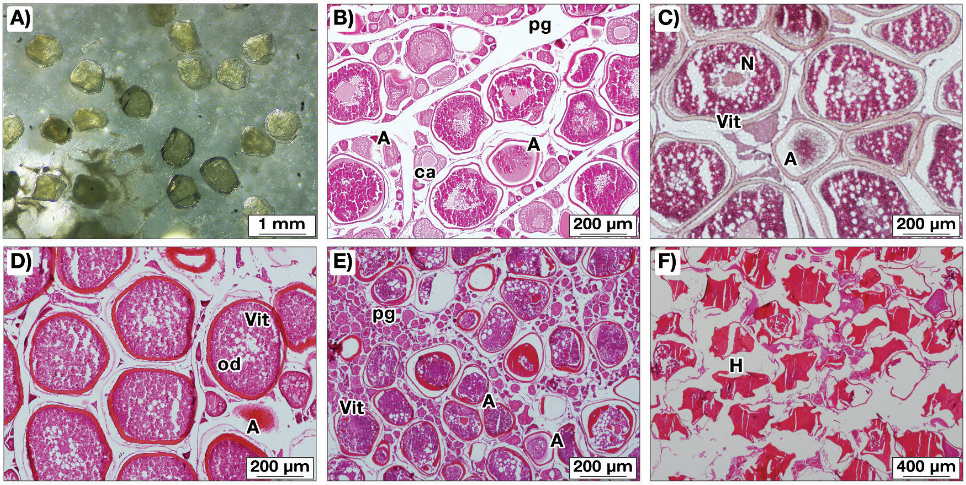

Figure 5. Stages of oocyte development in *Lagocephalus sceleratus*. Whole oocytes on the slide **A** and histological sections **B–E** from nucleolus to vitellogenesis; and **F** hydration: Primary growth (pg), cortical alveoli (ca), nucleus (N), vitellogenic oocytes (Vit), oil droplets (od), atretic oocyte (A), and hydrated oocytes (H). Scale bars 1 mm (**A**); 200 µm (**B–E**); 400 µm (**F**).

2,000 g. The relationships between fecundity vs. length, and oocyte number vs. body weight are provided in Suppl. material 1: Fig. S3.

GSI starts to increase in April and May, peaks in June (9%), then declines sharply in July (see Fig. 6, the top panel showing the GSI results of this study- Fethiye), suggesting that the main reproductive season of *L. sceleratus* in southwestern Turkey is late spring-early summer (May-June). This is confirmed by fishers' observations that spawning aggregations of *L. sceleratus* occur from the last days of May and span the month of June. Near Datça, Muğla, in 2020, the highest GSI values occurred in mid-June (10.2%) with numerous individuals caught while spawning. A second, minor spawning season is suggested by a small increase in GSI in September for both sexes. Fig. 6 illustrates that there is a tendency for the spawning season (i.e., the high GSI season) of *L. sceleratus* to become shorter, the further one gets from the Suez Canal; this graph assimilated the spawning seasons of *L. sceleratus* from the Mediterranean and other nearby regions with the results of this study at the top of the graph (Fethiye).

Condition factors were similar between sexes, and its monthly variability (not shown) was not very pronounced; it exhibited a weak peak in June (during peak spawning season) and another in November. The baseline of the HSI index was around 3–4%. The HSI index started to increase in November to peak at 8% in April, thus suggesting that reserves were taken from the liver to be used for gonadal development.

As in other fish species, observed maturity stages in *L. sceleratus* were a function of size (Figure 7), and the mean size at maturity, or L_m (i.e., the size at which 50% of the examined fish were mature, or L_{50}) for females and males are presented in Fig. 7. Here, the two features of interest are that there appears no clear pattern of one of the

