RESEARCH ARTICLE

Troubling travellers: are ecologically harmful alien species associated with particular introduction pathways?

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Abstract

Prioritization of introduction pathways is seen as an important component of the management of biological invasions. We address whether established alien plants, mammals, freshwater fish and terrestrial invertebrates with known ecological impacts are associated with particular introduction pathways (release, escape, contaminant, stowaway, corridor and unaided). We used the information from the European alien species database DAISIE (www.europe-aliens.org) supplemented by the EASIN catalogue (European Alien Species Information Network), and expert knowledge.

Plants introduced by the pathways release, corridor and unaided were disproportionately more likely to have ecological impacts than those introduced as contaminants. In contrast, impacts were not associated with particular introduction pathways for invertebrates, mammals or fish. Thus, while for plants management strategies should be targeted towards the appropriate pathways, for animals, management should focus on reducing the total number of taxa introduced, targeting those pathways responsible for high numbers of introductions. However, regardless of taxonomic group, having multiple introduction pathways increases the likelihood of the species having an ecological impact. This may simply reflect that species introduced by multiple pathways have high propagule pressure and so have a high probability of establishment. Clearly, patterns of invasion are determined by many interacting factors and management strategies should reflect this complexity.

Keywords

DAISIE, Europe, fish, ecological impact, introductions, invertebrates, mammals, pathways, plants

Introduction

The management of individual introduction pathways, and corresponding vectors, of alien species is a potentially powerful strategy to prevent new species introductions and thus reduce both the future costs to society as well as negative impacts on biodiversity (Carlton and Ruiz 2005, Hulme 2009, Essl et al. 2015). Pathway management is primarily aimed at eliminating or diminishing the propagule pressure of alien species and reflects the common wisdom that prevention and early action are more cost-effective than managing invaders after they have become established (Leung et al. 2002, Kaiser and Burnett 2010). Information on the pathways of introduction is increasingly incorporated in alien species databases (e.g. IUCN ISSG Global Invasive Species Database, www.issg.org/database, CABI Invasive Species Compendium, www.cabi.org/isc, and European Alien Species Information Network – EASIN, Katsanevakis et al. 2015) and country inventories (e.g. Kühn and Klotz 2003, García-Berthou et al. 2005, Nentwig 2007, Minchin et al. 2013, Roy et al. 2014). This provides an opportunity for comparative assessments of the role of pathways in biological invasions (Wilson et al. 2009, Bacon et al. 2012, 2014) and ultimately developing indicators based on trends in pathways (Rabitsch et al. 2016). This has led to a general framework for classifying pathways of introduction across taxa and environments that includes the identification of regulatory responsibilities (Hulme et al. 2008). A modified version of this general framework has recently been adopted by the UN Convention on Biological Diversity (CBD 2014). Some pathways are increasingly well studied, such as horticulture and forestry as a source for plant invasions (Mack and Erneberg 2002, Dehnen-Schmutz et al. 2007a,

b, Hanspach et al. 2008, Dawson et al. 2009, Pyšek et al. 2009, Essl et al. 2010, Smith et al. 2015, Pergl et al. 2016a, b), ballast water transport and aquaculture disseminating aquatic invaders (Galil et al. 2009, Mizrahi et al. 2015, Nuñes et al. 2015), live plants and plant products introducing pest insects and plant diseases (Roques 2010, Bacon et al. 2012, 2014, Liebhold et al. 2012, Eschen et al. 2015a, b), as well as snails (Bergey et al. 2014) and spiders (Nentwig 2015), the aquarium trade (Maceda-Veiga et al. 2013, Chucholl 2013), tourism (Anderson et al. 2015), the pet trade for terrestrial vertebrate invaders (Duncan et al. 2003, van Wilgen et al. 2010, Garcia-Diaz and Cassey 2014), and – more generally – the online trade (Kikillus et al. 2012, Humair et al. 2015). However, the role of pathways related to unintentional introductions has been difficult to quantify (Lee and Chown 2009, Pyšek et al. 2011, Bacon et al. 2012). Additionally, whether particular introduction pathways are associated disproportionally with the subsequent impacts of alien species has received little attention (Pyšek et al. 2011). Given the increasing rate at which alien species are being introduced around the world and predicted upward trends in the magnitude of major introduction pathways (Hulme 2015a), strategies to manage pathways based on their ultimate ecological risk are a priority. For example, several calls for identifying and managing pathways responsible for the introduction of species with high negative ecological and/or socio-economic impacts have been issued (EU 2014, CBD 2014).

Pathways of introduction and the subsequent impacts caused by invasive alien species (IAS) might be related in three ways (Essl et al. 2015). First, pathways that transport a high richness or abundance of species are more likely to lead to establishment and subsequent impact by a proportion of those species than pathways that carry fewer species or individuals. Second, certain pathways may introduce species into areas of conservation value, e.g. protected or remote areas where impacts may be particularly significant (Hulme 2011, Osyczka et al. 2012, Anderson et al. 2015). Third, some pathways may introduce more damaging species than others, particularly when pathogens are introduced as contaminants of their hosts (Roy et al. 2016). Therefore, identifying those pathways that are associated with impacts would help to prevent the emergence of new high-risk invaders. Yet, pathways and impacts have so far only been analysed together for a few taxonomic groups and particular pathways (e.g. Liebhold et al. 2012) and never across taxonomic groups. Lastly, taxa introduced by multiple pathways and introduced to different regions and habitats have a higher opportunity to become naturalized and then may have a greater probability of causing impact than those arriving on only one pathway (Küster et al. 2008).

Here we address the knowledge gap between impact and introduction pathways by relating for the first time the pathways of introduction of alien species spanning a range of taxonomic groups (plants, mammals, freshwater fish, and terrestrial invertebrates) in Europe to their ecological impacts. The aims of this study are: (i) to explore whether species with known ecological impacts differ in their pathway associations from those species for which no impact has been reported; (ii) to identify for particular alien taxonomic groups which pathways pose the greatest threat; and (iii) to explore whether species transported by multiple pathways are associated with a higher probability of

impact. More generally, the study presents a first attempt to identify the most relevant pathways of introduction of IAS with impact that can provide a data source for governments to fulfil their obligation under the Convention on Biological Diversity, and the recently adopted EU Regulation on IAS (EU 2014).

Methods

Data

Data from DAISIE (2009) database (www.europe-aliens.eu; Pergl et al. 2012) was used as a source of information on impacts of established alien species in Europe. It was also used as a basis for assignment of the pathways of their introduction to Europe for (i) vascular plants, (ii) freshwater fish, and (iii) mammals, while the EASIN catalogue (easin. jrc.ec.europa.eu; Katsanevakis et al. 2015) was used for pathway and impact classification of (iv) terrestrial invertebrates. The classification of introduction pathways follows the scheme of Hulme et al. (2008) that allows their comparison across taxonomic groups as well as between accidental and intentional introductions. Each species was assigned to one or more of the following pathway categories: (i) release (intentional introduction and release into the environment), (ii) escape (intentionally introduced as a commodity, but escaped from culture), (iii) contaminant (unintentional introduction with specified commodity), (iv) stowaway (unintentionally introduced attached to or within a transport vector), (v) corridor (unintentional spread via human transport infrastructures linking previously unconnected regions) or (vi) unaided (unintentional introduction by natural dispersal across political borders following a primary human-mediated introduction in a neighbouring region). The data do not differentiate between the pathways for initial introduction to Europe and those associated with movement among different European countries. Similarly, species are often listed as associated with more than one introduction pathway with no measure of their relative importance. In contrast to other taxonomic groups, the invertebrate data do not allow the exact area of origin to be identified for species that are native in a part of Europe and alien in another part and thus this group included only arrivals from other continents (classified as aliens *to* Europe in DAISIE 2009). Only species confirmed as established in at least one European country (DAISIE regions) were included in the analyses. As information on establishment status is incomplete for some regions of Europe, we also included species for which establishment could not be confirmed but that were found in five or more European regions.

As a second step, species for which introduction pathways had been identified were classified in two groups: those having an ecological impact and those for which no ecological impact had been recorded. For fish, mammals and plants, the information on ecological impacts was retrieved from DAISIE (Vilà et al. 2010). For invertebrates information in DAISIE and EASIN was updated with literature and expert opinion (M. Kenis, W. Rabitsch and A. Roques, unpublished data). Ecological impact was defined as an impact on native species or on the functioning of natural or semi-natural

ecosystems in Europe or in similar climatic and environmental conditions in other continents. There was no assessment of the type of impact or its magnitude.

Statistical analysis

We tested: (i) whether different pathways (release, escape, contaminant, stowaway, corridor and unaided) are associated with higher or lower probability of causing ecological impact and (ii) whether there is a relationship between the likelihood of impacts and the number of pathways through which a species has been introduced. All analyses were based on species counts that were analysed by generalized linear models with a log-link function and Poisson distribution of errors with control for overdispersion (if needed using quasi-Poisson distributions) (Crawley 2007). If the full model including the interaction with taxon was significant, then individual models for particular taxonomic groups were used. To test in which pathways the counts were lower or higher than expected by chance, adjusted standardized residuals of *G*-tests were compared with critical values of a normal distribution (Řehák and Řeháková 1986). The null expectations were thus that the proportion of species with and without ecological impact within an individual pathway is the same across all pathways and that number of species with and without impact are not related to number of pathways. The test for multiple pathways was done by summing up the number of pathways per species. All analyses were performed in R 3.0.2 (R Core Team 2015).

Results

Differences in pathway frequencies by taxonomic groups

There were 2529 vascular plant, 75 mammal, 107 fish and 1314 terrestrial invertebrate taxa (species or subspecies) with at least one pathway category assigned. The coverage of identified pathways for the taxa ranged from 98% for fish to 59% in plants (Table 1). The most frequently represented pathways differed between taxa. In plants, the most frequent pathway was escape, recorded for 58% of the total species number. Mammals had a high proportion of release and escape (49% and 41%, respectively). Among freshwater fishes, there were 43% escaped and 36% released species. In contrast, 76% of the terrestrial invertebrates were introduced as contaminants of commodities (Table 1).

Impact associated with pathways in different taxonomic groups

Among the established taxa with known introduction pathway, there were 250 vascular plants (6.2% of the total), 38 mammals (61.3%), 52 fishes (48.6%) and 80 terrestrial

Table 1. Percentages and observed counts (in brackets) of pathways identified for individual taxonomic groups. Totals show the percentage and number of alien species for which a pathway is known. Note that species can be associated with more than one pathway, so the counts do not add up to total. 'No pathway data' shows the percentage from all assessed taxa (total + no data) and the number of species that meet the criteria of establishment or widespread distribution in Europe, but for which there is no precise enough information on pathways.

	Release	Escape	Contaminant	Stowaway	Corridor	Unaided	Total	No pathway data
Plants	18.4 (638)	58.3 (2016)	19.4 (670)	2.7 (92)	0 (1)	1.2 (42)	59.4 (2529)	40.6 (1732)
Mammals	48.8 (40)	41.5 (34)	0 (0)	0 (0)	0 (0)	9.8 (8)	72.0 (54)	28.0 (21)
Fish	35.6 (74)	42.8 (89)	0 (0)	1.4 (3)	6.7 (14)	13.5 (28)	98.1 (105)	1.9 (2)
Terrestrial invertebrates	11.7 (156)	2.5 (34)	76.3 (1020)	9.0 (120)	0.4 (6)	0.0 (0)	75.0 (1314)	25.0 (438)

invertebrates (6.1%) with documented or strongly supposed ecological impact (Table 2). There was a significantly higher frequency of taxa with impact within mammals and fishes than in plants and invertebrates (two-way interaction taxon × impact χ^2 = 208.71; df = 3; P < 0.001). Overall, the frequency of ecological impacts differed significantly among pathways and taxa (three-way interaction taxon × pathway × impact: χ^2 = 29.11; df = 15; P = 0.015). Within the particular taxonomic group, the impacts were significantly different among pathways for plants (χ^2 = 32.54; df = 5; P < 0.001) but not so for invertebrates, mammals or fish. As discussed below, the results might be masked by lower statistical power of the test in these taxon groups due to high numbers of pathways with zeros and that mammals and fish are generally species-poor groups. For plants exerting ecological impact, the significant difference among pathways was mainly due to disproportionately more counts than expected for release, corridor and unaided pathways, and disproportionately fewer for contaminants (Table 2).

The role of multiple pathways

The maximum number of pathways recorded for species with ecological impact was four, represented by five plants (e.g. *Elodea canadensis* – Canadian waterweed, *Galinsoga parviflora* – gallant soldier, and *Senecio vernalis* – Eastern groundsel) and two fish (*Oncorhynchus gorbuscha* – pink salmon, and *O. mykiss* – rainbow trout). For mammals, the maximum number of pathways was three, recorded in six species (e.g. *Cervus nippon* – sika deer, *Ondatra zibethicus* – muskrat, *Procyon lotor* – raccoon). For terrestrial invertebrates with impact, the maximum number of pathways was two (*Lasius neglectus* – garden ant, and *Linepithema humile* – Argentine ant) (Fig. 1).

The taxonomic groups did not differ in their impact related to the number of pathways (three-way interaction taxon × number of pathways × impact: χ^2 = 8.01; df = 9; p=0.53), but pooled across taxa, having multiple pathways increased the probability of recording impact (χ^2 = 170.11; df = 3; P < 0.001). Taxa associated with only one

Table 2. Percentages and observed counts (in brackets) for ecological impact across pathways among taxonomic groups. Note that species can be associated with more than one pathway. Higher and lower values than expected by chance (based on *G*-tests) are indicated by asterisks (*<0.05, ** < 0.01, *** < 0.001) and corresponding sign (↓ observed counts lower than expected, ↑observed counts higher than expected). Significant effects of pathways are highlighted.

| Ecological Impact	Taxa with impact	Release		Escape		Contaminant		Stowaway		Corridor		Unaided	
		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Plants	6.2 (250)	15.5 (537)	↑2.9 (101)**	52 (1800)	6.2 (216)	17.7 (613)	↓1.6 (57)*	0 (0)	0 (1)	2.1 (74)	↑0.5 (18)*	0.9 (32)	↑0.3 (10)*
Mammals	61.3 (38)	14.6 (12)	34.1 (28)	7.3 (6)	34.1 (28)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.2 (1)	8.5 (7)
Fish	48.6 (52)	16.8 (35)	18.8 (39)	20.2 (42)	22.6 (47)	0 (0)	0 (0)	3.8 (8)	2.9 (6)	1.0 (2)	0.5 (1)	4.8 (10)	8.7 (18)
Terrestrial invertebrates	6.1 (80)	11.6 (152)	0.3 (4)	2.6 (34)	0.0 (0)	73.1 (961)	4.5 (59)	8.4 (111)	0.7 (9)	0.3 (4)	0.2 (2)	0.0 (0)	0.0 (0)
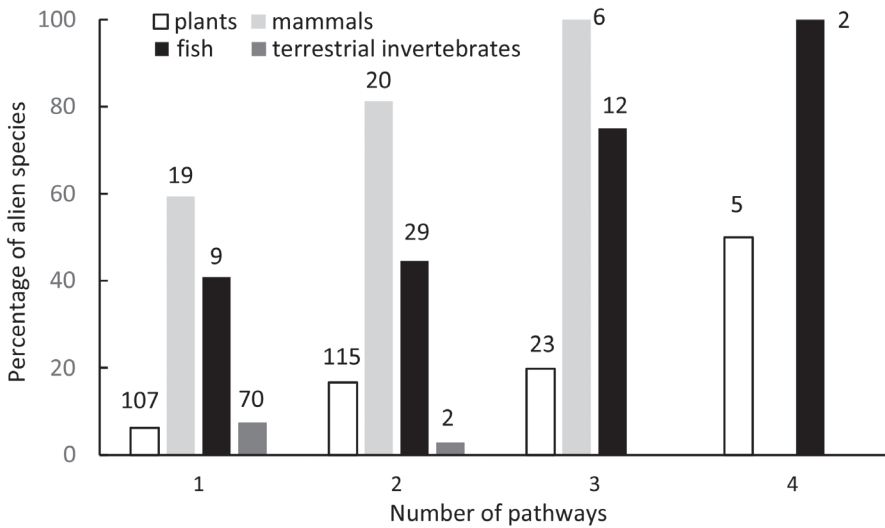
Figure 1. Percentage of alien species with impact in relation to the number of introduction pathways. The height of the bar indicates the percentage of the number of taxa with impact within the taxonomic group of species that are introduced via the given number of pathways. Numbers above bars indicate the numbers of species with impact for each taxonomic group and for the given number of pathways.

pathway were less likely to have an impact than expected by chance ($G = 3.47$, P < 0.001), while those associated with two and three pathways were more likely ($G = 4.45$, P < 0.001; $G = 2.62$, P < 0.01). The number of taxa without impact and introduced by four pathways was lower than expected by chance ($G = 2.89$, P < 0.01). Combinations of pathways per taxonomic group are shown in Appendix 1.

Discussion

Differences among taxonomic groups

The relationship between impacts and pathways differed with respect to taxonomic groups, but for most taxa no major significant differences among pathways were found. For plants, pathways vary in the proportion of species with impact they deliver, while for invertebrates, fish and mammals this was not the case. For example, among escaped mammals, in a group featuring prominent examples of escaped fur animals with high ecological impacts (*Neovison vison* – American mink, *Ondatra zibethicus* – muskrat), there were no significant differences between numbers of species with and without impacts. Further, the number of species with impact arriving by a given pathway is also important. For example, the absolute number of escaped plants with impact was twice as high as that of released plants with impact, despite the difference between the two pathways not being statistically significant. Similarly, fewer than expected species

of plants causing impact are introduced as contaminants, but absolute values for terrestrial invertebrates indicate a high importance of this pathway compared to release.

That pathways do not significantly affect the probability of impact of vertebrates may be related to the generally high invasion success of this group (Jeschke and Strayer 2005, Jeschke 2008), as well as to a high percentage of species with impacts compared to plants and invertebrates (Table 2). If vertebrates are introduced and establish there is a high probability of them having impact regardless of the pathway on which they arrive. Furthermore, identifying pathways causing negative environmental impacts by alien vertebrates may require more detailed analyses than for other taxonomic groups. For example, to assess the role of the pet trade, which is a subset of the escape category, it would be important to carry out analyses at a finer level than is currently the case. Considering intentional (release, escape) *vs* unintentional pathways (contaminant, stowaway, corridor and unaided) across all taxa, our results indicate that the latter are associated with impact less frequently than expected, and *vice versa*. In absolute numbers, unintentional pathways were more common for invertebrates, but not so for plants. However, the pattern is blurred by the fact that many species were introduced through several pathways, including both intentional and unintentional. For example, for plants, Pyšek et al. (2011) found that unintentionally introduced species invaded a wider range of semi-natural habitats than intentionally introduced species; hence the risk arising from unintentional introductions should not be underestimated.

An important question is whether species introduced by multiple pathways have an advantage because of a higher propagule pressure or an increased probability to reach a more diverse range of suitable sites. Unfortunately, robust data for propagule pressure that can be compared across individual pathways for the respective taxonomic groups are rarely available. If such data exist, they are limited to specific pathways such as direct release for biocontrol (Rossinelli and Bacher 2015) or landscaping (but see Lee and Chown 2009). Our knowledge thus mostly depends on proxies such as trade volume, numbers of botanic gardens, human population density or road density (Carlton and Ruiz 2005, Wilson et al. 2009, Kaluza et al. 2010, Pyšek et al. 2010; Hulme 2015b). Although some taxonomic groups such as invertebrates are highly dependent on one specific pathway, in general, the number of introduction pathways can be used as another proxy for propagule pressure. It appears that ecological impacts are more likely to occur if plants are introduced by multiple pathways. Besides profiting from increased propagule pressure, it is also possible that species introduced by multiple pathways have a greater chance of being introduced to a wider range of habitats or are also ecologically more versatile than those arriving on single pathways. In plants, the existence of multiple pathways usually includes escape from cultivation, reflecting the dominant role of horticultural introductions, which is for many species combined with introduction as contaminants. The combination of pathways that favours high impact fishes is release and escape, but these two pathways are also often accompanied with unintentional introductions. It seems that at least in these two taxonomic groups, the predisposition for opportunistic dispersal may be determined by the same traits as

the ability to escape from capture or cultivation. Still, there are fishes with severe impacts introduced by a single pathway, e.g. *Leuciscus leuciscus* (common dace), *Clarias gariepinus* (African sharptooth catfish), *Oreochromis niloticus* (Nile tilapia), *or Polyodon spathula* (American paddlefish). For terrestrial invertebrates, species with impact introduced as contaminants dominate, which highlights the importance of this pathway and the fact that this pathway is responsible for high propagule pressure. However, it is likely that a large number of these species are also introduced as stowaways but this pathway is hugely underestimated because it is so difficult to assess (A. Roques, unpublished data).

Relating impact to pathways: what data are available?

Although a simple yes/no classification of ecological impact provides basic information, it is evident that impacts manifest over a wide range of magnitudes, from local population declines to global extinctions, or from minor perturbations to massively adverse ecological and economic cascades. A yes/no impact classification lumps together species with low ecological impact, e.g. *Mahonia aquifolium* (Oregon-grape), with high-impact species such as *Fallopia* spp. (knotweeds) (Kumschick et al. 2015a). Similarly, while there are over 600 alien terrestrial invertebrate species (mostly insects) classified as having an ecological impact in DAISIE, an extensive literature survey of ecological impacts attributed to invasive insects found published records for less than 10 species in Europe (Kenis et al. 2009). On the other hand, using other impact criteria, Vaes-Petignat and Nentwig (2014) described impacts for 64 of the 77 most widely spread terrestrial arthropods alien to Europe. However, despite recent attempts to classify impacts more precisely (Nentwig et al. 2010, 2016, Kumschick and Nentwig 2011, Kenis et al. 2012, Blackburn et al. 2014, Jeschke et al. 2014, Kumschick et al. 2015b, Rumlerová et al. 2016), such information is rarely available for a large number of species. Low sample size is a constraint for the statistical analysis, particularly for alien mammals and fishes, and limits the power of finding relevant patterns despite the severe impacts that these two taxonomic groups are known to have on biodiversity (Kumschick et al. 2015a). Differences in the quality of impact data (Hulme et al. 2013) among taxonomic groups are not only due to species numbers or recorded impacts, but also result from the research activity (e.g. ease of study or attractiveness). The frequent impacts of released species may be due to the fact that some of those species are introduced for a purpose that requires having an ecological impact (e.g. plant species for dune stabilization, invertebrates for biocontrol) and are better scrutinized for any potential adverse (and unintended) impacts on native species. About 110 released invertebrates (mostly biocontrol agents) have been classified as having an ecological impact in Europe (DAISIE 2009). Only three are known to have some measurable negative impact on native species, the parasitoids *Cales noaki* and *Lysiphlebus testaceipes* (Kenis et al. 2009), and the harlequin ladybird *Harmonia axyridis* (Roy et al. 2012, 2016).

Management recommendations

The management of IAS with negative impacts on the environment and on human well-being is subject to efforts at national, continental and global levels (CBD 2014, Aichi Biodiversity Target 9). The categories used for the present analysis are consistent with the standard categorization of pathways of introduction of IAS presented by the CBD and recommended for identifying and prioritizing pathways (CBD 2014). At the European scale, the new EU Regulation on IAS, entered into force on 1 January 2015 (EU 2014, Genovesi et al. 2015), calls EU Member States to identify the pathways of unintentional introduction and spread of IAS of Union concern, and to effectively manage them through specific action plans.

The CBD and EU legislation confirm that policies are focusing on the prioritization of pathways in order to prevent the introduction of IAS (Meyerson and Reaser 2003, Hulme 2009, 2011). This covers managing or preventing the introduction of new species to a particular region and mitigating their impacts by regulation of intentional and unintentional introductions (Wittenberg and Cock 2001, Caffrey et al. 2014). To make pathway management work efficiently, it needs to be built on rigorous data on impacts of alien species, and how these interact with individual pathways. Some pathways and taxonomic groups, plants and invertebrates particularly, contribute disproportionally more to the overall risk from alien species with documented impacts, and these should receive increased attention. However, to fully assess the potential of each particular pathway, not only is it necessary to consider the proportion of species with negative impacts, but also the absolute number of species introduced along each pathway.

Using proportions as a measure emphasizes the release pathway as posing greater risk, regardless of the taxonomic group, while using absolute species numbers prioritizes the escape and contaminant pathways. The other pathways associated with arrival of IAS can be assumed to be less important for management and monitoring. Legislation, early warning systems and rapid response mechanisms should be primarily targeted at intentional introductions (release and escape) and species introduced unintentionally as contaminants (for which the pathway of arrival can be identified). An accurate identification of the pathways of introduction and spread of alien species is essential for efficient management of invasions, and in this regard it is important to adopt a standard terminology and categorization, as recommended by the CBD (Hulme et al. 2016); a standardized approach will be essential in enforcing the EU Legislation, to ensure that action by EU member states is coordinated. Also, the present study highlights that the proportions of alien species with negative ecological impacts are taxon-specific, a finding that should be reflected by legislation and pathway management. However, in many cases at the present level of understanding, the best predictor of the relevance of an introduction pathway is the total number of species that are associated with it. Furthermore, we showed that the results of this study are highly dependent on the availability of data and it is necessary to better reflect the scales of impacts ranging from minimal to massive to improve understanding and management of IAS.

