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Black, Grey and Watch Lists of alien species in the Czech Republic based on environmental impacts and management strategy

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Abstract

As legislation, research and management of invasive alien species (IAS) are not fully coordinated across countries or different stakeholder groups, one approach leading to more or less standardized activities is based on producing lists of prominent IAS that attain high level of concern and are a subject of priority monitoring and management. These so-called Black, Grey and Watch (alert) Lists represent a convenient starting point for setting priorities in prevention, early warning and management systems. It is important that these lists be based on transparent and robust criteria so as to accommodate interests and perception of impacts by groups of concerned authorities and stakeholders representing sectors as diverse as, e.g. forestry, horticulture, aquaculture, hunting, and nature conservation, and to justify possible trade restrictions. The principles for blacklisting need to be general enough to accommodate differences among taxonomic groups (plants, invertebrates, vertebrates) and invaded environments (e.g. aquatic, terrestrial, urban, suburban, seminatural), and must take into account invasion dynamics, the impact the IAS pose, and management strategies suitable for each particular invader.

With these assumptions in mind, we synthesize available information to present Black, Grey and Watch Lists of alien species for the Czech Republic, with recommended categorized management measures for land managers, policy makers and other stakeholders. We took into account differences in the listed species' distribution, invasion status, known or estimated environmental impact, as well as possible management options, and apply these criteria to both plants and animals. Species with lower impact, but for which some level of management and regulation is desirable, are included on the Grey List. Some potentially dangerous species occurring in European countries with comparable climatic conditions, as well as those introduced in the past but without presently known wild populations in the Czech Republic, are listed on the Watch list. In total, there are 78 plant and 39 animal species on the Black List, 47 and 16 on the Grey List, and 25 and 27, respectively, on the Watch List. The multilayered approach to the classification of alien species, combining their impacts, population status and relevant management, can serve as a model for other countries that are in process of developing their Black Lists.

Keywords
Alien species, Black list, Czech Republic, impact, legislative tools, management

Introduction

Impacts of invasive alien species and Black Lists: state of the art

Although only a small proportion of introduced species become naturalized or invasive and have a measurable impact (Lockwood et al. 2013; but see Ricciardi et al. 2013), biological invasions by alien species (introduced to regions outside their native distribution range due to human activities; Richardson et al. 2000, Blackburn et al. 2011) affect the majority of habitats, including semi-natural ones. Invasive alien species (IAS), with their widely documented impacts on biodiversity, ecosystem functioning and economy (Pyšek and Richardson 2010, Vilà et al. 2010, 2011, Pyšek et al. 2012c, Scalera et al. 2012, Follak et al. 2013, Blackburn et al. 2014, Jeschke et al. 2014) are recognized as one of the key components of global environmental change (MEA 2005). Costs due to IAS were estimated to reach up to 5% of global GDP (Pimentel et al. 2001, 2002). In Europe, recent estimates of direct costs due to IAS reach at least 12.7 billion € per year (Kettunen et al. 2009). It is also important to note that direct environmental and eradication costs associated with environmental weeds or pests are only a small fraction of costs caused to agriculture or forestry. Nevertheless, even these figures on overall costs for environmental weeds and pests illustrate the need for an urgent policy response at all scales, from national to international and global, supported by a corresponding scientific knowledge base; the fact that the majority of alien species are introduced intentionally or in association with imported/transported commodities (Hulme et al. 2008, 2009) provides an opportunity for interventions (Roques and Auger-Rozenberg 2006, Dehnen-Schmutz et al. 2007, Kenis et al. 2007).

In Europe, more than 12,000 alien plant and animal species are recorded (DAISIE 2009, www.europe-aliens.org) and the numbers of successfully establishing species continue to grow (Hulme et al. 2009, van Kleunen et al. 2015). Unfortunately, research, legislation, and management of IAS are not fully coordinated, neither within individual countries, nor continentally (Hulme et al. 2009), which leads individual countries to cope with alien species in different ways. The most common approach is based on producing lists of prominent IAS that receive much attention and are prioritized in terms of prevention, monitoring and management. These so-called Black, Grey and Watch (alert) Lists represent a convenient starting point for setting such priorities (European Commission 2014). The necessary condition for making such lists trustworthy is, however, a robust and transparent risk assessment, based on the impacts of individual species, allowing their scientifically defensible selection (Wittenberg and Cock 2001, Verbrugge et al. 2012, Lewis and Porter 2014). The transparency is important so as to accommodate interests and perception of impacts by groups of concerned authorities and stakeholders representing sectors as diverse as, e.g. forestry, horticulture, aquaculture, hunting and nature conservation, and to justify possible trade restrictions (Bayliss et al. 2013, Kelly et al. 2013, Ööpik et al. 2013). Therefore, when developing regional Black Lists, interests that differ among the above-mentioned sectors need to be taken into account. Many intentionally imported alien species are of a high economic value (DiTomaso et al. 2010, Richardson and Rejmánek 2011, Woziwoda et al. 2014), but can have negative impacts on native populations, species and communities due to a wide range of mechanisms and processes that have been described in the literature in the last decade (e.g. Levine et al. 2003, Gaertner et al. 2009, 2011, Mitchell et al. 2010, Pyšek and Richardson 2010, Vilà et al. 2010, 2011, Dodet and Collet 2012, Pyšek et al. 2012c, Scalera et al. 2012, Blackburn et al. 2014, Jeschke et al. 2014). However, although these processes are becoming reasonably well understood, there is still much uncertainty about which particular species will have an impact in specific environmental settings and how the invaded habitats and ecosystems will be impacted (Leung et al. 2012, Blackburn et al. 2014). Ideally, each intentional introduction of a new alien species should be thus preceded by a cost-benefit analysis of negative vs. positive effects on both the environment and socioeconomy (Keller and Drake 2009). The decision should then reflect the climatic and habitat match between the current range of the species and the region to which it is proposed for import, as well as information about previous invasion history and life history traits of the species itself, or its close relatives (Kolar and Lodge 2001, Keller and Springborn 2014).

IAS regulation in Europe and in the Czech Republic

The urgent need to tackle biological invasions, develop a common policy and establish an early warning system in Europe, has been recognized by the European Commission (see the Communication 'Towards an EU Strategy on Invasive Species', (COM (2008) 789 final) and EU Biodiversity Strategy to 2020 (http://ec.europa.eu/environment/nature/biodiversity/comm2006/2020.htm). Part of this activity is aimed at the new EU

Regulation on IAS COM (2013) 620 (European Commission 2014), which is an important legislation on invasive species threatening biodiversity and human well-being (Genovesi et al. 2015). Besides setting a framework for roles and responsibilities among the different bodies dealing with IAS it will include a list of species that pose the most significant threats (list of alien species of the Union concern) and thus should be prohibited from the import, sale, and use in Europe. This list will be prepared by the European Commission on the basis of the criteria set out in the Regulation; the EU member states participate in the process of the preparation of the list (by providing comments and proposals for individual IAS inclusion). Although national Black Lists may play an important role in the process of preparation of the EU list, so far only a few countries have developed their own Black Lists with some legislative support (Essl et al. 2011).

The development of national and regional Black Lists and identification of important species, based on using standard and transparent criteria, is a key aspect of the early warning and information systems. Some European countries or trade sectors (agriculture, aquaculture) already regulate the introduction and transport of selected species, based on risk assessments provided by the European Plant Protection Organisation (EPPO), European Food Safety Authority (EFSA) and UK Department for Environment, Food and Rural Affairs (DEFRA). An example of a working system is international cooperation in the field of agriculture pests (EPPO, DEFRA) which can serve as a template to be followed for the management of IAS in Europe in general (Brunel et al. 2013). Not only legislative tools are affecting the policy on IAS. To prevent the spread of alien species and restrict their trading, a significant component of policy and public involvement are voluntary codes of conduct developed for example for horticulture or sheltered under the Bern Convention (Heywood and Brunel 2011, Caffrey et al. 2014, Halford et al. 2014, Heywood 2014).

In the Czech Republic (78,866 km^2, 10.5 millions of inhabitants), as in many other European countries, there is an elaborate and legislatively well-anchored system of the approach to harmful organisms in agriculture. In the field of nature conservation, legislation is not sufficient and does not adequately respond to the current threats from biological invasions, but the issue of IAS has become in the last years one of the priorities in the Czech national strategic environmental documents (State Environmental Policy 2012–20, State Programme of Nature and Landscape Conservation 2009, Biodiversity Strategy 2005). These documents emphasize the need to focus on IAS, including development of priority lists of species for management, creating financial tools and preparation of new legislation, which will be encouraged by the adoption of the new IAS EU legislation.

Scoring species for Black Lists

Despite significant progress in producing lists of important alien species for individual countries (see review in Essl et al. 2011), a standard methodology for the complex assessment of their impacts only started to appear recently (e.g. Blackburn et al. 2014). Such a framework needs to be accompanied by a close cooperation between policy makers, researchers and practitioners in nature/biodiversity conservation and IAS management, to allow for harmonization of the information flow on IAS (Ricciardi et al. 2000, Kettunen et al. 2009, Shine et al. 2009, Caffrey et al. 2014).

Species with documented strong negative impacts, that threaten ecosystems, habitats or native biota, should be eradicated from the newly invaded sites as fast as possible, and further introductions of such species avoided (Convention on Biological Diversity 1992, Genovesi 2005). However, if resources are limited, the question remains which species, which locations and how (considering feasibility and control methods) should be targeted first, and this prioritization can be addressed by different methods (Humair et al. 2014).

The criteria for placing individual species into particular Black List categories need to be general enough to accommodate differences among various taxonomic groups (plants, invertebrates, vertebrates) and invaded environments (e.g. terrestrial, aquatic; urban, suburban, seminatural), take into account invasion dynamics, the environmental and socio-economic impact they pose and management strategy suitable for each particular invader. The existing Black Lists do not take differences between invaded habitats and management feasibility into account in their assessment, do not cover socio-economic impacts and are restricted to selected taxonomic groups (Essl et al. 2011). Some of the existing impact assessments, serving as a basis for Black Lists, multiply the impact scores by a given species' population status (Gederaas et al. 2012, http://ias.biodiversity.be) but as far as we know, there is no system that incorporates information on the type of invaded habitat and management feasibility into the Black List classification.

Aims of the study

In the Czech Republic, there is a thorough knowledge of biological invasions that has resulted in publications of comprehensive and updated lists of alien plants and animals (Pyšek et al. 2002, 2012b, Šefrová and Laštůvka 2005) with an indication of their invasion status using commonly accepted classification (Richardson et al. 2000, Pyšek et al. 2004, Blackburn et al. 2011). However, the classification of alien species based on management criteria has not been available up to now. Still, for any management planning, setting the priorities among species and habitats is crucial. In this paper we thus combine information on the potential environmental impact of alien species in the Czech Republic, their current or predicted population status, the feasibility of management, and type of invaded habitats. As a synthesis, we present Black, Grey and Watch Lists of alien species for the country, with recommended categorized management measures for land managers, policy makers and other stakeholders.

Data and classification approach

Data sources and species selection

The proposed Black and Grey Lists of alien species in the Czech Republic are based primarily on the existing inventories of plant (Pyšek et al. 2012b) and animal (Šefrová and Laštůvka 2005) alien species. The data from these lists were amended by recent updates of the alien biota in the Czech Republic for particular groups such as fishes (Musil et al. 2010), national museum collections or unpublished records (personal communications and databases). The Watch List of alien species includes those currently not present in the wild in the Czech Republic and occurring there only in captivity or cultivation, but reported from the wild in other European countries with similar climate and habitats. Existing lists of aliens in these comparable countries, as summarized in e.g DAISIE or Nobanis, were thus screened to generate the Watch List for the Czech Republic.

To minimize the possible subjective bias of experts assessing species on original lists, each species was reassessed according to the current state of its population status, invaded habitats, cultivation and farming history, impact on environment (ecology) and socio-economy and with respect to the knowledge of its effective management. The species sharing similar patterns of classification were then grouped into subgroups of Black and Grey Lists (see details below). Species included in Black Lists were those posing significant strong negative effects on the environment and where some management, if available and feasible, should be applied. Grey List was used for species with limited negative environmental impact, where monitoring and local management is also relevant. Species for Watch List were selected from those that may in the near future colonize the territory Czech Republic and whose monitoring and management, due to possible substantial negative environmental impact, is recommended.

The evaluation of alien species occurring in the Czech Republic was done for vascular plants, vertebrates and most invertebrate groups. As the classification of alien plant species in the Czech Republic is more elaborated than that of animals, in terms of their regional population dynamics or abundances (Pyšek et al. 2012a, b), the criteria for the Black List species' assessment were first developed for plants and then adapted for other taxonomic groups.

Criteria for classification

For each species included in the Black, Grey and Watch List based on the above criteria, the following information on their populations was assessed, if available, and used to classify species.

A. Mode of current spread:

1. Plants and animals that are intentionally released into the environment for landscaping, restoration or hunting (the 'release' pathway according to Hulme et al. 2008)

and distribution of the species is highly dependent on human activities. Without presence of humans activities the species will disappear in relatively short time.

2. Current spread is mostly spontaneous without direct contribution of humans. For this category it is not crucial if the initial occurrences resulted from past human activities (abandoned plantations, populations of animals escaped from cultures, contaminants) or results of spontaneous spread from other areas where they are alien. Without presence of human activities the species will remain in the landscape for relatively long time.
3. Combination of release and spontaneous spread.

B. Distribution:
Current distribution regardless of whether the species occurs as a result of release or spontaneous introduction. This categorization does not take into account abundance of the species. Both groups can be represented by dense or sparse populations. Especially in case of regionally widespread species, which are present in numerous, well established and continuously replenished populations, their local management cannot be usually efficient. However, in some cases local management may still be performed to reduce specific impacts, e.g. local and time-restricted trapping of *Neovison vison* (American mink) before the bird breeding season.

1. Regional: Present distribution of the species at a large scale or future expasion not strongly restricted by environmental constraints is expected. Clusters of local populations dispersed across country exchanging individuals due to the transport of propagules or active migration.
2. Local (isolated populations): current and also future distribution in localized area(s) within the Czech Republic. The distribution can be limited by e.g. climate or habitat specificity. The localized distribution makes management efficient if there are effective methods available.

C. Evaluation of environmental impact
Standardized assessment of environmental and socio-economic impact is not available for all alien species in the Czech Republic. Therefore it was assessed using the simplified rationale of GISS (Nentwig et al. 2010, Kumschick et al. 2012, Vaes-Petignat and Nentwig 2014) and the recently suggested unified classification of alien species based on the magnitude of their impacts (Blackburn et al. 2014). The black listing in this study is based primarily on the environmental impact of populations occurring in the outdoor environment, and excludes e.g. alien species only having significant economic impact as storage pests. Due to the lack of direct knowledge on impacts of many species in the Czech Republic, their impact was classified as "potential impact", taking into account any impact of the given species reported from climatically similar regions, and also considering interactions with, or impact of, ecologically similar species. The impact was classified based on expert judgement into three levels ranging from limited (minimal) to moderate and massive, with respect to whether it results in irreversible

negative changes to native populations, species or ecosystems (e.g. due to predation, competition, hybridization, ecosystem functioning). For impact assessment we used data from Kumschick et al. (2015), and Rumlerová et al. (unpublished).

D. Evaluation of socio-economic impact

Socio-economic impact and impact on humans was additionally assessed for taxa with considerable environmental impacts to support final reasoning of recommended management. The weight of socio-economic impact was used and ranked high in case of species like *Ambrosia artemisiifolia* (common ragweed), *Heracleum mantegazzianum* (giant hogweed), where strong negative impact on human health is significant or *Arion vulgaris* (Lusitanian slug), and *Varroa destructor* (varroa mite), which have direct effect on agriculture. The impact was classified based on expert judgement into three levels ranging from minimal to moderate (most weeds and pests) and massive.

E. Management options

Management options were assessed along axes representing the management itself, the context of invaded habitats, and population status. The species were classified according to the applicable management strategy (see details below and in Table 1).

Complete eradication is hardly feasible in the Czech Republic, an inland state surrounded by other countries, and can be only achieved, if at all, by intensive international cooperation followed by continuous sanitary measurements. Although complete eradication is usually feasible only on islands (e.g. Chapuis et al. 2004, Genovesi 2005, Simberloff et al. 2011), in some cases it is an ideal target to which efforts should be directed. In practice, complete eradication is possible only for populations of alien species that do not yet spread. For large infestations consisting of many metapopulations, complete eradication above some threshold is almost impossible due to enormous costs (Rejmánek and Pitcairn 2002, Pluess et al. 2012a, b). High cost of management can be justified only for newly detected occurrences of highly important alien species. Unfortunately, intentions behind eradication attempts are often led by wrong ideas to restore ecosystems to their “historical” state, which is often idealized. Eradication is sometimes initiated by the local public or little-informed conservation activists, and often is accompanied by damages to native communities.

Tolerance (resignation) means to refrain from any systematic attempts to manage the given alien species; although both lead to the same result, reasons for them are fundamentally different: tolerance is result of a decision based on the fact that the given IAS has a low impact, while resignation is an enforced attitude if there are no existing management options. The latter currently happens in e.g. mine disposal sites in northern Bohemia, where management is passive approach, and eradication efforts focused on a few selected plant species and habitats. Many newly introduced plants continuously spread as a result of restoration of brownfields and landscaping (Kabrna et al. 2014). Similarly, for some insects, e.g. *Harmonia axyridis* (harlequin ladybird), any management action is almost impossible.

Table 1. List of selected management options (detailed classification) applied to alien species.

Management option	Description	Recommendation
Tolerance/ resignation	This approach is relevant in many ecosystems/sectors (forestry, fishery) for several reasons. Many alien species occurring now in the landscape are of a high economic importance. This approach is also relevant for large populations of widespread alien species especially in urban and suburban environments. Direct eradication of such species is almost impossible or associated with enormous costs and likely to bring doubtful results.	Tolerance is applicable in several cases. In some urban and suburban areas we recommend to tolerate the species of a high economic value as well as species eradication of which is almost impossible because of their wide distribution. This tolerance should exclude areas of high conservation value where approaches including local eradication with subsequent change of local management can be applied. Tolerance cannot be used in rural landscape where primary aim is to prevent new alien populations from establishing. We recommend to tolerate e.g. large populations formed as a result of old abandoned plantations (e.g. *Robinia pseudoacacia*) or release (crayfish, white-tailed deer).
Eradication	Complete eradication of alien species at national scale. It is usually demanding in terms of financial, time and human labour resources, and would require transboundary coordination in case of species present also in neighbouring countries.	Complete eradication should be used primarily for small and pioneer populations where rapid response is likely to result in successful action. It is also applicable to small populations of relatively large animals where hunting or other effective control is feasible. Eradication is not recommended in urban and suburban environment where it usually fails for several reasons (public opinion, high propagule pressure). The complete eradication of several species currently posing strong negative socio-economic impact can be reasoned.
Containment	Local eradication or suppression of alien species' populations. Depending on infested area and habitat type, the costs can vary. Repeated and continuous management is necessary to meet the goals.	Containment is recommended only for sites with high conservation priorities or to lower the negative impact of selected alien species. Due to high costs and need to repeat the actions regularly it is not recommended in large areas, or urban and suburban environment. Containment can be used to reduce e.g. the propagule pressure.
Removal of populations from abandoned plantations and farming facilities	Removal of populations after cessation of their planting or farming, especially related to biofuel plants and animals bred in cages, fishponds or forest enclosures.	Complete eradication of the populations at local scale is recommended, as there is a high risk of escape into natural environment following the abandonment.
Prevention of spread to (semi-)natural environment	This management option refers mainly to revegetation activities in suburban zones (along road and railway corridors) and to species released for forestry, game hunting or fishery.	This option should be used in most cases to avoid conflicts of nature conservation with forestry, landscaping, agriculture and hunting. If a release of a species into the wild is considered, preference should be given to native or locally native taxa. Examples are e.g. brown vs rainbow trout, or red vs sika deer.
Change of management	Change of management is a widely used method applicable to a wide range of habitats. In rural landscapes such a recommended management (preferred by nature conservation) is similar to the traditional management (regular mowing, removal of shrubs, grazing). This management option includes also hunting and fishery practices.	In case of plants, change of the current management should be used to reduce the cover and therefore impact of local dominants. Important condition is that the management has to be permanent and resulting ecosystem must be of higher natural quality than the previous one. Change of management is relevant for a wide range of stakeholders including forestry, game hunting and fishery.

At present we are unable to stop the invasion of such species, let alone eradicate them completely.

Stratified approach reflects the local/regional context of the invasions and therefore represents, in the vast majority of cases, the optimal strategy. An example is the management of *Robinia pseudoacacia* (black locust) in the Czech Republic, whose planting can be allowed in areas where the stands do not represent an imminent threat to the landscape, but should be prohibited, and extant stands eradicated, from sites with nature conservation needs, such as in and around steppe habitats. Similarly, some economically important alien fish species are tolerated in aquaculture ponds (many of which are localities of high conservation value, and even listed among protected nature reserves and Natura 2000 sites), but in other localities might be subject to management. For example, the native *Salmo trutta* (brown trout) should be preferred over alien salmonid fish, such as *Oncorhynchus mykiss* (rainbow trout), in stream habitats, but alien fish species are less likely to pose a conservation problem in ponds used for recreational fishing. The stratified approach thus discriminates where and when the management of alien species is needed and efficient, and where the eradication is neither effective, nor necessary (e.g. in urban and suburban areas). The stratified management limits counterproductive and useless actions against alien species and places them into the framework of nature protection and traditional land use management.

Results

Although there are differences in life histories, population status and possible management options between plants and animals, in the proposed scheme for black-listing we were able to produce comparable Black, Grey and Watch lists for these groups together. In the Black List, species were assigned into three categories according to their impact, distribution, population dynamics and management strategy (Table 2). It is important to note that individual subgroups of Black Lists do not reflect the importance of the included species in the descending order. Species listed in the Grey List have lower impact than Black-Listed species, but still may require some level of management and regulation. The eradication of Grey-List species at a large scale is not a high priority, nevertheless their management is recommended in some restricted areas with nature protection concerns. Grey and Watch List species should be monitored for any rapid change in their distribution and possible impact, especially on the environment.

In total, there are 78 plant and 39 animal species on the Black List, 47 plant and 16 animal species on the Grey List, and 25 plant and 27 animal species on the Watch List (Appendix).

Table 2. Categories of Black and Grey Lists with indication of recommended management, handling restrictions, species examples and classifying criteria that are derived from environmental and socio-economic impact, population status and distribution of the target species. See Table 1 for details of the categories of recommended management.

Lists category	Grouping criteria	Population status, dynamics and distribution of target species	Recommended local management	Handling and release restrictions	No. of plant species	Plant examples	No. of animal species	Animal examples
BL1	High environmental and socio-economic impact.	Abundant, distributed in a wide range of habitats, throughout the country. Species showing high population growth rate and colonization potential.	Complete eradication; eradications or containment everywhere, disposal of abandoned plantations.	No release; application of trade regulations.	2	*Ambrosia artemisiifolia*, *Heracleum mantegazzianum*	3	*Neovison vison*, *Procyon lotor*, *Varroa destructor*
BL2	Moderate to massive environmental impact. Species depending highly on human actions that promote their spread.	Species often found as remnants of planting in gardens and plantations, or in case of animals introduced for hunting and fishing (released or escaped). Usually species with wide distribution, occurring in urban as well as in (semi-)natural habitats.	Stratified approach; instead of economically important species, alternative native species should be promoted. If necessary for economic activities in areas with low conservation value, keeping in capture could be permitted, with prerequisite of prevention escape, and removal of the captive population once the economic activity has ceased. In case of plants disposal of the remnants of abandoned plantations is needed.	No release, legislative regulations of trade and handling, regulation for planting in suburban and rural landscape, some of the economically important species (marked by *) can be planted outside areas of high natural value.	49	*Acer negundo*, *Ailanthus altissima*, *Robinia pseudoacacia*, *Asclepias syriaca*, *Helianthus tuberosus*, *Solidago* sp. *Symphyotrichum* sp., *Telekia speciosa*, *Pinus strobus*, *Quercus rubra*	8	*Cervus nippon*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Oncorhynchus mykiss*, *Ovis musimon*, *Salvelinus fontinalis*
BL3	Moderate to massive environmental impact. Current distribution results from spontaneous spread and unintentional introductions.	Species usually with wide distribution which results mainly from spontaneous spread. Species occur in urban as well as in (semi-)natural habitats.	Stratified approach; due to spontaneous distribution there is no need to tolerate in any area.	No release.	27	*Abutilon theophrasti*, *Bunias orientalis*, *Conyza canadensis*, *Echinochloa crus-galli*, *Iva xanthiifolia*, *Rumex alpinus*, *Senecio inaequidens*	28	*Ameiurus melas*, *Arion vulgaris*, *Cameraria ohridella*, *Dikerogammarus villosus*, *Harmonia axyridis*, *Myocastor coypus*, *Ondatra zibethicus*, *Trachemys scripta*

Lists category	Grouping criteria	Population status, dynamics and distribution of target species	Recommended local management	Handling and release restrictions	No. of plant species	Plant examples	No. of animal species	Animal examples
GL	Currently with limited environmental impact.	Scattered distribution throughout the country, resulting from spontaneous spread and escape from planting or captivity. Can be regionally or locally distributed.	Tolerance; outside areas of a high conservation value no need to take direct actions.	Where appropriate, change in management can be employed to reduce their distribution.	47	*Bidens frondosus*, *Erigeron annuus*, *Impatiens parviflora*, *Juglans regia*, *Lonicera caprifolium*, *Rubrivena polystachya*, *Sedum hispanicum*	16	*Ameiurus nebulosus*, *Astacus leptodactylus*, *Eriocheir sinensis*, *Fascioloides magna*, *Gyrodactylus cyprini*, *Rupicapra rupicapra*

Black and Grey Lists of alien species in the Czech Republic

There are in total 1454 alien vascular plant species recorded in the Czech Republic (36.6% of the total flora; Pyšek et al. 2012a, b), however, the vast majority of them do not have a measurable impact. This group of "low impact species" consist of species that (i) are unable to reproduce or develop viable populations outside cultivation (casuals); (ii) are naturalized but have not expanded their range for a long time, or even failed to persist and became rare (e.g. *Agrostemma githago*, common corn-cockle) and (iii) are locally naturalized, having potentially negative impact (e.g. *Celastrus orbiculatus*, oriental bittersweet), but their sparse distribution still makes management feasible. Within the last group belong species which are candidates for priority monitoring (e.g. biofuel plants like *Paulownia tomentosa*, princess tree). Alien plant species with potentially high risk of environmental and potential negative socio-economic impact thus recruit from naturalized species starting to spread (85 species), or species with continuing spread (61 species).

The assessment of fauna was based on several sources providing an overview of alien animal species occurring in the Czech Republic: 662 species from the DAISIE database (Pergl et al. 2012), 595 species from the catalogue of alien animal species (Šefrová and Laštůvka 2005), and 490 species from the list of alien terrestrial insects occurring in indoor and outdoor environments (Šefrová 2005 and unpublished database of Šefrová et al.). This screening resulted in a total of 680 alien animal species, the majority of which are terrestrial insects (490), followed by other terrestrial and aquatic invertebrates (110) and vertebrates (80). Of the alien terrestrial insects, 249 are known to be restricted to indoor spaces where stable temperature allows them to shelter from harsh winter conditions outside, and the same holds for the majority of arachnids and gastropods. These species, unable to escape into the outdoor environment, were thus not included in the assessment for the Black List. As a result, we identified 184 animal species that occur outdoors and have (or potentially may have) an environmental impact.