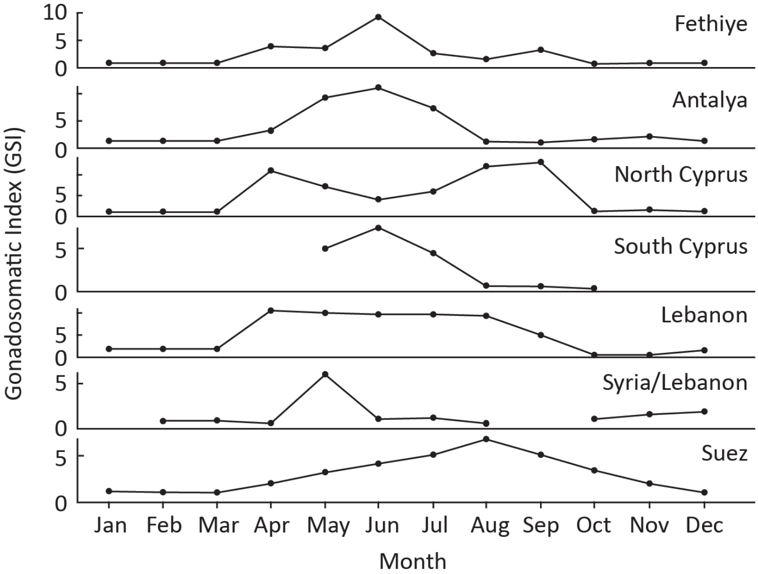

Figure 6. Seasonal variation of the Gonadosomatic Index (GSI) of *Lagocephalus sceleratus* in the Mediterranean and the Suez region, based on data by Sabrah 2006, (1st location at the bottom of the figure), Syria-Leb/Khalaf 2014 (2nd location), Lebanon/Boustany 2015 (3rd location), S. Cyprus/Rousou, 2014 (4th location), N. Cyprus/Akbora 2020 (5th location), Antalya Bay/Aydin 2011 (6th location), and Datça and Fethiye (top trend) from this study (values are averages of n = 14–340 fish per month, Suppl. material 1: Table S4). Note the trend toward a shorter spawning season as one moves North (upward from Suez).

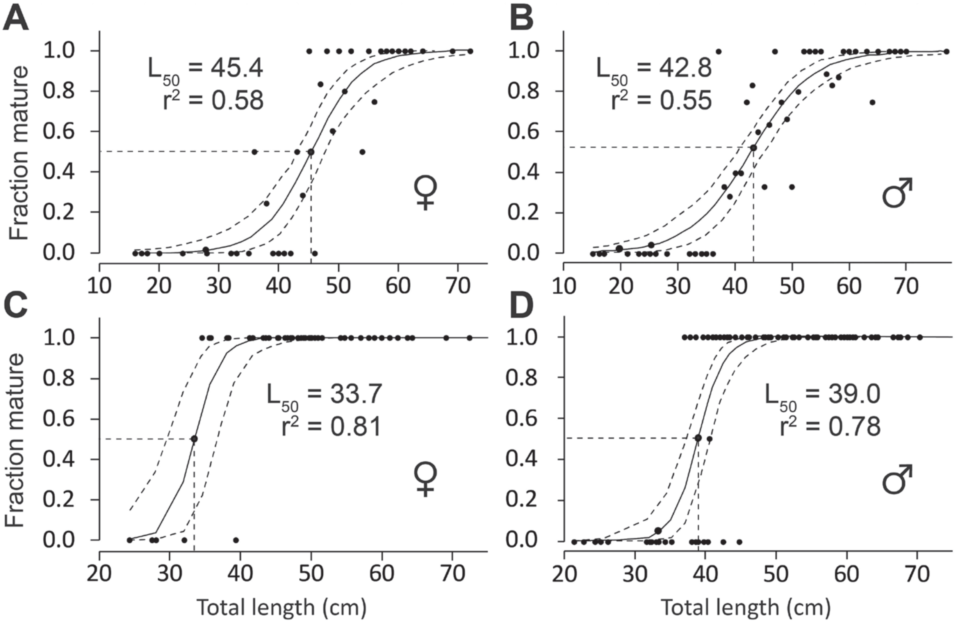

Figure 7. Maturity as a function of length in for *Lagocephalus sceleratus*. Note that mean length at first maturity is higher outside the spawning season (**A, B**) than inside (**C, D**) the spawning season, for both sexes.