Therefore, we encourage further work on the approach outlined here through more detailed analyses of individual pathways, their association with IAS, consideration of spatial and temporal variation in pathway trends (Padilla and Williams 2004, Copp et al. 2010, Maceda-Veiga et al. 2013, Hulme 2015b), inclusion of more detailed descriptions of the magnitude and/or types of impact (Nentwig et al. 2010, 2016, Blackburn et al. 2014, Kumschick et al. 2015a) and consideration of other taxa that were not included in the present study such as fungi, and considering interactions and synergies between pathways (Roy et al. 2016). As for most taxa it was not possible to detect major differences in the way in which IAS arrive and so until robust and comprehensive information on impact is available, we should not focus on subtle differences between the pathways of arrival for different taxonomic groups, but instead consider the most common pathways for all taxa and pathways that are most easily managed. Thus pathways that deliver many species should become a management priority.

Author contributions

J.P., P.P. and W.N conceived the ideas, AR, MK and WR revised data on impacts of insects and all authors contributed to writing the paper.

Acknowledgements

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Electronic appendix

A large number of possible combinations between pairs of individual pathways and a low number of observed species with particular pathway combinations prevented rigorous statistical testing of differences in the role of multiple pathways among taxonomic groups. Nevertheless, there was a clear trend for fishes, mammals and plants that the highest proportion of multiple pathways was associated with release and escape (intentional introductions). For terrestrial invertebrates, the highest proportion was found for the combinations "corridor and stowaways" (unintentional introductions with traded goods and their vectors without any biological meaning for the introduced species) (Table 3).

Generally, the patterns were highly taxon-specific. In fishes, most multiple pathways are associated with unaided spread. For terrestrial invertebrates where the very dominant pathway is contaminant – unaided pathway, multiple pathways are limited in frequency (Table 3). For mammals, also only few records were available and therefore only three combinations are covered, all showing high importance of intentional release and unintentional unaided spread. In plants, the combinations of several pathways are mostly associated with escape.

Table 3. Percentages and observed counts (in brackets) for pairs of introduction pathways for alien species with ecological impact among taxonomic groups. The most frequent combinations (above 3%) are highlighted. Pathways: rel – release, esc – escape, cont – contaminant, stow – stowaway, unaid – unaided.

	rel-esc	rel-cont	rel-stow	rel-corr	rel-unaid	esc-cont	esc-stow	esc-corr	esc-unaid	cont-stow	cont-corr	cont-unaid	stow-corr	stow-unaid	corr-unaid
Plants	3.9 (99)	0.4 (11)	0.2 (4)	0 (0)	0.1 (2)	1.9 (49)	0.6 (16)	0 (0)	0.3 (8)	0.5 (12)	0 (0)	0.3 (7)	0 (1)	0.2 (4)	0 (1)
Mammals	33.3 (18)	0 (0)	0 (0)	0 (0)	13 (7)	0 (0)	0 (0)	0 (0)	11.1 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fish	36.2 (38)	0 (0)	0 (0)	1.9 (2)	13.3 (14)	0 (0)	0 (0)	1.9 (2)	12.4 (13)	0 (0)	0 (0)	0 (0)	1 (1)	1 (1)	5.7 (6)
Terrestrial invertebrates	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2.7 (2)	0 (0)	0 (0)

SHORT COMMUNICATION

The European Alien Species Information Network on the Convention on Biological Diversity pathways categorization

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Citation: Tsiamis K, Cardoso AC, Gervasini E (2017) The European Alien Species Information Network on the Convention on Biological Diversity pathways categorization. NeoBiota 32: 21–29. https://doi.org/10.3897/neobiota.32.9429

Abstract

The adoption of a consistent alien species pathways categorization, hierarchy and terminology is crucial for increasing the interoperability of different online databases. In the present paper the European Alien Species Information Network (EASIN) classification system of pathways is compared and discussed with the classification scheme recently published by the Convention on Biological Diversity (CBD). Although the main pathway categories of the two classifications overall match, there are substantial differences in their subcategorization, with EASIN including 20 pathway subcategories while CBD considers 44 subcategories. In most cases, each EASIN subcategory pathway can correspond to two or more CBD subcategories. About 5,500 species listed in EASIN do not match directly with the CBD pathway sub-categories, most of which are terrestrial invertebrates. Aiming at achieving synchronization between the two classification systems and at facilitating the access to information to researchers and policy makers, EASIN is trying to align with the CBD pathway classification scheme. This alignment process requires the involvement of a large number of experts, especially from the terrestrial realm, and the adoption of commonly accepted definitions of the CBD pathways.

Keywords

alien, invasive, Europe, interoperability, pathway, EASIN, CBD

Invasive Alien Species (IAS) constitute one of the most important threats to biodiversity, causing severe ecological and socio-economic impacts (Ricciardi et al. 2013, Jeschke et al. 2014). A conservative estimate of the annual damage caused in the European Union (EU) by IAS is €12 billion (Kettunen et al. 2009). In addition, there is an increasing trend of new alien introductions (Essl et al. 2015, Roques et al. 2016). Aiming at protecting European biodiversity, the European Parliament and the Council adopted the EU Regulation no. 1143/2014 (EU 2014; hereafter referred to as the IAS Regulation) on the prevention and management of the introduction and spread of IAS, which entered into force on the 1st of January 2015, and a list of 37 invasive alien species of Union concern, by means of Implementing Regulation 1141/2016.

The European Alien Species Information Network (EASIN, http://easin.jrc.ec.europa.eu/) has been developed by the European Commission's Joint Research Centre (JRC) (Katsanevakis et al. 2012), and formally recognized as the information system supporting European Member States in the implementation of the IAS Regulation (EU 2014, Art. 25). EASIN aims at allowing easier access to data of alien species occurring in Europe, and to provide a one-stop shop to access all the information necessary to underpin alien species related policy and management decisions (Katsanevakis et al. 2013).

Information on alien species introduction pathways is fundamental for the reliability and effectiveness of alien species risk assessments, management, monitoring, and surveillance (EU 2011, Ojaveer et al. 2014, McGeosh et al. 2016). Pathways categorization is an essential aspect, and can benefit from applying consistent pathways classification, hierarchy, and terminology (Essl et al. 2015). To aid these efforts, a standardized pathways terminology and hierarchical classification has been proposed by Hulme et al. (2008) and largely adopted by EASIN in 2012 (Katsanevakis et al. 2012). Since then, EASIN datasets have been used for pan-European or regional assessments of pathways of alien species invasions, towards the fulfilment of the related targets of the Convention on Biological Diversity (CBD) and of European policies (Katsanevakis et al. 2015).

The increasing threat of IAS has lead CBD to a comprehensive review of the alien species pathways, adopting a note of the executive Secretary on the categorisation of identified pathways of introduction of IAS (CBD 2014), addressing the needs of Aichi Biodiversity Target 9 concerning the identification, prioritization and management of IAS by 2020, which is reflected in Target 5 of the EU Biodiversity Strategy (EU 2011). The CBD pathway analysis was based on Hulme et al. (2008) study, and integrates a set of international standards and guidelines, aiming at harmonizing the existing large number of pathways described. CBD (2014) scope is to develop a categorization of pathways using standard terminology applicable at a global scale, facilitating the interoperability of different online databases.

In the present paper a comparative analysis between the EASIN and the CBD pathway classification schemes is presented (Table 1), highlighting the need for harmonization to allow data interoperability. Both systems exhibit very good match (83%) regarding their main-category pathways ("release in nature", "escape from confinement", "transport contaminant", "transport-stowaway", "corridor"), since they are

Table 1. Comparison of sub-category pathways between CBD and EASIN classification systems; green colour corresponds to perfect match between the two systems; blue colour includes cases where an EASIN pathway corresponds to two (or more) CBD pathways; orange colour indicates a case where a CBD pathway corresponds to two EASIN pathways; red colour refers to cases where an EASIN pathway does not match with any CBD pathway or vice versa. The number of related species for each pathway in EASIN is also provided.

Category pathways	CBD sub-category pathways	EASIN sub-category pathways	EASIN No. of Species
Release in nature	Biological control	Biocontrol	181
	Erosion control/ dune stabilization (windbreaks, hedges, …)	Landscaping-Erosion control	64
	Landscape/flora/fauna "improvement" in the wild		
	Fishery in the wild (including game fishing)	Game animals	93
	Hunting		
	Introduction for conservation purposes or wildlife management		
	Release in nature for use (other than above, e.g., fur, transport, medical use)		
	Other intentional release	Other + Pets, Terrarium-Aquarium species	1102
Escape from confinement	Agriculture (including Biofuel feedstocks)	Cultivation and Livestock	780
	Farmed animals (including animals left under limited control)		
	Forestry (including afforestation or reforestation)		
	Fur farms		
	Horticulture		
	Aquaculture / mariculture	Aquaculture	171
	Botanical garden/zoo/aquaria (excluding domestic aquaria)	Zoos, botanical gardens	262
	Pet/aquarium/terrarium species (including live food for such species)	Pets, Terrarium-Aquarium species	246
	Ornamental purpose other than horticulture	Ornamental planting	1935
	Research and ex-situ breeding (in facilities)		
	Live food and live bait	Use of live food-bait	28
	Other escape from confinement		
Transport – contaminant	Contaminant nursery material	Trade of contaminated commodities	3382
	Contaminated bait		
	Food contaminant (including of live food)		
	Contaminant on animals (except parasites, species transported by host/vector)		
	Parasites on animals (including species transported by host and vector)		
	Contaminant on plants (except parasites, species transported by host/vector)		
	Parasites on plants (including species transported by host and vector)		
	Seed contaminant		
	Timber trade		
	Transportation of habitat material (soil, vegetation, …)		
		Aquaculture	228
		Packaging materials	56

Category pathways	CBD sub-category pathways	EASIN sub-category pathways	EASIN No. of Species
Transport - stowaway	Angling/fishing equipment		
	Container/bulk		
	Hitchhikers in or on airplane	Aviation	27
	Hitchhikers on ship/boat (excluding ballast water and hull fouling)	Shipping	921
	Ship/boat ballast water		
	Ship/boat hull fouling		
	Machinery/equipment		
	People and their luggage/equipment (in particular tourism)		
	Organic packing material, in particular wood packaging		
	Vehicles (car, train, …)	Land transport	297
	Other means of transport		
Corridor	Interconnected waterways/basins/seas	Lessepsian migrants	499
		Inland Canals	66
	Tunnels and land bridges		
		Railroads and Highways	38
Unaided	Natural dispersal across borders of invasive alien species that have been introduced through pathways the rest main pathways categories.	OTHER	981

both based on Hulme et al. (2008) concept. The only mismatch observed concerns the "unaided" category, adopted by CBD. EASIN uses instead "other" as a category which includes the "unaided" pathway. On the other hand, substantial differences are observed in the subcategorization of pathways: EASIN includes 20 pathway sub-categories, while the CBD includes 44 subcategories (Table 1), and thus offers more detailed information on species introduction channels. A perfect match is observed for 10 pathway subcategories between the two systems. More frequently, each EA-SIN subcategory corresponds to two or more CBD subcategories, or the opposite in one case, including 24 non-matching pathway subcategories considering both sides. A typical example is the EASIN "trade of contaminated commodities" pathway, assigned to about 3,400 species in EASIN, which could correspond to one or more among the ten related pathways of the CBD scheme (Table 1). Finally, there is no match at all concerning 14 subcategories included in both systems (Table 1).

A thorough comparison between the EASIN and CBD systems reveals that the sub-category pathways of about 5,500 alien species registered in EASIN (51% of the EASIN species with assigned pathway) do not match directly with the available CBD pathway subcategories. Similar comparisons were made by Essl et al. (2015) between the CBD scheme and GISD (Global Invasive Species Database), DAISIE (Delivering Alien Invasive Species Inventories for Europe) and GBNNSIP (Great Britain's Non-Native Species Information Portal) data, revealing higher levels of direct matching (from 79% to 99%). However, the number of species with assigned pathways in these databases is by far lower when compared with EASIN.
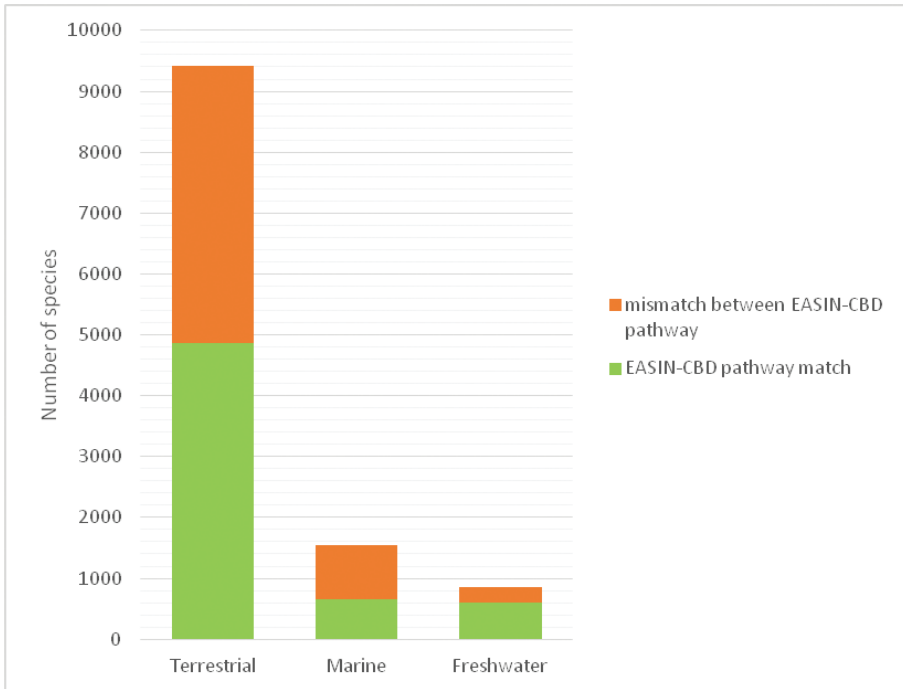
Figure 1. Pathway matching between EASIN and CBD sub-category classification systems for alien species included in EASIN per habitat.

A more detailed analysis of the EASIN data reveals that 52% of the terrestrial alien species included in EASIN match with the CBD classification. In comparison, the matching between the two systems is higher for the freshwater species of EASIN (70%), but lower for the marine species (43%). Still, the corresponding number of the EASIN alien species per habitat is much higher for the terrestrial group compared to the marine and freshwater (Figure 1). When it comes to alien species of EASIN per main taxonomic group, a low match is observed for the invertebrates (28%, Figure 2); most of the mismatched species of EASIN are assigned to the pathway "trade of contaminated commodities" and "shipping" (Figure 3). On the other hand, there is a relatively good match for plants (66%), although given the size of the group, a considerable number of them do not match with the CBD pathways (Figure 2), most of them assigned to "trade of contaminated commodities" and "cultivation and livestock" (Figure 3). A good match is observed for vertebrates (80%), with a low number of mismatched species (Figure 2), most of which are related with 'game animals" and 'shipping" (Figure 3).When it comes to fungi and chromista, there is a very low match for both of them (1% and 22%, respectively), although their species number is by far lower compared to the other main taxonomic groups (Figure 2). Almost all mismatching fungi are assigned to "trade of contaminated commodities", while most mismatching chromista to "shipping" (Figure 3).
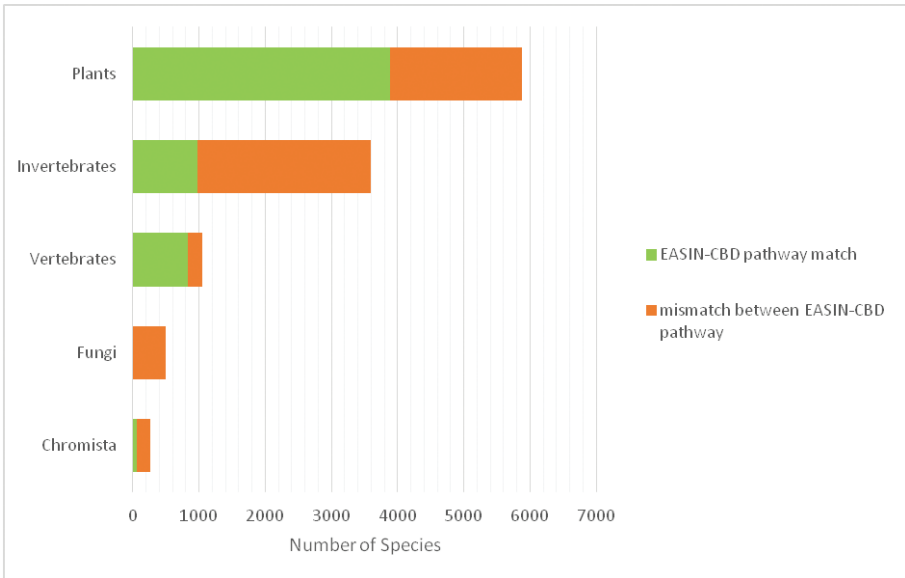
Figure 2. Pathway matching between EASIN and CBD sub-category classification systems for alien species included in EASIN per main taxonomic groups.
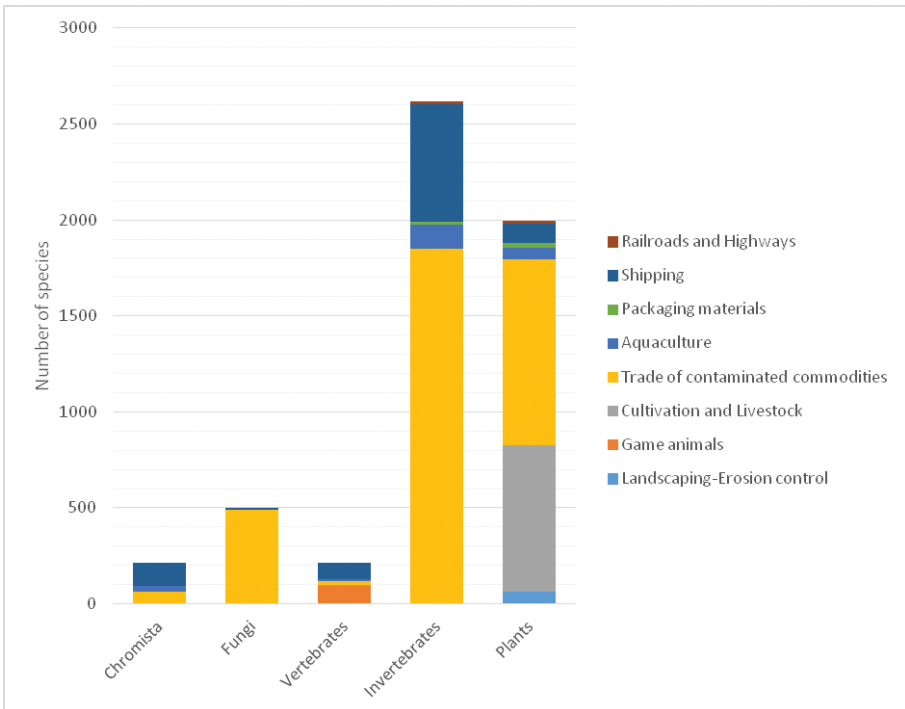

Figure 3. Number of EASIN alien species per main taxonomic group with assigned pathway not matching the CBD classification. The related number of alien species corresponding to each EASIN pathway is also depicted.

It should be noted that the accurate determination of an alien species pathway is not always an easy task and it may be characterised by high levels of uncertainty (Katsanevakis et al. 2013). Therefore, higher level of detail in subcategorization will inevitably lead to further difficulties in pathways accurate determination. For example, it is known that most of the marine aliens in European seas have been introduced through shipping (Nunes et al. 2014), but the accurate assignment of their pathway in terms of CBD subcategories related to shipping ("hitchhikers on ship-boat excluding ballast water and hull fouling" / "ship-boat ballast water" / "ship-boat hull fouling") could offer a real challenge even to experts. On the other hand, higher level of pathways distinction could allow a better definition of legislative instruments or tailored measures preventing the entry of new aliens.

In addition, there are some points in the CBD scheme that need further clarification. For example, the distinction between the CBD sub-category pathways "horticulture" and "ornamental purpose other than horticulture" is not clear and could result in different interpretations among stakeholders. This is especially important for alien land plants, where about 2,000 species in EASIN are assigned to the "ornamental planting" pathway (Table 1).

Still, the need for interoperability among pathway classification systems is today crucial, considering also the recent IAS Regulation implementation needs. Pathway terminology has historically varied among alien species databases (Hulme et al. 2008), restricting comparisons across data repositories (CBD 2014). In order to tackle this, JRC is revising the EASIN pathway classification system and harmonise it with the CBD scheme, aiming at enhancing interoperability and facilitate exchange of information amongst databases on alien species, but also to support the analysis of pathways and their relative importance for prioritising management and to facilitate the development of response options to tackle IAS.

The alignment of the EASIN species to the CBD pathway sub-categories is challenging and resources demanding due to the high number of EASIN species that need to be revised. In some cases this can be relatively simple; i.e. species assigned to "game animals" correspond either to "fishery in the wild" or to "hunting" in terms of the CBD classification. However, for species related with other pathway subcategories (e.g. 'trade of contaminated commodities") their alignment to the CBD system is more demanding and requires deep search in the scientific literature and any other relevant sources of information. Moreover, pathway assignment for less documented species is subject to uncertainty (Essl et al. 2015), setting the need for experts' judgement. Therefore, a large number of experts will be involved in the revision process of the EASIN pathways, covering a broad range of taxonomic groups and habitats. Emphasis should be given to the terrestrial species due to their large number, with the involvement of additional experts, especially when it comes to invertebrates (mostly insects), higher plants and fungi. In addition, uncertainty values attached to each pathway (Katsanevakis et al. 2013) will be included in the alignment process. Finally, it should be noted that the harmonization process of pathways requires a consensus from the scientific community on commonly accepted definitions and related interpretations of the CBD pathways, ensuring homogeneous alignment outcomes.

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REVIEW ARTICLE

Traits related to biological invasion: A note on the applicability of risk assessment tools across taxa

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Abstract

Biological invasions are occurring frequently and with great impact to agricultural production and other ecosystem services. In response to this, the Australian Weed Risk Assessment (AWRA) was created to assess the potential 'weediness' of plants based on answers to questions related to biogeography, undesirable attributes, and biology or ecology. This basic model has been expanded and adapted for use on other taxa, often without adequate validation. Since invasive insect crop pests are a major economic cost to agricultural production, there is interest in using an expanded model for insects. Here, we review traits related to invasiveness of insects based on a systematic review of the literature. We then compare the identified invasive traits of insects with those identified for plants in the AWRA. Using insects as a case study, we illustrate that although there is some overlap in invasive traits, there are many unique traits related to invasion for both insects and plants. For insects, these traits relate largely to social behaviour. This lack of congruence may also be the case for other taxa. To increase predictive power, a taxon-specific risk assessment tool and deliberate verification are required.

Keywords

Introduction

It is now widely accepted that invasive species are a major cause of global biodiversity loss, and as such, public interest in the topic has increased over recent decades (Didham et al. 2005). By way of increased transportation and international trade, biological invasions are occurring more frequently with increasingly undesirable costs to ecosystem services (Mack et al. 2000, Colautti et al. 2006). Although invasive species are defined in a number of ways with a variety of terms (Lockwood et al. 2013), often they are associated with 'harm' to the newly invaded environment (Mack et al. 2000). Because this is not always the case, and harm can be defined in many ways (Sagoff 2009), we follow Richardson et al. (2000b) and define 'invasive species' as those that have established and spread in a new geographic range.

In the United States alone, it has been estimated that 50 000 non-native species have been introduced, 4 500 of those being arthropods (Pimentel et al. 2005). Narrowed further, about 500 (11%) introduced insect and mite crop pest species have invaded (Pimentel et al. 2005), and the most economically important species of all agricultural pests are non-native (Mack et al. 2000). Approximately 95% of these arthropod introductions are accidental through entrance on plants, soil, ship ballast water, food sources, wood, etc (Pimentel et al. 2005, Rabitsch 2010). These crop pest introductions are estimated to cause US$13.5 billion dollars in damage annually in the United States due to crop loss and additional pesticide use (Pimentel et al. 2005). Economic impacts can also be indirect through restrictions on trade flow and market access changes (Roques et al. 2010). In comparison to the United States, 383 introduced insect species have been documented in the Czech Republic, of which 111 (29%) are considered either greenhouse or storage pests causing economic damage (Sefrova 2014). For just 10 nuisance invasive species (not just insect pests) in Canada, it was estimated that fisheries, forestry, and agriculture suffer a CDN$187 million loss annually (Colautti et al. 2006). In Europe, 1383 alien insects have been introduced and established to date, while the rate of introduction continues to accelerate (Roques et al. 2010). Despite substantial variation, species invasion is a global problem affecting a range of economically important services.

Government regulatory bodies have a legal responsibility to assess the risks of potential biotic invasions that could result in a detriment to plant resources, as dictated by the International Plant Protection Convention treaty (IPPC 1997). Thus, predictive pest risk assessment schemes have been created to assess invasion risks posed by plant species (e.g., Reichard and Hamilton 1997, Pheloung et al. 1999) based on the idea that certain life history traits increase the probability of invasiveness (Baker 1974). Using such schemes, plant species are evaluated for invasion risk according to the number and type of invasive traits they possess. For example, Reichard and Hamilton (1997) created a scheme to predict the invasion of woody plants in North America, yielding ~80% predictive success rate using life history and biogeographical attributes of a plant to predict invasion. In particular, reproductive attributes of the invader were important in predicting the invasive potential of woody plants (Reichard and Hamilton 1997).