There are 102 established (naturalized) but not invasive insect species that have not spread significantly or had already spread in the past and now are considered as a part of resident communities. Among the invasive insects, seven species have an impact on native insects and 41 can be classified also as pests in agriculture, forestry or horticulture. Of these, 28 species cause significant losses to the economy and are therefore permanently monitored and managed; monetary value of the damage to the environment, if at all possible to estimate based on current knowledge, is by an order of magnitude lower than that to economy.

In the list, we retained two invertebrate species known to have more devastating effect on agriculture than on biodiversity, *Arion vulgaris* (Lusitanian slug) and *Varroa destructor* (varroa mite), which potentially can also have a strong environmental impact. *Arion vulgaris* is generally widespread and may influence also natural communities by herbivory and competition with native gastropods; the environmental impacts of *V. destructor* are indirect, through its potential effect on the pollination by honeybees. In aquatic environments, the proportion of invertebrates with possible impact on native species or ecosystems is relatively high, with representatives from macrozoobenthic molluscs, such as *Dreissena polymorpha* (zebra mussel), or crustaceans, such as the amphipod *Dikerogammarus villosus* (killer shrimp), or invasive crayfish (*Orconectes limosus*, spiny-cheek crayfish; *Pacifastacus leniusculus*, signal crayfish).

Alien vertebrates are the smallest group in terms of species number, but host the highest proportion of species causing ecological impacts. There are marked differences among vertebrate groups. There is no alien bird with negative ecological impact in the Czech Republic, and only one reptile (*Trachemys scripta*, pond slider), which so far does not seem to be able to reproduce in the wild under the local climatic conditions. In contrast, fish and mammals with well documented or potential impact are quite common. Several of these fish (~10 spp.) and mammals (~15 spp.) are already widely distributed in the Czech Republic, and their complete eradication is not feasible. However, local/regional eradication or suppression by management action may be possible. It is therefore important to reduce new introductions and releases and strictly control the vicinity of farming and breeding facilities (e.g. deer parks, fishponds) to prevent or at least diminish escapes into nature.

The groups of alien species classified within the Black (BL1–3) and Grey Lists are characterized mainly by level of impact, type of spread (affecting the management and regulation). Species with high environmental and high socio-economic impact are in BL1. Species with high or medium environmental impact and almost negligible socio-economic impact are then classified according prevailing mode of their spread (BL2, BL3). Species, the environmental impact of which is limited at present, are included in the Grey List (GL). The detailed description of the groups is following:

Species group BL1: Species with the greatest impact and with the strongest regulations recommended/needed; their populations should be managed whenever possible although they are already present in large numbers in the Czech Republic and their complete eradication is not feasible. Whenever feasible, it is important to limit further spread of these species; for species where efficient management strategy is not available at present, research that may provide management options is warranted. The group includes two plant and three animal taxa. Plants listed in these category are rapidly spreading neophytes, an annual *Ambrosia artemisiifolia* (common ragweed) and monocarpic perennial *Heracleum mantegazzianum* (giant hogweed), having strong impacts on native biodiversity and/or posing direct threats to human health (allergy and photodermatitis) (Nielsen et al. 2005, Hejda et al. 2009, Pyšek et al. 2012a). Animal taxa comprise heterogeneous group of species which include *Varroa destructor*, a mite affecting bees, and two mammal species (*Neovison vison*, American mink; *Procyon lotor*, racoon). As *Varroa* has also significant socio-economic impact and is restricted to honey bee colonies, its distribution is monitored and management is already driven by state authorities.

Species group BL2: Species depending highly on human actions that promote their spread (mostly combination of release and spontaneous spread), both types of distribution, and mostly with moderate to massive environmental impact, but minimal socio-economic impact; 49 plant and 8 animal taxa. These species are often found as remnants of planting in gardens and plantations or in case of animals introduced for hunting and fishing, which facilitates their further spread. Instead of economically important species, alternative native species should be promoted. If necessary for economic activities in areas with low conservation value, keeping in capture could be permitted, with prerequisite of good prevention of escape, and removal of the captive population once the economic activity has ceased. Spontaneous populations outside urban areas or areas of captivity should be reduced by change of local management, or by local eradication campaigns when feasible. Specific focus should be on areas with high conservation value.

Species group BL3: Species whose current distribution results from spontaneous spread and unintentional introductions. They cover species with both types of distribution and impact ranging from limited to massive (Appendix). The recommended strategy for these species is stratified approach balancing between the local needs and the available resources for eradication. As none of the species is planted or released intentionally, the management and trade regulations can be more straightforward than in BL2. If locally necessary and there are known efficient eradication methods for the given species, eradication should be attempted. In urban and suburban environments species can be tolerated, but eradication or suppression by change of local management (land use) is recommended.

Species group GL: Species with limited environmental impact at present, distributed both regionally and locally, and with current distribution as a results of spontaneous or combined spread. For the listed species outside areas of a high conservation value there is no need to take actions against them, or restrict them. Change in management may be actively taken into account to reduce their distribution. This group consists of 47 plants and 16 animals, and is substantially formed of several weedy plant species and parasites.

Watch List of alien plant and animal species

The Watch List (Appendix) contains selected high-impact species that (1) have not yet been recorded from the Czech Republic but occur in other European countries with similar climatic conditions and habitats (and thus may be successfully introduced to or invade the Czech territory), (2) species that are at present kept in culture or enclosures only (such as *Capra aegagrus*, wild goat, or *Bison bison*, American bison), or (3) species introduced in the past but without presently known wild populations, which may be considered potential competitors for native species (several fish species). In case of plants this is analogous to species already present in e.g. gardens, parks or aquaculture (e.g. *Azolla filiculoides*, Pacific mosquitofern; *Paulownia tomentosa*, princess tree) which may in the future establish in the wild and became problematic. There are 25 plant and 27 animal taxa on the Watch List. For these species, as well as for some sparsely distributed species from the Black or Grey Lists, preventive actions against their introduction to and subsequent spread in the country, or uninvaded regions, are justified.

Discussion

This paper provides the first assessment of alien species in the Czech Republic in terms of their environmental impact, with direct habitat-related recommendations for land managers, policy makers and other stakeholders. Introduction and naturalization of a new species is a dynamic process (Blackburn et al. 2011, Richardson and Pyšek 2012, Lockwood et al. 2013), therefore the published lists of this kind are not and cannot be definitive. One of the important aspects of such a work is that it can stimulate discussion on the assessment of individual species as well suggestions of possible additions or deletions, from people involved in research, management, as well as general public.

It has to be highlighted that the proposed groups BL2 and BL3 within the Black List do not show the importance of the included species for prioritization of the management as their environmental impacts, though not negligible, may vary. The grouping is used mainly to differentiate between various management options in respect to particular site conditions. Furthermore, these lists are based on environmental rather than socio-economic impact. Thus, we did not include in the list pests causing heavy economic losses, like *Leptinotarsa decemlineata* (Colorado potato beetle), the impact of which is restricted exclusively to agriculture. In contrast, we included, for example, *Varroa destructor*, whose impact on commercial honey bees may have indirect environmental consequences through effects on pollination of many plant species.

Within the Grey List, we included also a taxon that, despite being a part of the alien fauna in the Czech Republic, does not require management in the wild but rather import restrictions. This is the case of the Chinese mitten crab (*Eriocheir sinensis*), a potential host of a serious pathogen that can be transmitted to freshwater crayfish, i.e., native species of conservation relevance (Svoboda et al. 2014). Due to its transient occurrence in the Czech Republic (during periodic migrations only), this species was not listed in group BL3 that includes alien crayfish species with the same capability but established in the country and thus eligible for local management. For the Chinese mitten crab, a legislative ban of release into the wild as well as regulation of trade and import of live individuals are recommended; if an import is considered, only dead animals for food market should be imported.

The system presented here follows the recommendations of IUCN that all newly introduced alien species should be treated as "guilty until proven innocent", following the precautionary principle (Genovesi 2005). The proper evaluation of a species is hindered by a possible lag phase between the introduction and naturalization (Williamson et al. 2005, Blackburn et al. 2011) and a wide range of possible impacts that are context-dependent (Pyšek et al. 2012c, Hulme et al. 2013, Horáčková et al. 2014). In reality, the recognition of problematic invasive alien species in early stages is very difficult and usually not possible until the species is widely distributed; at that stage, however, it is usually too late for its easy eradication (Pluess et al. 2012b).

Invasive alien species are responsible for many negative effects on native species and ecosystems, particularly in areas with a high conservation status (Foxcroft et al. 2013) where IAS management is costly and makes up a large proportion of the protected area management budget (Frazee et al. 2003). In contrast, in many ecosystems, human activities and resulting land-use change, such as increasing intensification of agriculture and urbanization, or abandonment of industrial areas, promotes existence of "novel" habitats where some alien species might be a valuable component (Hobbs et al. 2006, Gaertner et al. 2012). This is the case of green areas in and around cities where the native species diversity is reduced and vegetation is composed of a few dominant native species accompanied by aliens with a relative low cover. Urban areas are a significant source of alien species (Aronson et al. 2014, Kowarik et al. 2013), but they also fill important ecosystem services with wide socio-economic implications. Therefore, to eradicate or not is often not a simple decision, especially if one takes into account financial costs and feasibility of such a management action.

A separate issue related to alien species and our proposed Black, Grey and Watch Lists are recent developments in the area of biofuel plants and animal species imported for aquaculture and farming. It has been suggested that the traits of an ideal biofuel species are the same as those favouring invasiveness (Raghu et al. 2006, Buddenhagen et al. 2009, Smith et al. 2015). Some of the biofuel species (*Arundo donax*, giant cane; *Psidium cattleianum*, cattley guava) are even listed among 100 of the worst global invaders of the IUCN (Lowe et al. 2000). In the Czech Republic, the issue of importing and planting potentially invasive species is manifested by the biofuel or forestry species such as *Reynoutria* taxa, or *Quercus rubra* and *Paulownia tomentosa*, respectively. For such cases, we advocate a stratified approach based on the type of the invaded habitat, and habitat-related nature conservation needs. A knowledge-based and region-specific differentiated approach is much more suitable than efforts aimed at complete eradication, regardless of circumstances, which is in most cases hardly possible anyway (Rejmánek and Pitcairn 2002, Pluess et al. 2012a, b).

Our aim was to make the Lists on the one hand relatively comprehensive but on the other hand simple enough for later implementation into policy tools. Such an approach was reflected in the composition of the Watch List. It contains species that are not present in the Czech Republic but require attention (because they are already established and cause impact in the neighbouring countries or areas in Europe with similar climatic conditions, and their import is highly probable), but also species already present in the Czech Republic, but currently still restricted to cultivation, captivity or another kind of controlled environment. This allows for raising attention to those "knocking on the door" as well as those already cultivated/farmed species which should be monitored.

Implementing the Black Lists into legislative tools in the Czech Republic is, as in many other countries, constrained by limited integration of IAS-related agendas among different sectors and individual concerned bodies (e.g. nature protection, agriculture, forestry, aquaculture and fishery, hunting, pet industry and trade with various species and products, research, municipalities etc.). In the Czech Republic, the issue of IAS falls within the competence of the Ministry of Environment, but some activities which can on the one hand promote IAS (e.g. biofuel plants, horticulture), or on the other hand control them (e.g. phytosanitary and veterinary measures) are under the competence of other sectors, primarily the Ministry of Agriculture. Unfortunately, due to the different interests of each sector, cooperation between them is not very effective at present. These different interests lead to the inconsistency and weakening of the legislative instruments, unclear competences in the field of IAS, as well as to their ineffective management. Therefore, an essential condition of any progress in the Czech Republic is to communicate the goals and problems caused by IAS to the general public, stakeholders and policy makers to be able to successfully incorporate the legislative measures, and preventive and control management. Implementation of the new EU Regulation will significantly facilitate this process.

The lists presented here are the first attempt to provide basis for setting the priorities of policy and nature protection at the national level in the Czech Republic. The lists should also serve as a national starting point for discussion on priority IAS species at the EU level, based on the new EU Regulation on IAS (Caffrey et al. 2014, European Commission 2014). As the EU List has to take into account interests of individual member states, it will likely reflect to a large extent political interests rather than purely scientific assessment. Therefore national lists may provide a more flexible and effective way of dealing with invasive species. Compared to other existing Black and Grey Lists for other European countries (Essl et al. 2011, Gederaas et al. 2012, Nehring et al. 2013), our approach also takes into account invaded habitats and feasibility and meaningfulness of potential management; we believe that such a methodological approach to prioritization of species represents important advancement, transferable to other regions in Europe and elsewhere.

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References

Aronson MFJ, La Sorte FA, Nilon CH, Katti M, Goddard MA, Lepczyk CA, Warren PS, Williams NSG, Cilliers S, Clarkson B, Dobbs C, Dolan R, Hedblom M, Klotz S, Louwe

Koojimans J, Kühn I, MacGregor-Fors I, McDonnell M, Mörtberg U, Pyšek P, Siebert S, Sushinsky J, Werner P, Winter M (2014) A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. Proceedings of the Royal Society B 281: 20133330. doi: 10.1098/rspb.2013.3330

Bayliss H, Stewart G, Wilcox A, Randall N (2013) A perceived gap between invasive species research and stakeholder priorities. NeoBiota 19: 67–82. doi: 10.3897/neobiota.19.4897

Blackburn TM, Essl F, Evans T, Hulme PE, Jeschke JM, Kühn I, Kumschick S, Marková Z, Mrugała A, Nentwig W, Pergl J, Pyšek P, Rabitsch W, Ricciardi A, Richardson DM, Sendek A, Vilà M, Wilson JRU, Winter M, Genovesi P, Bacher S (2014) A unified classification of alien species based on the magnitude of their environmental impacts. PLoS Biology 12: e1001850. doi: 10.1371/journal.pbio.1001850

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26: 333–339. doi: 10.1016/j.tree.2011.03.023

Brunel S, Suffert M, Petter F, Baker R (2013) Interface between pest risk science and policy: the EPPO perspective. NeoBiota 18: 9–23. doi: 10.3897/neobiota.18.4049

Buddenhagen CE, Chimera C, Clifford P (2009) Assessing biofuel crop invasiveness: a case study. PLoS ONE 4: e5261. doi: 10.1371/journal.pone.0005261

Caffrey JM, Joe M, Baars J-R, Barbour JH, Boets P, Boon P, Davenport K, Dick JTA, Early J, Edsman L, Gallagher C, Gross J, Heinimaa P, Horrill C, Hudin S, Hulme PE, Hynes S, MacIsaac HJ, McLoone P, Millane M, Moen TL, Moore N, Newman J, O'Conchuir R, O'Farrell M, O'Flynn C, Oidtmann B, Renals T, Ricciardi A, Roy H, Shaw R, Weyl O, Williams F, Lucy FE (2014) Tackling invasive alien species in Europe: the Top 20 issues. Management of Biological Invasions 5: 1–20. doi: 10.3391/mbi.2014.5.1.01

Chapuis JL, Frenot Y, Lebouvier M (2004) Recovery of native plant communities after eradication of rabbits from the subantarctic Kerguelen Islands, and influence of climate change. Biological Conservation 117: 167–179. doi: 10.1016/S0006-3207(03)00290-8

Convention on Biological Diversity (1992) http://www.cbd.int

DAISIE (2009) Handbook of alien species in Europe. Springer, Berlin, 1–399.

Dehnen-Schmutz K, Touza J, Perrings C, Williamson M (2007) The horticultural trade and ornamental plant invasions in Britain. Conservation Biology 21: 224–231. doi: 10.1111/j.1523-1739.2006.00538.x

DiTomaso JM, Reaser JK, Dionigi CP, Doering OC, Chilton E, Schardt JD, Barney JN (2010) Biofuel versus bioinvasion: seeding policy priorities. Environmental Science & Technology 44: 6906–6910. doi: 10.1021/es100640y

Dodet M, Collet C (2012) When should exotic forest plantation tree species be considered as an invasive threat and how should we treat them? Biological Invasions 14: 1765–1778. doi: 10.1007/s10530-012-0202-4

Essl F, Nehring S, Klingenstein F, Milasowszky N, Nowack C, Rabitsch W (2011) Review of risk assessment systems of IAS in Europe and introducing the German-Austrian black list information system (GABLIS). Journal for Nature Conservation 19: 339–350. doi: 10.1016/j.jnc.2011.08.005

European Commission (2014) Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the Prevention and Management of the Introduction and Spread of Invasive Alien Species. Brussels.

Follak S, Dullinger S, Kleinbauer I, Moser D, Essl F (2013) Invasion dynamics of three allergenic invasive Asteraceae (*Ambrosia trifida*, *Artemisia annua*, *Iva xanthiifolia*) in central and eastern Europe. Preslia 85: 41–61.

Foxcroft LC, Pyšek P, Richardson DM, Genovesi P (Eds) (2013) Plant Invasions in Protected Areas – Patterns, Problems and Challenges. Springer, Dordrecht, 656 pp. doi: 10.1007/978-94-007-7750-7

Frazee SR, Cowling RM, Pressey RL, Turpie JK, Lindenberg N (2003) Estimating the costs of conserving a biodiversity hotspot: a case-study of the Cape Floristic Region, South Africa. Biological Conservation 112: 275–290. doi: 10.1016/S0006-3207(02)00400-7

Gaertner M, Breeyen AD, Hui C, Richardson DM (2009) Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. Progress in Physical Geography 33: 319–338. doi: 10.1177/0309133309341607

Gaertner M, Fisher J, Sharma G, Esler K (2012) Insights into invasion and restoration ecology: time to collaborate towards a holistic approach to tackle biological invasions. NeoBiota 12: 57–76. doi: 10.3897/neobiota.12.2123

Gaertner M, Richardson DM, Privett SDJ (2011) Effects of alien plants on ecosystem structure and functioning and implications for restoration: insights from three degraded sites in South African Fynbos. Environmental Management 48: 57–69. doi: 10.1007/s00267-011-9675-7

Gederaas L, Moen TL, Skjelseth S, Larsen L-K (Eds) (2012) Alien species in Norway – with the Norwegian Black List. The Norwegian Biodiversity Information Centre, Norway.

Genovesi P (2005) Eradications of invasive alien species in Europe: a review. Biological Invasions 7: 127–133. doi: 10.1007/1-4020-3870-4_12

Genovesi P, Carboneras C, Vilà M, Walton P (2015) EU adopts innovative legislation on invasive species: a step towards a global response to biological invasions? Biological Invasions 17: 1307–1311. doi: 10.1007/s10530-014-0817-8

Halford M, Heemers L, Dierickx M, Van Wesemael D, Mathys C, Mahy G (2014) Regulation and self-regulation instruments to prevent deliberate introductions of Invasive Alien Plants. A review with a focus on voluntary approaches. Discussion aper. AlterIAS Project http://www.alterias.be/images/stories/downloads/Article/discussion_paper_regulation_selfregulation_iap.pdf [accessed August 2014]

Hejda M, Pyšek P, Pergl J, Sádlo J, Chytrý M, Jarošík V (2009) Invasion success of alien plants: do habitats affinities in the native distribution range matter? Global Ecology and Biogeography 18: 372–382. doi: 10.1111/j.1466-8238.2009.00445.x

Heywood VH (2014) Voluntary codes of conduct for botanic gardens and horticulture and engagement with the public. EPPO Bulletin 44: 223–231. doi: 10.1111/epp.12112

Heywood VH, Brunel S (2011) Code of conduct on horticulture and Invasive Alien Plants. Nature and Environment no. 162. Council of Europe Publishing (Strasbourg).

Hobbs RJ, Arico S, Aronson J, Baron JS, Bridgewater P, Cramer VA, Epstein PR, Ewel JJ, Klink CA, Lugo AE, Norton D, Ojima D, Richardson DM, Sanderson EW, Valladares F, Vilà M, Zamora R, Zobel M (2006) Novel ecosystems: theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography 15: 1–7. doi: 10.1111/j.1466-822X.2006.00212.x

Horáčková J, Juřičková L, Jarošík V, Šizling A, Pyšek P (2014) Invasiveness does not predict impact: response of native land snail communities to plant invasions in riparian habitats. PLoS ONE 9: e108296. doi: 10.1371/journal.pone.0108296

Hulme PE, Bacher S, Kenis M, Klotz S, Kühn I, Minchin D, Nentwig W, Olenin S, Panov V, Pergl J, Pyšek P, Roques A, Sol D, Solarz W, Vilà M (2008) Grasping at the routes of biological invasions: a framework for integrating pathways into policy. Journal of Applied Ecology 45: 403–414. doi: 10.1111/j.1365-2664.2007.01442.x

Hulme PE, Pyšek P, Jarošík V, Pergl J, Schaffner U, Vilà M (2013) Bias and error in current knowledge of plant invasions impacts. Trends in Ecology & Evolution 28: 212–218. doi: 10.1016/j.tree.2012.10.010

Hulme PE, Pyšek P, Nentwig W, Vilà M (2009) Will threat of biological invasions unite the European Union? Science 324: 40–41. doi: 10.1126/science.1171111

Humair F, Edwards PJ, Siegrist M, Kueffer C (2014) Understanding misunderstandings in invasion science: why experts don't agree on common concepts and risk assessments. NeoBiota 20: 1–30. doi: 10.3897/neobiota.20.6043

Jeschke JM, Bacher B, Blackburn TM, Dick JTA, Essl F, Evans T, Gaertner M, Hulme PE, Kühn I, Mrugala A, Pergl J, Pyšek P, Rabitsch W, Ricciardi A, Richardson DM, Sendek A, Vilà M, Winter M, Kumschick S (2014) Defining the impact of non-native species. Conservation Biology 28: 1188–1194. doi: 10.1111/cobi.12299

Kabrna M, Hendrychová M, Prach K (2014) Establishment of target and invasive plant species on a reclaimed coal mining dump in relation to their occurrence in the surroundings. International Journal of Mining, Reclamation and Environment 28: 242–249. doi: 10.1080/17480930.2013.820390

Keller RP, Drake JM (2009) Trait-based risk assessment for invasive species. In: Keller RP, Lodge DM, Lewis MA, Shogren JF (Eds) Bioeconomics of invasive species. Oxford University Press, New York, 44–62.

Keller RP, Springborn MR (2014) Closing the screen door to new invasions. Conservation Letters 7: 285–292. doi: 10.1111/conl.12071

Kelly J, O'Flynn C, Maguire C (2013) Risk analysis and prioritisation for invasive and non-native species in Ireland and Northern Ireland. Report prepared for the Northern Ireland Environment Agency and National Parks and Wildlife Service as part of Invasive Species Ireland.

Kenis M, Rabitsch W, Auger-Rozenberg M-A, Roques A (2007) How can alien species inventories and interception data help us prevent insect invasions? Bulletin of Entomological Research 97: 489–502. doi: 10.1017/S0007485307005184

Kettunen M, Genovesi P, Gollasch S, Pagad S, Starfinger U, ten Brink P, Shine C (2009) Technical support to EU Strategy on Invasive Alien Species (IAS) – Assessment of the impacts of IAS in Europe and the EU. Institute for European Environmental Policy, London and Brussels.

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends in Ecology & Evolution 16: 199–204. doi: 10.1016/S0169-5347(01)02101-2

Kowarik I, von der Lippe M, Cierjacks A (2013) Prevalence of alien versus native species of woody plants in Berlin differs between habitats and at different scales. Preslia 85: 113–132.

Kumschick S, Bacher S, Dawson W, Heikkilä J, Sendek A, Pluess T, Robinson T, Kühn I (2012) A conceptual framework for prioritization of invasive alien species for management according to their impact. NeoBiota 15: 69–100. doi: 10.3897/neobiota.15.3323

Kumschick S, Bacher S, Evans T, Marková Z, Pergl J, Pyšek P, Vaes-Petignat S, van der Veer G, Vilà M, Nentwig W (2015) Comparing impacts of alien plants and animals using a standard scoring system. Journal of Applied Ecology 52: 552–561. doi: 10.1111/1365-2664.12427

Leung B, Roura-Pascual N, Bacher S, Heikkilä J, Brotons L, Burgman MA, Dehnen-Schmutz K, Essl F, Hulme PE, Richardson DM, Sol D, Vilà M, Rejmánek M (2012) TEASIng apart alien species risk assessments: a framework for best practices. Ecology Letters 15: 1475–1493. doi: 10.1111/ele.12003

Levine JM, Vilà M, D'Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society of London Series B-Biological Sciences 270: 775–781. doi: 10.1098/rspb.2003.2327

Lewis KC, Porter RD (2014) Global approaches to addressing biofuel-related invasive species risks and incorporation into U.S. laws and policies. Ecological Monographs 84: 171–201. doi: 10.1890/13-1625.1

Lockwood JL, Hoopes MF, Marchetti MP (2013) Invasion ecology. Second Edition. Wiley, Chichester, 466 pp.

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database. The IUCN Invasive Species Specialist Group (ISSG), Auckland, New Zealand, 1–12.

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: synthesis. Island Press, Washington, DC, 160 pp.

Mitchell CE, Blumenthal D, Jarošík V, Pulley EE, Pyšek P (2010) Controls on pathogen species richness in plants' introduced and native ranges: roles of residence time, range size, and host traits. Ecology Letters 13: 1525–1535. doi: 10.1111/j.1461-0248.2010.01543.x

Musil J, Jurajda P, Adámek Z, Horký P, Slavík O (2010) Non-native fish introductions in the Czech Republic – species inventory, facts and future perspectives. Journal of Applied Ichthyology 26: 38–45. doi: 10.1111/j.1439-0426.2010.01500.x

Nehring S, Essl F, Rabitsch W (2013) Methodik der naturschutzfachlichen Invasivitätsbewertung für gebietsfremde Arten. Version 1.2 BfN-Skripten 340: 1–46.

Nentwig W, Kühnel E, Bacher S (2010) A generic impact-scoring system applied to alien mammals in Europe. Conservation Biology 24: 302–311. doi: 10.1111/j.1523-1739.2009.01289.x

Nielsen C, Ravn HP, Nentwig W, Wade M (Eds) (2005) The giant hogweed best practice manual. Guidelines for the management and control of an invasive alien weed in Europe. Forest and Landscape Denmark, Hoersholm, 44 pp.

Ööpik M, Bunce RGH, Tischler M (2013) Horticultural markets promote alien species invasions: an Estonian case study of herbaceous perennials. NeoBiota 17: 19–37. doi: 10.3897/neobiota.17.4217

Pergl J, Nentwig W, Winter M, Bacher S, Essl F, Genovesi P, Hulme PE, Jarošík V, Kühn I, Pyšek P, Roques A, Roy D, Vilà M, Roy H (2012) Progress on DAISIE: ALIEN species inventories in Europe updated. In: Neobiota 2012, 7th European Conference on Biological Invasions, Pontevedra, Spain, 12–14 Sept 2012.

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. Agroecosystems and Environment 84: 1–20. doi: 10.1016/S0167-8809(00)00178-X

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connel C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2002) Economic and environmental threats of alien plant, animal, and microbe invasions. In: Pimentel D (Ed.) Biological invasions: economic and environmental costs of alien plant, animal, and microbe species. CRC Press, Boca Raton, FL, 307–329.

Pluess T, Cannon R, Jarošík V, Pergl J, Pyšek P, Bacher S (2012a) When are eradication campaigns successful? A test of common assumptions. Biological Invasions 14: 1365–1378. doi: 10.1007/s10530-011-0160-2

Pluess T, Jarošík V, Pyšek P, Cannon R, Pergl J, Breukers A, Bacher S (2012b) Which factors affect the success or failure of eradication campaigns against alien species? PLoS One 7: e48157. doi: 10.1371/journal.pone.0048157

Pyšek P, Chytrý M, Pergl J, Sádlo J, Wild J (2012a) Plant invasions in the Czech Republic: current state, introduction dynamics, invasive species and invaded habitats. Preslia 84: 575–630.