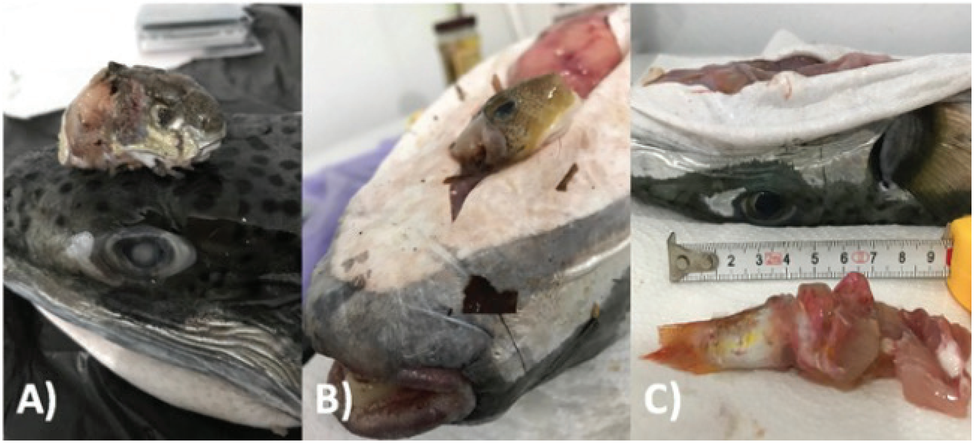

Figure 8. Stomach contents of *Lagocephalus sceleratus* presenting evidence of cannibalism (**A**) and predation on other invasive species, i.e., *Torquigener flavimaculosus* (**B**); and *Parapeneus forsskali* (**C**).

sexes reaching maturity earlier than the other, and perhaps more interestingly that in both sexes, Fig. 7 sexual maturity (L_m) is reached earlier during the spawning season than outside.

Prey

Of the 563 fish that had their stomach contents examined, 48 (9%) of the stomachs were empty, 58 (10%) had food residues, 253 (45%) had stomachs less than half full, 170 (30%) were over half full, and 34 (6%) were full. A total of 34 specimens (Suppl. material 1: Table S7) were found as prey items from 8 non-indigenous species (NIS); Of these, 23 were Tetraodontidae species: 10 juvenile *L. sceleratus*, 10 *Torquigener flavimaculosus*, two *Lagocephalus* spp., and one *L. suezensis* (Fig. 8). Other NIS were three *Pterois miles*, three *Parapeneus forsskali*, two *Siganus* spp., dozens of small gastropods (*Cerithium scabridum*) and one long-spine sea urchin (*Diadema setosum*). A total of 6% of *L. sceleratus* had consumed non-indigenous species.

Crustaceans and fish made up the majority of diets being found in 26% and 24% of stomachs, respectively, with cephalopod remains in 11%. There was no statistical difference between the taxonomic prey composition between juvenile and adult *L. sceleratus* ratios of crustaceans, fish and cephalopods ($p = 0.225$). For crustaceans, small crabs were the major taxon, with only a few stomachs containing shrimp remains, as expected, since crab shells take longer to be digested and/or evacuated. Of the crabs, *Carappa granulata* was found in two stomachs, one *Carcinus aestuarii*, one *Charybdis* sp., and one *Scyliarides latus*. For fish, those that could be identified were three *Pterois miles*, *Scorpaena* spp., *Epinephelus* spp., Mugilidae spp., *Atherina* spp., *Diplodus* sp., *Sparus aurata* and *Siganus* spp. Of cephalopods, there were about a dozen cases each of common squid (*Loligo vulgaris*), common octopus (*Octopus vulgaris*), one violet blanket octopus (*Tremoctopus violaceus*) and unidentified cephalopod beaks and ink (Suppl. material 1: Fig. S4). Three of the stomachs examined contained a lot of sand (between 8 to 10 g) suggesting that some individuals dig in the seafloor looking for food items. One stomach contained some seagrass *Posidonia oceanica*. In addition to food, a total of 48 fishing hooks were found, 9 pieces of fishing net (weighing between 4–7 grams, with one very large 20 cm × 15 cm net sample), and 2 pieces of metal wires.

From the IRI examination, 34 additional *L. sceleratus* stomachs were analysed, and 91% (31) of those had food in their stomachs (coefficient of vacuity: 23.5). The IRI results are presented in Suppl. material 1: Table S8, and prey fish taxa were identified as as *Lagocephalus* sp. and *Mullus* sp., and one *Octopus vulgaris*. The remaining shrimp, crab and cephalopod species could not be clearly identified to lower taxonomic groups. The trophic level of *L. sceleratus* was estimated as 4.15, which is the level assigned to tertiary consumers or carnivorous fish.

Discussion

L. sceleratus offers a trifecta of highly negative impacts due to its high toxicity, economic losses to fishers, and negative effects on native marine biodiversity. Their unique ability to puff and high toxicity likely contribute to their invasive success in the Mediterranean. Due to a nearly complete lack of population control, this species has expanded to all corners of the basin, putting people, fishers, fisheries and the native ecology at risk. Its conquest of the Mediterranean is one of the most successful marine invasions in modern history, comparable with that of the invasive Western Atlantic lionfish *Pterois volitans* and *Pterois miles*, the latter having also established itself as a Lessepsian species in the Mediterranean in 2012 (Bariche et al. 2013; Côté and Smith 2018).