Pheloung et al. (1999) expanded the decision tree method employed by Reichard and Hamilton (1997) to produce a computer-based spreadsheet checklist for invasive plants called the Australian Weed Risk Assessment (AWRA). The AWRA comprises 49 equally weighted questions, with sections on biogeography, undesirable attributes, and biology and ecology. The answers to these questions result in a score that informs the user about the potential 'weediness' of the plant, and from there a regulatory decision can be made. Pheloung et al. (1999) conclude that the AWRA can serve as a biosecurity tool to identify potentially invasive weeds, and can be modified for use in other locations. Currently the AWRA is used by the Department of Agriculture in Australia as a component of their multi-tiered WRA process (Department of Agriculture and Water Resources 2015). A comparison of the AWRA and other models (such as Reichard and Hamilton [2007]) found the AWRA to be the most accurate (Jefferson et al. 2004). The AWRA has since been modified and tested for invasive plants in New Zealand (Pheloung et al. 1999), the Hawaiian Islands (Daehler and Carino 2000), other Pacific islands (Daehler et al. 2004), Japan (Kato et al. 2006), the Czech Republic, and Florida (Gordon et al. 2008), with fairly consistent results. However, when the AWRA was tested for invasive plants in Canada, it was found to reject a high proportion of non-weedy species (McClay et al. 2010). Since Canada is characterized by cool, short growing seasons, simple alterations to the system that take cold-hardiness into account could increase the predictive power of the AWRA in Canada (McClay et al. 2010). Therefore, this system is generally accepted to function as a template for weed risk assessments across tropical and temperate geographies (Gordon et al. 2008).

Following the success of the AWRA, attempts have been made to create similar models for use with other taxa. Some models have evaluated potential invasive traits based on *a priori* hypothesized characteristics. For example, Causton et al. (2006) proposed a simple scoring system for identifying insects that are potentially invasive to the Galapagos Islands. However, it is not clear why Causton et al. (2006) chose the traits that they did, as the selection does not appear to be based on any systematic analysis. Similarly, Kolar and Lodge (2002) and Marchetti et al. (2004) do not provide reasoning for their selection of traits that were considered in their models for fish invasion. Additionally, the AWRA has been used as a basis for risk assessment schemes that are generalized for other non-native taxa (Table 1). Although this method might be useful because of its generality, it may not be valid if traits that are relevant to weediness in plants are not applicable to invasiveness in other taxa; its applicability remains unknown because the traits assessed in the AWRA have not been tested for relevance in other taxa.

The issue of transferability of invasive traits across taxa was investigated by Hayes and Barry (2008), who tested the significance of 115 invasive characteristics across seven taxonomic groups. Of the 49 studies included in their systematic review of predictors of invasion success, only two pertained to insects. Although they found some consistency in trait differences between native and invasive species, this was mainly only for plants. Overall, climate or habitat match was the only trait related to invasiveness *across* biological groups (Hayes and Barry 2008). Therefore, it is unclear whether a

Table 1. Examples detailing when the AWRA has been adapted for use on taxa other than plants.

Risk assessment model	Taxon	Reference
UK risk assessment scheme	Freshwater fish, marine fish, marine invertebrates, amphibian	Baker et al. 2008
FISK	Freshwater fish	Copp et al. 2005, Copp et al. 2008
MFISK	Marine fish	Copp et al. 2008
FI-ISK	Freshwater invertebrates	Copp et al. 2008
MI-ISK	Marine invertebrates	Coop et al. 2008
AmphISK	Amphibians	Copp et al. 2008
Infectious Agent Risk Assessment Module	Infectious agents	Copp et al. 2008
Generic Pre-screening Module	All other taxa	Copp et al. 2008

scheme created for use on plants can be generalized for use with other taxa. Traits that are related to invasiveness in plants may not generalize to other taxa, and if they do, their importance may not be similar across taxa.

Currently, there is no adequately validated, trait-based approach to risk assessment for insects, as there is for plants. Additionally, it remains unclear whether traits that are related to invasiveness in plants are generalizable to other taxa. Formal analyses are needed to determine traits predictive of invasiveness in taxa other than plants to ascertain the validity and generality of using a single risk assessment scheme across taxa. Because the AWRA has been expanded for use on other taxa, without validation, the aim of this paper is to compare questions in the AWRA with traits in the literature that are claimed to be related to insect invasion success.

As a first step in evaluating the generalizability of an invasion risk assessment scheme, we performed a systematic review of the literature for traits that are claimed to affect invasiveness in any insects. We compare these traits with those that are used to assess weediness in plants, and then discuss the potential validity of, and problems with, generalizing the AWRA for assessing the invasion risk of insects. We include all types of insects to gather the most trait data possible. This review, synthesis, and comparison of information is an important precursor to a larger project that will evaluate predictive traits and critical pathways of insect invasion with the overall objective of producing a comprehensive insect pest risk assessment scheme.

Methods

To determine whether there is congruence between traits related to invasion success in both plants and insects, we conducted a literature search that was completed in August 2015 using the Web of Science database (Thomson Reuters, New York, USA) and the following Boolean search adapted from Hayes and Barry (2008) in the "topic" function: (attributes OR correlates OR characteristics) AND (alien OR non-native OR non-indigenous OR exotic OR invasive) AND (invasion OR establishment) AND

(success OR predict) AND (insect OR invertebrate). The search resulted in approximately 3 500 results; from this, 125 articles were identified as relevant (i.e. they minimally discussed a biological or environmental trait of an invasive insect) by examining the article abstract. Papers were included in our analysis if they tested or claimed traits that were related to invasion success in insects (i.e. other classes of invertebrates were excluded). Papers that were included were then screened for experimental, observational, or anecdotal information pertaining to traits of invasive or native insects. These data were extracted and compiled into a spreadsheet to highlight whether differences existed between invasive and native insects, and between invasive plants and invasive insects. The data we included were: the trait being tested or claimed, the trait type (life history or environmental), the trait states (invasive vs. native), the reference, and what the significance or application of this result was.

Results and discussion

We identified a total of 79 traits that were claimed to have some relation to invasiveness in insects (Table 2). We grouped the most similar traits together to avoid repetition, and we assigned categorical nomenclature (Table 3 and 4). Traits that related to the same life processes were assigned to the same group. For example, the trait *dispersal* includes flight speed, flight distance, flight temperature, dispersal type, dispersal habitat, and colonization ability. Consolidation of similar traits resulted in a total of 29 trait groups that are allegedly related to invasion success in insects. These 29 trait groups were divided into life history (Table 3) and environmental traits (Table 4) and compared against plant traits used to assess weediness in the AWRA to determine if there are clear analogues between insect and plant invasiveness traits.

For insect invasion-related traits, it is noteworthy that some of the evidence is contradictory, i.e., a positive relation with invasiveness in some cases and a negative relation in others, and universal statements may not be accurate. For example, *body size* can either be positively or negatively associated with invasion (Table 2). It is self-evidently problematic to include contradictory traits in a risk assessment scheme based on universal statements.

Analogous insect and plant invasiveness traits

We identified 18 of 29 claimed invasive trait groups for insects that were represented by clear analogues of weedy traits in plants (Tables 1 and 2). This might lend some validity to a generalization of the AWRA for use on insects. However, whether these analogous traits infer invasiveness in insects in the same way, or to the same degree, as they do in plants has yet to be formally tested. By using decision tree modelling or similar methods to identify traits that are most important to invasion of insects, it would be possible to assess whether these traits hold similar ranks of importance

Table 2. Life history and environmental traits related to invasion, highlighting the suggested differences between invasive and native insects that were found through an extensive literature review.

Insect trait	Trait component	Differences between invasive and non-invasive insects	Type of evidence†
Life history traits			
Feeding guild	Feeding niche	Invasive insects predominantly sap feeders and detritivores (Kenis et al. 2007)	C
	Feeding guild	Herbivores more likely to establish than predators and parasitoids (Engelkes and Mills 2011); Parasitoids more likely to establish than predators (Kimberling 2004)	A, C
	Taxon	Diptera and Lepidoptera fastest to disperse, Coleoptera slowest (Paynter and Bellgard 2011) Invasive species predominantly Hemiptera (56.4 %), Lepidoptera (14.9 %), and Hymenoptera (12.9 %) (Matosevic and Zivkovic 2013); Invasive species predominantly Homoptera (39 %), Coleoptera (19 %), Lepidoptera (13 %), and Hymentoptera (13 %) (Mattson et al. 2007); Invasive species predominantly Coleoptera, Sternorrhyncha, and Psocoptera (Kenis et al. 2007); Homoptera and Lepidoptera most likely to establish (Peacock and Worner 2008)	C
	Feeding method	Miners, borers, and leaf-rollers disperse faster than external feeders, and root-, rosette-, and seed-feeders (Paynter and Bellgard 2011) Internal feeders more likely to establish than external feeders (Kimberling 2004) Insects that use single host species are more likely to establish than those that use multiple hosts (Mondor et al. 2007)	C
Diet breadth	Diet breadth or Host specificity	Invasive insects have a wide diet breadth (generalist) compared to natives (Moller 1996, Cervo et al. 2000, Kasper et al. 2004, Kimberling 2004, Moeser and Vidal 2005, Snyder and Evans 2006, Mondor et al. 2006, Ward and Masters 2007, Wilson et al. 2009, Orledge et al. 2010, Andersen et al. 2011)	A, C, E, O
	Voltinism (number of generations per year)	Dispersal rate increases as number of generations/year increases (Yan et al. 2005, Paynter and Bellgard 2011); Insects with multiple generations per year more likely to establish than insects with one generation per year (Kimberling 2004)	A, C
Generation onset	Adult emergence	Invasive insects emerge earlier than natives (Hack and Lawrence 1995, Pickett and Wenzel 2000, Gamboa et al. 2002, Gamboa et al. 2004, Boivin et al. 2008)	A, E, O
	Onset of egg laying	Invasive insects start laying eggs earlier than natives (Kajita and Evans 2010b, Laugier et al. 2013)	E, O
	Growth rate	Invasive insects have rapid growth rates compared to natives (Labrie et al. 2006, Ward and Masters 2007)	A, O
Development	Preimaginal (pre-adult) development time	Invasive insects have shorter preimaginal development time than natives (Cervo et al. 2000, Gamboa et al. 2004, Boman et al. 2008, Delatte et al. 2009); Invasive insects have a longer preimaginal development time than natives (Bonato et al. 2007)	A, E, O

Insect trait	Trait component	Differences between invasive and non-invasive insects	Type of evidence†
	Generation time	Invasive insects have shorter generation time than natives (Facon et al. 2011); Short generation times increase colonization success (Yan et al. 2005)	A, E
	Intrinsic rate of increase	Invasive insects have higher intrinsic rate of increase than natives (Crawley 1987, Duyck et al. 2007, Delatte et al. 2009, Orledge et al. 2010)	A, C, E, O
	Intrinsic death rate	Invasive insects have lower intrinsic death rate than natives (Duyck et al. 2007, Foucaud et al. 2013)	A, E, O
	Flight speed	Invasive insects have higher flight speeds than natives (Sun et al. 2013); Flight speed can enhance invasion (Lombaert et al. 2014)	A, E, O
	Flight distance	Invasive insects can fly longer distances than natives (Yan et al. 2005)	A
	Flight temperature	Invasive insects can fly within a broader range of temperatures than natives (Yan et al. 2005)	A
Dispersal	Dispersal type	Insects capable of flight more likely to disperse than wind-dispersed or crawling species (Moller 1996, Paynter and Bellgard 2011); Macropterous individuals increases dispersal ability (Niemela and Spence 1991)	A, C, O
	Dispersal habitat	Aquatic insects disperse faster than terrestrial insects (Paynter and Bellgard 2011); Permanent stream flow enhances invasion (Holway 1998)	C, O
	Colonization ability	Invasive insects have better colonization ability than natives (Harcourt et al. 1998, Yan et al. 2005)	A, O
Desiccation resistance	Desiccation resistance	Invasive insects more resistant to desiccation than natives (Parkash et al. 2014)	E, O
Mating behaviour	Copulatory behaviour	Invasive insects faster to copulate than natives (Laugier et al. 2013); Female invasive insects fertilized by more males than native females (Laugier et al. 2013); Invasive insects copulate more effectively than natives (Liu et al. 2007, Crowder et al. 2010)	A, E
Thermal resistance	Temperature tolerance	Invasive insects have lower temperature tolerance than natives (Wuellner and Saunder 2003, Bonato et al. 2007, Peacock and Worner 2008, Delatte et al. 2009, McGrannachan and Lester 2013, Parkash et al. 2014); Invasive insects have higher temperature tolerance than natives (Michaud 2002, Wuellner and Saunder 2003, Bonato et al. 2007, Delatte et al. 2009, Ju et al. 2013); Invasive insects have a higher lower developmental threshold than native insects (Jarošík et al. 2015)	C, E, O
Overwintering behaviour	Aggregate overwintering	Invasive insects overwinter in aggregate, whereas natives do not (Cottrell and Shapiro-Ilan 2003)	E, O
	Overwintering site	Invasive insects overwinter in sheltered habitat, whereas natives do not (Yan et al. 2005)	A
	Winter survival	Invasive insects have higher winter survival than natives (Inoue 2011, Raak-van den Berg et al. 2012)	E, O

Insect trait	Trait component	Differences between invasive and non-invasive insects	Type of evidence†
Body size	Body size	Invasive insects are smaller than natives (Crawley 1987, McGlynn 1999b, Gamboa et al. 2004, Ness et al. 2004, Cremer et al. 2006, Mondor et al. 2006, Cremer et al. 2008, Wilson-Rich and Starks 2010, Abril et al. 2013); Small insects more likely to establish than large insects (Lawton et al. 1986, Lester 2005); Invasive insects are larger than natives (Ings et al. 2006, Delatte et al. 2009, Kajita and Evans 2010a, Kajita and Evans 2010b); Large body size may promote invasion success (Moller 1996)	A, C, E, O
	Offspring mass	Invasive insect offspring mass smaller than native offspring mass (Armstrong and Stamp 2003)	E
Functional group	Functional group	Invasive insects predominantly cryptic, generalized Myrmicinae, and opportunists (McGlynn 1999a)	C
	Lifetime performance	Invasive insects have higher lifetime performance (product of hatching rate, larval survival, and subsequent fecundity) than natives (Facon et al. 2011); High progeny production increases colonization success (Yan et al. 2005)	A, E
	Egg laying behaviour	Insects that lay eggs in batches less likely to become invasive (Crawley 1987)	
	Egg size	Invasive insects lay larger eggs than natives (Duyck et al. 2007, Delatte et al. 2009); Invasive insects lay smaller eggs than natives (Kajita and Evans 2010a)	A, E, O
Fecundity characters	Fecundity	Invasive insects are fecund later than natives (Armstrong and Stamp 2003); Invasive insects allocate more resources to fecundity than natives (Curtis et al. 2005); Invasive insects have higher fecundity than natives (Pickett and Wenzel 2000, Michaud 2002, Duyck et al. 2007, Boivin et al. 2008, Delatte et al. 2009, Kajita and Evans 2010a, Kajita and Evans 2010b, Laugier et al. 2013); Invasive insects have higher net reproductive rate than natives (Duyck et al. 2007, Delatte et al. 2009); Invasive insects have higher gross reproductive rate than natives (Moller 1996, Duyck et al. 2007);	A, E, O
	Egg viability	Invasive insects have higher egg viability than natives (Michaud 2002)	E, O
	Juvenile survival	Invasive insects have higher juvenile survival than natives (Duyck et al. 2007, Delatte et al. 2009);	A, E, O
	Percentage paternity	Invasive insects have higher percentage paternity than natives (Laugier et al. 2013);	E
	Pupal mass	Invasive insect pupae are larger than natives (Duyck et al. 2007)	A, O
Competitive ability	Competitive ability, adaptive ability	Invasive insects can outcompete natives (Holway 1999, Thomson 2004, Snyder and Evans 2006, Rowles and O'Dowd 2007, De Barro and Bourne 2010, Wilson and Holway 2010, Sanders and Suarez 2011, Auger-Rozenberg and Roques 2012, Wilder et al. 2013); Interspecific competition may limit invasion (LeBrun et al. 2007); Invasive insects can use resources that natives cannot (Orledge et al. 2010); Invasive insects can use resources in their introduced range that they cannot in their native range (Yan et al. 2005, Sun et al. 2013); Invasive insects avoid predation through crypsis (Yan et al. 2005)	A, C, E, O

Insect trait	Trait component	Differences between invasive and non-invasive insects	Type of evidence[†]
Reproduction	Predation	Invasive insects prey upon native insects (Wilson and Holway 2010)	E, O
	Reproductive strategy	Invasive insects may be asexual, whereas natives reproduce sexually (Moller 1996, Mondor et al. 2006, Pecoud et al. 2008, Mikheyev et al. 2009, Caron et al. 2013)	A, C, E, O
Oviposition site	Oviposition site	Insects that oviposit internally more likely to establish than insects that oviposit outside of host (Kimberling 2004)	C
Sex ratio	Sex ratio	Invasive insects have female-skewed sex ratio, whereas natives do not (Michaud 2002, Liu et al. 2007)	E, O
Intraguild predation	Intraguild predation	Invasive insects are stronger intraguild predators than natives (Michaud 2002, Snyder et al. 2004, Roy et al. 2008a); Native insects consume more conspecific eggs than invasive insects (Michaud 2002); Invasive insects consume more heterospecific eggs than natives (Michaud 2002, Ware et al. 2009)	E, O
Foundress activity	Foundress activity	Invasive insect foundresses build and repair nests more often than natives (Armstrong and Stamp 2003); Invasive insect foundresses more aggressive towards offspring than natives (Armstrong and Stamp 2003)	E
Aggression	Level of aggression	Invasive insects show less intraspecific aggression compared to natives (Holway et al. 1998, Suarez et al. 1999, Le Breton et al. 2004, Errard et al. 2005, Cremer et al. 2008, Fournier et al. 2009, Perdereau et al. 2011, Sanders and Suarez 2011, Suhr et al. 2011, Ugelvig and Cremer 2012, Hoffmann 2014); Low intraspecific aggression may promote invasion success (Moller 1996); Invasive insects show more interspecific aggression than natives (Human and Gordon 1999, Cremer et al. 2006, Duyck et al. 2006, Snyder and Evans 2006, Rowles and O'Dowd 2007, Carpintero and Reyes-Lopez 2008, Fournier et al. 2009, Blight et al. 2010, Perdereau et al. 2011)	A, E, O
	Usurpation	Native insects attempt usurpation more often than invasive insects (Gamboa et al. 2002, Gamboa et al. 2004); Usurpation may increase establishment success (Moller 1996)	A, O
	Colony productivity	Invasive insect nests (combs) contain more cells than natives (Pickett and Wenzel 2000, Gamboa et al. 2002, Gamboa et al. 2004); Colony budding may increase establishment success (Moller 1996, Tsusui and Suarez 2003); Invasive insects produce more adults than natives (Gamboa et al. 2004)	A, E, O
Colony characteristics	Relatedness to queen	Invasive insects are less related to their queen than natives (Ross et al. 1996)	O
	Polygyne social form	Invasive insects are polygyne (multiple egg-laying queens per nest) (Yang et al. 2012)	O
	Unicoloniality	Invasive insects are unicolonial, whereas natives are multicolonial (Tsusui and Suarez 2003, Le Breton et al. 2004, Errard et al. 2005, Cremer et al. 2008, Suarez et al. 2008, Fournier et al. 2009, Wilson et al. 2009, Sanders and Suarez 2011, Ugelvig and Cremer 2012, Hoffmann 2014)	A, E, O
	Sociality	Social insects are likely to become invaders (Moller 1996)	A

Insect trait	Trait component	Differences between invasive and non-invasive insects	Type of evidence†
	Recognition cues	Invasive insects are more chemically similar than natives (Errard et al. 2005, Cremer et al. 2008, Brandt et al. 2009, Fournier et al. 2009, Perdereau et al. 2011); Invasive insects are more genetically similar than native insects (Tsusui and Suarez 2003, Suarez et al. 2008, Ugelvig and Cremer 2012)	A, E, O
	Colony longevity	Shift from small, annual colony to large, perennial colony can increase invasion success (Wilson et al. 2009)	E, O
Queen characteristics	Queen longevity	Queen longevity is greater in invasive insects than in natives (Gamboa et al. 2002)	O
	Queen number	Invasive insects have more queens per nest than natives (Ross et al. 1996, Tsusui and Suarez 2003, Abril et al. 2013); Invasive insects produce more gynes (reproductive female caste) than native insects (Pickett and Wenzel 2000, Ings et al. 2006)	A, E, O
	Nesting habitat	Ground nesting ants more likely to establish than arboreal ants (Suarez et al. 2005); Invasive insects nest in urban areas, whereas natives nest in rural areas (Downing 2012)	C, O
Nesting	Nest predation	Invasive insect nests suffer less predation than native nests (Cervo et al. 2000); Invasive insects more likely to re-nest after predation than natives (Gamboa et al. 2004)	A, O
	Nest reutilization	Invasive insects may reuse a nest, whereas natives seldom do (Cervo et al. 2000)	A
Environmental traits			
	Presence of predators	Presence of predators decreases invasive insect abundance and increases native insect development rate (Juliano et al. 2010); Dispersal increases as presence of parasitoids in native range increases (Paynter and Bellgard 2011); Absence of predators/parasitoids increases the likelihood of establishment (Lawton et al. 1986, Tsusui and Suarez 2003, Yan et al. 2005, Snyder and Evans 2006, Suarez et al. 2008, Ugelvig and Cremer 2012)	A, C, E
Natural enemies present	Rate of parasitism	Invasive insects parasitized less often than natives (Cervo et al. 2000, Gamboa et al. 2002, Gamboa et al. 2004, Allen et al. 2007, Cremer et al. 2008, Gray et al. 2008, Orledge et al. 2010, Comont et al. 2014); Invasive insects have higher parasite prevalence than natives (Jones and Brown 2014)	A, C, E, O
	Fungal susceptibility	Invasive insects less susceptible to fungal infections compared to natives (Cottrell and Shapiro-Ilan 2003)	E, O
	Immunocompetence	Invasive insects have a lower immune response than natives (Wilson-Rich and Starks 2010, Manfredini et al. 2013)	E, O
	Antimicrobial defence	Invasive insects have an efficient immune system (Vilcinskas et al. 2013)	E
Environmental matching	Host range	Invasive insects have wider host range than natives (Crawley 1987, Peacock and Worner 2008); Certain mutualistic interactions will enhance invasion success (Wilder et al. 2011); Presence of suitable host species increases invasion success (Brooks et al. 2012, Graziosi and Rieske 2012, Sun et al. 2013); Synchronization with host species increases invasion success (Harcourt et al. 1988); Phenological plasticity increases invasion success (Ward and Masters 2007)	A, C, E, O

Insect trait	Trait component	Differences between invasive and non-invasive insects	Type of evidence[†]
	Soil type	High-moisture soils promote insect invasion (Bolger 2007); Invasive insects more active at higher soil temperatures than natives (Human et al. 1998)	O
	Humidity	Invasive insects prefer high humidity, whereas natives do not (Walters and Mackay 2003); Invasive insects have more extreme high and low humidity tolerances than natives (Wuellner and Saunder 2003)	E, O
	Elevation	Invasive insects prefer low elevation, whereas natives prefer high elevation (Human et al. 1998, Arndt and Perner 2008, Fitzgerald and Gordon 2012)	O
	Climate matching	Invaded range must be climatically suitable for the invasive insect (Simberloff 1989, Holway 1998, Koch et al. 2006, Gray et al. 2008, Roura-Pascual et al. 2011, Sun et al. 2013)	A, C, E, O
	Light tolerance	Invasive insects have more extreme high and low light tolerances than natives (Wuellner and Saunder 2003)	O
	Habitat type	Invasive insects prefer dry cultivated fields over shrublands and plantations (Roura-Pascual et al. 2011); Invasive insects more abundant in cool, dry areas, whereas native insects are more abundant in warm, humid areas (Parkash et al. 2014); Invasive insects prefer open land, whereas natives prefer forests (Ishii et al. 2008); Invasive insects prefer agricultural lands (56.4 %), followed by parks and gardens (28.7 %), and woodlands and forests (14.9 %) (Matosevic and Zivkovic 2013)	C, E, O
Propagule pressure	Propagule pressure	Greater numbers of introduced propagules and greater numbers of introductions increase the probability of establishment (Beirne 1975, Simberloff 1989, Hopper et al. 1993, Hight et al. 1995, Memmott et al. 1998, Grevstad 1999, Memmott et al. 2005, Suarez et al. 2005, Walters and Mackay 2005, Ward and Masters 2007, Mikheyev et al. 2008, Tobin et al. 2013); Propagule size found not to affect establishment (Sagata and Lester 2009)	A, C, E, O
Disturbance	Environmental disturbance	Environmental disturbance decreases abundance of native species while increasing abundance of invasive species (Human et al. 1998, Zettler et al. 2004, Arndt and Perner 2008, Suarez et al. 2008, Fitzgerald and Gordon 2012); Environmental disturbance positively associated with invasion success (Simberloff 1989, Snyder and Evans 2006, Roura-Pascual et al. 2011); Environmental disturbance decreases the abundance of invasive species (Arndt and Perner 2008); Environmental disturbance negatively associated with invasion success (Yeates et al. 2012)	A, C, E, O
Resistance evolution	Resistance to insecticide	Invasive insect able to evolve resistance to insecticide, leading to exclusion of native insect (Crowder et al. 2010)	A, E
Biotic resistance	Biotic resistance	Insects able to invade due to lack of biotic resistance in the native assemblage (Niemelä and Spence 1991, Yan et al. 2005); Areas with high biotic resistance are less prone to invasion (Walters and Mackay 2005, Blight et al. 2010, Roura-Pascual et al. 2011)	A, C, E, O

Insect trait	Trait component	Differences between invasive and non-invasive insects	Type of evidence†
Foraging	Foraging rate	Foraging rate is greater in invasive insects than natives (Human and Gordon 1996, Holway et al. 1998, Gamboa et al. 2002, Ings et al. 2006, McGrannachan and Lester 2013)	E, O
	Predatory efficiency	Invasive insects more efficient at capturing prey than native insects (Labrie et al. 2006, Dejean et al. 2007); Invasive insects consume more prey than native insects (Labrie et al. 2006); Invasive insects are more efficient at exploiting resources than native insects (Sanders and Suarez 2011)	A, E, O
	Foraging distance	Invasive insects will travel farther than native insects to forage (Perdereau et al. 2011)	E, O
	Foraging behaviour	Invasive insects show flexible foraging behaviour (Wilson-Rankin 2014); Invasive insects forage throughout the year, whereas native insects do not forage during winter (Wuellner and Saunder 2003); Invasive insects start foraging earlier in the day than natives (Gamboa et al. 2004)	E, M, O
	Bait recruitment	Invasive insects recruit to bait faster than natives (Holway 1999, Dejean et al. 2007, Rowles and O'Dowd 2007, McGrannachan and Lester 2013); Invasive insects recruit to more bait types than natives (Human and Gordon 1996, Holway 1998, Holway 1999)	E, O

† A = Anecdotal information (no evidence given), C = correlational analysis (analyses using pre-existing data), E = experimental (standard experiment using treatments and controls), M = meta-analysis, O = observational (observational study with no experimental manipulation).