Pyšek P, Danihelka J, Sádlo J, Chrtek J Jr, Chytrý M, Jarošík V, Kaplan Z, Krahulec F, Moravcová L, Pergl J, Štajerová K, Tichý L (2012b) Catalogue of alien plants of the Czech Republic (2nd edition): checklist update, taxonomic diversity and invasion patterns. Preslia 84: 155–255.

Pyšek P, Jarošík V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M (2012c) A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. Global Change Biology 18: 1725–1737. doi: 10.1111/j.1365-2486.2011.02636.x

Pyšek P, Richardson DM (2010) Invasive species, environmental change and management, and health. Annual Review of Environment and Resources 35: 25–55. doi: 10.1146/annurev-environ-033009-095548

Pyšek P, Richardson DM, Rejmánek M, Webster G, Williamson M, Kirschner J (2004) Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon 53: 131–143. doi: 10.2307/4135498

Pyšek P, Sádlo J, Mandák B (2002) Catalogue of alien plants of the Czech Republic. Preslia 74: 97–186.

Raghu S, Anderson RC, Daehler CC, Davis AS, Widenmann RN, Simberloff D, Mack R (2006) Adding biofuels to the invasive species fire? Science 313: 1742. doi: 10.1126/science.1129313

Rejmánek M, Pitcairn MJ (2002) When is eradication of exotic pest plants a realistic goal? In: Veitch CR, Clout MN (Eds) Turning the tide: the eradication of invasive species. IUCN, Gland and Cambridge, 249–253.

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of nonnative species. Ecological Monographs 83: 263–282. doi: 10.1890/13-0183.1

Ricciardi A, Steiner WW, Mack RN, Simberloff D (2000) Toward a global information system for invasive species. Bioscience 50: 239–244. doi: 10.1641/0006-3568(2000)050[0239:TAGISF]2.3.CO;2

Richardson DM, Pyšek P (2012) Naturalization of introduced plants: ecological drivers of biogeographic patterns. New Phytologist 196: 383–396. doi: 10.1111/j.1469-8137.2012.04292.x

Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6: 93–107. doi: 10.1046/j.1472-4642.2000.00083.x

Richardson DM, Rejmánek M (2011) Trees and shrubs as invasive alien species: a global review. Diversity and Distributions 17: 788–809. doi: 10.1111/j.1472-4642.2011.00782.x

Roques A, Auger-Rozenberg MA (2006) Tentative analysis of the interceptions of non-indigenous organisms in Europe during 1995–2004. EPPO Bulletin 36: 490–496. doi: 10.1111/j.1365-2338.2006.01049.x

Scalera R, Genovesi P, De Man D et al. (2012) European code of conduct on Zoological gardens and aquaria and Invasive Alien Species. Council of Europe document T-PVS/Inf (2011) 26 rev., Strassbourg.

Šefrová H (2005) Introduced and invasive insect species in the Czech Republic and their economic and ecological impact (Insecta). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 53(5): 151–158. doi: 10.11118/actaun200553050151

Šefrová H, Laštůvka Z (2005) Catalogue of alien animal species in the Czech Republic. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 53(4): 151–170. doi: 10.11118/actaun200553040151

Shine C, Kettunen M, Genovesi P, Gollasch S, Pagad S, Starfinger U (2009) Technical support to EU Strategy on Invasive Species (IAS) – Policy options to control the negative impacts of IAS on biodiversity in Europe and the EU (Final module report for the European Commission). Institute for European Environmental Policy (IEEP), Brussels, Belgium.

Simberloff D, Genovesi P, Pyšek P, Campbell K (2011) Recognizing conservation success. Science 332: 419. doi: 10.1126/science.332.6028.419-a

Smith LL, Allen DJ, Barney J (2015) The thin green line: sustainable bioenergy feedstocks or invaders in waiting. NeoBiota 25: 47–71. doi: 10.3897/neobiota.25.8613

Svoboda J, Strand DA, Vrålstad T, Grandjean F, Edsman L, Kozák P, Kouba A, Fristad RF, Bahadir Koca S, Petrusek A (2014) The crayfish plague pathogen can infect freshwater-inhabiting crabs. Freshwater Biology 59: 918–929. doi: 10.1111/fwb.12315

Vaes-Petignat S, Nentwig W (2014) Environmental and economic impact of alien terrestrial arthropods in Europe. NeoBiota 22: 23–42. doi: 10.3897/neobiota.22.6620

van Kleunen M, Dawson W, Essl F, Pergl J, Winter M, Weber E, Kreft H, Weigelt P, Kartesz J, Nishino M, Antonova LA, Barcelona JF, Cabezas FJ, Cárdenas D, Cárdenas-Toro J, Castaño N, Chacón E, Chatelain C, Ebel AL, Figueiredo E, Fuentes N, Groom QJ, Henderson L, Inderjit, Kupriyanov A, Masciadri S, Meerman J, Morozova O, Moser D, Nickrent DL, Patzelt A, Pelser PB, Baptiste MP, Poopath M, Schulze M, Seebens H, Shu W, Thomas J, Velayos M, Wieringa JJ, Pyšek P (2015) Global exchange and accumulation of non-native plants. Nature 525: 100–103. doi: 10.1038/nature14910

Verbrugge LN, van der Velde G, Hendriks AJ, Verreycken H, Leuven R (2012) Risk classifications of aquatic non-native species: application of contemporary European assessment protocols in different biogeographical settings. Aquatic Invasions 7: 49–58. doi: 10.3391/ai.2012.7.1.006

Vilà M, Basnou C, Pyšek P, Josefsson M, Genovesi P, Gollasch S, Nentwig W, Olenin S, Roques A, Roy D, Hulme PE, DAISIE partners (2010) How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. Frontiers in Ecology and the Environment 8: 135–144. doi: 10.1890/080083

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters 14: 702–708. doi: 10.1111/j.1461-0248.2011.01628.x

Williamson M, Pyšek P, Jarošík V, Prach K (2005) On the rates and patterns of spread of alien plants in the Czech Republic, Britain and Ireland. Ecoscience 12: 424–433. doi: 10.2980/i1195-6860-12-3-424.1

Wittenberg R, Cock MJ (2001) Invasive alien species: a toolkit of best prevention and management practices. CABI Publishing, Wallingford, 240 pp.

Woziwoda B, Potocki M, Sagan J, Zasada M, Tomusiak R, Wilczyński S (2014) Commercial forestry as a vector of alien tree species – the case of *Quercus rubra* L. introduction in Poland. Baltic Forestry 20: 131–141.

Appendix

Table A1. List of species in the groups of Black (BL) and Grey (GL) Lists. For plants, life history is shown: a – annual, b – biennial, pe – perennial, s – shrub, t – tree, aq – aquatic, p – parasitic. Plant species marked by * may be tolerated outside nature valuable areas. Economically important species where replacement by native species or keeping in controlled conditions (e.g. fishponds, enclosures) is recommended, are marked by (+).

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environ-ment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environ-mental impact	Human (socio-economic) impact	Management strategy
plant	BL1	*Ambrosia artemisiifolia* L.	ambrozie peřenolistá	Asteraceae	terrestrial	a		Spontaneous	Local	Moderate	Massive	Complete eradication
plant	BL1	*Heracleum mantegazzianum* Sommier et Levier	bolševník velkolepý	Apiaceae	terrestrial	b pe		Spontaneous	Regional	Massive	Massive	Complete eradication
animal	BL1	*Neovison vison* (Schreber, 1777)	norek americký	Mustelidae	terrestrial (aquatic)	mammal		Spontaneous	Regional	Moderate	Limited	Complete eradication
animal	BL1	*Procyon lotor* (Linnaeus, 1758)	mýval severní	Procyonidae	terrestrial (aquatic)	mammal		Spontaneous	Regional	Moderate	Limited	Complete eradication
animal	BL1	*Varroa destructor* (Anderson & Trueman, 2000)	kleštík zhoubný	Varroidae	terrestrial	invertebrate		Spontaneous	Regional	Limited	Massive	Complete eradication
plant	BL2	*Acer negundo* L.	javor jasanolistý	Sapindaceae	terrestrial	t		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Ailanthus altissima* (Mill.) Swingle	pajasan žláznatý	Simaroubaceae	terrestrial	t		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Allium paradoxum* (M. Bieb.) G. Don	česnek podivný	Amaryllidaceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Amorpha fruticosa* L.	netvařec křovitý	Fabaceae	terrestrial	s		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Arrhenatherum elatius* (L.) J. Presl et C. Presl	ovsík vyvýšený	Poaceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Asclepias syriaca* L.	klejicha hedvábná, k. vatočník	Apocynaceae	terrestrial	pe		Released/spontaneous	Local	Moderate	Limited	Stratified approach
plant	BL2	*Azolla filiculoides* Lam.	azola americká	Salviniaceae	aquatic	a f aq		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Beta vulgaris* Altissima Group	řepa obecná cukrovka	Amaranthaceae	terrestrial	b a		Released	Regional	Limited	Moderate	Stratified approach
plant	BL2	*Buddleja davidii* Franch.	komule Davidova	Scrophulariaceae	terrestrial	s		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Colutea arborescens* L.	žanovec měchýřník	Fabaceae	terrestrial	s		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Cornus sericea* L. et *C. alba* L.	svída výběžkatá	Cornaceae	terrestrial	s		Released/spontaneous	Regional	Moderate	Limited	Stratified approach

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environ-ment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environ-mental impact	Human (socio-economic) impact	Management strategy
plant	BL2	*Cytisus scoparius* (L.) Link subsp. *scoparius*	janovec metlatý pravý	Fabaceae	terrestrial	s		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Echinocystis lobata* (Michx.) Torr. et A. Gray	štětinec laločnatý	Cucurbitaceae	terrestrial	a		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Echinops exaltatus* Schrad.	bělotrn statný	Asteraceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Echinops sphaerocephalus* L. subsp. *sphaerocephalus*	bělotrn kulatohlavý pravý	Asteraceae	terrestrial	pe		Released/spontaneous	Local	Moderate	Limited	Stratified approach
plant	BL2	*Fallopia aubertii* (L. Henry) Holub	opletka čínská	Polygonaceae	terrestrial	s		Released	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Fraxinus pennsylvanica* Marshall	jasan pensylvánský	Oleaceae	terrestrial	t		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Galega officinalis* L.	jestřabina lékařská	Fabaceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Galeobdolon argentatum* Smejkal	pitulník postříbřený	Lamiaceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Helianthus ×laetiflorus* Pers.	slunečnice pozdní	Asteraceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Helianthus pauciflorus* Nutt.	slunečnice tuhá	Asteraceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Helianthus tuberosus* L.	slunečnice topinambur	Asteraceae	terrestrial	pe		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Impatiens glandulifera* Royle	netýkavka žláznatá	Balsaminaceae	terrestrial	a		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Laburnum anagyroides* Medik.	štědřenec odvislý	Fabaceae	terrestrial	s t	incl. *L. x watereri* (Wettst.) Dippel, *L. alpinum* (Mill.) J. Presl	Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Lupinus polyphyllus* Lindl.	lupina mnoholistá, vlčí bob mnoholistý	Fabaceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Lycium barbarum* L.	kustovnice cizí	Solanaceae	terrestrial	s	*	Released	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Parthenocissus inserta* (A. Kern.) Fritsch	loubinec popínavý	Vitaceae	terrestrial	s		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Parthenocissus quinquefolia* (L.) Planch.	loubinec pětilistý	Vitaceae	terrestrial	s		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Physocarpus opulifolius* (L.) Maxim.	tavola kalinolistá	Rosaceae	terrestrial	s		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Phytolacca esculenta* Van Houtte	líčidlo jedlé	Phytolaccaceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environmental impact	Human (socio-economic) impact	Management strategy
plant	BL2	*Pinus nigra* J. F. Arnold subsp. *nigra*	borovice černá pravá	Pinaceae	terrestrial	t		Released/spontaneous	Local	Moderate	Limited	Stratified approach
plant	BL2	*Pinus strobus* L.	borovice vejmutovka, vejmutovka	Pinaceae	terrestrial	t		Released/spontaneous	Local	Massive	Limited	Stratified approach
plant	BL2	*Populus ×canadensis* Moench	topol kanadský	Salicaceae	terrestrial	t	*	Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Populus balsamifera* L.	topol balzámový	Salicaceae	terrestrial	t		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Prunus cerasifera* Ehrh.	slivoň myrobalán, myrobalán	Rosaceae	terrestrial	t s	*	Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Prunus serotina* Ehrh.	střemcha pozdní	Rosaceae	terrestrial	t s		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Pyracantha coccinea* M. J. Roem.	hlohyně šarlatová	Rosaceae	terrestrial	s t		Released/spontaneous	Local	Moderate	Limited	Stratified approach
plant	BL2	*Quercus rubra* L.	dub červený	Fagaceae	terrestrial	t		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Reynoutria ×bohemica* Chrtek et Chrtková	křídlatka česká	Polygonaceae	terrestrial	pe		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Reynoutria japonica* Houtt. var. *japonica*	křídlatka japonská pravá	Polygonaceae	terrestrial	pe		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Reynoutria sachalinensis* (F. Schmidt) Nakai	křídlatka sachalinská	Polygonaceae	terrestrial	pe		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Rhus typhina* (L.) Sudw.	škumpa orobincová	Anacardiaceae	terrestrial	s t		Released	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Robinia pseudoacacia* L.	trnovník akát, akát	Fabaceae	terrestrial	t	*	Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Rudbeckia laciniata* L.	třapatka dřípatá	Asteraceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Solidago canadensis* L.	zlatobýl kanadský	Asteraceae	terrestrial	pe		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Solidago gigantea* Aiton	zlatobýl obrovský	Asteraceae	terrestrial	pe		Released/spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL2	*Symphoricarpos albus* (L.) S. F. Blake	pámelník bílý	Caprifoliaceae	terrestrial	s		Released	Regional	Moderate	Limited	Stratified approach
plant	BL2	*Symphyotrichum novi-belgii* (L.) G. L. Nesom	astřička novobelgická, hvězdnice novobelgická	Asteraceae	terrestrial	pe	incl. all other closely related hybrids in this taxon complex (e.g. *S. lanceolatum*)	Released/spontaneous	Regional	Massive	Limited	Stratified approach

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environmental impact	Human (socio-economic) impact	Management strategy
plant	BL2	*Telekia speciosa* (Schreb.) Baumg.	kolotočník ozdobný	Asteraceae	terrestrial	pe		Released/spontaneous	Regional	Moderate	Limited	Stratified approach
animal	BL2	*Cervus nippon* Temminck, 1838	jelen sika	Cervidae	terrestrial	mammal	+	Released/spontaneous	Regional	Moderate	Limited	Stratified approach
animal	BL2	*Ctenopharyngodon idella* (Valenciennes, 1844)	amur bílý	Cyprinidae	aquatic	fish		Released	Regional	Moderate	Limited	Stratified approach
animal	BL2	*Hypophthalmichthys molitrix* (Valenciennes, 1844)	tolstolobik bílý	Cyprinidae	aquatic	fish		Released	Regional	Moderate	Limited	Stratified approach
animal	BL2	*Hypophthalmichthys nobilis* (Richardson, 1845)	tolstolobik pestrý	Cyprinidae	aquatic	fish		Released	Regional	Moderate	Limited	Stratified approach
animal	BL2	*Micropterus salmoides* (Lacépède, 1802)	okounek pstruhový	Centrarchidae	aquatic	fish		Released	Local	Limited	Limited	Stratified approach
animal	BL2	*Oncorhynchus mykiss* (Walbaum, 1792)	pstruh duhový	Salmonidae	aquatic	fish	+	Released	Regional	Limited	Limited	Stratified approach
animal	BL2	*Ovis musimon* (Pallas, 1811)	muflon	Bovidae	terrestrial	mammal	+	Released/spontaneous	Regional	Limited	Limited	Stratified approach
animal	BL2	*Salvelinus fontinalis* (Mitchill, 1815)	siven americký	Salmonidae	aquatic	fish	+	Released/spontaneous	Regional	Limited	Limited	Stratified approach
plant	BL3	*Abutilon theophrasti* Medik.	mračňák Theophrastův	Malvaceae	terrestrial	a		Spontaneous	Local	Limited	Moderate	Stratified approach
plant	BL3	*Alopecurus myosuroides* Huds.	psárka polní	Poaceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Amaranthus albus* L.	laskavec bílý	Amaranthaceae	terrestrial	a		Spontaneous	Local	Limited	Moderate	Stratified approach
plant	BL3	*Amaranthus powellii* S. Watson	laskavec zelenoklasý	Amaranthaceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Amaranthus retroflexus* L.	laskavec ohnutý, l. srstnatý	Amaranthaceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Bunias orientalis* L.	rukevník východní	Brassicaceae	terrestrial	b pe		Spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL3	*Cannabis sativa* var. *spontanea* Vavilov	konopí seté rumištní	Cannabaceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Cirsium arvense* (L.) Scop.	pcháč oset	Asteraceae	terrestrial	pe		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Conium maculatum* L.	bolehlav plamatý	Apiaceae	terrestrial	a b		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Consolida hispanica* (Costa) Greuter et Burdet	ostrožka východní	Ranunculaceae	terrestrial	a		Spontaneous	Regional	Limited	Moderate	Stratified approach
plant	BL3	*Conyza canadensis* (L.) Cronquist	turanka kanadská, turan kanadský	Asteraceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environ-ment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environ-mental impact	Human (socio-economic) impact	Management strategy
plant	BL3	*Cuscuta campestris* Yunck.	kokotice ladní	Convolvulaceae	terrestrial	a		Spontaneous	Local	Moderate	Moderate	Stratified approach
plant	BL3	*Digitaria ischaemum* (Schreb.) Muhl.	rosička lysá	Poaceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Echinochloa crus-galli* (L.) P. Beauv.	ježatka kuří noha	Poaceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Galinsoga parviflora* Cav.	pěťour malokvětý	Asteraceae	terrestrial	a		Spontaneous	Regional	Limited	Moderate	Stratified approach
plant	BL3	*Galinsoga quadriradiata* Ruiz et Pav.	pěťour srstnatý	Asteraceae	terrestrial	a		Spontaneous	Regional	Limited	Moderate	Stratified approach
plant	BL3	*Iva xanthiifolia* Nutt.	pouva řepňolistá	Asteraceae	terrestrial	a		Spontaneous	Local	Moderate	Moderate	Stratified approach
plant	BL3	*Orobanche minor* Sm.	záraza menší	Orobanchaceae	terrestrial	b pe p		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Oxalis corniculata* L. var. *corniculata*	šťavel růžkatý pravý	Oxalidaceae	terrestrial	a b pe		Spontaneous	Regional	Limited	Moderate	Stratified approach
plant	BL3	*Oxalis dillenii* Jacq.	šťavel prérijní	Oxalidaceae	terrestrial	a b pe		Spontaneous	Regional	Limited	Moderate	Stratified approach
plant	BL3	*Panicum miliaceum* subsp. *agricola* H. Scholz et Mikoláš	proso seté polní	Poaceae	terrestrial	a	*incl.* subsp. *ruderale* (Kitag.) Tzvelev	Spontaneous	Local	Moderate	Moderate	Stratified approach
plant	BL3	*Portulaca oleracea* L. subsp. *oleracea*	šrucha zelná pravá	Portulacaceae	terrestrial	a		Spontaneous	Regional	Limited	Moderate	Stratified approach
plant	BL3	*Rumex alpinus* L.	šťovík alpský	Polygonaceae	terrestrial	pe		Spontaneous	Local	Massive	Limited	Stratified approach
plant	BL3	*Rumex longifolius* subsp. *sourekii* Kubát	šťovík dlouholistý Šourkův	Polygonaceae	terrestrial	pe		Spontaneous	Local	Limited	Limited	Stratified approach
plant	BL3	*Senecio inaequidens* DC.	starček úzkolistý	Asteraceae	terrestrial	pe		Spontaneous	Regional	Massive	Limited	Stratified approach
plant	BL3	*Setaria faberi* R. A. W. Herrm.	bér ohnutý	Poaceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach
plant	BL3	*Setaria verticillata* (L.) P. Beauv.	bér přeslenitý	Poaceae	terrestrial	a		Spontaneous	Regional	Moderate	Moderate	Stratified approach
animal	BL3	*Ameiurus melas* (Rafinesque, 1820)	sumeček černý	Ictaluridae	aquatic	fish		Spontaneous	Local	Moderate	Limited	Stratified approach
animal	BL3	*Anguillicoloides crassus* Kuwah., Niimi & Itagaki, 1974	krevnatka úhoří	Anguillicolidae	aquatic	invertebrate		Spontaneous	Regional	Moderate	Moderate	Stratified approach
animal	BL3	*Arion vulgaris* Moquin-Tandon, 1855	plzák španělský	Arionidae	terrestrial	invertebrate		Spontaneous	Regional	Moderate	Massive	Stratified approach
animal	BL3	*Cameraria ohridella* Deschka & Dimic, 1986	klíněnka jírovcová	Gracillariidae	terrestrial	invertebrate		Spontaneous	Regional	Limited	Moderate	Stratified approach
animal	BL3	*Carassius gibelio* (Bloch, 1782)	karas stříbřitý	Cyprinidae	aquatic	fish		Spontaneous	Regional	Massive	Moderate	Stratified approach
animal	BL3	*Carassius langsdorfii* Temminck & Schlegel, 1846	karas ginbuna	Cyprinidae	aquatic	fish		Spontaneous	Regional	Moderate	Moderate	Stratified approach

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environmental impact	Human (socio-economic) impact	Management strategy
animal	BL3	*Corbicula fluminea* (O. F. Müller, 1774)	korbikula asijská	Cyrenidae	aquatic	invertebrate		Spontaneous	Regional	Moderate	Limited	Stratified approach
animal	BL3	*Diaspidiotus perniciosus* (Comstock, 1881)	štítenka zhoubná	Diaspididae	terrestrial	invertebrate		Spontaneous	Regional	Limited	Moderate	Stratified approach
animal	BL3	*Dikerogammarus villosus* (Sowinsky, 1894)	blešivec ježatý	Gammaridae	aquatic	invertebrate		Spontaneous	Regional	Massive	Limited	Stratified approach
animal	BL3	*Dreissena polymorpha* (Pallas, 1771)	slávička mnohotvárná	Dreissenidae	aquatic	invertebrate		Spontaneous	Regional	Massive	Moderate	Stratified approach
animal	BL3	*Eriosoma lanigerum* (Hausmann, 1802)	vlnatka krvavá	Aphididae	terrestrial	invertebrate		Spontaneous	Regional	Limited	Moderate	Stratified approach
animal	BL3	*Harmonia axyridis* (Pallas, 1773)	slunéčko východní	Coccinellidae	terrestrial	invertebrate		Spontaneous	Regional	Moderate	Moderate	Stratified approach
animal	BL3	*Hyphantria cunea* (Drury, 1773)	přástevníček americký	Arctiidae	terrestrial	invertebrate		Spontaneous	Local	Limited	Limited	Stratified approach
animal	BL3	*Khawia sinensis* Hsü, 1935	tasemnice	Lytocestidae	terrestrial	invertebrate		Spontaneous	Regional	Limited	Limited	Stratified approach
animal	BL3	*Lepomis gibbosus* (Linnaeus, 1758)	slunečnice pestrá	Centrarchidae	aquatic	fish		Spontaneous	Regional	Moderate	Limited	Stratified approach
animal	BL3	*Mus musculus / M. domesticus* Linnaeus, 1758	myš domácí/m. západoevropská	Muridae	terrestrial	mammal		Spontaneous	Regional	Limited	Massive	Stratified approach
animal	BL3	*Myocastor coypus* (Molina, 1782)	nutrie říční	Myocastoridae	terrestrial (aquatic)	mammal		Released/spontaneous	Regional	Limited	Limited	Stratified approach
animal	BL3	*Neogobius melanostomus* (Pallas, 1814)	hlaváč černotlamý	Gobiidae	aquatic	fish		Spontaneous	Regional	Moderate	Limited	Stratified approach
animal	BL3	*Nyctereutes procyonoides* (Gray, 1834)	psík mývalovitý	Canidae	terrestrial (aquatic)	mammal		Spontaneous	Regional	Limited	Limited	Stratified approach
animal	BL3	*Ondatra zibethicus* (Linnaeus, 1766)	ondatra pižmová	Arvicolidae	terrestrial (aquatic)	mammal		Spontaneous	Regional	Limited	Limited	Stratified approach
animal	BL3	*Orconectes limosus* (Rafinesque, 1817)	rak pruhovaný	Cambaridae	aquatic	invertebrate		Spontaneous	Local	Massive	Limited	Stratified approach
animal	BL3	*Oxycarenus lavaterae* (Fabricius, 1787)	ploštička lipová	Oxycarenus	terrestrial	invertebrate		Spontaneous	Regional	Limited	Limited	Stratified approach
animal	BL3	*Pacifastacus leniusculus* (Dana, 1852)	rak signální	Astacidae	aquatic	invertebrate		Spontaneous	Local	Massive	Limited	Stratified approach
animal	BL3	*Pseudorasbora parva* (Temminck & Schlegel, 1846)	střevlička východní	Cyprinidae	aquatic	fish		Spontaneous	Regional	Massive	Moderate	Stratified approach
animal	BL3	*Rattus norvegicus* (Berkenhout, 1769)	potkan	Muridae	terrestrial	mammal		Spontaneous	Regional	Moderate	Massive	Stratified approach
animal	BL3	*Rattus rattus* (Linnaeus, 1758)	krysa obecná	Muridae	terrestrial	mammal		Spontaneous	Local	Limited	Moderate	Stratified approach

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environmental impact	Human (socio-economic) impact	Management strategy
animal	BL3	*Sinanodonta woodiana* (Lea, 1834)	škeble asijská	Unionidae	aquatic	invertebrate		Spontaneous	Local	Limited	Limited	Stratified approach
animal	BL3	*Trachemys scripta* (Thunberg in Schoepff, 1792)	želva nádherná	Emydidae	aquatic (terrestrial)	reptile		Released	Regional	Limited	Limited	Stratified approach
plant	GL	*Amelanchier spicata* (Lam.) K. Koch	muchovník klasnatý	Rosaceae	terrestrial	s		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Angelica archangelica* L. subsp. *archangelica*	andělika lékařská, děhel lékařský	Apiaceae	terrestrial	b pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Artemisia annua* L.	pelyněk roční	Asteraceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Artemisia tournefortiana* Rchb.	pelyněk Tournefortův	Asteraceae	terrestrial	pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Artemisia verlotiorum* Lamotte	pelyněk Verlotů	Asteraceae	terrestrial	pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Atriplex sagittata* Borkh.	lebeda lesklá	Amaranthaceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Bassia scoparia* (L.) Voss subsp. *scoparia*	bytel metlatý pravý	Amaranthaceae	terrestrial	a	*incl. Bassia scoparia* subsp. *densiflora* (B. D. Jacks.) Ciruja et Velayos	Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Bidens frondosus* L.	dvouzubec černoplodý	Asteraceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Bromus carinatus* Hook. et Arn	sveřep kýlnatý	Poaceae	terrestrial	a pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Bryonia dioica* Jacq.	posed dvoudomý	Cucurbitaceae	terrestrial	pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Centaurea diffusa* Lam.	chrpa rozkladitá	Asteraceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Corispermum pallasii* Steven	velbloudník tenkokřídlý	Amaranthaceae	terrestrial	a		Spontaneous	Local	Limited	Limited	Tolerance
plant	GL	*Dipsacus strigosus* Willd. ex Roem. et Schult.	štětka větší	Dipsacaceae	terrestrial	b		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Dittrichia graveolens* (L.) Greuter	oman smradlavý	Asteraceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Duchesnea indica* (Jacks.) Focke	jahodka indická	Rosaceae	terrestrial	pe		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Dysphania pumilio* (R. Br.) Mosyakin et Clemants	merlík trpasličí	Amaranthaceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Eragrostis minor* Host	milička menší	Poaceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Erechtites hieraciifolius* (L.) DC.	starčkovec jestřábníkolistý	Asteraceae	terrestrial	pe		Spontaneous	Regional	Limited	Limited	Tolerance