Their marked expansion benefits from both a lack of human control (as fishing and sales of pufferfish are prohibited in most countries, including all countries of the EU), and limited predatory control (due to their ability to puff, and high TTX content). Their success is also likely enhanced by the overfishing that characterizes the Mediterranean basin, which has lost its top predators (Halouani et al. 2015). Indeed, with a Mediterranean trophic level estimate of 4.15, *L. sceleratus* can be considered a top predator, and may even be an apex predator due to a very pronounced regional loss of top predators due to overfishing (Demirel et al. 2020). Despite the overfished nature of the Eastern Mediterranean, *L. sceleratus* appears to be well-fed due to its generalist nature and sharp beak, with over 80% of samples having consumed a recent meal. It should also be emphasized here that *L. sceleratus* prey items very rapidly disintegrate into a ‘soup’ in their stomachs, and the species which were identified (other than tough shelled organisms) were very freshly swallowed and hence found in their esophagus.

Here we reveal the results of biological studies on their morphometrics and growth, reproduction, and diet before presenting some management advice and ideas for further directed research. The morphometric (LWRs) and growth studies conducted here produced results that were comparable to those of other authors. This also included the ratio L_{max}^{D}/L_{m}^{D}, which was statistically undistinguishable from estimates of this ratio in other teleosts (Pauly 1984; Amarasinghe and Pauly 2021; Meyer and Schill 2021). This implies that although *L. sceleratus* is unique in its invasive abilities and some biological features, its life-history is still constrained in the manner predicted by the Gill-Oxygen Limitation Theory (GOLT; Pauly 2019, 2021). However, one interesting finding that apparently has not been highlighted by other authors, or in other fish species that we are aware of, is that mean length at first maturity (L_m) of *L. sceleratus* is lower during the peak spawning season than outside of it (Fig. 7). This may occur in other teleosts, but to our knowledge, this feature has not been previously reported. Clearly, this should be examined further, notably when comparing non-indigenous and native fish, to test if perhaps this is an adaptation of successful non-indigenous species.

Using traditional biological sampling combined with fishers' knowledge improved the biological understanding of *L. sceleratus*, e.g, their spawning periodicity. We also found the HSI and GSI patterns to be asynchronous, which explains how its energy is stored and utilized (Torcu-Koç et al. 2020): peak HSI occurred in April, suggesting that the liver stores reserves up to that month; after which the reserves are used for gonadal development in the two months following. The June peak in condition factor, on the other hand, corresponds to the period prior to the major annual spawning event.

From its ovarian organization, *L. sceleratus* was identified as a group synchronous batch spawner from the presence of both previtellogenic oocytes (in a range of sizes) and larger vitellogenic oocytes (of larger similar sizes) in the ovaries during the peak spawning period. The presence of these two clearly different size groups of oocytes is defined as group asynchronous ovarian organization, with a heterogeneous population of oocytes in their primary growth stage together with a synchronous population of larger oocytes in the yolked stage, indicating further recruitment into the oocyte stock at any time during the spawning season (Hunter and Goldberg 1980; Murua et al. 2003). It is well identified that fecundity types of fish species exhibiting group synchronous ovarian organization have determinate fecundity, meaning the species has a fixed potential annual fecundity at the onset of spawning (Murua et al. 2003). The most important indication of fecundity type is the observation of atresia in different ovarian stages, but our histological samples consisted of mature ovaries from peak spawning season and did not exhibit atresia to clearly evaluate fecundity type. Therein, our findings slightly differ from the only other study on the reproduction of *L. sceleratus* in the Mediterranean coast, which reported their fecundity type as determinate with clear presence of atresia (Farrag et al. 2019). One possible explanation could be that our study was limited to the peak spawning season, which implied a lack of oocyte size changes before and after the spawning season. Also, Ganias et al. (2015) noted that the indeterminate pattern of the oocyte size-frequency distribution was continuous until almost the end of the reproductive period. It is commonly accepted that fecundity type is strongly related to environmental impacts, such as temperature and food availability, and is thus flexible (van Damme 2010). Considering this flexibility, it can be speculated that *L. sceleratus* is still adapting to its new habitats in the Mediterranean. Indeed, its various Mediterranean subpopulations have different spawning seasons, likely connected to different temperature cues, and their spawning seasons may change with continuous sea warming. The spawning season reported here for Muğla province, Turkey, which is restricted to late May and June, is the shortest spawning season reported from the Mediterranean (Fig. 6), and it supports the hypothesis of environmental factors determining fecundity types. We are confident that the fish we collected from Fethiye and Datça in this study are representative of the fish from the Muğla province, due to their slightly different reproductive season than the neighbouring Antalya province. As a recent invasive species, the *L. sceleratus* populations in the Mediterranean can be assumed to be relatively homogenous, as not enough time has occurred for much genetic differentiation to have evolved. Additionally, this study offers new insights into the dynamics of a different stock in Turkey. Clearly, further research should be directed at detailed spawning studies spanning an annual cycle, and by collecting samples daily, which would improve on the fecundity values reported here and allow for testing of this hypothesis.