Table 3. A comparison of life history traits related to insect invasion and traits considered in the Australian weed risk assessment for plants.

Insect trait	Invasive trait measures	Plant equivalent
Feeding guild	Feeding guild, feeding site, feeding niche, taxon, lifestyle category	Parasitic, allelopathic
Diet breadth	Diet breadth, host specificity	Broad climatic suitability (environmental versatility)
Desiccation resistance	Desiccation resistance	Broad climatic suitability (environmental versatility)
Thermal resistance	Temperature tolerance	Broad climatic suitability (environmental versatility)
Overwintering behaviour	Winter survival, aggregate overwintering, overwintering site	Broad climatic suitability (environmental versatility)
Generation onset	Generations per year, adult emergence, onset of egg laying	Minimum generative time
Development	Preimaginal development time, growth rate, generation time, intrinsic rate of increase, intrinsic death rate	Minimum generative time
Dispersal	Dispersal type, dispersal habitat, colonization ability, flight speed, flight temperature, flight distance	Dispersal mechanisms
Body size	Body size, offspring mass	Seed size
Functional group	Functional group	Plant type
Mating behaviour	Copulatory behaviour	Reproduction
Fecundity	Fecundity, egg viability, Net Reproductive Rate (NRR), egg size, Gross Reproductive Rate (GRR), percentage paternity, juvenile survival, lifetime performance, egg laying behaviour, pupal mass	Reproduction
Reproduction	Reproductive strategy	Reproduction
Competitive ability	Competitive ability, adaptive ability, predation	Climbing or smothering growth habit
Oviposition site	Oviposition site	None
Sex ratio	Sex ratio	None
Intraguild predation	Intraguild predation	None
Foundress activity	Foundress activity	None
Aggression	Aggression, usurpation	None
Colony characteristics	Recongnition cues, sociality, queen relatedness, colony productivity, polygyne social form, unicoloniality, colony longevity	None
Queen characteristics	Queen number, queen longevity	None
Nesting	Nesting habitat, nest predation, nest reutilization	None
None	None	Species has weedy races

Insect trait	Invasive trait measures	Plant equivalent
None	None	Species has a congeneric weed
None	None	Produces spines, thorns, or burrs
None	None	Unpalatable to grazing animals
None	None	Toxic to animals
None	None	Causes allergies or is otherwise toxic to humans
None	None	Prolific seed production
None	None	Evidence that a persistent propagule bank is formed (>1 yr)

Table 4. A comparison of environmental traits related to insect invasion and traits considered in the Australian WRA for plants.

Insect trait	Invasive trait measures	Plant equivalent
Natural enemies present	Presence of predators, rate of parasitism, fungal susceptibility, antimicrobial defense, immunocompetence	Effective natural enemies present
Environmental matching	Host range, climate matching, soil type, humidiry, elevation, light tolerance, habitat type	Species suited to climate, quality of climate match data, broad climatic suitability (environmental versatility)
Disturbance	Environmental disturbance	Broad climatic suitability (environmental versatility); tolerates or benefits from mutilation, cultivation, or fire
Propagule pressure	Propagule pressure	Dispersal mechanisms
Resistance evolution	Resistance to insecticide	None
Biotic resistance	Biotic resistance	None
Foraging	Foraging rate, foraging behaviour, predatory efficiency, foraging distance, bait recruitment	None
None	None	Species is highly domesticated
None	None	Species has become naturalized where grown
None	None	Native or naturalized in regions with extended dry periods
None	None	Species has a history of introductions outside its natural range
None	None	Species is naturalized beyond its native range
None	None	Garden/amenity/disturbance weed
None	None	Weed of agriculture/horticulture/forestry
None	None	Environmental weed
None	None	Creates a fire hazard to natural ecosystems
None	None	Is a shade tolerant plant at some stage in its life cycle
None	None	Grows on infertile soils
None	None	Forms dense thickets
None	None	Well controlled by herbicides

between plants and insects. Furthermore, certain insect trait groups can be measured through numerous proxies. For example, the insect *development* trait group comprises a number of measures related to development that are potentially indicative of invasion. In contrast, the AWRA has only one question related to plant development, called *minimum generative time*. The fact that more developmental characteristics were claimed to be related to invasion in insects does not necessarily mean that development is more important in the invasion success of insects than in plants. This discrepancy may mean that more questions could be developed relating to insect development in a modified pest risk assessment. Conversely, if the multiple insect development measures have similar reliability, the one that is easiest to measure (e.g., development time, rather than development plasticity) could be chosen for inclusion in the risk assessment. However, it is possible that some of these trait groups are more predictive than others, and as such, all else being equal, the measures that are most predictive should be included in a risk assessment if multiple traits are correlated. This same issue arises with other insect trait groups, particularly *generation onset*, *overwintering behaviour*, *fecundity characters*, *environmental matching*, *foraging*, and *colony characteristics*.

Unique invasiveness trait groups of insects

We identified 11 of 29 trait groups that seem to be uniquely related to insect invasion and have no clear analogue to plant traits. These trait groups involved both life history and the environment. This result suggests that a pest risk assessment developed for plant invasion may not be applicable for insects because traits that are important to insect invasion may be missing from the assessment. We next examine these unique insect life history and environmental trait groups in further detail.

Sex ratio: In sexually reproducing species, the intrinsic rate of population increase is generally limited by the number of females rather than the number of males. For example, *sex ratio*, specifically female dominance, can increase the successful establishment of biological control agents such as *Harmonia axyridis* (Asian lady beetle; Michaud 2002, Kimberling 2004). *Harmonia axyridis* has a female-skewed sex ratio that may give it intrinsic advantages over the native *Cycloneda sanguinea* (spotless lady beetle; Michaud 2002). A female-skewed sex ratio can compound the effect of high *per capita* fecundity, leading to explosive population growth, by which such invasive species may outcompete native species, or escape control by natural enemies. Aspects of plant reproduction are considered in the AWRA, such as self-fertilization and viable seed production, which may be distantly analogous to sex ratio in insects.

Oviposition site: According to Kimberling (2004), *oviposition site* can influence the establishment of alien insects whereby those who oviposit on or inside the host are more likely to establish. Although Kimberling does not discuss the reasoning for this association, we assume that larvae are not required to find a host upon hatching, so that individuals are more likely to achieve their developmental requirements. By contrast, eggs that are deposited elsewhere would be more susceptible to damage and

death before finding a suitable host, and individuals would be less likely to complete development. Although seed dispersal mechanisms are considered in the AWRA, *oviposition site* is not included because it is not relevant to plants.

Intraguild predation: Organisms that kill potential competitors within their feeding guild are referred to as intraguild predators. For example, the invasive *H. axyridis* is more likely than the native *Coccinella septempunctata* (seven-spot ladybird) to consume the cadavers of *Pandora neoaphidis* fungus-infected aphids (Roy et al. 2008a), a form of intraguild predation. Although study of other members of the aphidophagous and coccidophagous guilds is lacking, evidence indicates that *H. axyridis* may affect the population of *P. neoaphidis* more negatively than would *C. septempunctata*, leading to a greater competitive advantage for *H. axyridis*. In addition, *H. axyridis* will consume more heterospecific eggs (i.e., *Adalia bipunctata* eggs) than the native *A. bipunctata* will consume *H. axyridis* eggs (Ware et al. 2009). This also indicates a competitive advantage for the invasive insect over the native.

Resistance evolution: Because insecticides are commonly used to control invasive insects, the evolution of pesticide resistance would benefit species that are capable of evolving rapidly (Crowder et al. 2010). This trait has been recognized in the invasive biotype of the cryptic *Bemisa tabaci* (whitefly) species complex, which was able to displace other whiteflies competitively through adaptation to an insecticide (Crowder et al. 2010). The AWRA does not explicitly consider whether a plant is able to evolve resistance to herbicides; however, the trait *well controlled by herbicides* is included as a persistence attribute. Although it is possible that a plant will evolve resistance to an herbicide, a plant may resist control by an herbicide due to the specific mode of action; therefore, although these two traits may seem similar (*resistance evolution* and *well controlled by herbicides*), we consider them as different.

Biotic resistance: Native species richness can affect the extent to which biological invasions are likely to occur such that environments with greater species richness are often less easily invaded (Byers and Noonburg 2003). Although this may be a scale-dependent effect with multiple contributing factors such as disturbance, propagule pressure, and environmental stress (Byers and Noonburg 2003), environments with high *biotic resistance* by native fauna (i.e. areas with an abundance of similar species or containing competitors and predators) may provide protection against foreign invaders. For example, certain native ant species provide biotic resistance against the invasion of *Linepithema humile* (Argentine ant; Blight et al. 2010, Roura-Pascuala et al. 2011). Although there is evidence for biotic resistance against invading plants (Maron and Vila 2001), it is possible that this trait is not included in the AWRA because it could be a difficult parameter to measure. *Biotic resistance* may also be correlated to plant invasion through other explicit traits and therefore not warranted for inclusion in the AWRA. For example, at large scales, native and exotic plant diversity are positively related because they are driven by factors related to spatial heterogeneity (e.g. differences in soil measures such as soil depth and nitrogen; Davies et al. 2005).

Foraging: There is considerable diversity in the foraging abilities and behaviours of insects that affect their invasive potential. The *foraging* trait group includes the traits:

search efficiency, *foraging rate*, *bait recruitment*, *foraging behaviour*, *predatory efficiency*, and *foraging distance* (Table 2). For example, invasive ants are able to dominate native communities with respect to the rate and efficiency with which they forage, which may alter the food web structure in their favour (Human and Gordon 1996, Holway 1998, Holway 1999, Gamboa et al. 2002, Ings et al. 2006, Dejean et al. 2007, Rowles and O'Dowd 2007, McGrannachan and Lester 2013). Invasive ants have been found to forage at times of the year when native ants do not (Wuellner and Saunder 2003), and invasive wasps exhibit flexible foraging behaviour (Wilson-Rankin 2014). Overall, these traits imply dominance over the native community with respect to resource acquisition and may be important in invasion success. Although plants do not actively forage or recruit to bait, the AWRA does consider whether a plant is able to grow on infertile soils or fix nitrogen; these characteristics relate to the ability of the plant to acquire water and nutrients from the soil, which is related to but different from *foraging* behaviour in insects (but see e.g. Sutherland and Stillman 1988 for an alternative perspective).

Colony characteristics: Invasive ants (Holway et al. 2002), bees (Goulson et al. 2003), and wasps (Beggs et al. 2011) are some of the most widely studied invasive insects, and thus, many characteristics of social insect colonies are claimed to affect invasion potential. Traits included in this category are: greater *colony productivity* and *longevity*, decreased *relatedness to queen*, *polygyne social form*, *sociality*, *unicoloniality*, and *recognition cues* (Table 2). These traits are associated with colony structure. Certain traits lead to a competitive advantage for invasive insects; in general, large, unicolonial forms confer invasiveness in social insects by increasing the rates of colony growth and spread (Moller 1996, Tsusui and Suarez 2003). Loss of genetic diversity (Tsusui and Suarez 2003, Suarez et al. 2008, Ugelvig and Cremer 2012) and shifts in colony structure (Wilson et al. 2009) are also related to sustained rapid growth and dispersal of invasive social insects. This is thought to be the case because large supercolonies can be formed from many genetically similar individuals, making the colony more successful. It may be the case that plant coloniality (selfing, reproducing by extensive rhizomes) plays a similar role, although the mechanisms differ.

Foundress activity: Female founders (foundresses) can exemplify different behaviours within the colony. Armstrong and Stamp (2003) found that certain *foundress activity* (higher aggression towards offspring and higher nest repairing tendency) is related to invasiveness in *Polistes dominulus* (European paper wasp) as compared to the native *P. fuscatus* (northern paper wasp). It is unclear whether the aggression of *P. dominulus* foundresses leads to higher colony productivity, but the tendency of *P. dominulus* to be more opportunistic may increase its success as an invasive species (Armstrong and Stamp 2003). Prior to Armstrong and Stamp's (2003) work, it was thought that greater foundress activity would increase foraging behaviour of the workers (Reeve and Gamboa 1987), leading to higher productivity, and thus invasive potential.

Aggression: Aggression is thought to be related to insect invasiveness because it may lead to large, ecologically dominating supercolonies (Suhr et al. 2011). Individuals can display inter- and/or intraspecific aggression, which can lead to differences

in colony structure between native and invasive insects. For ants and termites, invasive species tend to have lower intraspecific aggression than native species (Holway et al. 1998, Suarez et al. 1999, Le Breton et al. 2004, Errard et al. 2005, Cremer et al. 2008, Fournier et al. 2009, Perdereau et al. 2011, Suhr et al. 2011, Ugelvig and Cremer 2012, Hoffmann 2014), suggesting that multiple invasive colonies may behave as a supercolony (Suhr et al. 2011). Interspecific aggression is also exhibited in invasive ants and *Ceratitis catoirii* (Mascarenes fruit fly; Human and Gordon 1999, Cremer et al. 2006, Duyck et al. 2006, Snyder and Evans 2006, Rowles and O'Dowd 2007, Carpintero and Reyes-Lopez 2008, Fournier et al. 2009, Blight et al. 2010, Perdereau et al. 2011). Low intraspecific aggression combined with high interspecific aggression can lead to ecological dominance of the invasive species while allowing individuals of the same species to amalgamate, possibly behaving as a supercolony (Suhr et al. 2011). Aggression may therefore be important in evaluating the potential invasiveness of certain insects and is easily measured between individuals (inter- or intraspecific) using a standard 1-1 assay (Holway et al. 1998).

Queen characteristics: Like *colony characteristics*, this category includes queen traits related to invasiveness: greater *queen number* and greater *queen longevity*. For example, an invasive colony of insects likely contains more queens (Ross et al. 1996, Tsusui and Suarez 2003, Abril et al. 2013), and these queens live longer (Gamboa et al. 2002) than do queens of native species. These invasive characteristics can lead to higher sustained progeny production and thus greater colony growth (Tsusui and Suarez 2003).

Nesting: The habitat used by nesting insects (Suarez et al. 2005, Downing et al. 2012), tendency of the nest to be predated (as a result of mimicry or habitat selection; Cervo et al. 2000), re-nesting after predation (Gamboa et al. 2004), and general nest reuse (Cervo et al. 2000) have all been claimed to be related to insect invasion in different ways. Invasive ants tend to be ground nesters rather than arboreal (Suarez et al. 2005), and invasive wasps tend to nest in urban rather than rural or natural habitats (Downing et al. 2012). Invasive ants and wasps may exploit nest resources not used by their native counterparts (Suarez et al. 2005), leading to their successful establishment. Invasive wasps also tend to encounter less nest predation as a result of their nest location choice (Cervo et al. 2000), and in the case of predation, are more likely to re-nest (Gamboa et al. 2004). Reuse of previous nests is also apparent in invasive wasps, conserving resources and saving time for foundresses (Cervo et al. 2000). These factors lead to a greater probability of establishment, and subsequently invasion, of these species by securing their persistence.

Many of the behavioural traits that are unique to insect invasion are also unique to social insects, which tend to dominate the insect invasion literature (e.g., Holway et al. 2002, Goulson et al. 2003, Kenis et al. 2009, Beggs et al. 2011). Social insects generally possess a suite of traits inherent to their lifestyle that also aids in the invasion process. As discussed above, certain ant species form supercolonies with genetically similar individuals, and these colonies may contain many reproductive females. This state, combined with high aggression, could allow the colony to dominate native species, further amplifying other traits that are important to invasion success, such as reproduction

and development. It is likely that understanding insect social form is critically important in determining the invasive potential of that insect, and therefore must be included in a risk assessment scheme. Because non-social invasive insects tend to be studied less often, there may be other behavioural traits that are important to invasion that are yet to be identified. These traits could be important for the predictive ability of a risk assessment scheme, but would possibly not be comparable to traits found in the AWRA.

Unique invasiveness traits of plants

Just as there are unique traits relating to invasion of insects, mainly relating to social behaviour, there are also a number of traits that are considered to be indicative of weediness in plants that do not generalize to insects. In total, 21 questions in the AWRA are not applicable to insects (Tables 3 and 4). Much of the discrepancy is found in the first four subsections of the AWRA.

Subsection one (three questions) of the AWRA deals with the domestication or cultivation of introduced plants. These questions refer to cases in which plants that have been introduced for horticultural or agricultural purposes, for example, escape cultivation, become naturalized, and then invasive. By contrast, invasive insects have rarely been introduced intentionally, with the exception of biocontrol agents that have become invasive, and so this would not apply to an insect model.

Subsection two (five questions) outlines climate and distribution. *Environmental matching* was identified as important for insect invasion (Table 2); however, the five questions in the AWRA are specific to Australian climatic conditions and should be modified for the specific region of interest (for example, Pheloung et al. 1999 modified the AWRA for use in New Zealand). Also, more *environmental matching* traits relating to abiotic factors were identified for insects than are included in the AWRA. This subsection would likely have to be expanded to apply appropriately to insects.

Subsection three (five questions) contains questions about the weediness of the plant elsewhere. This relates to the notion that success elsewhere can be a predictor of future invasiveness in areas with similar environmental conditions (Panetta and Mitchell 1991). It could also mean that the plant may have an increased probability of escape and spread because it is already naturalized. Whether an insect has been naturalized elsewhere may help to predict future invasiveness as certain species have been found to invade multiple areas (Samways 1999), but this was not identified as important for insect invasiveness in our search of the literature.

Subsection four (12 questions) lists undesirable physical and chemical traits of plants such as whether they produce thorns, spines, burrs, or toxic compounds. Many of these traits do not apply to insects because of their biology. Although it may be possible for an insect to possess mechanical/chemical defenses such as stinging or venom, these are not traits that are currently thought to be important for their invasion success, although they may be related to the ecological impact of the species, and thus would likely not be useful to include in an insect pest risk assessment.

Conclusion

Our systematic review of the invasion literature demonstrates that there are a number of differences in the traits that are claimed to be important for invasion in plants and insects. Species invasion is a complex process that involves both the invading species and its interaction with the biological and physical environment (Hayes and Barry 2008). Using insects as a case study, we have illustrated that expanding a pest risk assessment scheme originally developed for plants (such as the AWRA) may not appropriately capture the potential for invasiveness in other taxa because there are likely to be key differences in both the traits related to invasive behaviour and the importance of these traits. Given that this is the case for insects, it may also be the case for other important invasive taxa such as fish and mammals. Although invasive traits have been identified for plants and validated for a variety of regions (Gordon et al. 2008), consistent correlates of invasion success have yet to be comprehensively assessed across taxa (Hayes and Barry 2008).

Although our analysis identified a number of similar invasion traits for plants and insects, these traits may not carry the same importance in both taxa. For example, we identified many developmental traits that were claimed to be important to the invasion success of insects, while in the AWRA, few questions relate to the development of plants. Whether development, or any other trait, is more predictive of invasion in insects compared to plants would therefore have to be tested.

Furthermore, there are also traits that are unique to plants, as well as traits that are unique to insects. Therefore, the strength and predictive ability of an assessment scheme may be compromised by adapting an assessment for plants to other taxa without comprehensive validation and verification. For example, Coop et al. (2009) were required to further calibrate an invasion screening tool that was adapted from the AWRA to be used on fish. Many of the unique insect invasion-related traits identified were behavioural and were examined in social insects only. Many of these behavioural traits do not transfer directly to plants, but more importantly, non-social insects are largely absent from the insect invasion literature. It is unclear if additional or different traits might also be important to the invasion of non-social insects. The inclusion of behavioural traits may add to the predictive power of an insect risk assessment scheme, and more generally, this highlights a need for further research into invasion-related traits of non-social insects.

A reliable risk assessment scheme must reflect which traits are most strongly indicative of invasiveness for a given taxon. For a rapid risk assessment tool to be useful, consideration must also be given to understanding which traits are easily measured or commonly available in the scientific literature. For example, many of the suggested insect traits (Table 2) may be related to invasiveness but may be difficult to evaluate, especially for insects that are not well studied. The presence of many unanswered questions in any rapid risk assessment tool can compromise its validity and usefulness. Thus, the trade-off between simplicity and accuracy would also require assessment.

Future research development should aim to rate the importance and weight of specific traits related to invasion in taxonomic groups other than plants to develop

comprehensive pest risk assessment tools for other taxa. Currently, we are evaluating which traits are predictive of invasiveness in insects as a first step towards the development of such a tool for insects. Although in this analysis we found that 29 traits are related to invasion in insects, further analysis will inform which of these traits are most important in insect invasion. This approach will consolidate the trade-off between the most indicative and readily available trait information to produce a rapid and efficient design. From this we will know whether differences in invasive traits between taxa require that new risk assessment tools be created for other taxa, or if the approach taken thus far (i.e. making general risk assessments for all non-native taxa) is sufficient.

Acknowledgements

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RESEARCH ARTICLE

Defining the biosecurity risk posed by transported soil: Effects of storage time and environmental exposure on survival of soil biota

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Abstract

Soil frequently occurs as a contaminant on numerous sea, land and air transport pathways. It can carry unwanted invasive species, is widely recognized as a biosecurity risk, and is usually strictly regulated by biosecurity authorities. However, little is known about relative risk levels between pathways, thus authorities have limited capability to identify and target the riskiest soil pathways for management. We conducted a an experiment to test the hypotheses that biosecurity risks from soil organisms will increase both with declining transport duration and with increasing protection from environmental extremes. Soil was collected from two sites, a native forest remnant and an orchard, and stored on, in and under sea containers, or in cupboards, and assayed after 0, 3, 6 and 12 months for bacteria, fungi, nematodes and seeds.

Results showed that viability of *Pseudomonas* spp., bacteria, nematodes and plants declined over 12 months, irrespective of soil source. Also, mortality of most biota was higher when exposed to sunlight, moisture and desiccation than when protected. However, bacterial and fungal numbers were higher in exposed environments, possibly due to ongoing colonization of exposed soil by airborne propagules. The results were consistent with our observations of organisms in soil intercepted from airports and sea ports, and indicated there is potential to rank risks from transported soils based partly on transport duration and environmental exposure. This would help authorities to optimally allocate management resources according to pathway-specific risks.

Introduction

Protecting primary industries and native flora and fauna from invasive alien species (IAS) is critical to all nations' economic welfare and biodiversity (e.g. Pimentel et al. 2001; Early et al. 2016, Paini et al. 2016). Research that allows regulatory authorities to make rational evidence-based decisions about biosecurity risks (pathways and pest species) posed by arrivals of tourists, freight, machinery and biological material is essential to these aims. Soil frequently occurs as a contaminant on transported items, is widely recognised as a vector for non-native species, and is often the target of management practices that aim to minimise the spread of IAS (Catley 1980, Sequeira and Griffin 2014, Eschen et al. 2015, Migliorini et al. 2015). However, knowledge of the biosecurity hazards that can be vectored in transported soil, their relative survival rates on different pathways, and their establishment probabilities is currently insufficient to support optimal policy and management decisions (Brunel et al. 2014, Hulme 2015, Singh et al. 2015).

Comparisons between studies suggest that biosecurity risks from transported soil will vary depending on a range of biotic and abiotic factors including the taxa and life stages present, duration of transport, and exposure to environmental extremes during transport. For example, previous research suggests soil transported by sea, which will have had relatively long transport durations, contains fewer organisms than soil transported by air: Construction vehicles shipped by sea contained 0.002 nematodes/g (Hughes et al. 2010) and soil from sea containers contained 0.07 nematodes/g (Marshall and Varney 2000), while soil from air passengers' footwear contained 41 nematodes/g (McNeill et al. 2011). Similarly, 11% of seeds from construction vehicle soil were viable (Hughes et al. 2010) c.f. 69% in footwear soil (McNeill et al. 2011). McCullough et al. (2006) recorded a lower diversity of organisms from soil on cargo arriving by sea and air than from soil in luggage, and a recent study (McNeill et al. unpublished) recorded generally lower incidences and counts of organisms/g of soil from sea containers compared to air passengers' footwear (McNeill et al. 2011).