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environ-ment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environ-mental impact	Human (socio-economic) impact	Management strategy
plant	GL	*Erigeron annuus* (L.) Desf. subsp. *annuus*	turan roční pravý	Asteraceae	terrestrial	a	*incl. Erigeron annuus* subsp. *septentrionalis* (Fernald et Wiegand) Wagenitz	Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Erigeron strigosus* Muhl. ex Willd.	turan větevnatý	Asteraceae	terrestrial	a pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Erucastrum gallicum* (Willd.) O. E. Schulz	ředkevník galský	Brassicaceae	terrestrial	a b		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Erucastrum nasturtiifolium* (Poir.) O. E. Schulz	ředkevník potočnicolistý	Brassicaceae	terrestrial	b pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Geranium sibiricum* L.	kakost sibiřský	Geraniaceae	terrestrial	pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Hordeum jubatum* L.	ječmen hřívnatý	Poaceae	terrestrial	a		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Chenopodium striatiforme* J. Murr	merlík drobnolistý	Amaranthaceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Impatiens parviflora* DC.	netýkavka malokvětá	Balsaminaceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Juglans regia* L.	ořešák královský	Juglandaceae	terrestrial	t		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Lemna turionifera* Landolt	okřehek červený	Araceae	aquatic	a pe aq		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Lepidium densiflorum* Schrad.	řeřicha hustokvětá	Brassicaceae	terrestrial	a b		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Lepidium virginicum* L.	řeřicha virginská	Brassicaceae	terrestrial	a b		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Lonicera caprifolium* L.	zimolez kozí list	Caprifoliaceae	terrestrial	s		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Lunaria annua* L.	měsíčnice roční	Brassicaceae	terrestrial	b		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Mahonia aquifolium* (Pursh) Nutt.	mahónie cesmínolistá	Berberidaceae	terrestrial	s		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Melissa officinalis* (L.) Lam. subsp. *officinalis*	meduňka lékařská pravá	Lamiaceae	terrestrial	pe		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Oenothera glazioviana* Micheli	pupalka rudokališní	Onagraceae	terrestrial	b		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Oenothera pycnocarpa* G. F. Atk. et Bartlett	pupalka chicagská	Onagraceae	terrestrial	a b		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Oenothera rubricaulis* Kleb.	pupalka červenostonká	Onagraceae	terrestrial	b		Spontaneous	Regional	Limited	Limited	Tolerance

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environmental impact	Human (socio-economic) impact	Management strategy
plant	GL	*Rubrivena polystachya* (Wall. ex Meisn.) M. Král	rdesno mnohoklasé	Polygonaceae	terrestrial	pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Rumex triangulivalvis* (Danser) Rech. f.	šťovík trojmozolný	Polygonaceae	terrestrial	pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Sagittaria latifolia* Willd.	šípatka širolistá	Alismataceae	aquatic	pe aq		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Scutellaria altissima* L.	šišák vysoký	Lamiaceae	terrestrial	pe		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Sedum hispanicum* L.	rozchodník španělský	Crassulaceae	terrestrial	pe		Released/spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Senecio vernalis* Waldst. et Kit.	starček jarní	Asteraceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Sisymbrium loeselii* L.	hulevník Loeselův	Brassicaceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Solanum decipiens* Opiz	lilek vlnatý	Solanaceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Stellaria pallida* (Dumort.) Crép.	ptačinec bledý	Caryophyllaceae	terrestrial	a		Spontaneous	Regional	Limited	Limited	Tolerance
plant	GL	*Typha laxmannii* Lepech.	orobinec sítinovitý	Typhaceae	ne aquat??	pe		Released/spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Ameiurus nebulosus* (Lesueur, 1819)	sumeček americký	Ictaluridae	aquatic	fish		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Ashworthius sidemi* Schulz, 1933	vlasovka	Trichostrongylidae	terrestrial	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Astacus leptodactylus* Eschscholtz, 1823	rak bahenní	Astacidae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Dactylogyrus achmerowi* Gusev, 1955	žábrohlíst	Dactylogyridae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Eriocheir sinensis* H. Milne Edwards, 1853	krab říční	Varunidae	aquatic	invertebrate		Spontaneous	Local	Limited	Limited	Tolerance
animal	GL	*Eudiplozoon nipponicum* (Goto, 1891)	žábrohlíst	Diplozoidae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Fascioloides magna* (Bassi, 1875)	motolice obrovská	Fasciolidae	terrestrial	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Gyrodactylus cyprini* Diarova, 1964	žábrohlíst	Gyrodactylidae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Gyrodactylus kherulensis* Ergens, 1974	žábrohlíst	Gyrodactylidae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Gyrodactylus shulmani* Ling, 1962	žábrohlíst	Gyrodactylidae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Gyrodactylus sprostonae* Ling, 1962	žábrohlíst	Gyrodactylidae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Chelicorophium curvispinum* Sars, 1895		Corophiidae	aquatic	invertebrate		Spontaneous	Local	Limited	Limited	Tolerance

Taxon group	List categ.	Species (scientific name)	Czech name	Family	Environment	Life history/ taxon group	Note	Mode of current spread	Distribution	Environmental impact	Human (socio-economic) impact	Management strategy
animal	GL	*Proteocephalus longicollis* (Zeder, 1800)	tasemnice	Proteocephalidae	terrestrial	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Pseudodactylogyrus anguillae* (Yin & Sproston, 1948)	žábrohlíst	Ancyrocephalidae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Pseudodactylogyrus bini* (Kikuchi, 1929)	žábrohlíst	Ancyrocephalidae	aquatic	invertebrate		Spontaneous	Regional	Limited	Limited	Tolerance
animal	GL	*Rupicapra rupicapra* (Linnaeus, 1758)	kamzík horský	Bovidae	terrestrial	mammal		Spontaneous	Local	Limited	Limited	Tolerance

Table A2. Watch list (WL) of plant and animal species. For plants life history is shown: a – annual, b – biennial, pe – perennial, s – shrub, t – tree, aq – aquatic.

Taxon group	List category	Species (scientific name)	Czech name	Family	Environment	Life history/ taxon group
plant	WL	*Aesculus hippocastanum* L.	jírovec maďal ("koňský kaštan")	Sapindaceae	terrestrial	t
plant	WL	*Agrostis scabra* Willd.	psineček řídkokvětý	Poaceae	terrestrial	pe
plant	WL	*Amaranthus crispus* (Lesp. & Thévenau) N. Terracc.	laskavec kadeřavý	Amaranthaceae	terrestrial	a
plant	WL	*Amaranthus deflexus* L.	laskavec skloněný	Amaranthaceae	terrestrial	pe
plant	WL	*Azolla filiculoides* Lamk.	azola americká	Salviniaceae	aquatic	a f aq
plant	WL	*Cardamine chelidonia* L.	řeřišnice vlaštovičníkovitá	Brassicaceae	terrestrial	a pe
plant	WL	*Cotoneaster* sp.	skalník	Rosaceae	terrestrial	s
plant	WL	*Elodea canadensis* Michx	vodní mor kanadský	Hydrocharitaceae	aquatic	a f aq
plant	WL	*Elodea nutalii* Planchon	vodní mor americký	Hydrocharitaceae	aquatic	a f aq
plant	WL	*Eragrostis pilosa* (L.) P. Beauv.	milička chlupatá	Poaceae	terrestrial	a
plant	WL	*Glyceria striata* (Lam.) Hitchc.	zblochan žíhaný	Poaceae	terrestrial	pe
plant	WL	*Heracleum persicum* Fisch.	bolševník perský	Apiaceae	terrestrial	b pe
plant	WL	*Heracleum sosnowskyi* Manden.	bolševník Sosnovského	Apiaceae	terrestrial	b pe
plant	WL	*Lathyrus aphaca* L.	hrachor pačočkový	Fabaceae	terrestrial	a
plant	WL	*Lathyrus hirsutus* L.	hrachor chlupatý	Fabaceae	terrestrial	a
plant	WL	*Ludwigia × kentiana* E.J. Clement	zakucelka	Onagraceae	terrestrial (aquatic)	pe aq
plant	WL	*Ludwigia grandiflora* (M. Micheli) Greuter & Burdet	zakucelka velkokvětá	Onagraceae	terrestrial (aquatic)	pe aq
plant	WL	*Oenothera depressa* Greene	pupalka vrbolistá	Onagraceae	terrestrial	b
plant	WL	*Oenothera fallax* Renner	pupalka klamná	Onagraceae	terrestrial	b
plant	WL	*Oenothera issleri* Renner ex Rostański	pupalka Isslerova	Onagraceae	terrestrial	b
plant	WL	*Panicum miliaceum* subsp. *ruderale* (Kitag.) Tzvelev	proso seté rumištní	Poaceae	terrestrial	a
plant	WL	*Paulownia tomentosa* (Thunb.) Steud	pavlovnie plstnatá	Paulowniaceae	terrestrial	t
plant	WL	*Rudbeckia hirta* L.	třapatka srstnatá	Asteraceae	terrestrial	pe
plant	WL	*Sisymbrium volgense* E. Fourn.	hulevník povolžský	Brassicaceae	terrestrial	pe
plant	WL	*Spiraea* sp. (excluding native species)	tavolník	Rosaceae	terrestrial	s
animal	WL	*Anoplophora glabripennis* (Motschulsky, 1853)	kozlíček	Cerambycidae	terrestrial	invertebrate
animal	WL	*Babka gymnotrachelus* Kessler, 1857	hlaváč holokrký	Gobiidae	aquatic	fish
animal	WL	*Bison bison* (Linnaeus, 1758)	bizon americký	Bovidae	terrestrial	mammal
animal	WL	*Capra aegagrus* Erxleben, 1777	koza bezoárová	Bovidae	terrestrial	mammal
animal	WL	*Corbicula fluminalis* (O. F. Müller, 1774)	korbikula brakická	Cyrenidae	aquatic	invertebrate
animal	WL	*Dreissena bugensis* Andrusov, 1897	slávička	Dreissenidae	aquatic	invertebrate
animal	WL	*Gammarus tigrinus* Sexton, 1939	blešivec	Gammaridae	aquatic	invertebrate
animal	WL	*Ictiobus cyprinellus* (Vallensciennes, 1844)	kaprovec velkoústý	Catostomidae	aquatic	fish

Taxon group	List category	Species (scientific name)	Czech name	Family	Environ-ment	Life history/ taxon group
animal	WL	*Lasius neglectus* Van Loon, Boomsma & Andrásfalvy, 1990	mravenec	Formicidae	terrestrial	invertebrate
animal	WL	*Lepomis auritus* (Linnaeus, 1758)	slunečnice ušatá	Centrarchidae	aquatic	fish
animal	WL	*Lepomis cyanellus* (Rafinesque, 1819)	slunečnice zelená	Centrarchidae	aquatic	fish
animal	WL	*Misgurnus anguillicaudatus* Cantor, 1842	piskoř dálnovýchodní	Cobitidae	aquatic	fish
animal	WL	*Neogobius fluviatilis* (Pallas, 1814)	hlaváč říční	Gobiidae	aquatic	fish
animal	WL	*Orconectes immunis* (Hagen, 1870)	rak	Cambaridae	aquatic	invertebrate
animal	WL	*Orconectes juvenilis* (Hagen, 1870)	rak	Cambaridae	aquatic	invertebrate
animal	WL	*Orconectes virilis* (Hagen, 1870)	rak	Cambaridae	aquatic	invertebrate
animal	WL	*Perccottus glenii* Dybowski, 1877	hlavačkovec Glenův	Odontobutidae	aquatic	fish
animal	WL	*Ponticola kessleri* (Günther, 1861)	hlaváč Kesslerův	Gobiidae	aquatic	fish
animal	WL	*Procambarus acutus* Girard, 1852 / *zonangulus* Hobbs, Jr. & Hobbs III, 1990	rak	Cambaridae	aquatic	invertebrate
animal	WL	*Procambarus alleni* Faxon, 1884	rak floridský	Cambaridae	aquatic	invertebrate
animal	WL	*Procambarus clarkii* Girard, 1852	rak červený	Cambaridae	aquatic	invertebrate
animal	WL	*Procambarus fallax* (Hagen, 1870) f. *virginalis*	rak mramorovaný	Cambaridae	aquatic	invertebrate
animal	WL	*Psittacula krameri* Scopoli, 1769	alexandr malý	Psittacidae	terrestrial	bird
animal	WL	*Salvelinus alpinus* (Linnaeus, 1758)	siven severní	Salmonidae	aquatic	fish
animal	WL	*Sciurus carolinensis* Gmelin, 1788	veverka popelavá	Sciuridae	terrestrial	mammal
animal	WL	*Thymallus baicalensis* (Dybowski, 1874)	lipan bajkalský	Salmonidae	aquatic	fish
animal	WL	*Umbra pygmaea* DeKay, 1842	blatňák menší	Umbridae	aquatic	fish

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The influence of mowing regime on the soil seed bank of the invasive plant *Ambrosia artemisiifolia* L.

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Abstract

Ambrosia artemisiifolia is an invasive annual herb infamous for the high allergenicity of its pollen, which is related to increasing medical costs. Additionally, it can cause serious yield losses as agricultural weed. Common ragweed seeds accumulate in the soil and can remain therein viable for decades, which poses a problem for the sustainable management of these populations. A long term management should thus target a reduction of the soil seed bank. We observed the influence of four different mowing regimes on the ragweed soil seed bank at six roadside populations in eastern Austria. The mowing regimes were based on methods from common roadside management practice and specifically adapted to reduce seed production. After three years of application, the soil seed bank was indeed reduced by 45 to 80 percent through three of the four mowing regimes tested. Therefore, we suggest that the best mowing regime for the most effective reduction of the size of the soil seed bank is the one consisting of one cut just after the beginning of female flowering (around the 3rd week of August in Eastern Central Europe), followed by a second cut 2–3 weeks later.

Keywords

Common ragweed, invasive plant, management, mowing, roadside vegetation, seed bank, neophyte

Introduction

Invasive alien species (IAS) are evident threats to local and regional biodiversity (McGeoch et al. 2010, Vilá et al. 2010, SBSTTA 2014). Additionally, many IAS have severe economic impact (Jeschke et al. 2014) either as weeds that reduce agricultural yield (Oerke 2006) or by endangering human health (Reinhardt 2003, Salo et al. 2011). Control and eradication of IAS is of increasing importance for diversity conservation and environmental health (Pyšek et al. 2007, Shine et al. 2009, Smith et al. 2013).

Common ragweed (*Ambrosia artemisiifolia*) is an annual IAS, growing on disturbed sites like roadsides, fields, riversides and gardens. It is feared for the allergenic properties of its pollen, as well as a weed in agriculture, in both instances related to high financial costs (Coble et al. 1981, Buttenschøn et al. 2009, Rosenbaum et al. 2011, Smith et al. 2013). *A. artemisiifolia* is native to North-America and currently spreading through Europe and Asia (Kazinczi et al. 2008). In Europe, preferred habitats are summer crop fields in summer warm climates, but also ruderal places and roadsides.

The plant reproduces exclusively by seeds. One individual can produce up to 62000 seeds in North-America (Dickerson and Sweet 1971) or up to 18000 in Europe (Fumanal 2007). Ragweed seeds can enter primary dormancy and germinate next spring, or enter secondary dormancy after failure to germinate in spring (Bazzaz 1970, Baskin and Baskin 1980) and remain dormant in the soil seed bank for up to 39 years (Toole and Brown 1946). Ragweed dormancy is broken by stratification (Bazzaz 1970).

The persistent soil seed bank of *A. artemisiifolia* compromises the efficacy of any kind of control measure. Even if a control option succeeds in killing green plants aboveground, some part of the population remains dormant in the soil awaiting more favorable conditions to germinate. Another disadvantage of a persistent soil seed bank is that it acts as a source of further spreading of the weed in soil containments (Nawrath and Alberternst 2013, Karrer 2014). Soil is relocated from many habitats where the plant is growing, such as construction sites or roadsides to other sites. Therefore, aim of any sustainable long-term control of common ragweed should be a reduction of the soil seed bank in established populations.

Milakovic et al. (2014a and 2014b) and Bohren et al. (2008) found that seed production per plant could be influenced by carefully timed mowing. This study's goal is to test the effect of different cutting regimes applied for three years (Milakovic et al. 2014a) on the quantity and quality of the ragweed soil seed bank.

Regrowth of ragweed after mowing is well-documented (Barbour and Meade 1981, Bohren et al. 2005, Bohren et al. 2008, Meiss et al. 2008, Karrer et al. 2011, Patracchini et al. 2011, Simard and Benoit 2011, Tokarska-Guzik et al. 2011) and varies with season (Milakovic et al. 2014b). Timing and frequency of cutting has specific influences on the seed production of ragweed (Simard and Benoit 2011, Milakovic et al. 2014a). Higher ranked resprouts after cuts tend to produce only female flowers (Karrer et al. 2011) and, in consequence, preferably seeds that are incorporated into the soil seed bank.

Soil seed bank of plants varies by year and season. On undisturbed soil, the annual seed production of ragweed germinates to high percentages in early next spring (Dickerson 1968, Bassett and Crompton 1975, Fumanal et al. 2008, Kazinczi et al. 2008, Leitsch-Vitalos and Karrer unpubl.). Soil tillage incorporates new seeds into deeper layers of the soil (Buhler et al. 1997) and promotes long time persistency of ragweed seeds (Toole and Brown 1946).

The effects of different tillage systems were analyzed with respect to the composition of the soil seed bank of arable fields (Clements et al. 1996, Buhler et al. 1997, Cardina et al. 2000, Clay et al. 2006). Up to now, no study has considered the soil seed bank of ragweed for measuring the success of control options, even though the seeds in the soil make up a great portion of the population in annual weeds with a persistent soil seed bank. In this study, we used the soil seed bank of ragweed populations as long-term efficacy measure of non-chemical control options. We varied the mowing regime of ragweed roadside populations in Austria with respect to timing and frequency (Milakovic et al. 2014a) and analyzed the soil seed bank of ragweed at the beginning and at the end of the experiment.

Methods

We sampled the soil seed bank of six roadside populations in Eastern Austria before and after three years of application of management practices. Austrian arterial road verges are cut at least two times a year; a first cut in spring and a second cut between July and October. This resulted in a significant spread of common ragweed along arterial roads since 2000 (Karrer et al. 2011, Essl et al. 2009).

The cutting experiment was set up in 2009 in the heavily infested parts of Austria (Lower Austria, Styria and Burgenland) (Table 1). All populations have been naturalized for about one or two decades before the experiment.

Experimental design:
On each site, five experimental plots were installed on continuous spontaneous populations of *A. artemisiifolia* with coverages ranging from 5 to 25%. The plots were arranged along a line of 100 m, adjacent and parallel to the asphaltic surface of highways or arterial roads. Each plot sized 20 × 0.5 m and received one of the following treatments (mowing regimes), as defined in Milakovic et al. (2014a):

Treatment 1: not mown (control),
Treatment 2: first cut before the start of flowering (the last week of June), and second cut at the beginning of seed set (second week of September). Treatment 2 resembles the common roadside cutting regime in eastern Austria.
Treatment 3: first cut after the beginning of female mass flowering (third week of August), and second cut at the beginning of seed set (second week of September),
Treatment 4: first cut before the start of flowering (last week of June), second cut before the onset of male mass flowering (last week of July), and third cut at the beginning of seed set (second week of September),
Treatment 5: first cut before the start of flowering (last week of June), second cut after the beginning of female mass flowering (third week of August) and third cut at the beginning of seed set (second week of September).

Table 1. Location (coordinate system WGS84) and habitat characteristics (road type, road orientation, initial ragweed coverage (%)) of the experimental sites along arterial roads in Austria.

Site ID	Longitude (E)	Latitude (N)	Altitude (m)	Road type	Road orientation	Initial ragweed coverage
3	15°57'21.21"	46°42'59.81"	212	National	NW-SW	15
4	16° 3'9.65"	47°16'33.61"	381	Highway	SW-NE	5
5	16°50'41.91"	48°26'46.51"	170	National	N-S	14
6	16° 5'31.96"	47°42'17.61"	379	Highway	SW-NE	25
7	15°40'4.61"	48°10'54.87"	296	Highway	SW-NE	17
8	16°36'18.83"	48°18'40.06"	162	National	W-E	5

Soil seed bank sampling

All sites have been sampled for soil seed bank before the start of the mowing experiment in spring 2009 and after three years of the experiment in spring 2012. The sampling was always performed just before or at the very start of the germination period in the field. First sampling was done in March 2009 preceding the different treatment of the plots: 20 soil cores (depth 7cm, 285cm^3, equally distributed over 100m of the experiment plot) were taken at each site. After three years of applying the various treatments, in March 2012, 19 soil cores were taken per plot on each site.

The soil cores were analyzed for ragweed seed content using a wet sieving machine (Retsch). We counted all intact seeds and put them into wetted Petri dishes. In order to detect the proportion of viable seeds, first germination was induced by putting them into climate chambers at the following conditions: daylight for 8 hours at 30 °C and darkness for 16 hours at 15 °C. We stopped the germination trial after 4 weeks, left the dishes for drying out and stored them for 4 weeks at +4 °C in darkness, in order to overcome secondary dormancy by additional stratification. Afterwards, a second germination period was started at the same conditions like in the first session.

All seeds that did not germinate within the second germination session were tested for vitality by a standard staining (TTC-test with 1 % solution of 2,3,5 triphenyl tetrazolium chloride in pure water). For that, *Ambrosia*-achenes were first imbibed in tap water at room temperature for 24 hours. The achenes were then cut open with a scalpel to expose the embryo. The bigger part of the achene was used for testing, the other part was discarded. Achene halves were put into petri dishes, covered with TTC solution and left at 30 °C for 6 hours in absolute darkness. Finally seeds were evaluated under a dissecting microscope. All fully stained seeds were classified vital.

The soil seed bank samples in 2009 were taken from the whole sites that where covered consistently with *A. artemisiifolia*, and can therefore be used as baseline data for comparison to the soil seed bank counting at the differently treated plots three years later. That way, it is possible to observe the effect of the tested mowing regimes on the soil seed bank after three years of application.

Data were analyzed by GLM (generalized linear model) using Poisson distribution procedures and a log link in the package Statistica 10 (StatSoft 2011). Treatment was included in the model as independent categorical factor and seed number per m^2 as dependent variable. Pairwise differences between treatments were judged at 95% confidence intervals. We compared the overall most effective treatment with the initial seed bank of the populations of each site by Kruskal-Wallis Tests.

Results

Soil seed bank at different sites

In 2009, soil seed bank varied from 123 to 823 (522 in average) seeds per m^2 at all sites (Table 2), with germination rates varying from 53 to 100% (mean 80%). In 2012, soil seed bank at different sites varied from 0 to 1061 seeds per m^2. The germination rates were generally very high (mean 91%). From the 2012 samples, no seeds germinated during the second germination test, and no living seeds could be detected by the subsequent TTC test.

Soil seed bank in different treatments

After 3 years of applying different mowing regimes, significant differences in the soil seed bank under different treatments were found (Wald χ^2 (5) = 188795; p ≤ 0,01). The soil seed bank of treatment 1 (control, unmown) was three times higher than the soil seed bank of the population before the experiment (Figure 1). The soil seed bank of treatment 2 did not differ significantly from the soil seed bank of the population in 2009 (Figure 1). The soil seed bank of the treatments 3, 4 and 5 decreased by ca. 80%, 60% and 45%, respectively, compared to the magnitude order before the experiment (Figure 1). Efficacy of treatment 3 is obviously highest in controlling the ragweed populations sustainably. The soil seed bank decreased on all sites on the plots of treatment 3 (Figure 2), at most sites significantly (Table 3).

Table 2. Number of *Ambrosia artemisiifolia* seeds per m^2 (means and standard deviation (SD) calculated from 20 soil cores) in spring 2009 and in spring 2012 (calculated from 95 cores) at six experimental sites.

Site ID	Mean number of seeds/m² in 2009	SD	Germination rate (%)	Mean number of seeds/m² in 2012	SD	Germination rate (%)
3	467	652	66	1002	2069	98
4	467	699	53	394	1045	76
5	823	866	100	369	1102	98
6	541	702	77	1061	1181	98
7	123	246	90	205	565	86
8	713	836	95	0	-	-

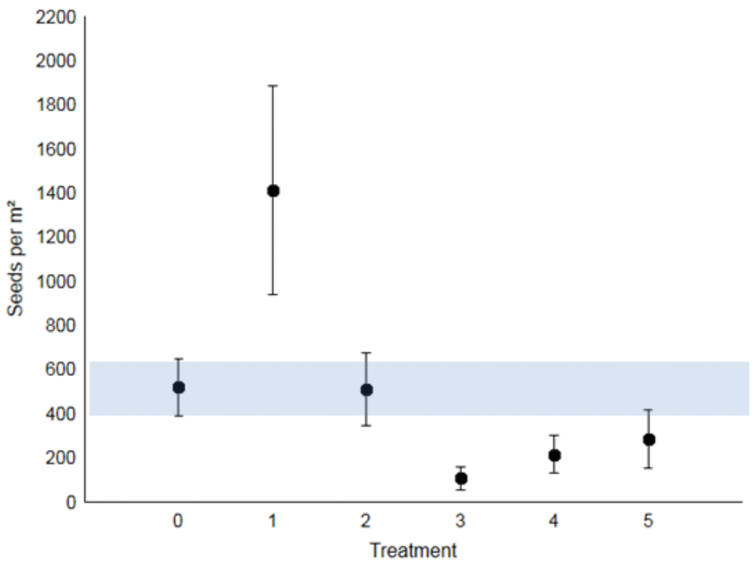

Figure 1. Means and confidence intervals of the number of seeds of *Ambrosia artemisiifolia* per m² (depth 7cm) after 3 years of different mowing treatments (1–5) in 2012 compared to the soil seed bank of the population before the experiment in 2009 ("Treatment" 0 = baseline)

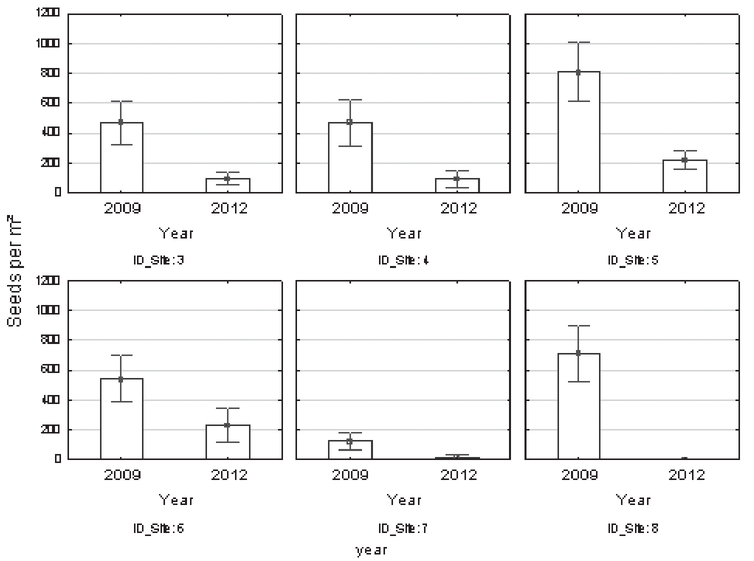

Figure 2. Mean numbers (and SE) of *A. artemisiifolia* seeds per m² (depth 7cm) in the plots of treatment 3 at six different sites in 2012 compared to the soil seed bank before the experiment in 2009

Table 3. Kruskal-Wallis test for the differences between the soil seed bank (seeds per m²) in plots of treatment 3 in 2012 and the soil seed bank of the respective populations in 2009, differentiated by sites.