The most important finding of this study is that *L. sceleratus* appears to prey on a wide range of other invasive species, and its control of them is its first positive documented ecological trait. The spines of lionfish (*P. miles*) found inside three *L. sceleratus* suggest that pufferfish are preying on lionfish. *L. sceleratus* also provide some control on other invasive species such as Red Sea goatfish, rabbitfish, other pufferfish species, their own species, and even the longspine sea urchin (*Diadema setosum*). The finding that their target prey composition is nearly equally comprised of fish and crustaceans, and a lesser extent of cephalopods, did not differ between juveniles and adults which contrasts the earlier findings of Kalogirou (2013) who found a dietary shift in prey from crustaceans to cephalopods to occur as *L. sceleratus* grows. This contrast may be due to density-dependent factors such as the increased abundances of pufferfish resulting in a marked reduction in cephalopods, which is commonly noted by fishers. Note also that their prey included toxic and venomous species, such as lionfish mentioned previously, scorpionfish and other pufferfish species. It was known that *L. sceleratus* is cannibalistic in its native range (EastMed 2010; Aydin 2011), and this study provides the first evidence of cannibalism in the Mediterranean. Their toxicity is also likely heightened by their newer cannibalistic trend, which may cause higher TTX concentrations due to bioaccumulation (Zhang et al. 2020). Cannibalism was not reported in earlier studies (Kalogirou 2013; Rousou et al. 2014), suggesting it may be a density-dependent factor which developed more recently, possibly induced by hunger. Thus, even juveniles should be considered as potentially toxic, as was recently reported.

Lagocephalus sceleratus are now in direct competition with small-scale fishers in the Eastern Mediterranean, consuming their catches, revenue, time and thus much of their livelihoods. Their increasing damage to fishing gear also negatively impacts their livelihoods. In Cyprus, fishers often use newspaper articles mentioning pufferfish damage to lobby for financial support. In the Muğla province of Turkey, Ünal (2013) found over 90% of small-scale fishers were no longer generating a net income from their work. Small-scale fishers from southern Turkey, already highly marginalized, with many being forced out of the profession due to declining catches and incomes, have to completely replace their fishing nets every few months at an added cost of over $2000 US due to pufferfish damage, which previously lasted them several years. As both an incentive with the benefit of aiding fishers offset the increasing costs from pufferfish, a bounty program was recently initiated in Turkey.

The Turkish government recently completed a pilot bounty project collecting *L. sceleratus* tails from the Turkish Mediterranean coast in December 2020. A total of 46,000 tails were collected for a reward price of US $0.60 each. A second bounty program was established on June 27, 2021 for a duration of three years, during which *L. sceleratus* will be, this time, collected in its entirety (@ US $0.60 each) so that proper disposal can be ensured (Mahir Kanyılmaz, Fisheries Directorate, Ankara, Turkey, pers. comm.). Even if this initiative is not effective at reducing abundances, it will still add some positive economic benefit to some fishers. To predict how much the population of *L. sceleratus* should be reduced to negate its impacts to native biodiversity through predation, its biomass, the biomass or abundance of its prey and its feeding rates must first be known. However, only one stock assessment from a small area has been completed in Turkey (Özbek et al. 2017). One study that modeled the required reduction of invasive lionfish densities to improve native fish communities found the reduction threshold to vary by site from 25–92% (Green et al. 2014).