Marshall and Varney (2000) suggested that soil organisms transported on the external surfaces of sea containers might suffer high mortality rates due to exposure to solar radiation and sea water. As transport duration is typically longer by sea than air, McNeill et al. (2011) further postulated that incidences and counts of soil taxa could also be influenced by the time elapsed from when imports become contaminated with soil, and when the soil is sampled to assess biosecurity hazards (hereafter referred to as 'soil age'). Unfortunately, observational studies can generally provide only imprecise estimates both of soil age, and of the environmental conditions that soil has been subjected to during transport. For example, container ships may visit several ports en-route to a destination, with soil contamination potentially occurring at any port

where containers are unloaded and reloaded. Moreover, the route that a sea container takes to New Zealand is often only traceable to the last two ports. Similarly, McNeill et al. (2011) could only roughly estimate the age of soil on air passengers' footwear by surveying passengers. Thus, controlled experiments offer better opportunities to measure factors influencing survival of soil biota.

Soil origin is also likely to influence the species that occur in transported soil (Fierer et al. 2007), and will be especially relevant to whether a particular high risk pest species with a restricted geographical distribution could be present. However, there is little published evidence to suggest that soil transported from different locations should show major community differences at higher taxonomic levels (Chu et al. 2010, Decaens 2010, Kuske et al. 1997). For example, McNeill et al. (2011) recorded bacteria, fungi, nematodes, seeds and arthropods in soil samples originating from a diversity of international locations. In addition, for reasons previously explained, the origin of transported soil is frequently impossible to identify and cannot be used for gauging biosecurity risks. Thus, our study focused on the roles of soil transport duration and storage method, rather than soil origin, on survival of soil biota. Nevertheless, our experiment included soil from two contrasting sites to check our assumption that soil organisms from different locations will show similar responses to transport duration and storage method.

Understanding the effects of environmental conditions and transport duration on organism survival is important for developing robust biosecurity risk assessment processes (World Trade Organisation 2010; Burgman et al. 2014). However, such knowledge is lacking for soil organisms (Singh et al. 2015), hence our replicated experimental study asked how different storage conditions and transport durations affect soil biota. Soil was collected, subjected to treatments that simulated differing conditions during transport, and subsampled throughout 1 year to monitor changes in soil organism incidence and abundance.

The results increase knowledge of how environmental exposure and transport time influence soil biota survival, inform pest risk assessments, and help prioritize risks from soil that occurs on different transport pathways. This will assist quarantine authorities to target management resources at the highest risks to improve biosecurity protection without additional cost.

Methods

Soil was collected on 23 August 2011 (winter) from two sites in Canterbury, New Zealand: A regenerating native forest reserve in Prices Valley, Banks Peninsula (43°46.014'S, 172°42.840'E) (soils 1-3); and an organic orchard at Lincoln (43°39.048'S, 172°27.354'E) (soils 4-6). The forest reserve was bounded by farmland and a road, and was fenced to exclude livestock. Soil was taken from three different locations at each site, and each of these six samples were treated separately throughout the experiment. In the native reserve, the three locations were approximately 10 m

apart and, in the organic orchard, the locations were approximately 5 m apart. At each location within a site, six spade square soil samples (14 cm × 14 cm) were taken to a depth of 5 cm at six randomly chosen points within a 3 m radius of an arbitrarily designated central point. Prior to extracting each spade square, vegetation was cut to ground level with scissors and loose litter was removed. The six spade squares were mixed in a stainless steel tray and transferred to a plastic bag. The spade was cleaned with 70% ethanol both between each site, and between each location within sites. Disposable laboratory gloves were worn at all times, and changed between sites.

In the laboratory, the soil was screened with a 10 mm sieve and a subsample was taken for chemical analysis, and for counting microbes and nematodes. The remaining soil from each site (n = 2) and location (n = 3 per site) was divided amongst stainless steel steam trays (external dimensions c. 400 mm × 240 mm × 50 mm; internal dimensions 300 mm × 200 mm × 50 mm), in which twenty 4 mm diameter drainage holes had been drilled into the base, then allocated to treatments (c. 700 g of soil per tray). The soil was spread evenly onto the tray surface and gently pressed with a stainless steel pan to lightly compact it.

Treatments

Each of the six soil samples was divided among the same eight treatments, which are summarised in Table 1 and described below. A data logger was allocated to each treatment to record temperature for the duration of the experiment. Treatments were divided between four cupboards and four sea containers. The four cupboards were placed in an unheated shed. The sea containers (3 m length × 2.4 m height × 2.5 m width) were cleaned with high pressure water, transported to an outdoor concrete pad at Lincoln (S43.6279, E172.4704), and situated c. 2 m apart.

Cupboard treatment

Six uncovered trays (two soil sources × three locations) containing soil were placed inside each of four cupboards, which were located indoors and kept at ambient temperature. Thus, these samples were protected from sun, wind and rain, and were expected to experience less temperature variation compared to samples assigned to the sea container treatments. There was low potential for additional organisms to disperse to these samples.

Sea container treatments

Six uncovered trays containing soil were placed in locations in, on and under each of four sea containers. The six samples placed within each container were protected from

Table 1. Summary of soil samples, treatments and assays to investigate survival of soil biota (bacteria, fungi, nematodes and plants).

Container	Treatments	Sites	Locations	Samples	Bioassay times
Sea container 1	Top, Inside, Under	2	3	6	4
Sea container 2	Top, Inside, Under	2	3	6	4
Sea container 3	Top, Inside, Under	2	3	6	4
Sea container 4	Top, Inside, Under	2	3	6	4
Cupboard 1	Inside	2	3	6	4
Cupboard 2	Inside	2	3	6	4
Cupboard 3	Inside	2	3	6	4
Cupboard 4	Inside	2	3	6	4

sun, wind and rain. They were expected to experience less temperature variation and lower rates of invasion by additional soil organisms. The six samples placed on top of each container were exposed to UV, wind and rain. They were expected to experience maximal temperature variation, and high rates of invasion by additional soil organisms such as windborne microbes and seeds. The six trays placed underneath each container were positioned in the fork lift cavities. They were protected from direct sunlight, and rain, but were exposed to wind and had potential to become wet. They were expected to experience moderate temperature variation and high rates of invasion by additional soil organisms such as windborne seeds and perhaps some arthropods.

Untreated controls

The sites from which the soil was originally sourced were resampled on the same dates the container samples were assayed on 28 November 2011 (spring), 27 February 2012 (summer) and 27 August 2012 (winter). The top 5 cm of soil was sampled from six randomly chosen points at each site (n = 2) and location (n = 3 per site). Soil from six spade squares per location was mixed in a stainless steel tray, then assayed in the same way as the sea container and cupboard treatments. These samples are hereafter referred to as 'fresh soil'.

Bioassay times

The incidence and abundance of soil organisms persisting within each tray was assayed on day 1 (23 August 2011, winter), then after 3 months (28 November 2011, spring), 6 months (27 February 2012, summer) and 12 months (27 August 2012, winter). This coincided with sampling of fresh soil.

Each tray was subdivided into five equal areas. At each bioassay time, one c. 20 g subsample of soil was taken from a predefined location within each of the five areas,

thereby providing c. 120 g of soil from each of the six trays per treatment for each assay. However, loss of soil from the trays situated on top of the sea containers meant that the amount collected for the 6 and 12 month bioassays had to be reduced.

Organisms assayed

Soil organisms were assayed as previously described in the study of soil on international air passengers' footwear (McNeill et al. 2011), then identified to the extent achievable with the resources and taxonomic expertise available. Assays included culturing of all culturable bacteria, *Pseudomonas* spp. bacteria, and fungi. *Pseudomonas* spp. were chosen as model bacteria for isolation because several species are important plant pathogens (Silby et al. 2011) and are categorised as regulated species by New Zealand's biosecurity authority, the Ministry for Primary Industries.

Counts of bacteria and fungi

Depending on the weight of each sample, a subsample of 1.05 g to 21.55 g of soil was taken to estimate bacterial and fungal densities (colony-forming units per gram, CFU/g). Each sample was diluted 10-fold using 1% peptone and sonicated for 3 minutes to facilitate mixing. Serial dilutions were plated onto three different media: 10% tryptic soy agar plates with 100 ml/L cycloheximide to determine total bacteria counts; water agar containing 100 mg/L streptomycin for total fungi counts; and Oxoid *Pseudomonas* agar, supplemented with Oxoid CFC (cetrimide 10 mg/L, fucidin 10 mg/L and cephalosporin 50 mg/L) to select for pseudomonads. Fungi were enumerated by serial dilution plating onto water agar containing 100 mg/L streptomycin. For bacteria, plates were incubated at 20 °C (light: dark, 0: 24) and colonies were counted after 7 days. For fungi, the plates were incubated at 20-25 °C and colonies were counted after 10 days. Counts for bacteria and fungi were taken from 186 of 192 samples. It was not always possible to count every plate due to the presence of high concentrations of bacteria or fungi. In these circumstances, experience was used to determine the best dilution result to use.

Nematodes

The amount of soil used for nematode extraction was 100 g for fresh soil, and ranged from 11.34 g to 25.3 g for soil subsampled from the trays, depending on the amount of soil available. Nematodes were extracted from the samples taken on 23 August 2011 following the method of Bell and Watson (2001). This used the Whitehead tray extraction method whereby each sample was placed on two-ply tissue paper, supported by two layers of nylon gauze within a shallow tray to which 500 ml of tap water was

added. The tray was left for 72 hours, after which the liquid was poured into a 1 L plastic beaker, left to settle for 4 hours, then gently reduced to 100 ml volume by removing the supernatant. The 100 ml samples were transferred to 100 ml plastic beakers and allowed to settle for 4 hours before reduction to a final volume of 20 ml. Nematodes were counted in a Doncaster dish (Doncaster 1962), to provide a total nematode count (fungal, bacterial, omnivore, predator and plant parasitic). For samples collected directly from the field sites, a quarter of the sample was counted then multiplied by four to give the estimated total. For those subsamples collected after 3, 6 and 12 months, all the nematodes extracted from the soil were counted. Plant parasitic nematodes were identified to genera based on the keys of Siddiqi (2000) for Tylenchida, and Bongers (1994) for other groups. The plant parasitic nematodes (PPN) were further identified, counted and assigned either to the Criconematidae family, or a plant parasitic genus (e.g. *Helicotylenchus*, *Heterodera*, *Paratylenchus* and *Pratylenchus*).

At the 3 month assay, nematode extraction employed both the Bell and Watson (2001) method and a misting method (Seinhorst 1950; De Waele et al. 1987). The misting method allowed faster throughput of multiple samples. Analysis comparing the two extraction methods on the 24 cupboard samples found no significant difference in nematode yields ($P = 0.724$ for the log total nematodes, and $P = 0.211$ for the the log plant parasitic nematodes) (L. Aalders, unpublished data). Therefore, the misting method was used to extract nematodes from subsequent samples. The misting method involved placing c. 25 g of soil in a mistifier funnel and misting for 30 s every 5 minutes for 72 hours at a water temperature of 20 °C. The mistifier funnel system consisted of a plastic tube (75 mm internal diameter), positioned vertically with a plastic mesh base (1 mm aperture) on which two layers of paper tissue (Kimwipes™, Kimberley-Clark Worldwide Inc.) supported the soil sample. This tube sat on top of a plastic funnel. The water from the overhead mister washed the nematodes through the soil and into a glass test tube. Another c. 20 g of soil was oven dried at 80 °C for 48 hours to measure soil moisture.

To enable comparison across treatments and with previous studies (e.g. McNeill et al. 2011), results are presented as nematodes per g of collected soil, rather than per g of dry soil. Soil samples from which nematodes had been extracted were then used to assess if the soil contained viable seeds (see below).

Plants

Because visual searches are an imperfect method for detecting all seeds in soil samples (McNeill et al. 2011), the soil that had been used for nematode extraction was also used to determine the number of viable seeds using germination tests. After the 72 h nematode extraction, the soil from each sample was laid in a 5 mm thick layer on a paper towel over a wet medium (potting mix) for small samples (<25 g), or on a layer of towels in a small metal tray for the larger samples (100 g). The soil was kept moist under natural light in a quarantine glasshouse (15–35 °C) for up to 12 weeks, and ob-

served 1–2 times per week for germinated seedlings. Seedlings were transplanted into sterile potting mix 1–2 days after emergence and grown on for identification to the lowest possible taxonomic level.

Aids to identification were a combination of web-based keys (e.g. http://www.efloras.org) and published literature on New Zealand native and introduced species (Webb et al. 1988; Edgar and Connor 2000; Champion et al. 2012; James et al. 2012).

Temperature

Temperature and humidity were recorded in each cupboard, and temperature on the top, inside on the floor, and under each sea container. The loggers located on the top of containers were housed within Stevenson-type screens. In addition, two extra loggers recorded humidity (and temperature) inside and under one of the containers. Temperature and humidity data were recorded either every 30 or every 60 minutes using Tinytag™ loggers (Gemini Data Loggers Ltd, Chichester, UK).

Soil chemistry

Soil for chemical analysis was collected at the start of the experiment by taking five 7.5 cm diameter × 5 cm deep cores from each location at each site. Any vegetation was removed with a box cutter. The cores from each location were hand mixed then bagged prior to being forwarded to a soil analysis laboratory. Soil pH, P (Olsen phosphate), K, sulphate sulphur (S SO_4), organic sulphur, total phosphorous, Cu, Co, Fe, Mn, Zn, organic carbon and organic matter were quantified. Soil was classified using Landcare Research's S-map series (http://smap.landcareresearch.co.nz/) (accessed 01 September 2016).

Analysis

Data analysis methods are briefly described here, and full details are given in the Suppl. material 1. Analysis was carried out using a Latin square design, blocked by treatment (cupboard, sea container, fresh) and site (forest, orchard), which allowed measurement of: variation between and amongst cupboards and sea containers; and interactions between site and treatment (e.g. soil with high organic matter content might dry more slowly, thus enhancing organism survival). Obtaining soil from three locations per site enabled comparisons between stored soil (treatments) and fresh soil (untreated controls).

This design has split-plot elements (the site effect is not replicated), nested random effects (soil location nested within site), crossed random effects (soil location nested within site is crossed with storage treatment location), and longitudinal measures (four repeated measurements in time). The null hypothesis was that there was no difference

between the treatment levels upon the effect of time upon the response variables. If the hypothesis was rejected for the response variables, then we explored possible relationships between temperature, treatment and the response variable.

The statistical model that we fitted used the following template.

$$f_L\,(y_{ijkml})=\beta_0+\omega_i+f_T\,(t,\tau_m)+\gamma_i+\delta_j+\psi_k+\varepsilon_{ijkmt} \qquad (1)$$

Here,

y_{ijkmt} is the response variable at duration t from soil location j within site i stored in site k under treatment m;

$f_L(\dots)$ is a selected transformation of the response variable, usually a natural logarithm;

β_0 is a constant intercept for the response variable;

t refers to the duration of storage;

τ_m refers to the treatments (m = 1,…,5)

$f_T\,(t,\tau_m)$ is some smooth function of the duration and the treatment that is constrained so that all treatment effects are identical at $t = 0$;

ω_i is a fixed effect representing site (forest / orchard);

$\gamma_i \sim N(0, \sigma_\delta^2)$; i = 1, 2 is the site random effect, which may be confounded with ω_i;

$\delta_i \sim N(0, \sigma_\delta^2)$; j =1, …, 6 is the location random effect, with 3 from each site;

$\psi_i \sim N(0, \sigma_\delta^2)$; k = 1, …, 9 is the storage treatment location random effect, where locations 1 – 4 are sea containers (corresponding to treatments 1 – 3 only), 5 – 8 are cupboards (corresponding to treatment 4), and 9 is the fresh soil treatment; and $\varepsilon_{ijkmtt} \sim N(0, \sigma^2)$ is a random error.

It is reasonable to expect that the response variables will vary with site, so they should be included in the model, as ω_{ii}. However, in order that the three locations within each site not be considered as independent realizations of the predictor variable, we included site as a random effect in the model (γ_{ii}). Including site as a random effect induced the needed correlation between samples from the same site to reflect the design. The soil samples were taken from two sites only, so any test of soil origin, or factors that interact with soil origin, are pseudo-replicated. The tests can not be interpreted as representing all possible soil samples from forests or orchards, although see Discussion. It was sometimes necessary to include those terms in the model as fixed effects in order to accurately capture the variability (see online materials).

This model was applied to seven response variables, namely (i) relative bacteria count per unit soil volume, (ii) fungi, (iii) *Pseudomonas*, (iv) nematodes, (v) plant seeds corrected for soil volume (vi) plant species corrected for soil volume and (vii) the raw number of plant species not corrected for soil volume. A similar model was applied to the response variable defined as the binomial proportion of nematodes that were plant-parasitic nematodes, for which we used only the observations that had non-zero counts of nematodes.

The fixed effects were tested using likelihood ratio tests, with the cut off for statistical significance set at 0.01 in order to loosely compensate for the multiplicity of tests. The

random intercepts were not tested; they were included in order that the model faithfully represent the experimental design. Initially a full model was fitted, and diagnostic graphics were checked. The full model included treatment, site, and storage duration as a linear effect. The outcome of the check was generally the selection of some form of transformation for the response variable, e.g., natural log. We next applied backwards elimination to assess whether site should be retained as a fixed effect, noting that the site fixed and random effects are confounded. A backwards elimination approach was then applied to see how duration should enter the model, including: not at all, as a linear term, as a quadratic term, and as a constrained two-knot spline, with knots set at 3 months and 6 months, which is equivalent in degrees of freedom to a cubic polynomial function, but performed better visually. Invariably, the most complex temporal model was selected by the test. Duration was treated using cubic splines, because extrapolation using the cubic polynomial was unrealistic, and using a category for duration ignores the relative location of the observations in time. For example, the first three measures were separated by 3 months whereas the last measure is after 6 months. The best model was then assessed using graphical diagnostics, and adjustments made if needed. The fitted models were then overlaid upon scatterplots of the data that were augmented with smooth means, as a visual aid to the testing and modelling process.

Although it was commonly true in the analyses that the model assumptions were ratified by graphical diagnostics, sometimes the distribution of the residuals was a little more skewed than symmetric. In these instances, we were reasonably confident that the sample size was sufficiently large that the Central Limit Theorem would hold, and that the test assumptions would be robust to the departure from the nominal assumptions.

The following reasoning for assessing the importance of interactions between random effects was used. There are no reasons to assume that the random effects would interact with duration (see above) and beyond the effects of the interaction of treatment with duration, so the random interactions were not formally tested. However, graphs were constructed to act as diagnostics for this assumption. If there was an important interaction between the random effect and duration, then we assumed that it would be visible in these graphics. In cases where this occurred, we formally tested the extended term using a whole-model likelihood ratio test, and if it was significant, repeated the fixed effects test suite with the new random effects structure. In no case did the test of the fixed effects alter from the original result in any important way; hence, these results were not pursued further. Interactions between fixed and random effects were not tested further.

If the estimated soil location random effect was non-zero, then the effects of soil chemistry upon the response variable was informally assessed by examining scatterplots of the estimated soil location random effects against the soil chemistry variables. More formal analysis did not seem appropriate owing to the large number of potential soil variable predictors. These results are not included here.

If the interaction between treatment and duration was statistically significant, then further analysis was undertaken to assess the effect of temperature. The analysis was performed by taking the temporal differences of the response variable, or transformations of the response variable where appropriate (e.g. natural log for count data and empirical

logit for proportion data) and assessing the effect of the interaction of duration with both treatment and temperature upon the change from assay time to assay time.

All analyses were carried out using the open source statistical environment R (R Core Team 2014). We obtained 95% confidence intervals of the estimated random effects (not shown here), using a parametric bootstrap algorithm that is provided by the lme4 package (Bates et al. 2014).

Results

Earthworms and arthropod larvae (e.g., grass grub, *Costelytra zealandica*) were observed in the soil during its initial collection. Some were inadvertently transferred to the trays, but none were alive in the 3 month assay. For the trays located under the sea containers, colonisation by spiders occurred within 3 months, a dead bird was found in one tray after 12 months, and rodent droppings were observed at intervals throughout the experiment.

Full results from the statistical analysis are in the Suppl. material 1, and are summarised here as follows. Each response variable is presented with a graphical summary that plots duration against the raw data with smoothed means, constructed using a loess algorithm, augmented by dashed lines that represent the best-fitting statistical model. In each case, the results are contrasted with results from the fresh soil that was assayed at the same time.

For all response variables, the most complex model with duration was required. Sample site was statistically important for several variables (namely fungi, nematodes, plant count and plant species count, see online materials).

Bacteria

Modelled and mean observed changes in bacteria across time are presented in Figure 1. The fresh soil retained high counts throughout the 12 months of the study. The cupboard and inside-container treatments had similar trajectories for both forest and orchard soil, dropping in the 12 months to a tenth of the original count or less. Soil located on top of the sea containers retained the initial high counts, and in some cases had levels higher than fresh soil. Soil located under the container suffered a short-term drop, but returned to the same levels as the container-top soil and the fresh treatment. There was no detectable difference between soil sites.

Pseudomonas species

Modelled and mean observed changes in *Pseudomonas* spp. counts across time are presented in Figure 2. Counts from fresh soil remained reasonably constant throughout the 12 months. For stored soils, mortality was highest in cupboards and on top of containers
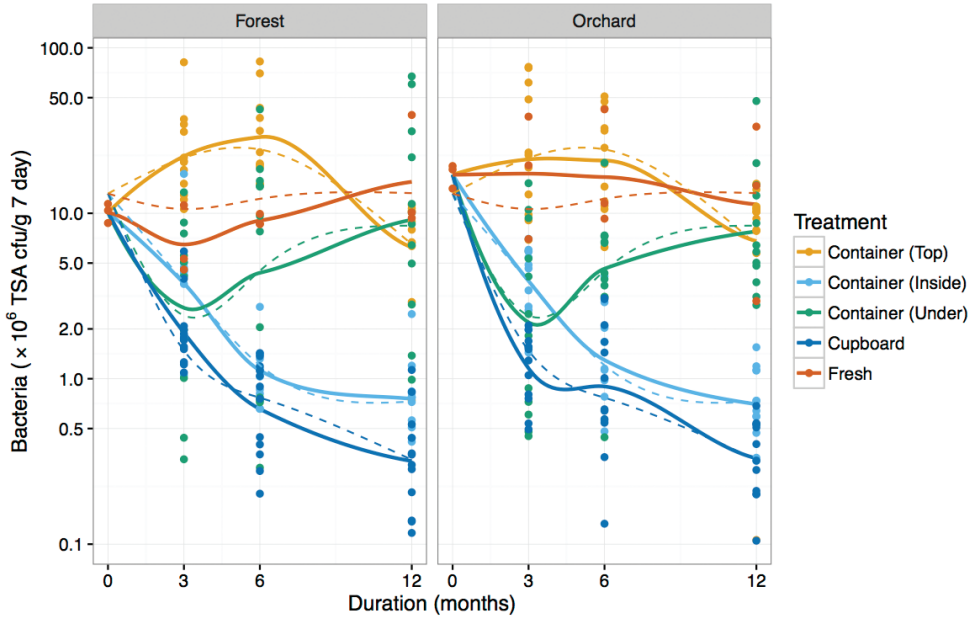
Figure 1. Scatterplot of mean bacterium counts (cfu/g) at 0, 3, 6 and 12 months measured in soil recovered from on, in and under four sea containers and inside four cupboards. Solid lines show the smoothed means and dashed lines predictions from the preferred model.
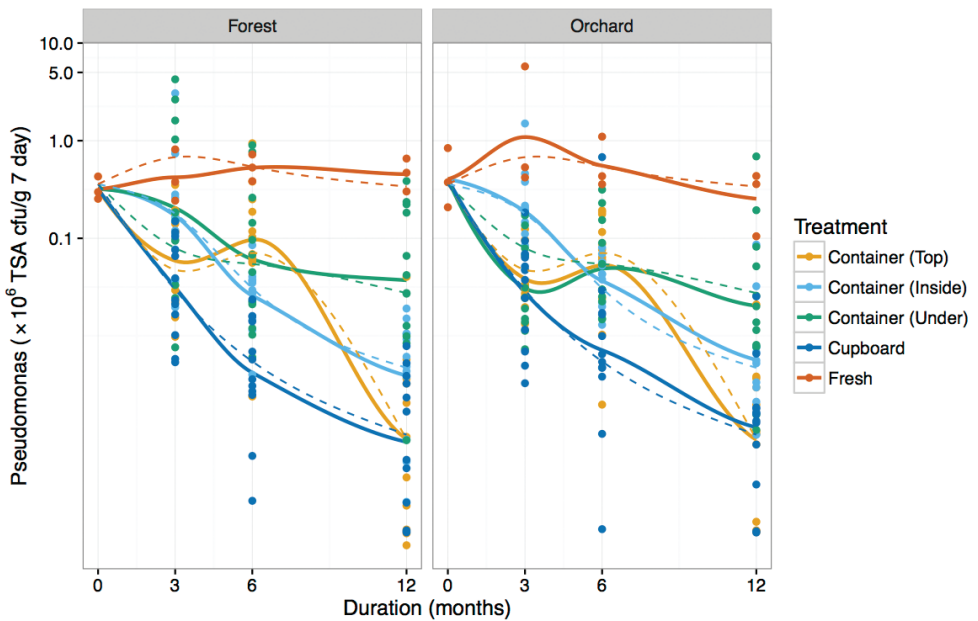
Figure 2. Scatterplot of mean *Pseudomonas* spp. counts (cfu/g) at 0, 3, 6 and 12 months measured in soil recovered from on, in and under four sea containers and inside four cupboards. Solid lines show the smoothed means and dashed lines predictions from the preferred model.
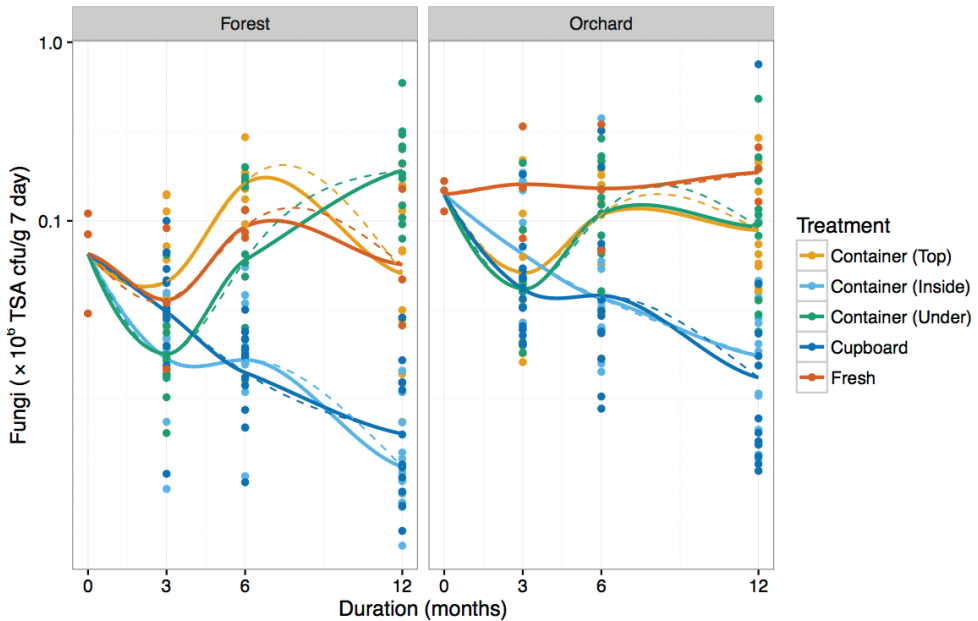
Figure 3. Scatterplot of mean fungus counts (cfu/g) at 0, 3, 6 and 12 months measured in soil recovered from on, in and under four sea containers and inside four cupboards. Solid lines show the smoothed means and dashed lines predictions from the preferred model.

after 12 months, which both descended to about a tenth of the original counts. *Pseudomonas* spp. counts in soil located under containers, followed by soil inside containers, were intermediate between fresh soil and cupboard soil except at 6-month period. Counts in soil located on top of containers showed a temporary plateau at 6 months.