Site ID	H	p
3	5.72	**<0.05**
4	6.65	**<0.01**
5	7.54	**<0.01**
6	3.04	0.08
7	3.74	0.53
8	14.7	**<0.001**

Discussion

The number of ragweed seeds per m² found in populations along Austrian roadsides before the start of treatments in 2009 indicate that those are all well-established populations that cannot be controlled by a one time management action. The aboveground assimilating part of the *A. artemisiifolia* population varied between the sites at the beginning of the experiment (Table 1) but showed similar dynamics to the soil seed bank towards the end of the experiment. Compared to the soil seed bank of other ruderal habitats (waste lands and set-asides) our roadside populations showed relative low seed densities. Fumanal et al. (2008) describe seed densities ranging from 510–3324 seeds per m² in the upper 5 cm of soil. This indicates that the Austrian roadside populations are relatively young but 'active' populations. Corresponding to the high population turnover rates, most seeds accumulate in the uppermost soil layer and germinate at high rates to produce many new seeds every generation. The fraction of old seeds from former population establishment phases that might have lower germination rates, seems to be relatively low as the overall germination rates of the seeds in the soil is considerably high (Table 2).

The seed bank densities of ragweed along Austrian highways are generally lower than in European arable fields (Vitalos and Karrer 2008). Habitat types that have been infested by ragweed for decades, like abandoned fields in N-America, have a load of 0–200 ragweed seeds per m² even when sampling only the persistent soil seed bank in summer (Rothrock et al. 1993). Bigwood and Inouye (1988) found on average 36 ragweed seeds per m² in the upper soil (0–8 cm) and 57.6 seeds per m² at a depth of 8–16 cm in an old field in Maryland (US). Raynal and Bazzaz (1973) counted means of 64 ragweed seeds per m² in maize fields on former forest soil and 4.8 seeds per m² on former prairie soil, when analyzing the upper soil (0–5 cm) in early spring; autumn samples did not contain ragweed seeds. Considering that the Austrian ragweed seed populations along highways are concentrated at the upper horizons of the road shoulder soil, they can be classified as very active and contribute to an increasing infestation.

Because most management options act on the green parts of the plant, they are not sustainable. The most desired aspect of ragweed control is the successful elimination of persistent seeds from the soil. The results of this long term experiment show, that the soil seed bank can be diminished vigorously by a sophisticated mowing management. The mowing regime should consist of a first cut in August, just at the first appearance of female flowers, and a second cut in early September, just before fertility of the female flowers on the regrowth from the base (Milakovic et al. 2014a). According to our results, we suggest to rate this mowing regime as the most sustainable and environmentally friendly control option, because it progressively leads to indirect depletion of the soil seed bank. This way the ragweed populations decline and can be managed easier. Hence the biologically most effective control measure of pulling out the remaining few plants by hand (Bohren et al. 2008) might become economically feasible.

We advise analyzing the soil seed bank of ragweed before installing a field experiment or defining a management regime for ragweed control, as well as after the activity. Thus sustainability can be proven. The knowledge about the status of soil seed bank is particularly important for ragweed populations growing on roadsides, as the upper soil is prone to transportation elsewhere, which contributes to further dispersal of ragweed seeds and creates new populations.

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References

Barbour BM, Meade JA (1981) The effects of cutting date and height on anthesis of common ragweed (*Ambrosia artemisiifolia* L.). Proceedings of the Annual Meeting Northeast Weed Science Society 35: 82–86.

Baskin JM, Baskin CC (1980) Ecophysiology of secondary dormancy in seeds of *Ambrosia artemisiifolia*. Ecology 61: 475–480. doi: 10.2307/1937410

Bassett IJ, Crompton CW (1975) The biology of Canadian weeds 11 – *Ambrosia artemisiifolia* L. and *Ambrosia psilostachya* DC. Canadian Journal of Plant Science 55: 463–476. doi: 10.4141/cjps75-072

Bazzaz FA (1970) Secondary dormancy in the seeds of the common ragweed *Ambrosia artemisiifolia*. Bulletin of the Torrey Botanical Club 97: 302–305. doi: 10.2307/2483650

Bigwood DW, Inouye DW (1988) Spatial pattern analysis of seed banks: an improved method and optimized sampling. Ecology 69: 497–507. doi: 10.2307/1940448

Bohren C, Delabays N, Mermillod G, Keimer C, Kündig C (2005) *Ambrosia artemisiifolia* in der Schweiz–eine herbologische Annäherung. AgrarForschung 12: 71–78. http://www.agrarforschungschweiz.ch/archiv_11de.php?id_artikel=933

Bohren C, Delabays N, Mermillod G, Baker A, Vertenten J (2008) *Ambrosia artemisiifolia* L: Optimieren des Schnittregimes. Agrarforschung 15: 308–313. http://www.agrarforschungschweiz.ch/artikel/2008_07_1393.pdf

Buhler DD, Hartzler RG, Forcella F (1997) Implications of weed seedbank dynamics to weed management. Weed Science 45: 329–336. http://www.jstor.org/stable/4046027

Buttenschøn RM, Waldispühl S, Bohren C (2009) Guidelines for management of common ragweed, *Ambrosia artemisiifolia*. http://xwww.agrsci.dk/ambrosia/outputs/ambrosia_eng.pdf [last accessed March 2015]

Cardina J, Herms CP, Doohan DJ (2000) Crop rotation and tillage system effects on weed seedbanks. Weed Science 50: 448–460. doi: 10.1614/0043-1745(2002)050[0448:CRATSE]2.0.CO;2

Clements DR, Benott DL, Murphy SD, Swanton CJ (1996) Tillage effects on weed seed return and seedbank composition. Weed Science 44: 314–322. http://www.jstor.org/stable/4045684

Coble HD, Williams FM, Ritter RL (1981) Common Ragweed (*Ambrosia artemisiifolia*) Interference in Soybeans (Glycine max). Weed Science 29: 339–342. http://www.jstor.org/stable/4043252

Clay SA, Kreutner B, Clay DE, Reese C, Kleinjan J, Forcella F (2006) Spatial distribution, temporal stability, and yield loss estimates for annual grasses and common ragweed (*Ambrosia artimisiifolia*) in a corn/soybean production field over nine years. Weed Science 54: 380–390. doi: 10.1614/WS-05-090R1.1

Dickerson CT (1968) Studies on the germination, growth, development and control of common ragweed (Ambrosia artemisiifolia L.). Dissertation, Cornell Univ. Ann Arbour, Michigan.

Dickerson CT, Sweet RD (1971) Common ragweed ecotypes. Weed Science 19: 64–66. http://www.jstor.org/stable/4041819

Essl F, Dullinger S, Kleinbauer I (2009) Changes in the spatiotemporal patterns and habitat preferences of *Ambrosia artemisiifolia* during its invasion of Austria. Preslia 81: 119–133. www.preslia.cz/P092Essl.pdf

Fumanal B, Chauvel B, Bretagnolle F (2007) Estimation of pollen and seed production of common ragweed in France. Annals of Agricultural and Environmental Medicine 14: 233–236. http://www.aaem.pl/pdf/14233.pdf

Fumanal B, Gaudot I, Bretagnolle F (2008) Seed bank dynamics of an invasive plant: *Ambrosia artemisiifolia* L. as a case study. Seed Science Research 18: 101–114. doi: 10.1017/S0960258508974316

Jeschke JM, Bacher B, Blackburn TM, Dick JTA, Essl F, Evans T, Gaertner M, Hulme PE, Kühn I, Mrugala A, Pergl J, Pyšek P, Rabitsch W, Ricciardi A, Richardson DM, Sendek A, Vilà M, Winter M, Kumschick S (2014) Defining the impact of non-native species. Conservation Biology 28: 1188–1194. doi: 10.1111/cobi.12299

Karrer G, Milakovic I, Kropf M, Hackl G, Essl F, Hauser M, Mayer M, Blöch C, Leitsch-Vitalos M, Dlugosch A, Hackl G, Follak S, Fertsak S, Schwab M, Baumgarten A, Gansberger M, Moosbeckhofer R, Reiter E, Publig E, Moser D, Kleinbauer I, Dullinger S (2011) Ausbreitungsbiologie und Management einer extrem allergenen, eingeschleppten Pflanze – Wege und Ursachen der Ausbreitung von Ragweed (Ambrosia artemisiifolia) sowie Möglichkeiten seiner Bekämpfung. Final report, BMLFUW, Wien, 329 pp.

Karrer G (2014) Das österreichische Ragweed Projekt – übertragbare Erfahrungen? Julius-Kühn-Archiv 445: 27–33. doi: 10.5073/jka.2013.445.003

Kazinczi G, Béres I, Novák R, Bíró K (2008) Common ragweed (*Ambrosia artemisiifolia* L.): A review with special regards to the results in Hungary: I. Taxonomy, origin and distribution, morphology, life cycle and reproduction strategy. Herbologia 9: 55–91.

McGeoch MA, Butchart SHM, Spear D, Marais E, Kleynhans EJ, Symes A, Chanson J, Hoffmann M (2010) Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. Diversity and Distributions 16: 95–108. doi: 10.1111/j.1472-4642.2009.00633.x

Meiss H, Munier-Jolain N, Henriot F, Caneill J (2008) Effects of biomass, age and functional traits on regrowth of arable weeds after cutting. Journal of Plant Diseases and Protection XXI: 493–499. https://www.researchgate.net/publication/230691479_Effects_of_biomass_age_and_functional_traits_on_regrowth_of_arable_weeds_after_cutting

Milakovic I, Fiedler K, Karrer G (2014a) Management of roadside populations of invasive Ambrosia artemisiifolia by mowing. Weed Research 54: 256–264. doi: 10.1111/wre.12074

Milakovic I, Fiedler K, Karrer G (2014b) Fine tuning of mowing regime, a method for the management of the invasive *Ambrosia artemisiifolia* L. at different population densities. Weed Biology and Management 14: 232–241. doi: 10.1111/wbm.12051

Nawrath S, Alberternst B (2013) Aktionsprogramm Ambrosia-Bekämpfung in Bayern: Ergebnisse aus sechs Jahren Monitoring. ANLiegen Natur 35: 44–58. http://www.anl.bayern.de/publikationen/anliegen/doc/an35205nawrath_et_al_2013_ambrosia.pdf [last accessed March 2015]

Oerke EC (2006) Crop losses to pests. Journal of Agricultural Science 144: 31–43. doi: 10.1017/S0021859605005708

Patracchini C, Vidotto F, Ferrero A (2011) Common ragweed (Ambrosia artemisiifolia) growth as affected by plant density and clipping. Weed Technology 25: 268–276. doi: 10.1614/WT-D-09-00070.1

Pyšek P, Cock MJW, Nentwig W, Ravn HP (2007) Ecology and Management of Giant Hogweed (*Heracleum mantegazzianum*). CABI International, Wollingford, 352 pp. doi: 10.1079/9781845932060.0000

Raynal DJ, Bazzaz FA (1973) Establishment of early successional plant populations on forest and prairie soil. Ecology 54: 1335–1341. doi: 10.2307/1934196

Reinhardt F, Herle M, Bastiansen F, Streit B (2003) Economic Impact of the Spread of Alien Species in Germany. Environmental Research of the Federal Ministry of the Environment, Nature Conservation and Nuclear Safety, Research Report 201 86 211 UBA-FB 000441e, Texte Nr. 80/2003. Federal Environmental Agency (Umweltbundesamt), Berlin, 229 pp.

Rosenbaum KK, Bradley KW, Roberts CA (2011) Influence of Increasing Common Ragweed (*Ambrosia artemisiifolia*) or Common Cocklebur (*Xanthium strumarium*) Densities on Forage Nutritive Value and Yield in Tall Fescue Pastures and Hay Fields. Weed Technology 25: 222–229. doi: 10.1614/WT-D-10-00114.1

Rothrock PE, Squiers ER, Sheeley S (1993) Heterogeneity and size of a persistent seedbank of *Ambrosia artemisiifolia* L. and *Setaria faberi* Herrm. Bulletin of the Torrey Botanical Club 120: 417–422. doi: 10.2307/2996745

Salo PM, Calatroni A, Gergen PJ, Hoppin JA, Sever ML, Jaramillo R, Arbes SJ, Zeldin DC (2011) Allergy-related outcomes in relation to serum IgE: results from the National Health and Nutrition Examination Survey 2005–2006. Journal of Allergy and Clinical Immunology 127: 1226–1235. doi: 10.1016/j.jaci.2010.12.1106

SBSTTA (Subsidiary Body on Scientific, Technical and Technological Advice of the CBD) (2014) Review of work on invasive alien species and considerations for future work. SBSTTA 18 Recommendation XVIII/6: http://www.cbd.int/recommendation/sbstta/default.shtml?id=13331 [last accessed March 2015]

Shine C, Kettunen M, ten Brink P, Genovesi P, Gollasch S (2009) Technical support to EU strategy on invasive alien species (IAS) Recommendations on policy options to minimize the negative impacts of invasive alien species on biodiversity in Europe and the EU. Final report for the European Commission, Institute for European Environmental Policy (IEEP), Brussels, Belgium, 35 pp.

Simard M-J, Benoit DL (2011) Effect of repetitive mowing on common ragweed (*Ambrosia artemisiifolia* L.) pollen and seed production. Annals of Agricultural and Environmental Medicine 18: 55–62. http://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-cd-4c08b7-27dc-439d-a420-995a9de0669a/c/fulltext-8.pdf

Smith M, Cecchi L, Skjøth CA, Karrer G, Šikoparija B (2013) Common ragweed: A threat to environmental health in Europe. Environment International 61: 115–126. doi: 10.1016/j.envint.2013.08.005

Tokarska-Guzik B, Bzdęga K, Koszela K, Żabińska I, Krzuś B, Sajan M, Sendek A (2011) Allergenic invasive plant *Ambrosia artemisiifolia* L. in Poland: threat and selected aspects of biology. Biodiversity: Research and Conservation 21: 39–48. doi: 10.2478/v10119-011-0008-8

Toole HE, Brown E (1946) Final results of the Durvel buried seed experiment. Journal of Agricultural Research 72: 201–210.

Vilà M, Basnou C, Pyšek P, Josefsson M, Genovesi P, Gollasch S, Nentwig W, Olenin S, Roques A, Roy D, Hulme PE, DAISIE partners (2010) How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. Frontiers in Ecology and the Environment 8: 135–144. doi: 10.1890/080083

Vitalos M, Karrer G (2008) Seed bank dynamics of Ambrosia artemisiifolia in Austria. p. 17 In: Proceedings from the 2nd International Symposium “Intractable Weeds and Plant Invaders”, September 2008, Osijek, Croatia.

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Small but tough: What can ecophysiology of croaking gourami *Trichopsis vittata* (Cuvier, 1831) tell us about invasiveness of non-native fishes in Florida?

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Abstract

Trichopsis vittata (Cuvier, 1831) is a small, freshwater gourami (Fam: Osphronemidae) native to southeast Asia. It was first detected in Florida in the 1970s and seems to have persisted for decades in a small area. In this study, we documented *T. vittata*'s ecophysiological tolerances (salinity and low-temperature) and qualitatively compared them to published values for other sympatric non-native species that have successfully invaded much of the Florida peninsula. *Trichopsis vittata* survived acute salinity shifts to 16 psu and was able to survive up to 20 psu when salinity was raised more slowly (5 psu per week). In a cold-tolerance experiment, temperature was lowered from 24 °C at 1 °C hr^{-1} until fish died. Mean temperature at death (i.e., lower lethal limit) was 7.2 °C. *Trichopsis vittata* seems as tolerant or more tolerant than many other sympatric non-native fishes for the variables we examined. However, *T. vittata* is the only species that has not dispersed since its introduction. Species other than *T. vittata* have broadly invaded ranges, many of which include the entire lower third of the Florida peninsula. It is possible that tolerance to environmental parameters serves as a filter for establishment, wherein candidate species must possess the ability to survive abiotic extremes as a first step. However, a species' ability to expand its geographic range may ultimately rely on a secondary set of criteria including biotic interactions and life-history variables.

Keywords

Trichopsis, ecophysiology, low-temperature tolerance, salinity tolerance, invasiveness

Introduction

Destructive (sometimes catastrophic) ecological impacts have been attributed to the introduction and establishment of non-native fishes across the globe (Canonico et al. 2005; Pelicice and Agostinho 2009; Vitule et al. 2009). However, the severity of negative consequences of non-native fish invasions varies greatly amongst taxa. Variation in the ability of species to establish and spread (i.e., 'invasiveness' *sensu* Rejmánek et al. 2002) has provided clues to underlying ecological attributes correlated with invasiveness (García-Berthou 2007). Understanding the characteristics associated with invasiveness is especially important in predicting potential establishment and spread of newly introduced species or those considered a threat for introduction. Most studies aimed at discriminating ecological features of invasive species quantify, collate and report life-history, ecophysiological, and other data for species that have become invasive (Kolar and Lodge 2002; García-Berthou 2007). Less abundant are data on species that were introduced and died out over time, or those that were introduced and established but did not become invasive. Data on those non-invasive species can be difficult to obtain when species were not intentionally introduced (e.g., via stocking). Population dynamics of fishes that were not introduced intentionally (e.g., aquaculture escapes) may not be closely monitored. Nonetheless, the fate of these populations is important as they may provide clues to allow researchers to be able to identify characteristics unique to invasive fishes from those shared between invasive and non-invasive species.

In Florida, there are dozens of non-native fish species that have established and spread widely within the state, especially in the southern half of the peninsula. However, a few species have established but remain localised (Shafland et al. 2008; USGS-NAS 2014). Croaking gourami *Trichopsis vittata* (Cuvier, 1831) was first collected in 1978 and was considered extirpated in the 1990s; however, a localised population was rediscovered in 2013 (Schofield and Pecora 2013; Fig. 1). The species may have persisted in a relatively small area for several decades where it escaped detection. Failure of a species to spread widely after establishment may be due to many factors, such as ecophysiological intolerance or biotic interactions with predators and/or competitors. Little is known regarding the ecophysiology of *T. vittata*, other than the fact that it is an air-breather, making it capable of living in anoxic waters. Other ecophysiological attributes (e.g., tolerance to salinity, extreme temperature) were unknown before this report. Herein, we investigate two ecophysiological parameters for *T. vittata* thought to be conducive to invasiveness in Florida (cold- and salinity-tolerance). We compare those (along with hypoxia-tolerance) to published reports for other non-native fishes with much larger geographic ranges within the State. We ask: Can *T. vittata*'s small geographic range be explained by its relative lack of ecophysiological 'toughness' (i.e., ability to withstand environmental extremes)? In other words, are fishes more tolerant to cold temperatures, low oxygen and salinity predicted to have larger invasive geographic ranges? We hypothesised that *T. vittata*'s small non-native range could be related to a lack of tolerance of ecophysiological variables, and expected it to be less tolerant to environmental variables than sympatric non-native fishes with large ranges.

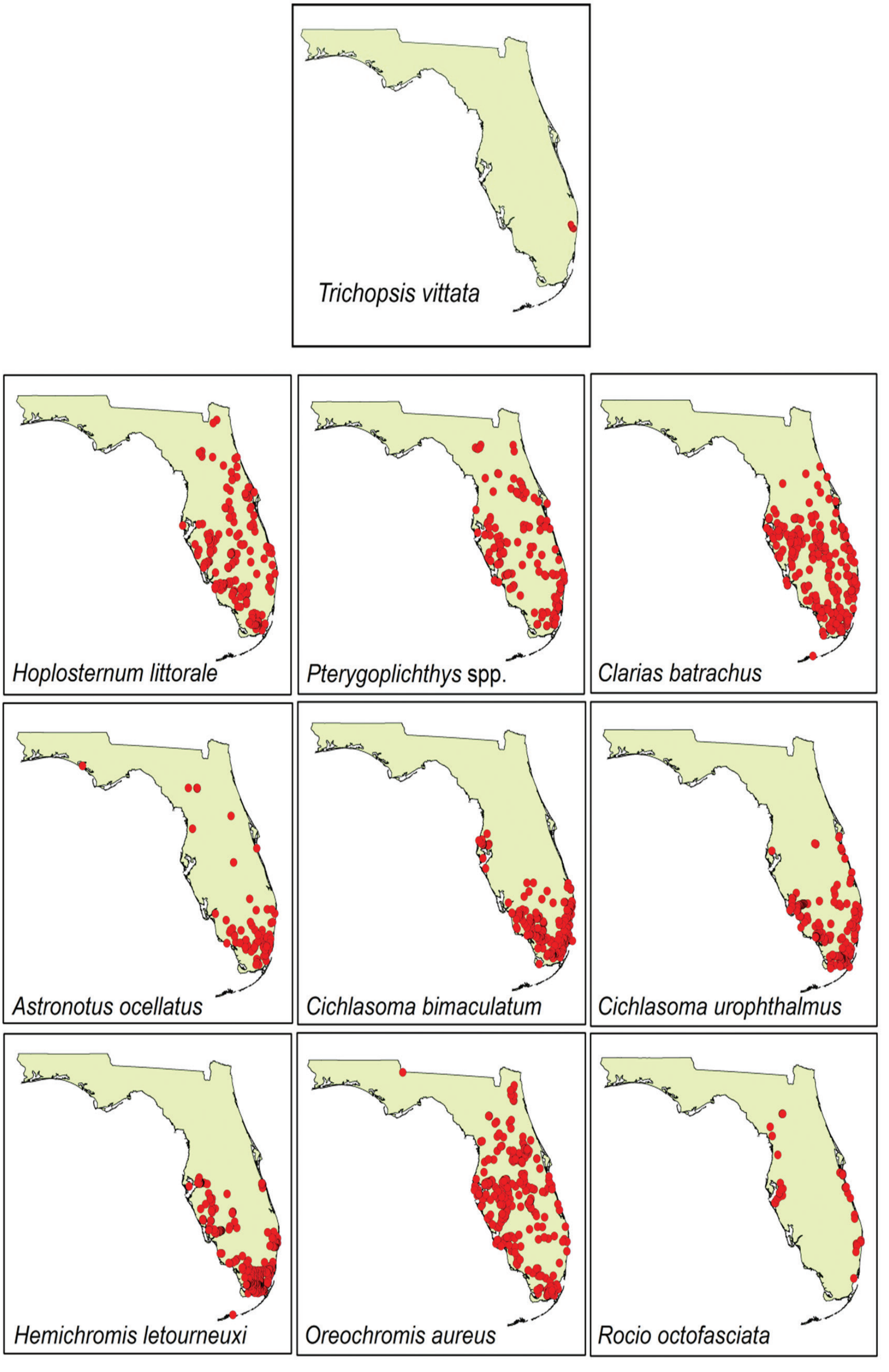


Figure 1. Geographic range of selected non-native fishes in Florida. Occurrence data (red dots) are from USGS-NAS (2014).

Methods

Specimens of *T. vittata* were collected with dip nets in March and April 2014, from Loxahatchee National Wildlife Refuge, Florida, USA. Fish were transported to the USGS laboratory in Gainesville, Florida within 48 hours of capture. Upon arrival, fish were treated with Pond Rid-Ich® Plus™ (Kordon LLC, Hayward, CA, USA) and erythromycin antibiotic. In the laboratory, fish were held indoors in 380 L fiberglass tanks with aerated well water (0.2 psu, hereafter termed "0" psu, 21-25 °C) and were fed daily with commercial flake food. Individuals were held in these conditions for about one month before experiments began. Before each experiment, fish were measured (± 0.1 cm total length [TL]), weighed (± 0.1 g) and placed into individual plastic bins (17 × 14 × 11 cm) filled with 8 cm of well water. Bins were equipped with small plastic plants, lids to prevent escape and were blinded on three sides to prevent fish from seeing each other. Because gourami are obligate air breathers, no air was provided except for the low-temperature tolerance experiment, where an airstone was placed in each bin to aid in mixing of the water for even temperature distribution throughout the bin. For both salinity experiments, fish were kept in individual bins inside a temperature-controlled room set at 26 C for the duration of the experiments. Salt water was pre-mixed to various salinities using well water and aquarium salt (Crystal Sea® Marinemix, Marine Enterprises International, Baltimore, MD, USA) before water changes using YSI Professional Plus Multiparameter meter (YSI Inc., Yellow Springs, Ohio, USA; ± 0.05 psu). For all experiments, when death was confirmed, water temperature was measured using a hand-held digital thermometer (EXTECH® waterproof thermometer model #39240, EXTECH Instruments®, Nashua, NH, USA). Individual fish were used only once in one experiment (low-temperature tolerance, chronic salinity-tolerance or acute salinity-tolerance).

Low temperature tolerance

The low-temperature tolerance experiment was conducted in April 2014 inside an environmental chamber in which temperature could be controlled by continuously decreasing the air temperature at a constant (i.e., linear) rate. Two endpoints were determined: loss of equilibrium and death (i.e., lower lethal limit). Loss of equilibrium (LOE) was defined as the fish's inability to right itself after being gently prodded, and death was defined as the extended lack of movement by the fish after it was gently prodded while in the water. Twenty-five fish were used and were not fed during the duration of the experiment. Fish were placed in the environmental chamber in individual bins and left undisturbed for 72 hours at 24 °C to acclimate. The experiment began by decreasing the air temperature by 1 °C hr^{-1} to produce an equivalent decline in water temperature. Control fish (n = 5) were immediately moved from the environmental chamber to a stable "warm room" at 24 °C (± 1 °C) until the end of the testing period, when all experimental fish (n = 20) had succumbed to death. Each hour, air temperature in the chamber was manually adjusted to produce a constant decrease of water temperature at the rate of 1 °C per hour. Temperature of each bin was measured with a hand-held digital thermometer every 20 minutes. All fish were checked for LOE and death every 20 minutes; time and temperature were recorded when LOE and death were confirmed.

Chronic salinity tolerance

An initial pilot study was conducted on n = 20 individuals to establish a general range of salinity tolerance and determine what experimental salinity levels would be used for the experiment. For the chronic salinity tolerance experiment, fish were allocated randomly to one of five treatments (0 [control] = 8 replicates, 20 psu = 11 replicates, 22.5 psu = 12 replicates, 25 psu = 12 replicates, 27.5 psu = 13 replicates). Fish were held initially for 48 hours in well water after which salinities were gradually increased at a rate of 2.5 psu every 2-3 days (5 psu per week) until fish reached the predetermined target salinity. Once the last experimental fish reached its target salinity, all fish remained in their respective salinities for an additional 30 days or until death. Fish reached their target salinities in a staggered (time-wise) fashion; however, each time the salinities were changed in one or more of the treatments, water changes were performed for all of the fish (including controls) to maintain similarity of handling across treatments. Fish were fed twice per week with a mixture of flake food and pellets on days before water changes. Fish were checked 1-2 times per day, seven days per week for death.

Acute salinity tolerance

To determine how *T. vittata* responded to acute salinity changes, fish were transferred directly from well water (0 psu) into various salinity treatments: 0 [control] = 8 replicates, 14, 16, 18, 20 psu = 10 replicates each. Similar to the chronic-salinity tolerance experiment, values for salinity treatments were derived from a pilot study. After being transferred to their respective treatments, fish were left in bins for seven days or until death. Fish were checked for mortalities every hour for the first six hours and then once per day for the remainder of the experiment.