After discussing the bounty program with twenty small-scale fishers from the Muğla province of Turkey, we strongly believe that this new bounty will not be effective at reducing their population enough to negate their effects. At present, small-scale fishers refuse to target this species due to the low reward and high costs of fishing gear damage; however, large-scale fishers may return specimens for reward if many are caught in a net at once. Alternatively, to control this species, we suggest a better solution would be to hire select commercial fishers, equip them with more resistant fishing nets, and have them specifically target *L. sceleratus* in their spawning season where they tend to aggregate. One Turkish fishing gear technology expert, Dr. Zafer Tosunoğlu, Ege University, who was contacted for advice on the most applicable net material to specifically target *L. sceleratus* suggested using Dyneema netting (used for catamaran trampoline netting), which is the strongest netting fibre currently available, 15 times stronger than steel, and should minimize fishing gear damage. Also, emerging 'genetic biocontrol' may be applied (Teem et al. 2020) to control *L. sceleratus* and other invasives.

The development of commercial applications for invasive species such as *L. sceleratus* may financially support their ongoing removal (Giakoumi et al. 2019). Applications in the bio-medical industry include using their skin as antimicrobial wound dressing (Iswariya et al. 2016), their teeth as regenerative dental implants (Thiery et al. 2017), and TTX for extreme pain relief (Hagen et al. 2008, 2017). *L. sceleratus* skin is also being trialed as a type of exotic fish leather (Alla et al. 2017). Since each surveyed fisher despises this species as it causes them a great deal of stress from fishing gear and fish losses, the possible development of a commercial market for pufferfish would not likely encourage any fishers to undertake fishing this species over the long-term to secure their new stream of income, as sometimes occurs after commercialization. Regardless, this invader appears to be here to stay in the Mediterranean.

This NIS top predator in the Mediterranean threatens local biodiversity, human health, fishing communities and potentially even tourism. Since it is currently lacking control on its population in most of the Mediterranean Sea, we suggest that removal through targeted fishing during its spawning period is the best control recommendation for decision-makers. Removals needs to be prioritized but can be expensive to fund, which is why commercialization of this species could help financially sustain their long-term control. The current commercial solutions, which would use a highly invasive species to benefit our teeth and skin, heal our wounds, alleviate our pain and protect our feet, if successful, could represent the largest turnaround in the history of marine invasions.

Prior to this study, most Mediterranean research on this species either studied its growth, or toxicity. This study added to this body of knowledge by determining the spawning strategy and reproductive ecology of *L. sceleratus*, factors relating to its growth, and its position within the trophic web, its density-dependent cannibalistic nature, and its potential ability to help control subsequent invasions. The diversity of their prey can be used as inputs for ecosystem modeling efforts, which, along with improved biomass estimates, can help to understand how much should be removed to help improve the state of native biodiversity. Further directed research needed to better understand and hence manage this invasion should involve mapping its various spawning habitats and seasons, its larval ecology and growth, its feeding rates, DNA stomach content analysis, a forum to update on their interactions with humans, and baseline stock assessments along the entire Levantine coast.

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Supplementary material 1

Appendix 1, Figures S1–S4, Tables S1–S8

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Data type: docx. file

Explanation note: **Appendix 1.** The Gill-Oxygen Limitation Theory. **Figure S1.** Plot of L_{max}^{D} vs. L_{m}^{D} (for D = 0.6) in different populations of *L. sceleratus*. **Figure S2.** Length-weight relationships for A) female and B) male *Lagocephalus sceleratus*. **Figure S3.** Relationships, in *Lagocephalus sceleratus* between fecundity and total length, total weight and gonad weight during peak spawning in June 2020. **Figure S4.** Stomach contents of *Lagocephalus sceleratus* showing freshly ingested cephalopods: *Loligo vulgaris* (A), *Octopus vulgaris* (B); and parts of *Tremoctopus violaceus* (C). **Table S1.** Sex ratios for *L. sceleratus* from Mediterranean and Suez Canal studies. **Table S2.** Length-weight relationships of *Lagocephalus sceleratus* from this study in southwestern Turkey. **Table S3.** Some length-weight parameters with sex, length range, length type and sample size (N) for *L. sceleratus* from Mediterranean and other studies. **Table S4.** Length-frequency data of *Lagocephalus sceleratus* collected in Southwestern Turkey from June 2019 to November 2020. **Table S5.** Growth parameters estimates for *Lagocephalus sceleratus* in the Mediterranean Sea (L in cm). **Table S6.** Mean length at first maturity (Lm) and maximum length (Lmax) for *L. sceleratus* at various locations of the Suez Canal and Mediterranean Sea. **Table S7.** Frequency of non-indigenous species (NIS) preyed upon by *L. sceleratus* in this study. **Table S8.** IRI Results of prey items for 34 sampled *L. sceleratus*.

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