Fungi

Modelled and mean observed changes in fungus cfu/g across time are presented in Figure 3. Fungus counts in soil on top of and under containers was comparable to fresh soil, with little mortality occurring during the experiment. Fungi from soil held inside either the cupboard or the container showed the highest mortality after 6 months, with counts dropping about ten-fold over the 12 months. The orchard site had more fungi than the forest, but in the absence of replication we cannot ascribe this difference to site.

Nematodes

Modelled and mean observed changes in nematode counts over time are presented in Figure 4. In the day zero sample, there were means of 22 and 33 total nematodes/g of
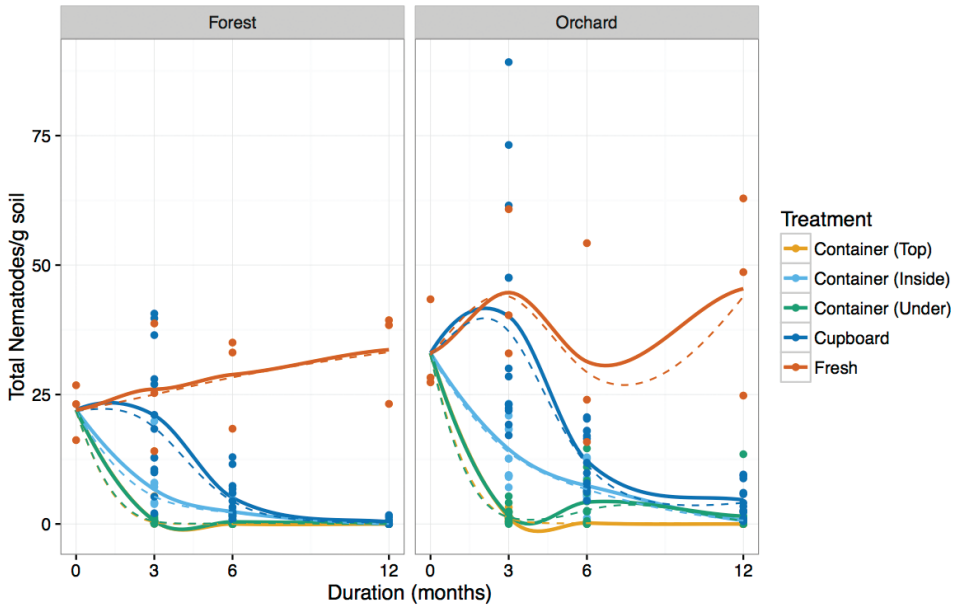
Figure 4. Scatterplot showing changes in mean total nematodes/ g soil at 0, 3, 6 and 12 months measured in soil recovered from on, in and under four sea containers and inside four cupboards. Solid lines show the smoothed means and dashed lines predictions from the preferred model.

soil for forest and orchard soil, respectively. Counts declined in all storage treatments over time. Fresh soil collected at 3, 6 and 12 months had higher counts than stored soil. With stored soil, nematode counts were higher in orchard soil than forest soil, with survival in cupboards generally higher than in sea containers over the 12 months.

Mortality was highest in soil located either on or under the sea containers, with approximately 7.5- and 12-fold declines in total nematodes for the orchard and forest soil, respectively, after 3 months. Thereafter, total nematode numbers were negligible for the rest of the experiment. By comparison, for soil held inside the sea containers, there was only a 2- and 3-fold decline in nematode numbers after 3 months for the orchard and forest soil, respectively. At 6 months, there were 5- and 6-fold declines in numbers for the orchard and forest soils, respectively. Total nematode counts were close to zero after 12 months.

For soil held in cupboards, nematode counts did not change for the first 3 months, but after 6 months there were 4- and 5-fold declines in the orchard and forest soil, respectively. At 12 months, this decline had increased, to a 55-fold decrease in forest soil compared to 7-fold decrease in orchard soil.

Plant-parasitic nematodes

PPN recovered were from the genera *Pratylenchus*, *Paratylenchus*, *Heterodera* and *Helicotylenchus*, and the family Criconematidae. There was a higher diversity of
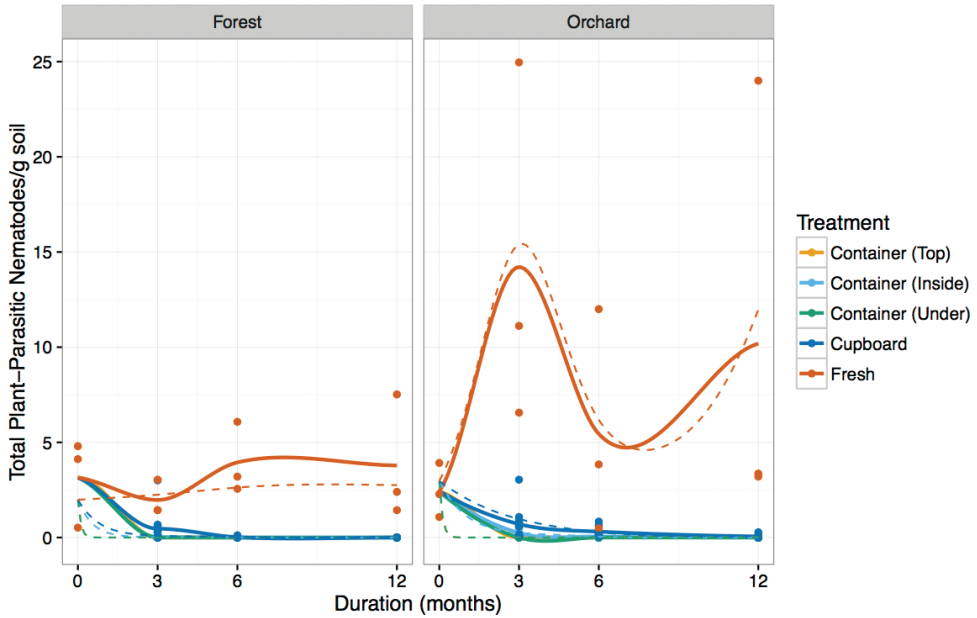
Figure 5. Scatterplot showing changes in mean plant-parasitic nematodes/ g soil at 0, 3, 6 and 12 months measured in soil recovered from on, in and under four sea containers and inside four cupboards. Solid lines show the smoothed means and dashed lines predictions from the preferred model.

plant parasitic nematodes in the forest soil (*Pratylenchus, Paratylenchus, Globodera, Helicotylenchus, Rotylenchus* and Criconematidae) than the orchard soil (*Pratylenchus, Paratylenchus* and *Heterodera* spp.). In addition, many plant parasitic species from the forest soil could only be identified to genus. Changes in the count of PPN across time are presented in Figure 5. In the day zero sample, there were means of 3 and 2.4 PPN/ g of soil for forest and orchard soil, respectively. Fresh soil collected from the source sites at 3, 6 and 12 months had higher plant parasitic nematode counts than stored soil (Figure 5). As observed for total nematodes, PPN declined rapidly in stored soil, with the rate of decline highest in soil situated on or under sea containers, and lowest in soil in cupboards. PPN were not detected in soil positioned on or under sea containers from 3 months onwards. Survival was highest in soil in cupboards, and better in orchard than forest soil. PPN were present in orchard soil after 12 months, with a 40-fold decline over that period. In comparison, in forest soil, PPN were not detected after 6 months, showing a 122-fold decline over that period. There was a decline in PPN bio-diversity for all storage treatments over time, both relative to the original soil samples and compared with the two sites were resampled at 3, 6 and 12 months. Mean number of taxa found in soil from the forest and orchard over the 12 months averaged 3.9 ± 0.23 and 2.0 ± 0.12, respectively. By comparison, in stored soil, the mean number of taxa in soil where PPN were present, averaged (± SEM) 1.5 ± 0.29 and 1.1 ± 0.08 after 3 months for cupboard and sea container soil, respectively. By 6 months,
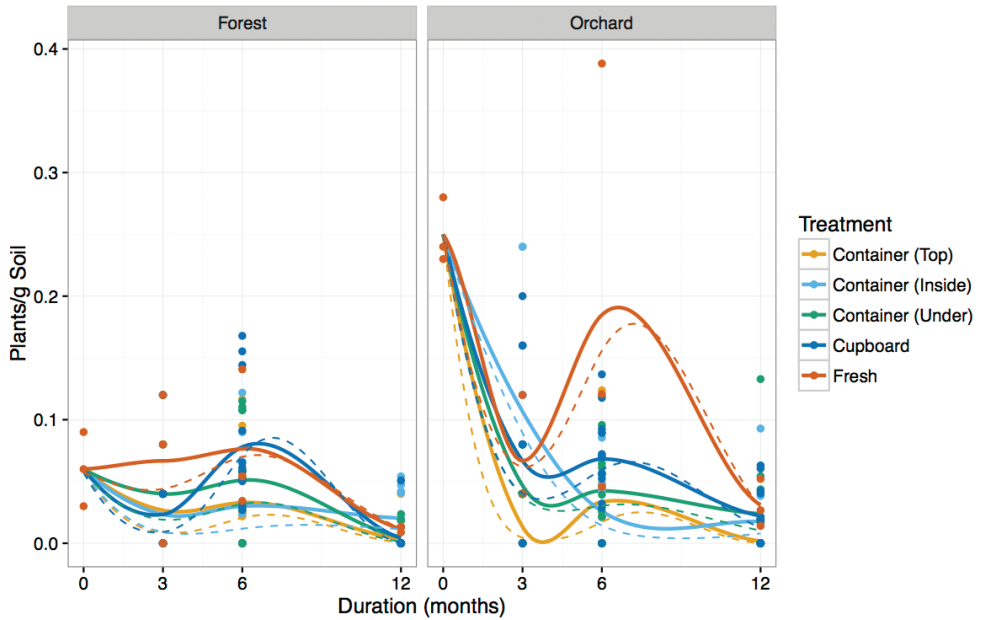
Figure 6. Scatterplot showing changes in mean plants/g soil grown from soil collected at 0, 3, 6 and 12 months recovered from on, in and under four sea containers and inside four cupboards. Solid lines show the smoothed means and dashed lines predictions from the preferred model.

the mean number of taxa recovered from cupboard and sea container soil was 1.1 ± 0.07 and 1.0 ± 0.0 after 6 months and 1.0 ± 0.0 and 0 after 12 months, respectively. *Pratylenchus* species were the dominant genus (84%) recovered from stored soil from 3-12 months irrespective of location.

Plants

Overall, the orchard soil had more seeds/g soil than forest soil, but in the absence of replication we cannot ascribe this difference to site. The number of plant species per soil was similar, with 29 and 38 species in the forest and orchard soils, respectively, but the composition of plant species between the two sites was markedly different. In the forest soil, 13 native and 16 exotic species were found, while in the orchard soil one native and 37 exotic species were present. Only 13 species were common to both sites (Suppl. material 2), all exotic invasive weed species, with *Solanum nigrum* L. (black nightshade) being the most frequently recorded species in both soils.

Soil in relatively exposed locations on or under sea containers did not recruit significantly more new plant species than soil in relatively protected locations inside cupboards or sea containers (data not shown).

Changes in plant counts per g of soil over time are presented in Figure 6. In the day-zero sample, there were means of 0.3 and 0.1 plants/g of soil for forest and orchard soil, respectively. Fresh soil had higher total plant numbers and plants/g of soil than stored soil. The number of plants that germinated declined significantly after 12 months for soil in all storage treatments from both sites. Soil stored on top of containers showed the greatest decline, while soil stored elsewhere showed declines that were similar to one another. Plant species count (not shown) showed a similar response, regardless of whether raw counts were used, or species per g of soil.

Soil chemistry

The forest soil is a Taitapu deep silt loam described as a recent gley soil, while the orchard soil is a Wakanui deep silt loam and described as a mottled immature pallic soil. Both soils comprise 15-35% clay. Based on the USDA Soil Series Classification, the Taitapu soil is a Typic Fluvaquent and the Wakanui soil an Udic Dystocrept. They had similar pH, but P, K, total phosphorus and Cu were higher in the orchard soil, while Fe was higher in the forest soil. Soil chemistry had no significant effect on counts of any taxa. Details of the soil chemical analysis are in the Suppl. material 3.

Temperature

Temperatures (Figure 7) varied with season and treatment, being lowest during winter (June-August) and highest in summer (December-February). Throughout the year, the lowest minimum temperature was recorded on top of containers (-6.5 °C), with a maximum temperature of 30.7 °C. The highest temperature fluctuations and maximum temperatures occurred inside containers with a range of -5.3 to 36.9 °C. Temperatures under containers were consistently bounded by temperatures in other treatments and ranged from -5.0 to 29.1. Temperatures varied least inside cupboards (0.8-26.0 °C).

Synthesis

Figure 8 summarises how relative numbers of each taxon changed with storage duration in each storage location. Nematodes, plants and *Pseudomonas* spp. bacteria exhibited one set of characteristic responses to the treatments, and fungi and other bacteria exhibited another. When stored in exposed locations, survival of nematodes, plants and *Pseudomonas* spp. bacteria rapidly declined to less than c. 25% of the original numbers after 3 months and less than c. 10% after 12 months (Figure 8). When stored in protected locations, the decline to c. 25% of the original numbers took c. 6 months rather than three (Figure 8). Fungi and bacteria (other than *Pseudomonas* spp.) showed
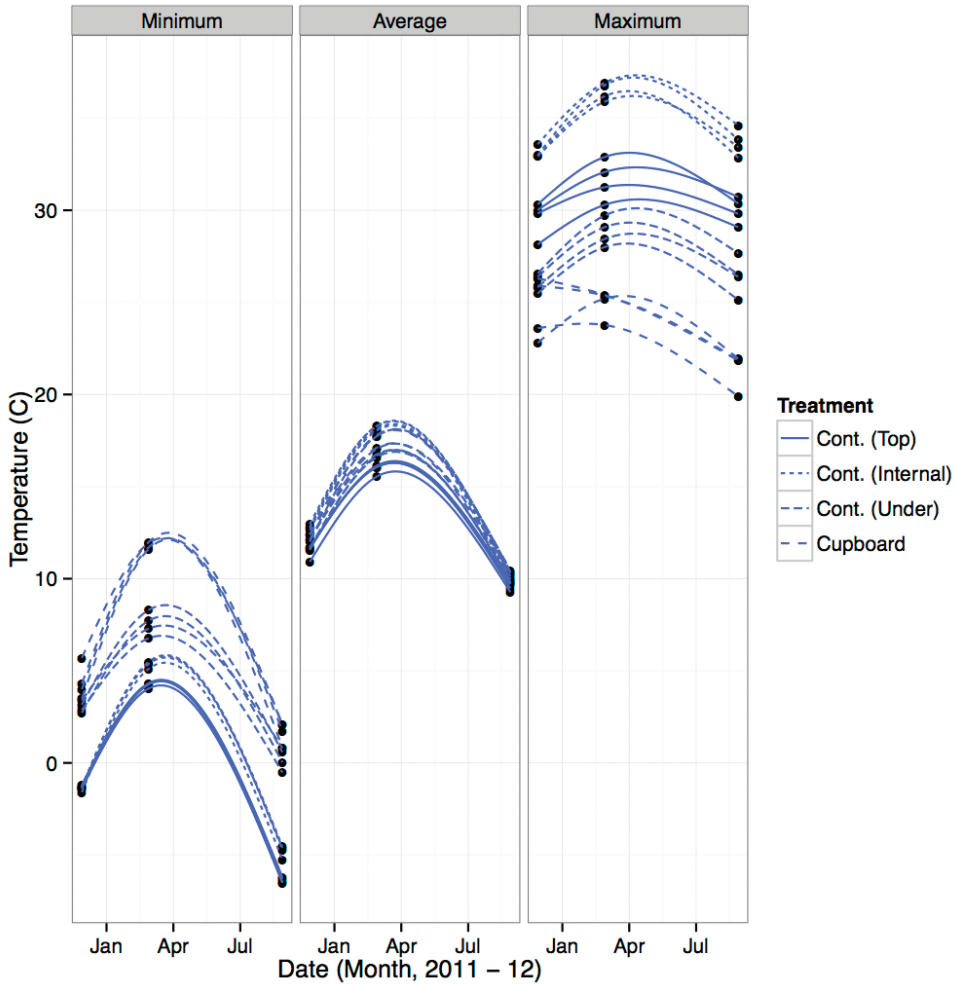
Figure 7. The minimum, average and maximum temperatures recorded on, in and under four sea containers and inside four cupboards over a 12 month period. Lines of the same colour represent different replicates of the same treatment.

the same patterns as the other taxa when stored in interior locations. However, when stored on top of or under containers, populations of fungi and non-*Pseudomonas* bacteria fluctuated widely with storage time, though only fungi stored under containers remained above their original levels after 12 months.

Discussion

This research is the first to estimate the effects of storage time and environmental exposure on soil-borne taxa that could be biosecurity hazards. The results showed clear patterns that
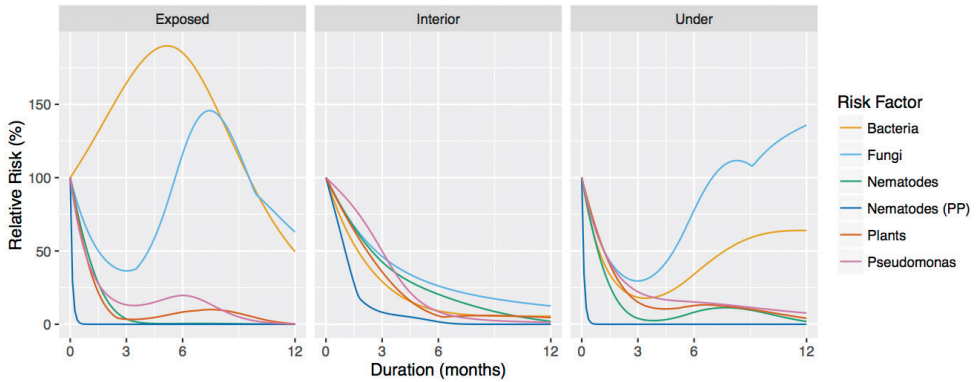
Figure 8. Changes in relative amounts of biota against duration, by storage location. Where the relative amount differed by soil origin, the maximum was chosen. In the legend, 'Nematodes (PP)' represents plant parasitic nematodes.

should prove useful for targeting management resources at soil contaminants that are the most hazardous, particularly when combined with results of other studies.

Unlike other taxa, fungi and non-*Pseudomonas* bacteria in soil stored in exposed locations showed large population fluctuations during storage. It is uncertain if these fluctuations were due to growth and mortality of the fungi and bacteria originally present in the samples – perhaps associated with fluctuations in populations of algae and other food substrates – or if the taxa concerned colonised the soil after it was originally collected (S. Wakelin, AgResearch, personal comm.). Molecular analysis of the samples we retained from this experiment that are currently stored at -80 °C might help to clarify which of these processes was most important. This issue is pertinent because, if fungi and bacteria present in transported soil tend to be recent colonisers, then they are more likely to be local rather than introduced taxa, and often may not be biosecurity hazards.

We expected soil stored in exposed locations would be invaded by additional organisms such as windborne seeds as the experiment proceeded, but the results showed both that soil stored in exposed locations contained similar densities of viable seeds to soil stored in protected locations, and that seed viability declined with storage duration. It is possible the similarity between the exposed and protected treatments was an artefact of our experimental setup: For example, our sea containers were situated on a paved surface, approximately 20 m from the nearest vegetation, thus reducing propagule pressure; and the trays that contained the soil had 50 mm high rims which may have impeded seed entry. However, it is more likely this result is indicative of real-world soil contaminants on sea containers because it is similar to seed count data obtained from a recent study of soil intercepted from sea containers at New Zealand ports (McNeill et al. in prep.). Moreover, our experimental result also matched the 0.03 seeds that germinated per gram of soil intercepted from construction vehicles in Antarctica (Hughes et al. 2010). Thus, we tentatively conclude that soil contaminants on sea containers represent small, difficult-to-hit targets for windborne seeds, so seed

counts per gram of soil will seldom increase during transport, while seed viability will generally decline. It is even possible that in some circumstances seed counts per gram of soil will also decline with transport duration due to granivory by birds, rodents and arthropods.

The organisms contained in soil from each of our sources (orchard and forest) showed nearly identical responses to storage duration and location. Although soil source was unreplicated, these results were consistent with our expectation, based on previous observations (Chu et al. 2010, Decaens 2010, Kuske et al. 1997, McNeill et al. 2011), that higher taxonomic groups will usually be shared between soil sources, and those obtained from different locations will show similar responses to storage duration and location.

It is interesting to compare our results from fresh orchard and forest soil to those from a study of soil on international air passengers' footwear (McNeill et al. 2011). Air passengers' footwear gave similar counts per gram of soil for bacteria and nematodes, and even higher values for seeds and fungi. This is consistent with short transport duration (low soil age), low environmental exposure, and relatively high risks from soil transported on airline pathways.

In general, our results supported the hypotheses that some soil organisms transported on the external surfaces of shipping containers will suffer high mortality rates due to environmental exposure (Marshall and Varney 2000), and that duration of soil transport will negatively influence soil organism survival (McNeill et al. 2011). This suggests there is potential to rank risks presented by soil on different pathways by comparing environmental exposure and transport duration between pathways, thus enabling management to mitigate the highest risks. Risks will also vary depending on the diversity and also the life stages of taxa (e.g. cysts, eggs and seeds/spores in dormant stages) vectored with soil. Also the risks are likely to vary between taxa as some genera and/or species may have better survival rates than others. To further develop and test this approach, we need measurements of the incidence and abundance of soil organisms from a greater range of pathways (including conveyances), and better pathway-specific estimates of soil age.