Analyses

Cold-tolerance of *T. vittata* was compared to published values for other previously tested non-native fishes. We statistically compared four species that are sympatric with *T. vittata* (e.g., are found in Loxahatchee NWR) and are widely distributed across south Florida (*Cichlasoma bimaculatum* [Linnaeus 1758], *Cichlasoma urophthalmus* [Günther 1862], *Hoplosternum littorale* [Hancock 1828], *Hemichromis letourneuxi* Sauvage 1880; Fig. 1). These species were tested in our laboratory using the same technique, acclimation temperature, experimental equipment and rate of temperature decrease used here for *T. vittata* (Schofield et al. 2010; Schofield and Huge 2011; Schofield unpub. data). We only compared data for individuals tested in freshwater and acclimated to 24 °C. Mean temperature at death (lower lethal limit) for these species was compared with one-way analysis of variance (ANOVA), and Dunnett's T3 *post-hoc* test was used to discriminate homogeneous subsets. Levene's test was used to test for heteroscedasticity.

One-way ANOVA was used to compare fish mass among salinity treatments, and Levene's test was used to check for heteroscedasticity. Life expectancy was estimated with the Kaplan-Meier product-limit estimator (Kaplan and Meier 1958) and the log-rank test was used to compare survivorship curves (Savage 1956; Cox and Oakes 1984). For the acute-salinity challenge, all treatments began at the same time (time = 0). However, for the chronic-salinity experiment, fish reached their target salinities sequentially (i.e., staggered over time). Thus, for the chronic-salinity experiment the day the fish reached their target salinity was designated as time = 0 for that treatment. We set our alpha level for statistical significance at 0.05. All data were analysed using SPSS version 13.0.

Results

Environmental variables measured while collecting *T. vittata* on several occasions (including fish used in this experiment) are provided in Table 1. *Trichopsis vittata* used in the cold-tolerance study averaged 0.73 g (+ 0.67 standard deviation [SD]; range 0.20–3.00 g; n = 25), and 3.7 cm TL (+ 0.99 SD; range 2.5–5.9 cm). Fish lost equilibrium at 10.2 °C (+ 0.68 SD; range 8.2–11.2 °C) and died at 7.2 °C (+ 0.68 SD; range 6.4–8.8 °C). *Trichopsis vittata* was the second-most cold-tolerant species tested (after *H. littorale*), and exhibited greater tolerance to low temperatures than all cichlids (one-way ANOVA F = 49.46, df = 4, P <0.001, Fig. 2).

For the chronic salinity-tolerance experiment, fish mass averaged 0.93 g (+ 0.28 SD; range 0.30–1.5 g; n = 56) and mean TL was 4.2 cm (+ 0.55 SD; range 3.0–5.1 cm; n = 56). Fish mass did not vary significantly by treatment (one-way ANOVA F = 0.11, df = 4, *P* = 0.58). At the end of the experiment, survival was 100% at the control salinity (0 psu), 63% at 20 psu, 25% at 22.5 psu, and 8% at 25 psu (Fig. 3a). All fish at 27.5 psu died by the 24th day after reaching their 27.5 psu salinity goal. Because the majority of the data for the 20 psu treatment was censored (i.e., the majority of fish in this treatment survived the challenge), it was not possible to compute a survival estimate. Mean (95% Confidence Interval [CI]) survival estimates for other treatments are: 18 days (11–25) at 22.5 psu, 10 days (4–16) at 25 psu, 7 days (3–11) at 27.5 psu. All treatments were significantly different from the control except 20 psu (although the *P*-value was close to significance; log-rank statistic = 3.41; *P* = 0.065)

The mean mass of fish used in the acute salinity-tolerance experiment was 0.81 g (+ 0.31 SD; range 0.30–1.6 g; n = 48) and mean length was 4.1 cm TL (+ 0.54 SD; range 3.0–5.2 cm). Fish mass did not vary significantly by treatment (one-way ANOVA F =

0.98, df = 4, *P* = 0.43). After the acute salinity change, *T. vittata* at 20 psu exhibited 60% mortality within the first four hours and 100% mortality within the first six hours (mean survival estimate = 4.5 hrs; 4.1–4.9 hrs 95% CI). The 18 psu treatment group displayed 70% mortality after 24 hours, with no fish surviving longer than 48 hours (mean survival estimate = 30 hrs; 21.5–38.5 95% CI; Fig. 3b). At salinities of 0 and 14, survival was 100% and at 16 psu, it was 90% at the end of the experiment. No survival estimates were calculated for these three treatments as survival was so high (and subsequently most of the data were censored). Survival was equivalent for 0 and 14 psu (100%) and did not differ significantly between 0 and 16 psu (log-rank statistic = 0.80; *P* = 0.37) nor 14 and 16 (log-rank statistic = 1.00; *P* = 0.32).

Table 1. Environmental variables measured while collecting *Trichopsis vittata* on several occasions from Loxahatchee National Wildlife Refuge. Fish for experiments in this report were collected in March and April 2014. N/A = Not Available.

Collection date	Temperature (°C)	Salinity (psu)	Dissolved Oxygen (mg L^{-1})	pH
7 March 2014	20.6	0.07	0.87	N/A
23 April 2014	25.1	0.21	0.74	7.18
24 April 2014	21.8	0.22	0.67	7.12
31 March 2015	21.2	0.17	3.14	7.27

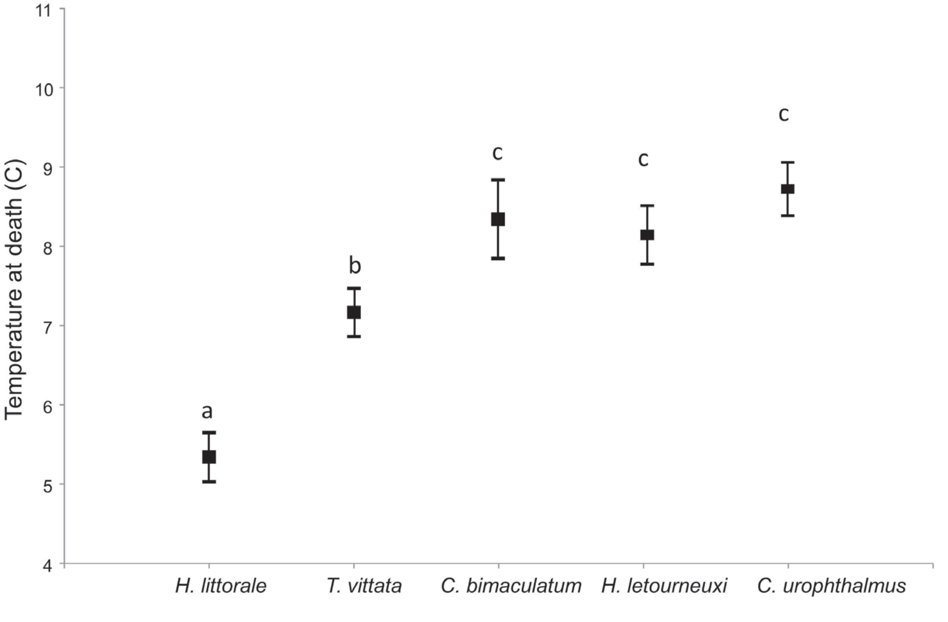
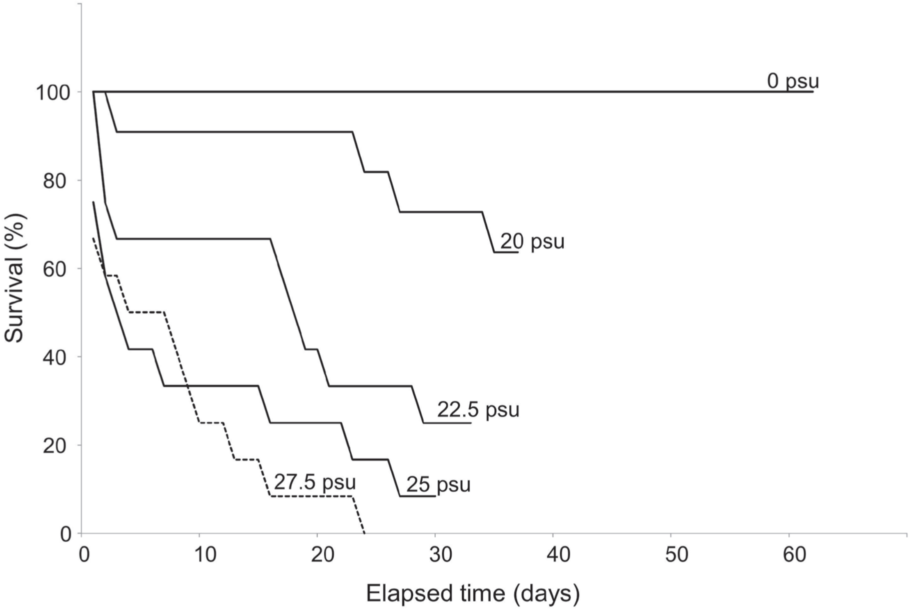


Figure 2. Mean temperature (+ 2 SE) at which fishes died in cold-temperature tolerance experiments (i.e., lower lethal limit). Letters denote significant differences (one-way ANOVA with Dunnett's T3 *post-hoc* test; see text for details). References for data sources are given in Table 2.



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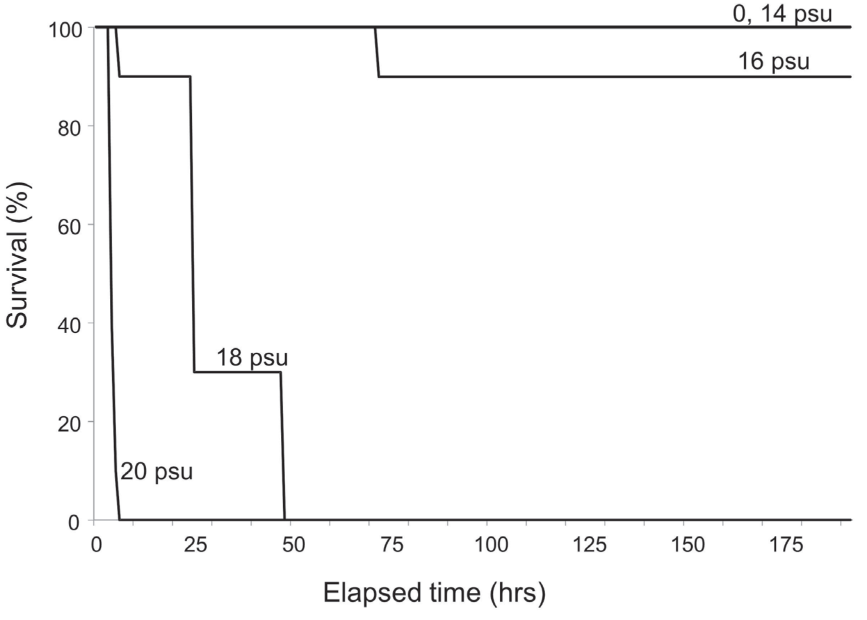

b

Figure 3. Salinity tolerance of *T. vittata*. Results from **a** chronic and **b** acute salinity-tolerance trials.

Discussion

Trichopsis vittata has been known from Florida since the 1970s, when an established population was discovered within 10 km of its current range (Courtenay et al. 1984, 1986; Schofield and Pecora 2013). Its introduction source is unknown; however, at the time of discovery it was speculated that it had escaped from nearby aquarium fish farms (Courtenay et al. 1984, 1986). Over time, the species was thought to have been extirpated (Shafland 1996; Shafland et al. 2008) until its recent rediscovery (Schofield and Pecora 2013). No fish-monitoring programmes cover urban areas in this region of Florida, so it is unclear how long the fish was established before its recent collection at Loxahatchee NWR. Furthermore, it is unclear whether the species had died out and was subsequently re-introduced or whether this is a remnant population. Nevertheless, its ability to persist in this small range for many decades makes it an interesting candidate for study. The purpose of this investigation was to document ecophysiological attributes of the species and qualitatively compare them to sympatric species, to see if perhaps reduced ecophysiological tolerance might be related to the lack of geographic expansion.

In general, our hypothesis (low ecophysiological toughness ≈ small geographic range) was not supported. Ecophysiological traits of *T. vittata* and nine sympatric non-native fishes known from Florida freshwaters were tabulated (Table 2). Sympatric non-native fishes include ones that have been established since the 1950s (*Pterygoplichthys* spp., *Astronotus ocellatus* [Agassiz 1831], *Cichlasoma bimaculatum*), 1960s (*Clarias batrachus* [Linnaeus 1758], *Hemichromis letourneuxi*, *Oreochromis aureus* [Steindachner 1864]), 1970s (*Rocio octofasciata* [Regan 1903]), 1980s (*C. urophthalmus*) and 1990s (*H. littorale*; see Shafland et al. 2008, Schofield and Loftus 2014 for establishment timelines; Table 2, Fig. 1). Tolerance of these species to hypoxia and low-temperatures was graphically compared (Fig. 4). *Trichopsis vittata* was more tolerant of cold than many sympatric non-native fishes, leading us to believe it could tolerate habitats north of its current range; however, it has not expanded its range in any direction. Furthermore, its ability to breathe atmospheric air (via a labyrinth organ) imparts an ability to live indefinitely in water devoid of oxygen. It should be able to tolerate a variety of marginal habitats such as shallow pools, vegetation-choked swamps, and habitats with low light levels as it does at Loxahatchee NWR and in its native range (Rainboth 1996). As for salinity, we documented herein that *T. vittata* was tolerant to acute shifts in salinity to 16 psu and gradual shifts to 20 psu. This level of tolerance is lower than published values for most cichlids, but greater than many non-cichlid invasive fishes (Table 2). Nonetheless, it is a species that is probably tolerant enough to occupy freshwater tidal or low-salinity estuarine areas, or use them as salt bridges for dispersal. Yet it has not moved into coastal areas even though the current population is less than 20 km from the Atlantic coast. In summary, while *T. vittata* seems as tough or tougher than other sympatric non-native fishes (in terms of ecophysiology; Table 2, Fig. 4), it has not been able to capitalise on these advantages and expand its geographic range as the others have. It is possible that tolerances to environmental parameters are not directly correlated with geographic range for this group of species, but instead serve as

Table 2. Ecophysiological traits for *Trichopsis vittata* and several species of sympatric non-native fishes. For hypoxia tolerance, 'extremely tolerant' = can survive in water with no oxygen (<0.02 mg L^{-1}) for prolonged periods, as long as surface access is allowed.

Species	Hypoxia tolerance	Chronic salinity tolerance	Acute salinity tolerance	Cold tolerance (°C)	Max body size in FL (cm TL)
Hoplosternum littorale	air-breather	unknown for adults	7 psu (larvae Mol 1994)	5.7 (Schofield and Huge 2011) 10 (Gestring et al. 2009)	26.2 (Gestring et al. 2009)
Pterygoplichthys spp.	air-breather	unknown	10 psu (Capps et al. 2011)	9-11 for *P. multiradiatus*; 4-6 for *P. disjunctivus* (Gestring et al. 2010)	50.4 (Gestring et al. 2010)
Clarias batrachus	air-breather	unknown	Unknown	9.8 (Shafland and Pestrak 1982)	52.9 (Shafland 1996)
Trichopsis vittata	air-breather	20 psu (this study)	16 psu (this study)	7.2 (this study)	6.5 (Schofield and Pecora 2013)
Astronotus ocellatus	extremely tolerant (Almeida-Val et al. 2000)	14 psu (Gutierre et al. in press)	16 psu (Gutierre et al. in press)	12.9 (Shafland and Pestrak 1982)	34.8 (Shafland 1996)
Cichlasoma bimaculatum	unknown	unknown	unknown	8.6 (Schofield and Huge 2011) 8.9 (Shafland and Pestrak 1982)	18.6 (Shafland 1996)
Cichlasoma urophthalmus	extremely tolerant (Schofield et al. 2009)	> 37 psu (Stauffer and Boltz 1994)	unknown	8.7 (Schofield unpub. data) 14-15 (Stauffer and Boltz 1994)	28.8 (Idelberger et al. 2011)
Hemichromis letourneuxi	extremely tolerant (Schofield et al. 2007)	50 psu (Langston et al. 2010)	20 psu (Langston et al. 2010)	8.1 (Schofield unpub. data) 9.5 (Shafland and Pestrak 1982)	11.2 (Shafland 1996)
Oreochromis aureus	unknown	52 psu (Suresh and Lin 1992)	unknown	6.2 (Shafland and Pestrak 1982)	54.3 (Shafland 1996)
Rocio octofasciata	extremely tolerant (Obordo and Chapman 1997)	unknown	unknown	8.0 (Shafland and Pestrak 1982)	13.7 (Jennings 1986)

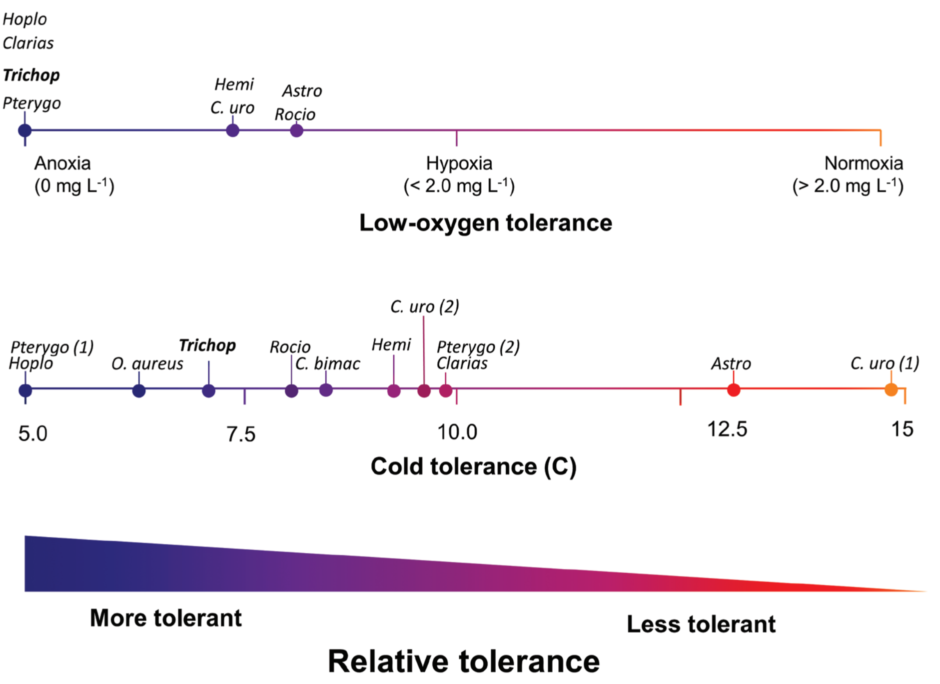

Figure 4. Graphic representation of relative ecophysiological 'toughness' for *T. vittata* and sympatric non-native fishes. References for cold and low-oxygen tolerance are given in Table 2. Two values are presented for cold tolerance of *C. urophthalmus* as two separate reports provided dissimilar data (Table 2). Two values are given for *Pterygoplichthys* spp. corresponding to two different species (Table 2). "*O. aureus*" = *Oreochromis aureus;* "*Pterygo*" = *Pterygoplichthys* spp.; "*Hoplo*" = *Hoplosternum littorale*; "*Clarias*" = *Clarias batrachus*; "*Trichop*" = *Trichopsis vittata*; "*Hemi*" = *Hemichromis letourneuxi*; "*C. uro*" = *Cichlasoma urophthalmus*; "*Astro*" = *Astronotus ocellatus*; "*Rocio*" = *Rocio octofasciata*; "*C. bimac*" = *Cichlasoma bimaculatum*.

a filter for establishment, wherein candidate species must possess the ability to survive abiotic extremes as a first step (Peterson et al. 2004). Once fish have passed through this step, invasiveness (at least in terms of geographic spread) may ultimately rely on a secondary set of criteria including biotic interactions and life-history variables.

The intriguing combination of high abiotic tolerance and low invasiveness in *T. vittata* may support the biotic-abiotic constraining hypothesis (Quist et al. 2003), wherein abiotic environmental variables structure population levels until overridden by biotic ones (e.g., predation, competition). For example, Quist et al. (2003) showed that variation in walleye *Stizostedion vitreum* (now *Sander vitreus* [Mitchill, 1818]) populations in Kansas reservoirs could be explained by environmental variables until a critical threshold for biotic interactions was reached. In that case, once the density of a predator (*Pomoxis annularis* Rafinesque, 1818) was exceeded, then biotic interactions overrode abiotic influences and *S. vitreum* population dynamics were related to *P. annularis* density. Similarly, Weber and Brown (2011) showed that variation in density of native fish populations were related to environmental variables until a threshold density of *Cyprinus carpio* Linnaeus, 1758 was reached and then biotic interactions overrode abiotic ones. As for *T. vittata*, future research on its co-occurrence with competitors and predators may shed light on the relative influences of abiotic versus biotic constraints.

There are many other factors that could explain the lack of geographic range expansion for *T. vittata.* Some of the most obvious candidates include body size, diet and their interaction. *Trichopsis vittata* is smaller than other sympatric non-native fishes and occupies a relatively low position on the predation spectrum (i.e., primarily consumes small invertebrates; Rainboth 1996). This combination of attributes separates *T. vittata* from the other non-native fishes that are either: 1) large-bodied species that consume benthic algae and detritus (e.g., *Pterygoplichthys* spp., *O. aureus*) or 2) large- to medium-sized fish predators (cichlids, *C. batrachus*). One species that does not fit this pattern is *H. letourneuxi*, which consumes both invertebrates and fish and does not reach a large body size (Table 2), yet is extremely invasive (Kline et al. 2013; Fig. 1). Protection from bony dermal plates may confer an additional advantage to catfishes (*H. littorale*, *Pterygoplichthys* spp.) and bolster their ability to spread geographically. Other factors that could affect invasiveness include biotic resistance (e.g., Thompson et al. 2012), specific requirements for egg/larval development or nesting, multiple introductions (Collins et al. 2002), predation susceptibility (e.g., Rehage et al. 2009) and propagule pressure (Colautti et al. 2006). Application of modern modelling techniques may allow researchers to identify which variables are most important for an invader's success (and spread) and the critical thresholds for those variables (e.g. Kolar and Lodge 2002).

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References

Almeida-Val VM, Val AL, Duncan WP, Souza FCA, Paula-Silva MN, Land S (2000) Scaling effects on hypoxia tolerance in the Amazon fish *Astronotus ocellatus* (Perciformes:

Cichlidae): contribution of tissue enzyme levels. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 125: 219–226. doi: 10.1016/S0305-0491(99)00172-8

Canonico GC, Arthington A, McCrary JK, Thieme ML (2005) The effects of introduced tilapias on native biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems 15: 463–483. doi: 10.1002/aqc.699

Capps KA, Nico LG, Mendoza-Carranza M, Arévlo-Frías W, Ropicki AJ, Heilpern SA, Rodiles-Hernández R (2011) Salinity tolerance of non-native suckermouth armoured catfish (Loricariidae: *Pterygoplichthys*) in south-eastern Mexico: implications for invasion and dispersal. Aquatic Conservation: Marine and Freshwater Ecosystems 21: 528–540. doi: 10.1002/aqc.1210

Colautti RI, Grigorovich IA, MacIsaac HJ (2006) Propagule pressure: a null model for biological invasions. Biological Invasions 8: 1023–1037. doi: 10.1007/s10530-005-3735-y

Collins TM, Trexler JC, Nico LG, Rawlings TA (2002) Genetic diversity in a morphologically conservative invasive taxon: multiple introductions of swamp eels to the southeastern United States. Conservation Biology 16: 1024–1035. doi: 10.1046/j.1523-1739.2002.01182.x

Courtenay WR Jr, Hensley DA, Taylor JN, McCann JA (1984) Distribution of exotic fishes in the continental United States. In: Courtenay WR Jr, Stauffer JR Jr (Eds) Distribution, Biology and Management of Exotic Fishes. Johns Hopkins University Press, Baltimore, MD, 41–77.

Courtenay WR Jr, Hensley DA, Taylor JN, McCann JA (1986) Distribution of exotic fishes in North America. In: Hocutt CH, Wiley EO (Ed.) The Zoogeography of North American Freshwater Fishes. John Wiley and Sons, New York, NY, 675–698.

Cox DR, Oakes D (1984) Analysis of survival data. Monographs on statistics and applied probability. Chapman and Hall, New York, NY.

García-Berthou E (2007) The characteristics of invasive fishes: what has been learned so far? Journal of Fish Biology 71 (Supplement D): 33–55. doi: 10.1111/j.1095-8649.2007.01668.x

Gestring KB, Shafland PL, Stanford MS, Eisenhauer RL (2009) Status and selected life history attributes of the illegally introduced Brown Hoplo (*Hoplosternum littorale*) in Florida. Florida Scientist 72: 37–47.

Gestring KB, Shafland PL, Stanford MS (2010) Status of the exotic Orinoco sailfin catfish (*Pterygoplichthys multiradiatus*) in Florida. Florida Scientist 73: 122–137.

Gutierre SMM, Schofield PJ, Prodocimo V (in press) Salinity and temperature tolerance of an emergent alien species, the Amazon fish *Astronotus ocellatus*. Hydrobiologia.

Idelberger CF, Stafford CJ, Erickson SE (2011) Distribution and abundance of introduced fishes in Florida's Charlotte Harbor estuary. Gulf and Caribbean Research 23: 13–22.

Jennings DP (1986) Characterization of a localized Jack Dempsey, *Cichlasoma octofasciatum*, population in Alachua County, Florida. Florida Scientist 49: 255–259.

Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 53: 457–481. doi: 10.1080/01621459.1958.10501452

Kline JL, Loftus WF, Kotun K, Trexler JC, Rehage JS, Lorenz JJ, Robinson M (2013) Recent fish introductions into Everglades National Park: an unforeseen consequence of water management? Wetlands 34: 175–187. doi: 10.1007/s13157-012-0362-0

Kolar CS, Lodge DM (2002) Ecological predictions and risk assessment for alien fishes in North America. Science 298: 1233–1236. doi: 10.1126/science.1075753

Langston JN, Schofield PJ, Hill JE, Loftus WF (2010) Salinity tolerance of the African jewelfish *Hemichromis letourneuxi*, a non-native cichlid in south Florida (USA). Copeia, 475–480. doi: 10.1643/CP-09-069

Mol JH (1994) Effects of salinity on distribution, growth and survival of three Neotropical armoured catfishes (Siluriformes—Callichthyidae). Journal of Fish Biology 45: 763–776.

Obordo CO, Chapman LJ (1997) Respiratory strategies of a non-native Florida cichlid, *Cichlasoma octofasciatum*. Florida Scientist 60: 40–52.

Pelicice FM, Agostinho AA (2009) Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir. Biological Invasions 11: 1789–1801. doi: 10.1007/s10530-008-9358-3

Peterson MS, Comyns BH, Rakocinski CF (2004) Defining the fundamental physiological niche of young estuarine fishes and its relationship to understanding distribution, vital metrics, and optimal nursery conditions. Environmental Biology of Fishes 71: 143–149. doi: 10.1007/s10641-004-0302-1

Quist MC, Guy CS, Stephen JL (2003) Recruitment dynamics of walleyes (*Stizostedion vitreum*) in Kansas reservoirs: generalities with natural systems and effects of a centrarchid predator. Canadian Journal of Fisheries and Aquatic Sciences 60: 830–839. doi: 10.1139/f03-067

Rainboth WJ (1996) FAO Species Identification Field Guide for Fishery Purposes: Fishes of the Cambodian Mekong. Food and Agriculture Organisation of the United Nations, Rome.

Rehage JS, Dunlop KL, Loftus WL (2009) Antipredator responses by native mosquitofish to non-native cichlids: an examination of the role of preynaiveté. Ethology 115: 1046–105. doi: 10.1111/j.1439-0310.2009.01694.x

Rejmánek M, Richardson DM, Barbour MG, Crawley MJ, Hrusa GF, Moyle PB, Randall JM, Simberloff D, Williamson M (2002) Biological invasions: politics and the discontinuity of ecological terminology. Bulletin of the Ecological Society of America 83: 131–133.