An important aspect of risk analysis relevant to soil contaminants that has seldom been studied is between-pathway variation in the likelihood that organisms vectored by soil will be transported to habitats suitable for their establishment. Soil on plants imported for planting would presumably present a particularly high risk in this regard because environmental exposure and transport duration will likely be low and, unless the soil is removed and quarantined, there will be a high probability that IAS will be transferred with the plants to habitats suitable for organism establishment (Miglionini et al. 2015). Organisms vectored in soil adhering to footwear might also have high potential for transfer to suitable habitats through treading in unpaved areas, while, at first glance, establishment opportunities for organisms in sea container soil perhaps seem less. Proper investigations of such possibilities would contribute greatly to improved management of biosecurity risks from transported soil.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors contribution

MM: Developed the research concept, led and carried out the sampling, contributed to manuscript writing. CP: Developed the research concept, contributed to sampling and manuscript writing, AR: Analyzed data and contributed to manuscript writing. LA: Carried out the extraction and identification of nematodes and contributed to manuscript writing, NR: Carried out the extraction and identification of Pseudomonas and contributed to manuscript writing, SY: Carried out the isolation and countijng of bacteria and fungi and contributed to manuscript writing, CD: Carried out the rearing and identification of plants and contributed to manuscript writing; TJ: Assisted in rearing and identification of plants, NB: Assisted in identification of nematodes

Acknowledgements

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Supplementary material 1

Description of the data analysis methods and results

Authors: Mark McNeill, Craig Phillips, Andrew Robinson, Lee Aalders, Nicky Richards, Sandra Young, Claire Dowsett, Trevor James, Nigel Bell

Data type: data analysis methods and results

Supplementary material 2

Plant species common to both soil sources

Authors: Mark McNeill, Craig Phillips, Andrew Robinson, Lee Aalders, Nicky Richards, Sandra Young, Claire Dowsett, Trevor James, Nigel Bell

Data type: species description

Supplementary material 3

Soil chemistry of the two soil sources

Authors: Mark McNeill, Craig Phillips, Andrew Robinson, Lee Aalders, Nicky Richards, Sandra Young, Claire Dowsett, Trevor James, Nigel Bell

Data type: soil chemistry

RESEARCH ARTICLE

Quantifying errors and omissions in alien species lists: The introduction status of *Melaleuca* species in South Africa as a case study

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Abstract

Introduced species lists provide essential background information for biological invasions research and management. The compilation of these lists is, however, prone to a variety of errors. We highlight the frequency and consequences of such errors using introduced *Melaleuca* (*sensu lato*, including *Callistemon*) species in South Africa as a case study. We examined 111 herbarium specimens from South Africa and noted the categories and sub-categories of errors that occurred in identification. We also used information from herbarium specimens and distribution data collected in the field to determine whether a species was introduced, naturalized and invasive. We found that 72% of the specimens were not named correctly. These were due to human error (70%) (misidentification, and improved identifications) and species identification problems (30%) (synonyms arising from inclusion of *Callistemon*, and unresolved taxonomy). At least 36 *Melaleuca* species have been introduced to South Africa, and field observations indicate that ten of these have naturalized, including five that are invasive. While most of the errors likely have negligible impact on management, we highlight one case where incorrect identification lead to an inappropriate management approach and some instances of errors in published lists. Invasive species lists need to be carefully reviewed to minimise errors, and herbarium specimens supported by DNA identification are required where identification using morphological features is particularly challenging.

Introduction

Species lists form the basis for much of the current research on biological invasions (e.g. the Global Naturalized Alien Flora Database of van Kleunen et al. 2015). Such lists are also essential for guiding legislation, as input to decision making and risk assessment, and in the formulation of management policies and strategies (McGeoch et al. 2012; Latombe et al. 2016). Because resources required to address the threat of invasive species are limited, objective categorization of species is required to prioritize resource allocation according to species, areas and introduction pathways (McNeely et al. 2001, Nel et al. 2003, Wilson et al. 2013). Accurate lists of alien species, with data on their introduction status, are thus crucial resources, not just for the regions for which they are compiled, but also globally (Wilson et al. 2011). But, as with any information derived from a variety of sources, the compilation of lists is prone to a number of errors which can then be perpetuated in various ways (McGeoch et al. 2012). To address these concerns, it is thus essential that the error rates in species lists are assessed and ways to detect them are identified.

Pyšek et al. (2002) include alien taxa and their status in a flora of the Czech Republic. This well-compiled list lends itself to comparison with other regions and is an important example allowing for determinants and patterns of plant invasions at a global scale to be studied (Pyšek et al. 2004). Such lists are the essential building blocks on which assessments of the status of invasions in a country should be built (Latombe et al. 2016). By comparison, even though South Africa has a reasonably well-funded national programme for controlling invasive species, especially plants, research on lesser known invasive groups has only recently been given special attention (Wilson et al. 2013), and there is no comprehensive list of introduced and invasive species yet (Faulkner et al. 2015). A list of regulated invasive plant species was published in 2014 and this forms the basis for management plans and regulation (Department of Environmental Affairs 2014). However, this regulatory list is incomplete and contains several errors (per. obs.). Moreover, more species will need to be added as surveillance progresses, as more species demonstrate invasiveness, impacts are evaluated, and as errors in the list are discovered (Rouget et al. 2016).

For plants, herbaria are indispensable resources and reference sources for much botanical research which requires reliable species identifications, including the compilation of introduced species lists (Glen 2002). Funding for taxonomy and the upkeep of herbaria is declining worldwide (Smith et al. 2008, Guerin 2013, Pyšek et al. 2013) and is a concern that can be compounded because expertise for alien species is less likely to exist in any particular country. Herbarium specimens, upon which comprehensive lists are ideally based (Pyšek et al. 2013), require curation as taxa are revised or new information becomes available, e.g. from molecular and other studies

(e.g., Le Roux et al. 2010). Many alien taxa are underrepresented, remain unidentified for considerable periods of time, or are misidentified in herbaria (Pyšek et al. 2013). In this paper we explore the scale of this problem using taxa in the genus *Melaleuca* (*sensu* Craven (2006) and Brophy et al. (2013)) in South Africa as a case study.

The genus *Melaleuca* has not been distributed around the world as extensively as some other tree groups (e.g. *Eucalyptus,* a sister genus in the Myrtaceae) (Rejmánek and Richardson 2011). However, seven species are listed as invasive in the USA and South Africa (Rejmánek and Richardson 2013), including one of the world's poster-child plant invaders, *Melaleuca quinquenervia*, which has invaded large areas and caused major damage in the Everglades region in Florida (Richardson and Rejmánek 2011). The genus has about 290 species consisting of shrubs and trees, a number of which are planted in many parts of the world, largely as ornamentals, but also for timber, honey, bark and plant extracts (Brophy et al. 2013). Widespread cultivation of *Melaleuca* species is relatively recent, especially when compared to other genera in the Myrtaceae such as *Eucalyptus*, and records of naturalization and invasions in South Africa (Jacobs et al. 2014, 2015) and other parts of the world (Rejmánek and Richardson 2013) are comparably recent. Several species are recorded as weedy within Australia (Randall 2007), perhaps indicating that these (mostly) fire-adapted species could pose a risk to areas with similar fire-prone areas, such as the Cape Floristic Region of South Africa which has been invaded by many other woody plants from Australia (Wilson et al. 2014b).

In 2009, the discovery of several naturalised populations of *Melaleuca* species in South Africa prompted an evaluation of the introduction status for the entire group in the country (Wilson et al. 2013). Taxa such as *Melaleuca armillaris* subsp. *armillaris*, *M. viminalis* subsp. *viminalis* and *M. citrina* have been widely planted in South Africa and also warranted further study. This also provided an opportunity to reassess the accuracy of current published lists.

Here, we compile a list of *Melaleuca* species recorded as present in South Africa and determine the invasive status of each species. We use herbarium specimens to do this, while also noting the extent to which they are accurately identified and the types of errors which occur. We discuss consequences of errors and omissions and make recommendations on how these could be avoided and addressed.

Methods

Taxonomy

Generic limits in the tribe Melaleuceae have been the subject of much recent study (Brown et al. 2000, Wilson et al. 2005, Craven 2006, Edwards et al. 2010, Udovicic and Spencer 2012, Craven et al. 2014). We follow Craven (2006), Edwards et al. (2010) and Brophy et al. (2013) in adopting a broad concept of *Melaleuca*, i.e. including *Callistemon*. The further expansion of the genus *Melaleuca* to include *Beaufortia*, *Calothamnus*, *Conothamnus*, *Eremaea*, *Lamarchea*, *Petraeomyrtus*, *Phymatocarpus*, and *Regelia*

(Craven et al. 2014), has not yet been fully evaluated by the Australian taxonomic community, and these taxa are excluded from consideration for this study. Many *Melaleuca* species (especially those formerly recognised as *Callistemon*) are morphologically similar which makes them difficult to identify using morphological features. Several cultivars have been developed for some *Melaleuca* species in the *Callistemon* group and difficulty in identifying such specimens in South Africa is perhaps due to horticultural selection and the existence of hybrid and both sexual and apomictic species within the group (Craven 2009, Brophy et al. 2013).

Review of herbarium specimens and error classification

Herbarium specimens from the Compton herbarium (NBG) were examined to check whether specimen identifications were correct, and to provide accurate identifications where necessary. To do this, we used the taxonomic literature to compare morphological characters on the specimens with descriptions and taxonomic keys (in particular Craven and Lepschi 1999 and Brophy et al. 2013). Photographs and high-resolution scans of the specimens were taken for verification and future reference. Specimen identifications were checked against referenced herbarium specimens housed at the Australian National Herbarium (CANB; herbarium codes follow Thiers (2016)). The identifications of all specimens were subsequently confirmed by a taxonomic authority for *Melaleuca* (B.J. Lepschi).

Herbarium specimens were examined in 2013; any specimens accessioned or re-identified after this date were not included in the analysis. McGeoch et al. (2012) proposed an uncertainty classification that separates epistemic and linguistic errors into ten sub-categories. In this study we focussed on two of these sub-categories—human error and species identification. In keeping with terminology from McGeoch et al. (2012), we define the word "error" to be inclusive of actual and potential errors. For example, although a species name on a specimen was not currently accepted but no obvious mistake in listing arose from this yet, it was still recorded. As per McGeoch et al. (2012) scheme the human errors we discovered in this study were: misidentifications, and improved resolution of the identification (e.g. *Melaleuca* sp. identified as *M. parvistaminea*, or *M. armillaris* as *M. armillaris* subsp. *armillaris*). The only species identification error was unresolved taxonomy. A description of the different errors and how they were determined is shown in Table 1, as well as the frequency and relative proportions of the errors. Because the inclusion of *Callistemon* in an expanded *Melaleuca* is still under debate, synonyms where the genus name *Callistemon* changed to *Melaleuca* were placed under the species identification error type (instead of human error as per McGeoch et al. 2012 treatment). No synonyms outside of this situation were found and therefore synonyms relating to human error were absent from our dataset.

We also looked to see if there were any historical trends in the errors by comparing the years when taxa with particular errors were collected to the years when taxa with no errors were collected using Mann-Whitney U tests in R.

Table 1. Result of analysis of confirmed herbarium records (n=111), indicating the breakdown of correctly identified specimens with various error types. For full details see Suppl. material 1: Appendix. The errors identified here are error type 1 (i.e. human error, indicated as HE) and type 3 (i.e. species identification indicated as SI) as per McGeoch et al. (2012); synonyms are included in type 3 here (see Methods). The table only includes samples from the Compton Herbarium, Kirstenbosch (NBG).

Status	Description	Number of herbarium specimens	Examples
Correctly identified	The identification on the herbarium specimen was the same as determined by an expert in the group (the author: BL)	31	Seven specimens of *Melaleuca styphelioides* and five specimens of *M. hypericifolia* correctly identified
Misidentification (HE)	The identification on the herbarium specimen was to a currently accepted species, but not the correct one	31	*Melaleuca parvistaminea, M. armillaris* subsp. *armillaris* and *M. cuticularis* were misidentified as *M. ericifolia*
Further identification (HE)	The identification on the herbarium specimen could be refined, either by providing the specific epithet or the subspecific epithet	25	Several specimens (e.g. *M. rugulosa*) only identified to genus level; *M. armillaris* could be identified further to subspecies level
Unresolved taxonomy (SI)	The taxonomy used to identify the herbarium specimen was not resolved at that time, so any name provided will have some uncertainty around it.	2	Several names misapplied to *Melaleuca quinquenervia* (prior to 1968)
Synonym (SI)	The identification was confirmed, but the name on the herbarium specimen was not the most current accepted name	22	Nine specimens of *Callistemon rigidus* (a synonym of *C. linearis*, also a synonym of *Melaleuca linearis* var. *linearis*), *Callistemon viminalis = Melaleuca viminalis* subsp. *viminalis*

List compilation

Once correct identification for all specimens had been confirmed, we used these specimens as the source for compiling a list of species present in South Africa. We also used a list of cultivated plants based on herbarium records in southern Africa (Glen 2002), and a list of forestry trees and their uses in South Africa (Poynton 2009). The minimum residence time in South Africa was determined from the date on the oldest herbarium specimen for each species.

Naturalized populations were reported by a variety of conservation agencies, with the reports collated by the South African National Biodiversity Institute's Invasive Species Programme and through the Southern African Plant Invaders Atlas (Henderson et al. 2007; Wilson et al. 2013).

We collected height data as an estimate of age, presence/absence of reproductive structures and GPS coordinates for each plant. Using these data we were able to determine whether a species is sustaining itself, whether it is reproducing and/or spreading, hereby indicating the status of each species as introduced, naturalized or invasive according to the subcategories proposed by Blackburn et al. (2011).

Results

Review of herbarium specimens

A summary of the errors found is in Table 1 with details of each specimen that required a name change in Suppl. material 1: Appendix. Examples of the types of errors on are shown in Figure 1. Of the 111 specimens examined, only 31 specimens carried a currently accepted name (excluding current names for specimens that were incorrectly identified). Misidentifications made up the largest proportion of errors, while poorly resolved taxonomy was the reason for two specimens being incorrectly named. All synonyms required at least the genus name to be changed.

There was no significant effect of date of collection on whether an error was noted, or on particular errors types (dates of collection varied between 1907 and 2013).

List compilation

Our analysis of herbarium specimens and the lists in Glen (2002), also based on herbarium collections, is summarised in Table 2 (no additional species were found in Poynton's (2009) list), with species that did not have confirmed herbarium records discussed in Table 3. Thirty-six species are confirmed present in South Africa, of which ten species are naturalized – five of these are invasive (Fig. 2; Table 2). Five naturalized species were categorised as C3 according to Blackburn et al. (2011), indicating that individuals were surviving, reproducing and populations were self-sustaining, but less than 100 m from planting sites (Richardson et al. 2000; Wilson et al. 2014). *Melaleuca linearis* var. *linearis*, *M. hypericifolia*, *M. rugulosa* and *M. viminalis* subsp. *viminalis* are invasive, surviving and reproducing a significant distance from the site of original introduction, but not over a wide extent (D2). *Melaleuca parvistaminea* is invading several sites (E) near the towns of Tulbagh and Wolseley in the Western Cape province (Fig. 2c). There are a few separate invasive populations spread over ~10,000 ha, with a total of around 30 000 plants (Fig. 3; Jacobs et al. (2014)).

Discussion and conclusions

There are a number of ways that errors can be generated during the compilation of species lists (McGeoch et al. 2012), but here we show the challenges that exist at a fundamental stage of the listing process. Importantly, since only a subset of herbaria were analysed in detail, there could be additional errors (and in fact additional species) present in South Africa. The high proportion of misidentifications (Table 1) is concerning, indicating the difficulties encountered when dealing with novel species and highlighting the need for expertise on specific non-indigenous taxa. Synonymy, however, does not necessarily imply

Figure 1. Examples of the types of errors found on the herbarium specimens examined, **a** Misidentification: *Melaleuca salicina* misidentified as *Callistemon pallidus* **b** Improved identification: *Callistemon* sp. was further identified as *Melaleuca rugulosa* **c** Synonymy: *Callistemon rigidus* is a synonym of *C. linearis* but is currently accepted as *Melaleuca linearis* var. *linearis*, and **d** unresolved taxonomy: prior to 1968, *Melaleuca quinquenervia*, along with several other broad-leaved species were included under *M. leucadendra sensu lato*.

Table 2. List of 36 _Melaleuca_ species in South Africa for which there is a confirmed herbarium record in either the Compton Herbarium, Kirstenbosch (NBG) or in the cultivated collection in the National Herbarium (PRE). Note that several other collections were searched, but no additional species could be discovered. Invasive status is according to Blackburn et al. (2011), with interpretation for trees from Wilson et al. (2014). All species were used as ornamentals although older records are often associated with historic forestry sites and arboreta. Later records were sourced mainly from gardens and nurseries. Recently used synonyms are listed and are intended to aid recognition of some species.

Species	Recently used synonym / misapplied name	Earliest record	Status in South Africa	Notes and references
Melaleuca alternifolia (Maiden & Betche) Cheel		1974	Introduced B2	
Melaleuca armillaris (Sol. ex Gaertn.) Sm. subsp. _armillaris_		1930	Naturalized C3	Widely cultivated ornamental. Potentially invasive.
Melaleuca brachyandra (Lindl.) Craven	_Callistemon brachyandrus_ Lindl.	1968	Introduced B2	
Melaleuca bracteata F.Muell.		1981	Introduced B2	
Melaleuca citrina (Curtis) Dum.Cours.	_Callistemon citrinus_ (Curtis) Skeels	1932	Naturalized C3	Bromilow (2010). Cultivars and hybrids also introduced. Cited in Réjmanek and Richardson (2013). Also recorded in Southern African Plant Invaders Atlas at Honingklip farm (3419AC) in 1998, but plants have been removed
Melaleuca cuticularis Labill.		1902	Introduced B2	
Melaleuca decora (Salisb.) Britten	_Melaleuca genistifolia_ Sm.	1963	Introduced B2	
Melaleuca decussata R.Br.		1954	Introduced B2/B3	
Melaleuca diosmifolia Andrews		1933	Introduced B2	
Melaleuca elliptica Labill.		1963	Introduced B2	Observed in the deer park on the slopes of Devil's Peak, Table Mountain, Cape Town. Possibly naturalized, but no supporting evidence.
Melaleuca flammea Craven	_Callistemon acuminatus_ Cheel	1986	Introduced B2	
Melaleuca fulgens R.Br.		1952	Introduced B2	
Melaleuca huegelii Endl. subsp. _huegelii_		1945	Introduced B2	
Melaleuca hypericifolia Sm.		1902	Invasive D2	Hickley et al. (2017). Field data at Hout Bay indicate spread > 100 m.
Melaleuca incana R.Br. subsp. _incana_		1967	Introduced B2	
Melaleuca incana subsp. _tenella_ (Benth.) Barlow		1981	Introduced B2	
Melaleuca lanceolata Otto		1982	Introduced B2	

Species	Recently used synonym / misapplied name	Earliest record	Status in South Africa	Notes and references
Melaleuca lateritia A.Dietr.		1954	Introduced B2	
Melaleuca linariifolia Sm.		1958	Introduced B2	
Melaleuca linearis Schrad. & J.C.Wendl. var. *linearis*	*Callistemon linearis* (Schrad. & J.C.Wendl.) Colvill ex Sweet, *C. rigidus* R.Br.	1902	Invasive D2	Several plants found at Kluitjeskraal and 56 plants (30–130 cm height range) were found at two sites in Grahamstown.
Melaleuca nesophila F.Muell.		1967	Introduced B2	
Melaleuca nodosa Sm.		1961	Introduced B2	
Melaleuca pachyphylla (Cheel) Craven	*Callistemon pachyphyllus* Cheel	1983	Introduced B2	
Melaleuca paludicola Craven	*Callistemon sieberi* DC.	2011	Introduced B2	
Melaleuca parvistaminea Byrnes		1933	Invasive E	Invading a wetland system, Jacobs et al. (2014). Misidentified as the morphologically similar *M. ericifolia* Sm.
Melaleuca phoenicea (Lindl.) Craven	*Callistemon phoeniceus* Lindl.	1981	Introduced B2	
Melaleuca quinquenervia (Cav.) S.T.Blake	*Melaleuca leucadendra* L.	1928	Naturalized C3	Jacobs et al. (2015)
Melaleuca rhaphiophylla Schauer		1984	Introduced B2	
Melaleuca rugulosa (Schltdl. ex Link) Craven	*Callistemon rugulosus* (Schltdl. ex Link) DC.	1961	Invasive D1/D2	Devil's Peak, Cape Town. Spread > 500 m. ~20 adults. Seedlings growing in firebreak.
Melaleuca salicina Craven	*Callistemon salignus* (DC.) Colvill ex Sweet	1932	Naturalized C3	
Melaleuca squarrosa Donn ex Sm.		1994	Introduced B2	
Melaleuca stypphelioides Sm.		1902	Naturalized C3	145 plants at Kluitjeskraal near the town Wosleley (60–450 cm height range)
Melaleuca subulata (Cheel) Craven	*Callistemon subulatus* Cheel	2013	Introduced B2/ Naturalized C3	Near water body 10km NE of Villiersdorp, possibly at Rockview dam near Grabouw
Melaleuca teretifolia Endl.		1967	Introduced B2	
Melaleuca thymifolia Sm.		1907	Introduced B2	
Melaleuca viminalis (Sol. ex Gaertn) Byrnes subsp. *viminalis*	*Callistemon viminalis* Sol. ex Gaertn subsp. *viminalis*	1948	Invasive D2	Widely planted with several localized sites of naturalization. Spreading along Kaaimans river ~3 km East of George

Figure 2. Examples of naturalized *Melaleuca* species in South Africa. **a** naturalized *M. quinquenervia* plants showing seed capsules opening after fire **b** *M. viminalis* subsp. *viminalis* naturalized along a stream in an urban setting **c** *Melaleuca parvistaminea* invading a conservation area that was previously under pine plantation, and **d** *M. linearis* var. *linearis* is invasive at another site previously under plantation with *M. parvistaminea* in background. Photos: **a, c** is E van Wyk, **b** is LEO Jacobs, **d** is DM Richardson.

human error, but rather that the use of an outdated or otherwise superseded taxonomy can lead to errors in interpretation, or incorrect estimates of numbers by subsequent users (McGeoch et al. 2012). In this study however, synonymy arose rather from differing perceptions of *Callistemon*, than from human error. Although the effect of synonymy is potentially large (McGeoch et al. 2012), the checking of synonymies is commonly practised. However, a rudimentary training in taxonomic principles is necessary for any practitioner dealing with scientific names. It is of concern that the inclusion of *Callistemon* in an expanded *Melaleuca* is still under debate. All synonym issues found in our study at least
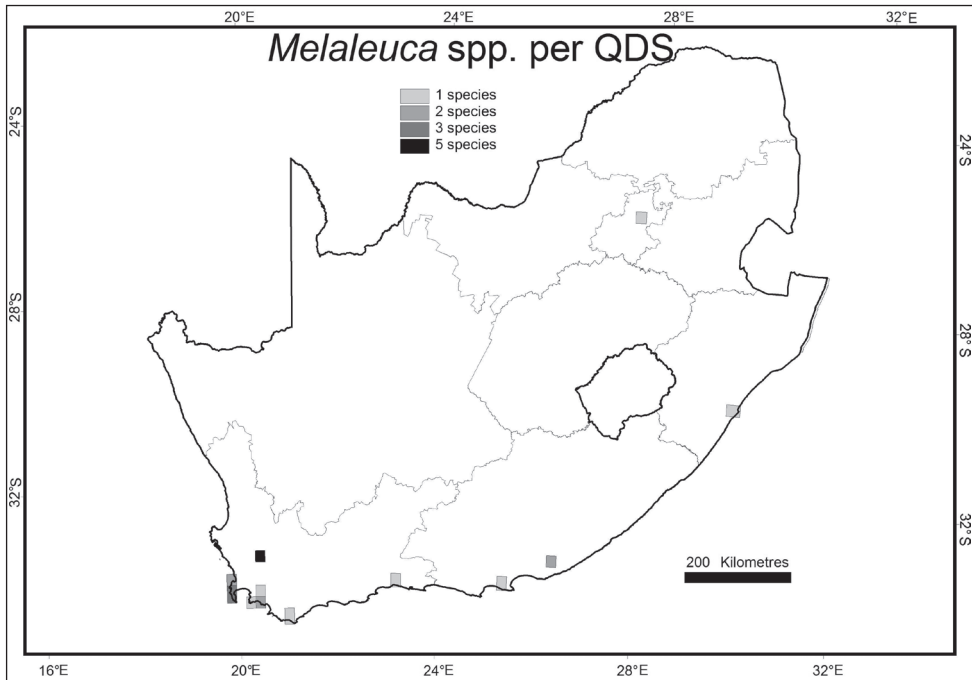
Figure 3. Localities of naturalized *Melaleuca* species in South Africa at the resolution of quarter-degree squares (QDS). Darker shading indicates a higher number of species. Grey borders are province boundaries.

Table 3. List of *Melaleuca* species recorded in South Africa for which there is no confirmed herbarium record.

Species	Earliest record	Source of information	Notes
Melaleuca hamulosa Turcz.	Unknown	Glen (2002)	No specimens found in PRE cultivated collection
Melaleuca glauca (Sweet) Craven [recorded as *Callistemon speciosus* (Sims) DC.]	Unknown	Glen (2002)	No specimens found in PRE cultivated collection
Melaleuca paludosa (Sweet) Craven [recorded as *Callistemon glaucus* (Bonpl.) Sweet]	1979	South African Plant Invaders Atlas (SAPIA)	Probably a misidentification. The only species found at the reported locality in Grahamstown is *M. linearis*.
Melaleuca pauperifolia F.Muell.	Unknown	Glen (2002)	No specimens found in PRE cultivated collection
Melaleuca wilsonii F.Muell.	1998	South African Plant Invaders Atlas (SAPIA, Australia's Virtual Herbarium (MEL 2053098A)	Land owner at Honingklip near Botrivier in the Western Cape reports historic occurrence of "bottlebrushes" but no *Melaleuca* species occur at this site as of 2011.
Melaleuca nervosa (Lindl.) Cheel	Unknown	Gibbs (1998)	One tree recorded at Damara Farm near Malmesbury. Several *Acacia* species trials were also carried out at this site

required the genus name to be updated. Lists not taking this into account could generate errors of a greater magnitude than errors relating only to the specific epithet.