Savage IR (1956) Contributions to the theory of rank order statistics – the two-sample case. Annals of Mathematical Statistics 27: 590–615. doi: 10.1214/aoms/1177728170

Schofield PJ, Huge DH (2011) Low-temperature tolerance of two non-native fishes (*Hoplosternum littorale* [Hancock 1828], *Cichlasoma bimaculatum* [Linnaeus 1758]) established in Florida. Florida Scientist 74: 73–83.

Schofield PJ, Loftus WF (2014) Non-native fishes in Florida freshwaters: A literature review and synthesis. Reviews in Fish Biology and Fisheries. doi: 10.1007/s11160-014-9373-7

Schofield PJ, Pecora DH (2013) Croaking gourami, *Trichopsis vittata* (Cuvier, 1831), in Florida, USA. BioInvasions Records 2: 247–251. doi: 10.3391/bir.2013.2.3.12

Schofield PJ, Loftus WF, Brown ME (2007) Hypoxia tolerance of two centrarchid sunfishes and an introduced cichlid in karstic Everglades wetlands of southern Florida, U.S.A. Journal of Fish Biology 71 (Sup D): 87–99. doi: 10.1111/j.1095-8649.2007.01686.x

Schofield PJ, Loftus WJ, Fontaine JA (2009) Salinity effects on behavioural response to hypoxia in the non-native Mayan cichlid (*Cichlasoma urophthalmus*) from Florida Everglades wetlands. Journal of Fish Biology 74: 1245–1258. doi: 10.1111/j.1095-8649.2009.02192.x

Schofield PJ, Loftus WF, Kobza RM, Cook MI, Slone DH (2010) Tolerance of nonindigenous cichlid fishes (*Cichlasoma urophthalmus*, *Hemichromis letourneuxi*) to low temperature: laboratory and field experiments in south Florida. Biological Invasions 12: 2441–2457. doi: 10.1007/s10530-009-9654-6

Shafland PL (1996) Exotic fishes of Florida – 1994. Reviews in Fisheries Science 4: 101–122. doi: 10.1080/10641269609388581

Shafland PL, Pestrak JM (1982) Lower lethal temperatures for fourteen non-native fishes in Florida. Environmental Biology of Fishes 7: 149–156. doi: 10.1007/BF00001785

Shafland PL, Gestring KB, Stanford MS (2008) Florida's exotic freshwater fishes – 2007. Florida Scientist 71: 220–245.

Stauffer JR Jr, Boltz SE (1994) Effect of salinity on the temperature preference and tolerance of age-0 Mayan cichlids. Transactions of the American Fisheries Society 123: 101–107. doi: 10.1577/1548-8659(1994)123<0101:EOSOTT>2.3.CO;2

Suresh AV, Lin CK (1992) Tilapia culture in saline waters: a review. Aquaculture 106: 201–226. doi: 10.1016/0044-8486(92)90253-H

Thompson KA, Hill JE, Nico LG (2012) Eastern mosquitofish resists invasion by nonindigenous poeciliids through agonistic behaviors. Biological Invasions 14: 1515–1529. doi: 10.1007/s10530-012-0176-2

Vitule JRS, Freire CA, Simberloff D (2009) Introduction of non-native freshwater fish can certainly be bad. Fish and Fisheries 10: 98–108. doi: 10.1111/j.1467-2979.2008.00312.x

Weber MJ, Brown ML (2011) Relationships among invasive common carp, native fishes and physicochemical characteristics in upper Midwest (USA) lakes. Ecology of Freshwater Fish 20: 270–278. doi: 10.1111/j.1600-0633.2011.00493.x

USGS-NAS (2014) US Geological Survey: Nonindigenous Aquatic Species database. http://nas.er.usgs.gov

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RESEARCH ARTICLE

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Distribution and abundance of exotic earthworms within a boreal forest system in southcentral Alaska

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Abstract

Little is known about exotic earthworms (Oligochaeta: Lumbricidae) in Alaska outside its southeastern panhandle. This study documents the distribution of exotic earthworms in the relatively undisturbed Kenai National Wildlife Refuge (KNWR), a large, primarily wilderness refuge in southcentral Alaska. We sampled 69 sites near boat launches, along road corridors, and in low human impact areas > 5 km from the road, finding three species of earthworms (*Dendrobaena octaedra*, *Dendrodrilus rubidus*, and *Lumbricus terrestris*). Most road sites (90%) and boat launches (80%) contained earthworms; half (50%) of low human impact sites contained earthworms. Distance to roads was the only significant factor in predicting earthworm occurrence; soil pH, soil moisture, leaf litter depth, and vegetation cover were not. The disparate distributions of these three species suggest that within the KNWR road construction and vehicle traffic played a role in dispersal of the widespread, abundant *Dendrobaena octaedra* and uncommon *Dendrodrilus rubidus*; bait abandonment appeared to be the primary method of introduction of *Lumbricus terrestris*. While the distribution of harmful anecic earthworms in KNWR is currently limited, the prohibition of *Lumbricus* spp. as bait within conservation units in Alaska may be warranted.

Keywords

Lumbricidae, earthworm invasion, taiga, bait abandonment, non-native species

Introduction

Pleistocene glaciations extirpated native earthworms from much of North America, leaving landscapes devoid of earthworms until the introduction of exotic earthworms (Oligochaeta; Lumbricidae) during European settlement (Hale et al. 2005, 2006, Frelich et al. 2006, Holdsworth et al. 2007a, 2007b). The effects of exotic earthworms on forest ecosystems are well documented (Hale et al. 2005, 2006, Frelich et al. 2006, Holdsworth et al. 2007a, 2007b) and vary by feeding strategy. Leaf litter-dwelling, small-sized epigeic species are least destructive, consuming and mixing the top organic layers into textured, homogeneous litter. Endogeic species burrow through the top soil horizon; their physical effects on ecosystem ecology are greater than epigeic worms but less than anecic worms. Anecic earthworms penetrate deep into the soil, transporting surface litter into the mineral layer (Addison 2008) and increasing soil porosity and water infiltration (Anderson 1988). Removal of leaf litter and deposition of casts on the soil surface by anecic earthworms can also increase soil erosion and nutrient run-off (Edwards and Bohlen 1996).

Material transport by anecic worms, their large adult size, and dense populations have led to substantial ecosystem changes in some parts of North America (Frelich et al. 2006). Earthworms can accelerate litter decomposition (Hale et al. 2006, Suárez et al. 2006, Holdsworth et al. 2007a, 2007b, Addison 2008) and reduce plant species richness (Hale et al. 2006, Holdsworth et al. 2007a, 2007b). Suárez et al. (2006) found that litter remaining in earthworm-invaded areas in New York was 30-60% less than in reference plots. Holdsworth et al. (2007a) found in a Wisconsin forest that exotic earthworms reduced plant species richness in heavily invaded plots by 17%. Similarly, Hale et al. (2006) documented a negative relationship between exotic earthworm diversity and plant diversity in a Minnesota hardwood forest.

Most studies of exotic earthworms have occurred in temperate regions (Hale et al. 2006, Suárez et al. 2006, Holdsworth et al. 2007a, 2007b, Addison 2008); less is known about the distribution and effects of earthworms in subarctic boreal forests (Cameron et al. 2007, Cameron and Bayne 2009, Sanderson et al. 2012). In northern Alberta, Cameron et al. (2007) found boat launches and roads had the highest probability of earthworm occurrence. Their results suggested vehicle transport and bait abandonment as primary mechanisms of earthworm introduction.

As for most invasive species, human activities, particularly road construction and unintentional transport, likely increase the rate of spread for exotic earthworms above their natural dispersal rate of 5-10 meters a year (Gundale et al. 2005, Addison 2008). Consequently, exotic earthworms more likely occur near roads due to availability of habitats disturbed by road construction and maintenance that allow for potential establishment, as well as the creation of dispersal corridors (Cameron et al. 2009). Vehicles themselves function as dispersal vectors for earthworm cocoons, which are sticky, mucus coated sacks containing developing embryos (Gundale et al. 2005). Several species such as *Lumbricus terrestris* (anecic) and *Lumbricus rubellus* (epi-endogeic) are sold commercially as fishing bait and are possibly introduced into ecosystems when anglers discard unused bait (Cameron et al. 2007).

Seventeen species of earthworms are known to occur in Alaska (see records in Gates 1972, 1974, Reynolds et al. 1974, Reynolds and Wetzel 2008, Reynolds 1977, 1980, Berman and Marusik 1994, Costello et al. 2011, Rinella et al. 2014, and Suppl. material 1: Alaska earthworm records). Of these, 14 are exotic worms introduced from the Palearctic and have become established. *Eisenia fetida* (Savigny, 1826), a Palearctic species, is commonly used for indoor vermicomposting in Alaska, but due to its low cold tolerance (Greiner et al. 2011, Meshcheryakova and Berman 2014), it is unlikely to become established in Alaska. Two species of earthworms found in southeast Alaska (*Arctiostrotus* sp. and *Sparganophilus* sp.) may be native to Alaska or may have been transported from elsewhere in North America.

Factors such as pH and temperature likely limit earthworm distribution, especially in boreal regions like Alaska (Chan and Mead 2003, Addison 2008). Earthworms are usually associated with soil pH of 5-7.4, although *D. octaedra* inhabits soil pH as low as 2.8-3.6, and *L. rubellus* has been found in areas with pH ≥ 3.0 (Addison 2008). Survival of earthworms in low temperature areas depends on the species and stage of development (Greiner et al. 2011, Meshcheryakova et al. 2014). Meshcheryakova and Berman (2014), by comparing cold hardiness and present distributions of earthworm species in Siberia, concluded that varying cold tolerance of the species considered contributed toward their present distribution ranges.

A rapidly warming climate in Alaska is likely improving environmental conditions for earthworms. Wetlands in Alaska are warming and drying (Klein et al. 2005, Riordan et al. 2006, Berg et al. 2009) and average winter temperatures have warmed 3.5 °C in the last 50 years (Karl et al. 2009). Drying wetlands and warmer winters may provide increasingly suitable habitat for exotic earthworms. Addison (2008) suggested that even small increases in winter temperatures will lead to large increases in earthworm habitat.

The present study documents species composition, distribution, and habitat correlates for earthworms in the Kenai National Wildlife Refuge, a conservation area in southcentral Alaska. A secondary goal is to examine relationships between earthworm occurrence and distance from human-disturbed areas, such as roads and popular fishing areas. The final goal is to identify factors potentially limiting earthworm distribution, such as pH and soil moisture, which are likely to change as the climate continues to warm on the Kenai Peninsula.

Methods

Study area

Located on the Kenai Peninsula in southcentral Alaska, USA (60°N, 150°W), the Kenai National Wildlife Refuge (KNWR) covers 777,000 ha. Mountains and glaciers characterize the southeastern KNWR (Figure 1). The Kenai Lowlands, mantled by glacial deposits that vary in texture and are capped by silt loam derived from post-

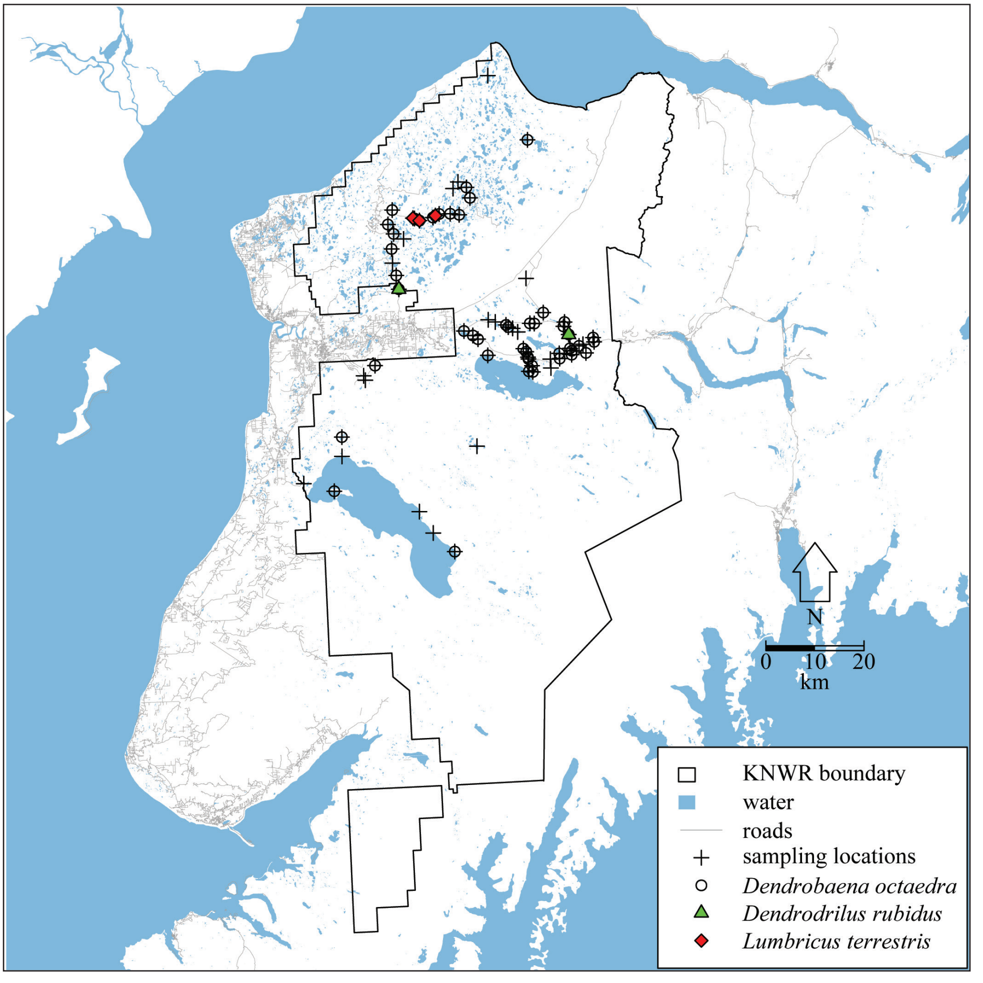

Figure 1. Map of sampling locations and earthworm occurrences by species.

glacial windblown loess, cover the western portion of KNWR. The Lowlands consist of wetlands and mixed boreal forest (Klein et al. 2005) dominated by black spruce (*Picea mariana*), white spruce (*Picea glauca*), birch (*Betula papyrifera*), and quaking aspen (*Populus tremuloides*). The climate is boreal with a maritime influence. Temperatures are rarely greater than 26 °C in summer or less than -18 °C in winter. The frost-free growing season varies from 71-129 days depending on location, with about 480 mm of total precipitation per year (U.S. Fish & Wildlife Service 2010).

While most of the KNWR is currently managed as congressionally designated Wilderness, over 130 historic cabins have been inventoried on the Refuge along with other historic resources associated with mining, trapping, oil development, and road construction, mostly in the north of the refuge. Commercial mining and fishing in the area occurred in the late 1800s and early 1900s. Oil exploration began in the northern part of KNWR in the Swanson River area in the mid-1950s, resulting in 2,900 km of bulldozed seismic lines. There are also 240 km of utility and transmission lines and 180 km of established trails within the Refuge (U.S. Fish & Wildlife Service 2010). Fire and associated suppression activities have also been prevalent within the refuge in the past. Major fires of unknown origin occurred in 1871, 1883, 1891, and 1910. Two large, human-caused fires (1947: 125,000 ha and 1969: 35,000 ha) resulted in replacement of mature spruce forests by a mosaic of young mixed conifer-deciduous forest in various stages of succession. Many historic disturbances provided opportunities for earthworm introduction and establishment on the KNWR.

Experimental design

Earthworms were sampled throughout the KNWR during July and August 2011 at 69 total sites representing three levels of human impact. These levels of human impact were characterized by explicit vectors of introduction: (1) boat launches (n = 20), (2) road corridors (n = 20); and (3) low impact areas (> 600 m from any road or facility and 50 m from any trail or river; n = 29). The sampling site locations were chosen within a GIS (ArcGIS v.10.1) by first identifying suitable areas for each impact level and then randomly selecting sample sites. Three 0.09 m^2 plots were established at each site to sample earthworm occurrence. At road and boat launch sites, plots were placed two, three, and five meters from the road or edge of a boat launch. The plots were located approximately 10 m apart when possible, establishing a wider area for detecting earthworm occurrence. This protocol was modified at four of the low impact sites accessed by float plane, where only a single 0.09 m^2 quadrat was sampled at each site.

Plot level sampling

We sampled earthworm abundance at each plot using a 30 cm × 30 cm quadrat. Within each quadrat we removed and hand-sorted surface organic material for earthworms. We extracted additional earthworms with a liquid mustard solution of 40 g ground mustard powder in 3.8 L water (Lawrence and Bowers 2002). Earthworms were collected and stored in 70% ethanol. Specimens were deposited in the entomology collection of the Kenai National Wildlife Refuge (coden: KNWR) and specimen data were made available via Arctos (http://arctos.database.museum/).

We identified earthworm specimens to species level when possible based on visual observations of external morphology (Reynolds 1977). Juveniles were grouped into two categories: (1) *Lumbricus* spp. and (2) other immature. In addition, six specimens collected at geographically remote sites were identified to species level using the mitochondrial COI barcoding gene to confirm taxonomic identification using the Basic Local Alignment Search Tool (BLAST) and the BOLD identification engine (http://www.boldsystems.org). We submitted sequence data to BOLD where they are publicly available.

We measured leaf litter depth by clearing a small area and measuring the vertical depth of the leaf layer with a 30 cm ruler within each plot. Soil pH, (Soil pH Meter, HANNA, RI), and moisture (Digital Moisture Meter, General, NY) were measured in the field. In each plot, we estimated the percentage cover of litter, grass, forbs, moss, and lichen. General forest type (deciduous, mixed, conifer) of the area was determined from field observations and a GIS land cover layer.

We estimated ash-free dry biomass (g) from the length (mm) of each preserved specimen with the allometric equation of Hale (Hale et al. 2004):

$$g = e^{2.2853\ln(mm)-11.9047}$$

Hale (2004) found that the allometric equations for *Octolasion tyrtaeum*, *Lumbricus* spp., and *Dendrobaena octaedra* were not significantly different from one another, allowing one equation for all species.

Lumbricus spp. distribution sampling

Anecic *Lumbricus* species are potentially more damaging than other genera (Eisenhauer et al. 2007). Consequently, at sites where *Lumbricus* spp. were found, we used three transects to estimate the extent of local distribution. One transect was perpendicular to the initial site and the other two at approximately 45 degree angles from the site. At 10 m intervals along each transect we sampled three plots for earthworm presence using liquid mustard extraction within a 0.25 m^2 quadrat. To delineate the boundaries of this infestation, we continued sampling until we failed to find earthworms in all three plots at two consecutive 10 m intervals. Earthworms were collected and stored in 70% ethanol and later identified in the lab.

Statistical analysis

Individual plots served as replicates for each site, but we used site level data for most analyses by averaging plot level data. A site was considered to contain earthworms if individuals were detected in at least one plot. Soil moisture and leaf litter depth were square-root transformed for normality. We calculated remoteness for each site as a measure of distance from the nearest road. This distance, Y (m), was calculated in GIS by using true surface distance, as it accounted for elevation changes and also masked out lakes. To approximate a normal distribution for analyses, we transformed this distance using $\ln(Y + 1)$.

All analyses were performed using R, version 3.1.2 (R Core Team 2014). We assessed independence of earthworm presence and human impact level (road, boat launch, low impact) and vegetation type (conifer, deciduous) using chi-square tests of independence. Correlations of independent variables were examined with the corr.test function of the psych package, version 1.5.1 (Revelle 2015), accepting default arguments.

Prior to occupancy modeling, principal components analysis (PCA) was used to reduce the dimensionality of the eight habitat variables soil pH, soil moisture, leaf litter depth, and moss, grass, lichen, litter, and forb cover percentages. PCA was performed using the PCA function from the FactoMineR package (Husson et al. 2015), version 1.29. We used the estim_ncp function, also from the FactoMineR package, to determine the optimal number of dimensions to use in the PCA.

Results of the PCA were included in occupancy models (MacKenzie et al. 2003, 2006) using the occu function of the unmarked package, version 0.10-4 (Fiske and Chandler 2011). Detection probability was assumed to be constant. Site occupancy was modeled using all 2^5=32 permutations of first-order terms for impact level (factor with three levels), forest type (factor with two levels), distance to roads, and values from the first two PCA components. Finally, we obtained parameter importance and AICc model-averaged estimates of coefficients of the independent variables from the full set of candidate models using the importance and modavg functions from the AICcmodavg package, vesion 2.0–3 (Mazerolle 2015).

We used MANOVA to determine the effects of the impact level and earthworm occurrence on the three dependent variables of soil pH, soil moisture, and leaf litter depth.

Results

Distribution and abundance

We found three exotic earthworm species, *Dendrobaena octaedra*, *Dendrodrilus rubidus*, and *Lumbricus terrestris*, on KNWR. We failed to detect *Lumbricus rubellus*, known from one location on KNWR (http://arctos.database.museum/guid/KNWR:Ento:6755), even though one of our sampling sites was only ~ 48 meters from this known occurrence. Specimen records are included in Suppl. material 2: Specimen records.

The six individuals that were genetically identified using the COI gene showed > 96% probability of identity to their respective species based on a BLAST results. BOLD process ID's for sequenced specimens are also included in Suppl. material 2: Specimen records.

No site had more than two species confirmed as present. Only four sites (three boat launch sites one road site) contained two species of earthworms, while the majority of sites contained only one species. *Dendrobaena octaedra* was the most widespread, occurring at 48 (70%) of 69 sites. Most immature earthworms appeared to be *D. octaedra* based on morphology. *Dendrodrilus rubidus* occurred at two sites geographically distant from one another. *Lumbricus terrestris* occurred at three sites, all of which were boat launches located adjacent to one another in the northern part of the Refuge (Figure 1). Along transects surveyed at these sites, we found that *L. terrestris* had dispersed only 30 to 110 m from the boat launches.

Overall, earthworms occurred at 49 (71%) of the 69 sampled sites. Nearly all road sites (18 of 20 total sites, 90%) had earthworms in at least one plot, as did most boat launches (17 of 20 total sites, 85%). In contrast, only half (14 of 29 total sites, 48%) of the low-impact sites contained earthworms.

Earthworms occurred more frequently at roads and boat launch sites than expected, but much less frequently at low impact sites than expected (Table 1, chi-square test of independence, $\chi^2 = 11.18$, df = 2, p = 0.004, $n = 69$ observations). Earthworms were found more frequently than expected at sites dominated by deciduous trees and shrubs and less frequently than expected at conifer-dominated sites ($\chi^2 = 13.3$, df = 1, p = 0.0003, $n = 65$, Table 2).

Where earthworms were present, the mean density of earthworms was (28.1 ± 4.4 individuals/m^2), with mean densities ranging from 23.9 ± 4.5 at road sites to 33.1 ± 6.1 at boat launches (Table 3). At the three sites where transects were surveyed for *L. terrestris*, the mean density of this species was 37.4 ± 7.0 individuals/m^2. Log-transformed total earthworm densities (excluding absences) did not differ significantly among impact levels (one-way ANOVA: F = 1.57, $p = 0.219$).

Table 1. Observed and expected values for earthworm occurrence in boat launch, road, and low impact sites from a chi-square test of independence.

	Boat launch	Road	Low impact
Earthworms present	16 (13.9)	18 (13.9)	14 (20.2)
Earthworms absent	4 (6.1)	2 (6.1)	15 (8.8)

Table 2. Observed and expected values for earthworm occurrence at sites in conifer forests and deciduous trees/shrubs from a chi-square test of independence.

	Conifer	Deciduous
Earthworms present	7 (13.3)	41 (34.7)
Earthworms absent	11(4.7)	6 (12.3)

Table 3. Mean densities (individuals extracted/m^2 ± SE) by species and human impact level.

Species	Boat launch	Road	Low impact	Total density
Dendrobaena octaedra	26.4 ± 6.4	23.9 ± 4.5	26.9 ± 11.9	25.6 ± 4.4
Dendrodrilus rubidus	3.7 ± 3.7	7.4 ± 7.4	-	5.6 ± 1.9
Lumbricus terrestris	7.4 ± 7.4	-	-	7.4 ± 7.4
Unidentified immatures	43.2 ± 3.2	-	-	43.2 ± 3.2
Total density	33.1 ± 6.1	24.3 ± 4.7	26.9 ± 11.9	28.1 ± 4.4

Table 4. Mean biomass (mg/m^2 ± SE) of earthworms by species and human impact level.

Species	Boat launch	Road	Low impact	Total
Dendrobaena octaedra	105 ± 30	114 ± 19	193 ± 61.4	135 ± 23
Dendrodrilus rubidus	32.4	52.8	-	42.6 ± 10.2
Lumbricus terrestris	5651	-	-	5651
Unidentified immatures	1891	-	-	1891
All species	652 ± 353	105 ± 36	193 ± 61.4	361 ± 144

Where earthworms were found, ash-free dry biomass (AFD) of earthworms showed moderate variation (0.36 ± 0.14 AFD g/m^2, n = 49; Table 3) and was greatest at boat launches due to the presence of *Lumbricus* (0.65 ± 0.35 g/m^2, n = 16), lowest at roads (0.11 ± 0.04 g/m^2, n = 18), and moderate at low impact sites (0.19 ± 0.06 g/m^2, n = 14). At the three sites where transects were surveyed for *L. terrestris*, the mean AFD of this species was 4.2 ± 1.8 g/m^2. Log-transformed total earthworm biomass did not differ significantly among impact levels (one-way ANOVA: F = 0.818, p = 0.448). In summary, we found the highest density and biomass of earthworms at boat launches, and the least of both abundance metrics along roads.

PCA and occupancy modeling

There were many significant correlations among the habitat variables (Table 5). Depending on the method used for determining the best number of principal components to include, the optimal number was estimated to be two or three. We chose to include two components because of the relatively small sample size of our dataset (n = 65). The first two principal components that emerged from the PCA together accounted for 63% of the total variability in the original data. The first component accounted for 35% of the variability with positive loadings from soil pH, soil moisture, and grass cover and negative loadings for moss, lichen and forb cover. The second component accounted for 28% of the variability in the original variables with positive loadings from soil moisture and grass cover and negative loadings from leaf litter cover and leaf litter depth (Table 6).

The model-averaged overall estimates of occupancy and detection probability of earthworms were, respectively, 0.83 ± 0.07 and 0.68 ± 0.04. Among the impact levels, the occupancy estimate was highest at road sites (0.90 ± 0.09) and lowest at remote sites (0.73 ± 0.16), although confidence intervals of occupancy at all three human impact levels overlapped considerably (Table 7). Earthworms were more likely to occupy hardwood-dominated sites (0.91 ± 0.06) than conifer-dominated sites (0.47 ± 0.16).

The top-ranked occupancy model had a weight of 0.31 and included terms for forest type and distance to roads (Table 8). The second-ranked model, with a weight of 0.16, included terms for forest type and the impact levels boat launch and road distance. The evidence ratio between these two models suggested the highest-ranked model was 1.9 times more likely to be the most parsimonious model than the second-ranked model, but a Δ AICc < 2 indicated that the two models were nearly equivalent (Symonds and Moussalli 2011). In fact, the combination of the road and boat launch terms, both reflecting categories of sites very close to roads, conveyed much of the same information as the distance term.

Forest type was included in all highly-ranked models (importance value of 0.98, Table 9, Table 9). Its value as parameterized (conifer as intercept, hardwood as dummy variable) was consistently positive (95% CI: 0.76, 4.14), meaning that earthworms were more likely to occur under hardwoods than under conifers. Distance to roads

Table 5. Correlation matrix for variables used in principal component analysis. *Correlation is significant at the 0.05 level (2-tailed).**Correlation is significant at the 0.01 level (2-tailed).

	litter cover	**moss cover**	**grass cover**	**forbs cover**	**lichen cover**	**soil pH**	**soil moisture**
moss cover	**-0.39****						
grass cover	**-0.54****	**-0.45****					
forbs cover	-0.12	**0.24***	**-0.28***				
lichen cover	-0.22	**0.51****	**-0.28***	0.01			
soil pH	0.15	**-0.62****	**0.51****	**-0.33****	**-0.47****		
soil moisture	**-0.38****	-0.17	**0.53****	-0.17	**-0.30***	**0.26***	
litter depth	**0.53****	**-0.43****	**-0.24***	-0.18	-0.19	0.00	**-0.26***

Table 6. Factor analysis loadings for components: (n = 65).

Variable	dim1	dim2
litter cover	0.075	-0.905
moss cover	-0.816	0.380
grass cover	0.719	0.566
forbs cover	-0.445	0.082
lichen cover	-0.672	0.185
soil pH	0.823	-0.042
soil moisture	0.517	0.564
litter depth	0.161	-0.780

Table 7. Model-averaged estimates of occupancy (Ψ) and detection probability (p). Uncond. SE: unconditional stand error.

Parameter	Estimate	Uncond. SE	95% CI
Ψ	0.83	0.07	0.63, 0.93
$\Psi_{\text{boat launch}}$	0.84	0.08	0.59, 0.95
Ψ_{road}	0.90	0.09	0.42, 0.99
Ψ_{remote}	0.73	0.16	0.38, 0.94
Ψ_{conifer}	0.47	0.16	0.19, 0.76
Ψ_{hardwood}	0.91	0.06	0.72, 0.97
p	0.68	0.04	0.59, 0.76

was the only other parameter with an importance value greater than 0.5. Even though its model-averaged 95% confidence interval included zero, the parameter estimate for distance to roads was negative in all models in which it was included, indicating that the likelihood of earthworm occurrence decreased with increasing distance from roads.

Table 8. Top five occupancy models for earthworm occurrence based on the AICc. K: the number of estimated parameters.

Model (occupancy)	Log-likelihood	K	AICc	Δ AICc	Akaike weight
hardwood + distance	-111.86	4	232.39	0	0.31
hardwood + boat launch + road	-111.38	5	233.78	1.39	0.16
hardwood + distance + Dim2	-111.7	5	234.42	2.03	0.11
hardwood + distance + Dim1	-111.71	5	234.44	2.05	0.11
hardwood + distance + boat launch + road	-111.22	6	235.88	3.5	0.05

Table 9. Model-averaged parameters on logit scale from models of earthworm occurrence. Estimate: model-averaged parameter estimates. SE: Unconditional standard errors.

Parameter	Importance	Estimate	SE	95% CI
hardwood	0.98	2.45	0.86	0.76, 4.14
distance	0.69	-0.30	0.16	-0.62, 0.02
boat launch	0.37	1.54	1.10	-0.61, 3.69
road	0.37	2.86	1.78	-0.62, 6.34
Dim1	0.26	0.08	0.24	-0.39, 0.55
Dim2	0.26	0.16	0.33	-0.48, 0.8

Effects of earthworm presence on soil properties

The presence of earthworms did not affect leaf litter depth or other measured soil properties. In a two-way MANOVA of the three factors *impact level*, *forest type*, and *earthworm occurrence* on the three dependent variables of *soil pH*, *soil moisture*, and *leaf litter depth*, the combined dependent variables differed among impact levels (Pilliai's trace = 0.804, F= 11.7, $p < 0.001$) and between forest types (Pilliai's trace = 0.243, F = 5.47, $p = 0.002$), but the combined variables did not differ between sites where earthworms were present or absent (Pilliai's trace = 0.037, F = 0.648, $p = 0.588$). Follow-up univariate ANOVA tests and Bayesian model averaging confirmed that neither *soil pH*, *soil moisture*, nor *leaf litter depth* were affected by the presence or biomass of earthworms.

Discussion

Exotic earthworms were found to inhabit 90% of road corridors and 85% of boat launch sites, but only 48% of low impact sites. These results suggest that human traffic influences earthworm presence in the KNWR. Similarly, Cameron and Bayne (2009) found a higher probability of earthworm occurrence at boat launches and roads compared to forest interiors and remote shorelines in Alberta, Canada. Gundale et al. (Gundale et al. 2005) found exotic earthworms at all non-wilderness sites (fishing, timber harvest, road) in Michigan, but at only 50% of wilderness sites with no history of logging.

The road system in the KNWR, while poorly developed compared to conservation units in the contiguous U.S., is fairly extensive compared to other Federal conservation units in Alaska, constituting ~1% of the refuge (100 m buffer either side of all refuge roads gives 6,420 ha). The paved 35 km Sterling Highway and graveled 31 km Skilak Lake Road together bisect the KNWR. These two unpaved roads provide connectivity to many of the 2,900 km of seismic lines (U.S. Fish & Wildlife Service 2010) and three active oil fields that have been laid down on the landscape over the past six decades. Road age has been linked to earthworm presence in northern Alberta, where Cameron and Bayne (2009) found that older road corridors (average age = 46 years) were significantly more likely to have earthworms than younger ones. The few roads on the KNWR were built in the 1950s, suggesting that they likely contributed to the dispersal of exotic earthworms, although sites on Tustumena Lake in the southern KNWR suggest that roads and survey lines are not necessary for earthworm invasion.

We found *Dendrobaena octaedra* to be the most widespread (adults at 70% of study sites) and abundant species (25.6 ± 4.4 ind./m^2) of earthworm on KNWR. This species is most likely introduced and spread by vehicles because its small size and epigeic habits (i.e., inhabit near-surface of the leaf litter) likely increase its chances of dispersal by human activities. *Dendrobaena octaedra* is a prominent invader throughout North America, often both the most widespread and densest exotic earthworm (Cameron et al. 2007).

Dendrodrilus rubidus was found at only two locations geographically distant from each other, suggesting independent introduction events and perhaps multiple vectors. In an unrelated sampling effort, we have also collected *Dd. rubidus* in the subalpine zone on the southern portion of the refuge at a site accessible only by floatplane or by foot (http://arctos.database.museum/guid/KNWR:Ento:7100), again suggesting another independent introduction.

Dendrodrilus rubidus is a fairly common earthworm that appears to be present more in northern hardwood and coniferous forests throughout Alaska and Canada (Cameron et al. 2007, Addison 2008, Costello et al. 2011) than in hardwood forests in the Midwest and other areas of the contiguous United States (Hale et al. 2005, Suárez et al. 2006, Holdsworth et al. 2007b). Like *D. octaedra*, it is tolerant of both acidic conditions and frost and, as an epigeic species, likely impacts the forest floor ecosystems less than anecic species (Addison 2008).

In contrast, *L. terrestris* is an anecic species that lives deep in the soil (Hale et al. 2005, Suárez et al. 2006, Addison 2008) and is commonly sold as fishing bait. We found *L. terrestris* at three boat launches within 5 km of one another, all at lakes popular for sport fishing (Figure 1). This peculiar distribution and the fact that *L. terrestris* is sold for bait locally (e.g, http://arctos.database.museum/guid/KNWR:Ento:6753) suggests direct bait abandonment as the main method of introduction on the KNWR. Additional species may be expected to arrive at boat launches because fishing bait can contain other species as well (Tiunov et al. 2006).

Though we did not detect *L. rubellus* in our sampling effort, it is presently known to occur on KNWR at only one site, a boat launch on a popular fishing lake. As with *L. terrestris*, it was most likely introduced by bait abandonment.

In this study, the most important factors determining earthworm occurrence appeared to be forest type (conifer versus hardwoods) followed by distance from roads. We found that earthworms were more likely to be found at sites dominated by deciduous trees and shrubs than at sites dominated by conifers. On KNWR, conifer dominated sites tend to have acidic soils covered by a thick moss carpet, conditions unfavorable to most earthworm species. In contrast, hardwood sites tend to have less acidic soils covered by deciduous leaf litter, providing more ideal conditions for most earthworm species.

Our finding that half (48%) of the low impact sites (> 600 m from any road or facility, and 50 m from any trail or river) contained earthworms was relatively higher than Cameron and Bayne (2009), who noted 8-35% of their remote transects (300-500 m in the forest interior) contained earthworms, but similar to Gundale et al. (2005) who found 50% of wilderness areas without earthworms. The difference between occurrence patterns in the above studies and KNWR, with its remote sites far from roads supporting earthworms, is perhaps due to boat and float plane access into more remote regions of the KNWR. Similarly, Holdsworth et al. (2007b) found that of all habitat and distance variables, distance to roads was the only significant predictor of earthworm occurrence in a Wisconsin hardwood forest for most earthworm groups. Holdsworth et al. (2007b) noted that *Dendrobaena* species are early colonizers among earthworm assemblages. The highest occurrence of *Dendrobaena* near roads suggests that the KNWR may be in the early stages of earthworm colonization.

Besides surface vehicle access, other anthropogenic influences likely contribute to earthworm presence in KNWR, especially in more remote areas. These remote site invasion vectors are not easily identified. *Dendrobaena octaedra* was found throughout the study area, most likely introduced by road construction, but also possibly by seismic exploration, fire suppression activities, and mechanical tree crushing for moose browse in the northern part of the KNWR during the 1970s. There, extensive seismic lines, mostly in the northern part of the Refuge, have been in place since as early as the 1950s, and many remain visible on the landscape today as animal, hiking, and snowmobile trails, as well as illegal access routes for all-terrain vehicles (U.S. Fish & Wildlife Service 2010). Numerous prescribed fires and wildfires within the KNWR, together with associated control and suppression efforts using heavy equipment, provided additional opportunities for earthworm establishment.

There are also non-anthropogenic vectors that can spread earthworms such as birds (D. Saltmarsh, *pers obs*.) and streams. In southeast Alaska, Costello et al. (2011) found that earthworms appear to disperse along streams. They showed that several earthworm species could survive ≥ 6 days submerged in a stream.

Factors such as soil pH likely also limit earthworm distribution. Most earthworms prefer soil pH of 5–7.4 (Addison 2008). While earthworms were found in the present study at sites with slightly higher pH (5.74 ± 0.13, n = 48 sites) than sites without earthworms (5.32 ± 0.23, n = 21), the distribution observed was most likely due to the distance from human impacts rather than pH. The average pH of the low impact sites was significantly different from other site types, likely due to the high number of low impact sites dominated by conifers compared to boat launch and road sites. Addison (2008) cited references documenting earthworms under fairly acidic conditions: *D. octaedra* has been found in areas of Canada with a pH as low as 2.8-3.6; *Dd. rubidus* and *L. terrestris* have been recorded in areas with pH of 3.0-3.4, suggesting that even low impact sites on KNWR were well within the range of tolerance for both species.

Earthworm densities showed substantial variation with a mean value (28 earthworms/m^2) comparable to other studies. Cameron et al. (2007) found densities along transects in Alberta of 0-35 earthworms/m^2, averaging 2-41 earthworms/m^2. González et al. (2003) found average density in a Colorado aspen forest was 44.4 earthworms/m^2. Boat launches had the highest density of earthworms, likely due to introduction from both roads and bait abandonment, as well as close proximity to campgrounds (Cameron et al. 2007).

We found no evidence that earthworms were affecting the soil properties pH, soil moisture, and leaf litter depth on KNWR. Likely explanations are the dominance of the epigeic *D. octaedra*, moderate densities of earthworms, and that these may have been young infestations. In Minnesota, Hale et al. (2005) found that the *Dendrobaena* group alone did not remove the forest floor or change other soil parameters, while *L. terrestris* resulted in the complete removal of surface litter and the lowest percentage of organic matter in the A horizon. They also found that fine root density, total fine root biomass, and nutrient availability were lower in *L. terrestris* dominated areas compared to others. These observations in other systems suggest that *D. octaedra* has a lesser impact on forest floor ecology than *L. terrestris*, so ecological impacts may not be apparent within the *D. octaedra* dominated KNWR. Moderate densities and biomass of earthworms as well as the potential that these populations have not had many years to work the soil are additional reasons that they have not yet measurably altered soil properties on KNWR.

In temperate studies, earthworm invasions appear to follow a predictable successional sequence, beginning with early invasion by epigeic species, such as *D. octaedra*, and epi-endogeic species, like *L. rubellus.* Subsequently endogeic and anecic species like *L. terrestris* colonize (Hale et al. 2005, Tiunov et al. 2006, Addison 2008). Gundale et al. (2005) confirmed this sequence in Michigan where they found communities consisting of just one or two species that were almost exclusively composed of *D. octaedra* and *L. rubellus.* This was similarly observed by Suárez et al. (2006) in New York where the edge of earthworm distribution was dominated by *L. rubellus*, followed by communities dominated by *L. terrestris.* This sequence can largely be explained by the differences in species traits such as reproductive strategy, fecundity, cold tolerance, and colonization rates. *D. octaedra* is partheogenic, has high cocoon production (Dymond et al. 1997), is extremely frost tolerant and can withstand over-winter freezing in all stages of development down to at least -14 °C. Together, these traits in *D. octaedra* aid in its success as an initial invader (Holmstrup 1994, Bindesbøl et al. 2007).

Even though differences in cold tolerance do limit the distributions of earthworm species at larger scales (Meshcheryakova et al. 2014), the extent our study area, confined to the central lowlands of the western Kenai Peninsula, did not cover enough of a climatic gradient for consideration of temperature as a determinant of earthworm invasion success.

Regionally in Alaska, the distribution of permafrost and cold winter temperatures, as well as soil moisture and pH, likely limit earthworms' potential Alaskan distribution. Where earthworms can survive, historic and current human activity and land use practices, and the composition of particular source populations, likely determine earthworm occurrence. The fact that all earthworm records in Alaska up to the present time have been from southern Alaska (see Suppl. material 1: Alaska earthworm records) despite rates of earthworm introductions that are likely comparable in southern and Interior Alaska indicates that the harsher, colder climate of the Interior precludes successful invasions by most earthworms. However, some of the more cold-hardy species present in the far north of the Palearctic, including *D. octaedra* and *Dd. rubidus*, may be able to survive in Interior Alaska based on the physiological and distributional data presented by Meshcheryakova et al. (2014).

Both *D. octaedra* and *Dd. rubidus* are parthenogenic, frost-hardy species, traits that, combined with their ability to tolerate acidic soils and exploit poor litter quality, contribute to their success in colonizing large areas. As with many "weedy" species, parthenogenesis facilitates rapid reproduction from very low densities, characteristic of rare dispersal events, where a single individual can establish an entire population (Tiunov et al. 2006). The small body size of these species also facilitates spread by vectors such as tires more often than *Lumbricus* and other anecic species. Given its wide distribution on the KNWR and its particular ecological traits, *D. octaedra* will likely be able to colonize large areas of permafrost-free Alaska, an expanding region as climate warms (Osterkamp 2005). While *D. octaedra* has limited impacts compared to other exotic earthworm species, its presence could portend an invasion by a larger assemblage of earthworms and commensurate changes in soil properties if Alaska follows the same colonization sequence seen elsewhere in northern North America.

Conclusion

As there are no effective strategies for removing exotic earthworms once they are established, preventing invasion and slowing their dispersal are the only viable ways to reduce their overall impacts. While we found that *D. octaedra* was widespread, most of the KNWR was free of the more damaging *Lumbricus* species. Most of the KNWR can be kept free of *Lumbricus* species for many years due to the extremely slow natural dispersal rate of these worms. Because the main vectors of earthworms on the KNWR appeared to be vehicles and bait abandonment, logical methods for slowing the spread of species already on the landscape and preventing the introduction of additional earthworm species would be to minimize vehicular activity in areas currently devoid of earthworms and to explicitly prohibit the use of earthworms as live bait.

As the first study of earthworm diversity and distribution in the southcentral Alaska region, we established patterns of distribution likely to hold true regionally and we set the stage for considering ways to limit the further introductions of exotic earthworms in Alaska.

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References

Addison JA (2008) Distribution and impacts of invasive earthworms in Canadian forest ecosystems. Biological Invasions 11: 59–79. doi: 10.1007/s10530-008-9320-4

Anderson JM (1988) Spatiotemporal effects of invertebrates on soil processes. Biology and Fertility of Soils 6: 216–227. doi: 10.1007/BF00260818

Berg EE, Hillman KM, Dial R, DeRuwe A (2009) Recent woody invasion of wetlands on the Kenai Peninsula Lowlands, south-central Alaska: a major regime shift after 18000 years of wet Sphagnum–sedge peat recruitment. Canadian Journal of Forest Research 39: 2033–2046. doi: 10.1139/X09-121

Berman DI, Marusik YM (1994) On *Bimastos parvus* (Oligochaeta: Lumbricidae) from Yukon Territory (Canada), with discussion of distribution of the earthworms in northwestern North America and northeastern Siberia. Megadrilogica 5: 113–116.

Bindesbøl A-M, Bayley M, Damgaard C, Holmstrup M (2007) Life-history traits and population growth rate in the laboratory of the earthworm *Dendrobaena octaedra* cultured in copper-contaminated soil. Applied Soil Ecology 35: 46–56. doi: 10.1016/j.apsoil.2006.05.010

Cameron EK, Bayne EM (2009) Road age and its importance in earthworm invasion of northern boreal forests. Journal of Applied Ecology 46: 28–36. doi: 10.1111/j.1365-2664.2008.01535.x

Cameron EK, Bayne EM, Clapperton MJ (2007) Human-facilitated invasion of exotic earthworms into northern boreal forests. Ecoscience 14: 482–490. doi: 10.2980/1195-6860(2007)14[482:HIOEEI]2.0.CO;2

Chan K-Y, Mead JA (2003) Soil acidity limits colonisation by *Aporrectodea trapezoides*, an exotic earthworm. Pedobiologia 47: 225–229. doi: 10.1078/0031-4056-00186

Costello DM, Tiegs SD, Lamberti GA (2011) Do non-native earthworms in Southeast Alaska use streams as invasion corridors in watersheds harvested for timber? Biological Invasions 13: 177–187. doi: 10.1007/s10530-010-9800-1

Dymond P, Scheu S, Parkinson D (1997) Density and distribution of *Dendrobaena octaedra* (Lumbricidae) in aspen and pine forests in the Canadian Rocky Mountains (Alberta). Soil Biology and Biochemistry 29: 265–273. doi: 10.1016/S0038-0717(96)00052-1

Edwards CA, Bohlen PJ (1996) Biology and Ecology of Earthworms (3rd ed). Chapman & Hall, London, 426 pp.

Eisenhauer N, Partsch S, Parkinson D, Scheu S (2007) Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthropods and vegetation. Soil Biology and Biochemistry 39: 1099–110. doi: 10.1016/j.soilbio.2006.12.019

Fiske I, Chandler R (2011) unmarked: an R package for fitting hierarchical models of wildlife occurrence and abundance. Journal of Statistical Software 43: 1–23. http://www.jstatsoft.org/v43/i10

Frelich LE, Hale CM, Scheu S, Holdsworth AR, Heneghan L, Bohlen PJ, Reich PB (2006) Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions 8: 1235–1245. doi: 10.1007/s10530-006-9019-3

Gates GE (1972) Contributions to North American earthworms (Annelida: Oligochaeta). No. 3. Toward a revision of the earthworm family Lumbricidae IV. The *trapezoides* species group. Bulletin of the Tall Timbers Research Station 12: 1–146.

Gates GE (1974) Contributions on North American earthworms (Annelida). No. 10. Contributions to a revision of the Lumbricidae. X. *Dendrobaena octaedra* (Savigny, 1826) with special reference to the importance of its parthenogenetic polymorphism for the classification of earthworms. Bulletin of the Tall Timbers Research Station 15: 15–57.

González G, Seastedt TR, Donato Z (2003) Earthworms, arthropods and plant litter decomposition in aspen (*Populus tremuloides*) and lodgepole pine (*Pinus contorta*) forests in Colorado, USA: The 7th international symposium on earthworm ecology, Cardiff, Wales, 2002. Pedobiologia 47: 863–869. doi: 10.1078/0031-4056-00272

Greiner HG, Stonehouse AMT, Tiegs SD (2011) Cold tolerance among composting earthworm species to evaluate invasion potential. American Midland Naturalist 166: 349–357. doi: 10.1674/0003-0031-166.2.349

Gundale MJ, Jolly WM, Deluca TH (2005) Susceptibility of a northern hardwood forest to exotic earthworm invasion. Conservation Biology 19: 1075–1083. doi: 10.1111/j.1523-1739.2005.00103.x

Hale CM, Frelich LE, Reich PB (2006) Changes in hardwood forest understory plant communities in response to European earthworm invasions. Ecology 87: 1637–1649. doi: 10.1890/0012-9658(2006)87[1637:CIHFUP]2.0.CO;2

Hale CM, Frelich LE, Reich PB, Pastor J (2005) Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. Ecosystems 8: 911–927. doi: 10.1007/s10021-005-0066-x

Hale CM, Reich PB, Frelich LE (2004) Allometric Equations for Estimation of Ash-free Dry Mass from Length Measurements for Selected European Earthworm Species (Lumbricidae) in the Western Great Lakes Region. The American Midland Naturalist 151: 179–185. doi: 10.1674/0003-0031(2004)151[0179:AEFEOA]2.0.CO;2

Holdsworth AR, Frelich LE, Reich PB (2007a) Effects of earthworm invasion on plant species richness in northern hardwood forests. Conservation Biology 21: 997–1008. doi: 10.1111/j.1523-1739.2007.00740.x

Holdsworth AR, Frelich LE, Reich PB (2007b) Regional extent of an ecosystem engineer: earthworm invasion in northern hardwood forests. Ecological Applications 17: 1666–1677. doi: 10.1890/05-2003.1

Holmstrup M (1994) Physiology of cold hardiness in cocoons of five earthworm taxa (Lumbricidae: Oligochaeta). Journal of Comparative Physiology B 164: 222–228. doi: 10.1007/BF00354083

Husson F, Josse J, Le S, Mazet J (2015) FactoMineR: multivariate exploratory data analysis and data mining. http://CRAN.R-project.org/package=FactoMineR

Karl TR, Melillo JM, Peterson TC (Eds) (2009) Global Climate Change Impacts in the United States. Cambridge University Press, New York. http://downloads.globalchange.gov/usimpacts/pdfs/climate-impacts-report.pdf

Klein E, Berg EE, Dial R (2005) Wetland drying and succession across the Kenai Peninsula Lowlands, south-central Alaska. Canadian Journal of Forest Research 35: 1931–1941. doi: 10.1139/x05-129

Lawrence AP, Bowers MA (2002) A test of the 'hot' mustard extraction method of sampling earthworms. Soil Biology and Biochemistry 34: 549–552. doi: 10.1016/S0038-0717(01)00211-5

MacKenzie DI, Nichols JD, Hines JE, Knutson MG, Franklin AB (2003) Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. Ecology 84: 2200–2207. doi: 10.1890/02-3090

MacKenzie DI, Nichols JD, Royle JA, Pollock KH, Bailey LL, Hines JE (2006) Occupancy estimation and modeling. Elsevier, New York.

Mazerolle MJ (2015) AICcmodavg: Model selection and multimodel inference based on (Q) AIC(c). http://CRAN.R-project.org/package=AICcmodavg

Meshcheryakova EN, Berman DI (2014) Cold hardiness and geographic distribution of earthworms (Oligochaeta, Lumbricidae, Moniligastridae). Entomological Review 94: 486–497. doi: 10.1134/S0013873814040046

Osterkamp TE (2005) The recent warming of permafrost in Alaska. Global and Planetary Change 49: 187–202. doi: 10.1016/j.gloplacha.2005.09.001

R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/

Revelle W (2015) psych: procedures for psychological, psychometric, and personality research. Northwestern University (Evanston, Illinois). http://CRAN.R-project.org/package=psych

Reynolds JW (1977) The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Royal Ontario Museum, Toronto, 141 pp. doi: 10.5962/bhl.title.60740

Reynolds JW (1980) The earthworm family Sparganophilidae (Annelida, Oligochaeta) in North America. Megadrilogica 3: 189–204.

Reynolds JW, Clebsch EEC, Reynolds WM (1974) Contributions to North American earthworms (Oligochaeta) No. 13. The earthworms of Tennessee (Oligochaeta). I. Lumbricidae. Bulletin of the Tall Timbers Research Station 17: 1–133.

Reynolds JW, Wetzel MJ (2008) Terrestrial Oligochaeta (Annelida: Clitellata) in North America, including Mexico, Puerto Rico, Hawaii, and Bermuda. Megadrilogica 12: 157–208.

Rinella DJ, Walker C, Merrigan D (2014) Exotic earthworms as a trophic subsidy to native salmonids. http://www.uaf.edu/files/ces/pests/cnipm/annual_invasive_species_c/15th_annual_meeting_ancho/Rinella-Daniel.pdf

Riordan B, Verbyla D, McGuire AD (2006) Shrinking ponds in subarctic Alaska based on 1950–2002 remotely sensed images. Journal of Geophysical Research: Biogeosciences 111: G04002. doi: 10.1029/2005JG000150

Sanderson LA, Mclaughlin JA, Antunes PM (2012) The last great forest: a review of the status of invasive species in the North American boreal forest. Forestry. doi: 10.1093/forestry/cps033

Suárez ER, Fahey TJ, Yavitt JB, Groffman PM, Bohlen PJ (2006) Patterns of litter disappearance in a northern hardwood forest invaded by exotic earthworms. Ecological Applications 16: 154–165. doi: 10.1890/04-0788

Symonds MRE, Moussalli A (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behavioral Ecology and Sociobiology 65: 13–21. doi: 10.2307/41413993

Tiunov AV, Hale CM, Holdsworth AR, Vsevolodova-Perel TS (2006) Invasion patterns of Lumbricidae into previously earthworm-free areas of northeastern Europe and the western Great Lakes region of North America. Biological Invasions 8: 1223–1234. doi: 10.1007/s10530-006-9018-4

U.S. Fish & Wildlife Service (2010) Comprehensive Conservation Plan: Kenai National Wildlife Refuge. U.S. Fish & Wildlife Service, Region 7 (Anchorage, Alaska). http://www.fws.gov/alaska/nwr/planning/kenpol.htm

Supplementary material 1

Alaska earthworm records

Authors: Deanna Marie Saltmarsh, Matthew L. Bowser, John M. Morton, Shirley Lang, Daniel Shain, Roman Dial

Data type: occurrence

Explanation note: Earthworm records from Alaska exclusive of data from the present study are compiled. All literature items cited are included in the References section of the manuscript.

Supplementary material 2

Specimen records

Authors: Deanna Marie Saltmarsh, Matthew L. Bowser, John M. Morton, Shirley Lang, Daniel Shain, Roman Dial

Data type: occurrence

Explanation note: Occurrence data are provided for earthworm specimens collected. Data field definitions are those used by Arctos (http://arctos.database.museum/, http://arctosdb.org/).

Supplementary material 3

Analysis dataset

Authors: Deanna Marie Saltmarsh, Matthew L. Bowser, John M. Morton, Shirley Lang, Daniel Shain, Roman Dial

Data type: measurement

Explanation note: This spreadsheet file contains all original measurements and derived metrics used in the analyses. It is arranged in a relational format. The sheet labeled site_data contains all site-level data, including original data and some derived metrics; the plot_data sheet contains plot-level data. The earthworm_lengths sheet contains all of the earthworm length measurements and, by implication, the occurrence data. The two response_data sheets hold data derived from the first three sheets that were used in subsequent analyses.