Lists therefore require the application of taxonomic expertise on taxa not native to a particular region (Pyšek et al. 2013). The knowledge generated from these lists form the basis for informing end users, (e.g. quarantine officials, conservation agencies) that perform crucial functions in stemming the tide of biological invasions and informing future research (e.g. identifying biological control agents) requiring accurate species identifications. Herbaria have often served as barometers for new and rediscovered alien plant species. They also provide a reference source for research or conservation initiatives that require accurate species names. The ongoing decline of resources being allocated to the maintenance of herbaria worldwide will adversely affect many research fields including invasion biology (Guerin 2013, Pyšek et al. 2013). We strongly believe that part of the funding for invasive species management needs to be allocated to the maintenance and functioning of herbaria and other collections as they are an essential resource for the work (this has begun to be supported in South Africa but further sustained resources need to be devoted to this). The same could be suggested for other fields of botanical research.

While genetic verification of species identifications is proving to be a reliable means of verifying a species, classical taxonomy still remains crucial to the identification of new species to a region (Pyšek et al. 2013). In the absence of molecular data suitable for use in species identification, identifications based on morphology are usually adequate (Pyšek et al. 2013). For these reasons, and an uncertain taxonomy in some cases, we found morphological identification based on published descriptions and keys the best approach to reviewing herbarium specimens of *Melaleuca*. Because suitable molecular data is often lacking, we recommend that DNA barcoding efforts should prioritise potentially invasive genera, so that species can be accurately identified in regions where expertise on that group is likely absent. Species identification issues due to uncertain or unresolved taxonomy can be avoided by continued taxonomic research (Edwards et al. 2010). This research will likely be conducted in the country of origin and therefore cross-border communication and collaboration between taxonomists are essential (Smith et al. 2008, Pyšek et al. 2013). Errors could be avoided by either collaborating with researchers from regions where alien species are native, thus tying into a strategic response of the *Global Invasive Alien Species Strategy* (McNeely 2001) or by investing in local taxonomic expertise on key alien groups. There are several ways in which these groups could be identified based on known patterns of invasion. Minimum residence time, invasiveness in other regions and weedy species are data obtainable from herbarium specimens and could thus be used to identify these groups.

Identification errors noted in this study have had direct implications. *Melaleuca parvistaminea* was initially misidentified in 2011 as the morphologically similar *M. ericifolia*. *Melaleuca parvistaminea* was only formally described in 1984 and collections prior to this were treated within the broad concept for *M. ericifolia*. Some *M. armillaris* subsp. *armillaris* specimens were also misidentified as *M. ericifolia* (e.g. NBG0269364). *Melaleuca ericifolia* is regarded as being predominantly clonal rather than reseeding. This affected management actions, through unforeseen profuse recruitment via seed

after clearing and the absence of clonal spread and resprouting (Jacobs et al. 2014). The incorrect name was perpetuated into Richardson and Rejmánek's (2011) global list of invasive trees and shrubs, but corrected in an update of this list (Rejmánek and Richardson 2013). Although this was not investigated, it is possible that publications citing *Melaleuca* species from Richardson and Rejmánek (2011) could carry this mistake forward.

Effective pre-emptive control efforts rely heavily on whether alien species are listed as invasive in that region or are known to be invasive elsewhere (Mack 1996). As a result of debate surrounding generic limits in the tribe Melaleuceae, especially regarding the recognition of *Callistemon* as a segregate genus (Craven 2006; Udovicic and Spencer 2012, Edwards et al. 2010, Craven et al. 2014), species lists included in the recently published *Alien and Invasive Species Regulations in South Africa* (DEA 2014) may generate errors due to synonymy issues. For example, the regulations list *Callistemon rigidus*, which is now treated as a synonym of *C. linearis* if one accepts the separation of the two genera (see Council of Heads of Australasian Herbaria 2016); if a broad concept of *Melaleuca* is adopted, then the taxon should be listed as *Melaleuca linearis*. Moreover, several species have been omitted from the regulations, e.g. *Melaleuca parvistaminea,* a species which is clearly invasive and poses a considerable environmental threat (Jacobs et al. 2014). Recognition of situations like these requires adequate taxonomic expertise and familiarity with the group in question.

Hybridization and horticultural selection for some *Melaleuca* species, especially those in the *Callistemon* group can further complicate accurate identification (Brophy et al. 2013). Hybrids and several cultivars exist for some taxa and it is not clear whether some hybrids or cultivars are more invasive than others. Moreover, some *Melaleuca* species, such as *M. linearis*, are apomictic and may further contribute to species identification problems.

We identified ten species of *Melaleuca* naturalised in the Western Cape province of South Africa, but invasions of taxa in this genus are at an early stage, and there is likely to be a high level of invasion debt (*sensu* Rouget et al. 2016). Unlike other invasive Australian tree and shrub species (e.g. *Acacia* and *Eucalyptus*), *Melaleuca* species were never widely disseminated in South Africa for forestry or dune stabilisation. *Melaleuca quinquenervia* was introduced and widely disseminated for a variety of reasons, including ecosystem engineering, in the USA (Dray et al. 2006). No wide scale plantings took place in South Africa. *Melaleuca* introductions and plantings in South Africa have been for ornamental purposes, mostly in the last few decades. Because naturalized populations are still small there is still the opportunity to eradicate several species if action is taken quickly and with sufficient resources. Besides the small populations, other factors that suggest that eradication is feasible are the short-lived serotinous seed banks, the effectiveness of available herbicides (Jacobs et al. 2014, van Wyk and Jacobs 2015), limited dispersal capability (inferred from Rejmánek and Richardson 2011) and a focused, national programme with a mandate to respond to incursions (Wilson et al. 2013). The high level of errors in identification which we found in this study, however, highlights the urgent need to assess and improve the accuracy of alien species lists.

Acknowledgements

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Supplementary material 1

Appendix and Supplementary Figures

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Data type: Table (Appendix 1), map (supplementary figures)

Explanation note:

Appendix: Herbarium specimens requiring name changes, indicating accession numbers, original species name, corrected species name and types of errors.

Supplementary Figures: Distribution at the quarter-degree cell scale of nine *Melaleuca* species naturalized in South Africa. No map is given for *Melaleuca citrina* because historic sites of naturalization could not be confirmed or plants were no longer there.

RESEARCH ARTICLE

Evaluating simplistic methods to understand current distributions and forecast distribution changes under climate change scenarios: an example with coypu (*Myocastor coypus*)

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Abstract

Invasive species provide a unique opportunity to evaluate factors controlling biogeographic distributions; we can consider introduction success as an experiment testing suitability of environmental conditions. Predicting potential distributions of spreading species is not easy, and forecasting potential distributions with changing climate is even more difficult. Using the globally invasive coypu (*Myocastor coypus* [Molina, 1782]), we evaluate and compare the utility of a simplistic ecophysiological based model and a correlative model to predict current and future distribution. The ecophysiological model was based on winter temperature relationships with nutria survival. We developed correlative statistical models using the Software for Assisted Habitat Modeling and biologically relevant climate data with a global extent. We applied the ecophysiological based model to several global circulation model (GCM) predictions for mid-century. We used global coypu introduction data to evaluate these models and to explore a hypothesized physiological limitation, finding general agreement with known coypu distribution locally and globally and support for an upper thermal tolerance threshold. Global circulation model based model results showed variability in

coypu predicted distribution among GCMs, but had general agreement of increasing suitable area in the USA. Our methods highlighted the dynamic nature of the edges of the coypu distribution due to climate non-equilibrium, and uncertainty associated with forecasting future distributions. Areas deemed suitable habitat, especially those on the edge of the current known range, could be used for early detection of the spread of coypu populations for management purposes. Combining approaches can be beneficial to predicting potential distributions of invasive species now and in the future and in exploring hypotheses of factors controlling distributions.

Introduction

Understanding species distributions and forecasting potential distributional changes with changing climates is a common goal in ecology. Invasive species provide a unique opportunity to evaluate factors controlling distribution using introduction information to evaluate different hypotheses. Species distribution models (SDM) have a wide range of applications ranging from conservation to invasive species management. There are several different approaches to developing SDMs, including mathematical models, defined a priori, that causally relate a species presence to the environment, and statistical models based on direct correlations between observations of the species and the environment (Dormann et al. 2012).

Correlative models assume that the species being modeled is in equilibrium with its environment, that the current distribution represents basic habitat requirements of the species, and that these requirements are constant through time (Pearson and Dawson 2003). These assumptions are known to be unrealistic, especially for invasive species that are often still spreading in their invaded ranges (Araújo and Peterson 2012; Elith et al. 2010). Correlative models are relatively simple to parameterize, requiring location data for a species and associated environmental data (Kearney and Porter 2009). Ecophysiological based models, on the other hand, are often more difficult to parameterize because they generally require detailed information about the physiological requirements of the species. However, ecophysiological based models may be more appropriate for forecasting species distributions under climate change scenarios due to their causal nature, and the simple and reasonable assumption that physiologically limiting mechanisms are maintained in the models (Dormann 2007).

The coypu (*Myocastor coypus* [Molina, 1782]) is a large, semi-aquatic, invasive rodent native to South America south of 23° latitude (Ehrlich 1967; Woods et al. 1992). The native range includes southern Brazil, Bolivia, Paraguay, Uruguay, Argentina, and central and southern Chile (Gosling and Baker 1991). The coypu has been introduced around the world over the last century for fur farming (Carter and Leonard 2002), but has also been released as a game animal and as a management strategy to control

aquatic vegetation (Bounds and Carowan 2000). Feral populations quickly established throughout the introduced range when individuals escaped from farms or were released when fur markets declined. Coypu are now established on every continent except Australia and Antarctica (Carter and Leonard 2002). In the USA, 15 states are considered to have stable or increasing coypu populations (Bounds and Carowan 2000). The global scale introduction, with sufficient time to allow spread in many areas, can be used as an experiment to test if thermal boundaries on coypu distribution exist.

The coypu is a generalist herbivore, with a diet that includes all types of plant material, including leaves, stems, roots, and bark (Willner et al. 1979). Coypu over-utilize preferred species (Borgnia et al. 2000), but are able to change food habits seasonally based on availability of food sources (Abbas 1991; Wilsey et al. 1991). Thus, it is not believed that food or habitat are limiting factors in their distribution. In their introduced range, coypus experience high mortality during periods of sustained freezing temperatures due to both physiological constraints and the lack of available food resources during these events (Doncaster and Micol 1989; Gosling et al. 1981; Willner et al. 1979). There is no evidence of an upper thermal limit for coypus, but we can explore this theory using observed data from the native range and introduction success around the globe.

Using coypu as a test case, we examined and compared the utility of using a very simplistic ecophysiological based model versus a correlative model to predict current and future coypu distribution. We used independent regional and global distribution information to validate the two approaches. Specifically, our objectives were to: 1) evaluate the relationship between known physiological limitations and geographical distribution, 2) evaluate a hypothesized physiological limitation using native range and introduction success information, 3) predict future distribution based on climate change scenarios, and 4) evaluate the benefit of using both modeling approaches. Given the economic and ecological impacts of coypu in invaded ranges, secondary objectives were to develop a current model of potential suitable habitat for coypu within the USA and globally and to investigate possible distribution changes under potential climate change to inform management strategies.

Methods

Occurrence data

Global occurrence records for coypu were downloaded from the Global Biodiversity Information Facility (GBIF; gbif.org; March 4, 2011). The data were inspected, and records with a resolution greater than 30 minutes, our model resolution, were removed due to accuracy issues. We also removed presence locations in countries or states with a status of never established or extinct, retaining only those with a status of country of origin, escape or release, range expansion, or eradicated as defined by a global review of coypu distribution (Carter and Leonard 2002).

Environmental data

For the USA, we used monthly mean, minimum and maximum temperature data at a 4-km spatial resolution between 2003 and 2007 for our analyses (PRISM Group 2007). This time frame is biologically meaningful in that it matches the average lifespan of an individual, and is data-driven in that it matches the time frame of the sub-watershed scale (hydrologic unit code [HUC] 12) data used to validate the model in the Pacific Northwest, USA. Global environmental data were obtained from World-Clim (Hijmans et al. 2005). These data were averaged by month between 1950 and 2000 at a 30 arc second resolution. Thus, the national-scale climate data had a fine temporal resolution (monthly data) that matched some data collection whereas the global climate data had a finer spatial resolution with a coarse temporal resolution (50-year average).

Ecophysiological based modeling

We developed ecophysiological based models at the continental USA and the global scale, based on known physiological constraints on coypu. This species has known winter temperature tolerances that are thought to be the primary limiting factors on their distribution, at least in temperate regions (Gosling et al. 1983). Gosling et al. developed a population simulation model based on observed relationships between sequences of freezing days (defined as minimum temperature < 0 °C and maximum temperature < 5 °C) and body fat, litter frequency, litter size, and mortality. This model showed that a sequence of freezing days resulted in population declines due to adverse effects on the four measured characteristics, and Doncaster and Micol (1990) reported a 71% decrease in population density after canals were frozen for 20 consecutive days in France. In addition, coypu heavily depend on aquatic environments and are limited to environments within the transition zone between aquatic and upland environments (D'Adamo et al. 2000; Doncaster and Micol 1989; Guichón et al. 2003). We used this information on known coypu requirements (sensitivity to cold temperatures and need for aquatic environment) to define a model of habitat suitability rather than allowing a statistical model to detect relationships between habitat suitability and coypu presence.

For the continental USA we developed two different ecophysiological based models using monthly climate data from the PRISM data set at 4-km resolution; one using a five-year period (2003 to 2007) and another using a three-year period (2005 to 2007), hereafter referred to as US 5yr and US 3yr. We used two different time periods to assess the importance of inter-annual climatic variability on predicted distribution. We calculated the number of months within each time period that had a minimum temperature of less than 0 °C and a maximum temperature of less than 5 °C. Given the negative relationship between coypu populations and sequences of freezing days, we defined any month with average values meeting these criteria as unsuitable for coypu survival. To address the water limitation we developed a layer of arid locations

by identifying locations in the USA with annual precipitation less than 250 mm, based on PRISM average annual precipitation from 2003 to 2007.

For the global ecophysiological model, we used WorldClim monthly data averaged from 1950 to 2000 at a 30 arc second resolution (~1 km), hereafter referred to as Global 50yr. Unsuitable environments were defined as locations with any month meeting the criteria of average minimum temperature less than 0 °C and average maximum temperature less than 5 °C. We again masked out arid regions, defined as areas with annual precipitation less than 250 mm based on the WorldClim average annual precipitation layer.

Correlative modeling

We used the VisTrails software (Freire et al. 2006) with the Software for Assisted Habitat Modeling (SAHM) package (Morisette et al. 2013) to develop correlative models of global coypu distribution using a Generalized Linear Model (GLM). We used GLMs because this technique creates simple models and has been recommended for model generalization (i.e., transferability to novel environment or time periods, Heikkinen et al. 2012). We generated background points using two different methods: a random generation of 10,000 locations within countries from which our data set had location records, referred to as GLM country, and a targeted background approach using location data for muskrats (*Ondatra zibethicus*), referred to as GLM targeted (Suppl. material 1: Figure 1). We downloaded muskrat data from GBIF and cleaned it by dropping records that had a spatial resolution greater than 30 min, removing fossil records and removing records from countries not known to have muskrats. The target background approach of using locations for similar species is recommended when using a presence-background method where the data are likely to have sampling bias (Phillips et al. 2009). Coypu and muskrats are sympatric species because both rodents are aquatic, are herbivores, are burrowers, and have similar global distributions (Ruys et al. 2011). By using a targeted background approach, biases in the presence locations are also assumed to occur in the background and thus cancel each other to some degree, similar to presence and absence data collected using the same methodology.

The number of environmental variables from the global WorldClim data set used in the GLM was limited to six based on the known physiology of coypu and included mean diurnal range, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, precipitation seasonality, and precipitation of warmest quarter. Environmental variables were reduced by removing one of each pair of highly correlated environmental variables (maximum of Spearman rank coefficient, Pearson's product moment or Kendall tau rank; |r| > 0.7 following the recommendation of Dormann et al. (2013)) and biological knowledge of the species.

Using a threshold defined as maximizing sensitivity plus specificity divided by two, we created binary predictions of suitable and unsuitable habitat for the correlative models.

Liu et al. (2013) recommended this threshold because it is transferable between methods that use presence-absence and presence-background. The binary predictions were then used to create equal-weight ensemble predictions of habitat suitability for coypu. We created two sets of ensemble models, three for the USA and one for the globe. The USA ensembles included an ensemble of the correlative models (GLM country and GLM targeted), another of the three ecophysiological based models (US 3yr, US 5yr, and global 50yr), and another of all five models. The global ensemble model was created using all three global models including the global 50yr, the GLM country, and GLM targeted models.

Model evaluation

The models were evaluated using zonal statics at two scales; sub-watershed hydrologic unit code (HUC12) and the USA state boundaries. Standardized spatial surveys completed by on-the-ground fish and wildlife biologists for Oregon and Washington provided coypu density estimates at the HUC12 level and were used as an independent model validation (Sheffels 2013). Using the binary model predictions, we calculated zonal statistics using ArcGIS version 10.0 (ESRI, 2011) for each HUC12. If any location within a HUC12 was classified as suitable by the model, the entire HUC12 was defined as suitable. HUC12 coypu density estimates were grouped within four density classes, >100, 11–100, 1–10, and 0 individuals, and the percent of HUC12 units that classified as suitable for each model were calculated for each density class. The models were also evaluated using zonal statistics identifying the number of USA states by coypu status (i.e., never established/extinct, present, no data and eradicated) identified by Carter and Leonard (2002). Again, if a state had any locations within it identified as suitable by the model, the state was defined as suitable, while a state was defined as unsuitable if it did not have any suitable habitat (i.e., no suitable locations within entire state).

We evaluated the global models using two additional methods. Similar to the state level evaluation, we used country level zonal statistics compared to the coypu status identified by Carter and Leonard (2002) for countries. Countries were classified into two coypu occurrence statuses: present (status of present or eradicated) and absent (status of never established or extinct). A country was classified as suitable habitat if any location within the country was predicted suitable based on the model. In addition, we used independent georeferenced locations as another evaluation metric. These independent records were compiled by searching the social media site 'YouTube' for the keywords: 'bieberratte' and 'wasserratte' (German), 'beverrat' (Dutch), 'castorino' (Italian), 'coypu' (British English, Spanish), 'nutria' (American English, Italian), 'ragondin' (French), Нумрия (Russian, Kyrgyzstani, Uzbekistani). The coypu in the videos had to be a naturally occurring population and the location of where the video was taken provided. Videos were examined to make certain that other species were not being misidentified as coypus. Videos where coypu were held as pets or in other confined situations such as fur farms, zoos or aquaria or for which location

could not be determined were excluded. For videos not in English or French we used 'Google Translate' as an approximate translation tool to determine the circumstances and location. Since our focus was documenting the range of coypu, once presence was determined in a particular location we excluded videos from those regions on future searches. Finally, we used documents from national reports such as the 'Red Book Data and Invasive Species Korea' or personal communications from trusted researchers to verify regional presence. We used R version 2.15 (R Core Team, 2012) and the caret package (Kuhn, 2013) to calculate sensitivity, specificity and percent correctly classified for each global model based on the model predictions and either the classification by Carter and Leonard (2002) or the independent locations.

Forecasting distributions

We applied our ecophysiological based rule-set to future climate data. We obtained historic data from the Maurer data set (Maurer et al. 2007), which covers the USA at 1/8th degree (~12km). These data were the reference data set used to downscale the global climate projections (GCMs) from the World Climate Research Programme's Coupled Model Intercomparison Project phase 5 (CMIP5) projections using the monthly bias correction spatial disaggregation (BCSD) technique (Reclamation 2013). These downscaled GCMs (listed in Suppl. material 1: Table 1) provided monthly projections of total precipitation and monthly average temperature. We quantified the amount of suitable habitat (i.e., the number of pixels meeting the criteria of monthly minimum temperature >0 °C or monthly maximum temperature >5 °C) for the Maurer dataset (1950 to 2013) and the 12 downscaled GCMs available (1950 to 2013 for historic comparison; 2014 to 2100 as climate forecasts). We calculated the forecasts for all four representative concentration pathways (RCPs), which describe four different greenhouse gas concentration trajectories. We also generated maps of suitable habitat based on the Maurer dataset for 2003 to 2013 and an ensemble of downscaled GCMs for 2006 to 2016 to assess how well the GCMs performed currently when compared to the observations on which they were calibrated. Because the forecasted GCMs began in 2006, we were unable to have complete overlap in the decades for comparison. We applied our criteria for suitable habitat to the GCMs for the period 2040 to 2050 to assess how coypu distribution may change in the future. For this forecast we only used the 4.5 RCP, as RCPs do not begin to diverge significantly until after mid-century.

Results

Model results

For the ecophysiological based models, we produced layers with the number of months for each cell that did not meet the required temperature criteria. For the US 5yr model,

the number of months with unsuitable temperature conditions ranged from 0 to 41, while for the US 3yr model the maximum number of unsuitable months was 28.

The GLM country model retained all six environmental variables in model fitting, while the GLM targeted model dropped average annual precipitation and mean diurnal range. Average minimum temperature of the coldest month was the most important predictor in both models, with a logistic shape where suitability began to steeply increase from zero around -10 °C and climbing to 1.3 °C before reaching an index value defined as suitable. Both models retained temperature of the warmest month, with a generally positive relationship when considered with other variables. However, a function considering that predictor alone revealed a hump shaped relationship. Internal cross validation produced good assessment metrics for both models. The GLM country model had a cross-validation area under the receiver operating characteristic curve (AUC) of 0.94 and a true skill statistic (TSS) of 0.76, while the GLM targeted model had a cross-validation AUC value of 0.91 and TSS of 0.70. To produce binary maps, the GLM country threshold was 0.14 and the GLM targeted threshold was 0.44.

USA Assessment

All models performed well when compared to the HUC12 coypu density data (Table 1). All HUC12 sub-watersheds with a coypu density greater than 100 individuals per sub-watershed were predicted by all models to contain suitable habitat. The percentage of coypu density HUC12 classes of 11-100 and 1-10 predicted as suitable by the models were also high (> 87%), while HUC12 areas with a reported density of 0 had a much lower percentage predicted as suitable (Table 1). Model predictions compared to USA state-level classifications showed the models were better at correctly identifying states with established coypu populations than in predicting states that had populations that are now extinct or states where coypu have never been established (Fig. 1). Only the US 3yr and US 5yr models predicted a state classified as having an established coypu population as unsuitable (state of Delaware), while the other three models correctly classified all states with coypu status as present. For states with no data on population status, three to 12 of them were predicted to contain suitable habitat.

The ecophysiological based ensemble model results show greatest agreement in suitability in the southeastern USA from Texas to North Carolina and along the Pacific coast from Washington to southern California (Fig. 2a). Currently, coypu are not in California. We hypothesize this is due to a geographic barrier to their expansion south from Oregon. The waterways containing coypu in Southwestern Oregon are not hydrologically connected to the ones in Northern California and mountain ranges separate the two. The greatest area of model discrepancy was along the border between Tennessee and Arkansas, where the US 3yr model predicted a more northern distribution limit compared to the US 5yr and global 50yr models (Fig. 2a). These

Table 1. Model correct classification by coypu density class. Numbers represent the percent of sub-watersheds (Hydrologic Unit Code 12s) in Washington and Oregon classified as suitable by each model (generalized linear models [GLM]; row) and coypu density class (>100, 11–100, 1–10, and 0 individuals; column).

	>100	11–100	1–10	0
GLM country	100	100	93	16
GLM targeted	100	100	95	18
Global 50yr	100	98	87	15
US 3yr	100	99	91	12
US 5yr	100	99	89	12
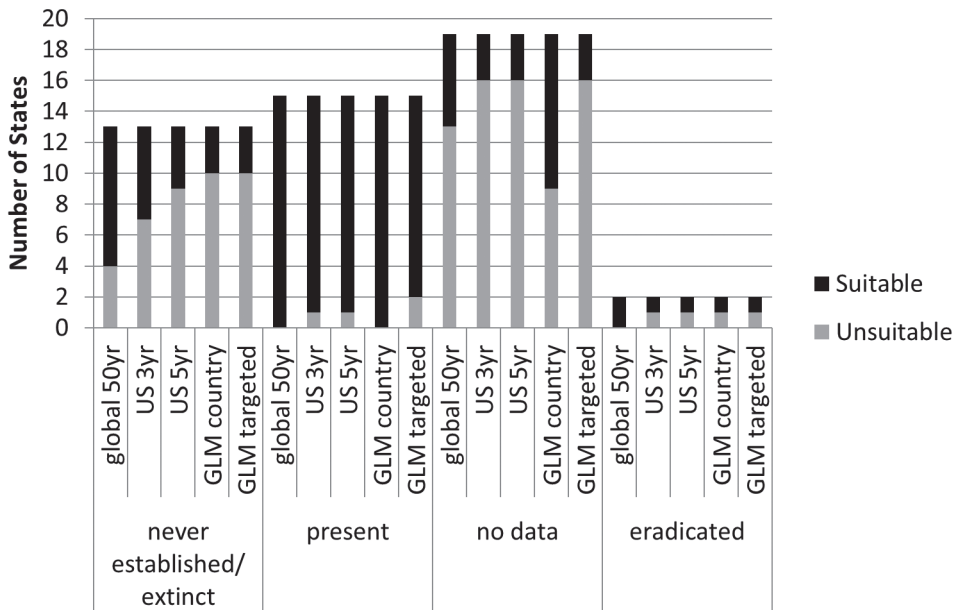
Figure 1. USA state assessment of the five models. The assessment includes the number of USA states classified with at least some suitable (1) or no suitable (0) coypu (*Myocastor coypus* [Molina, 1782]) habitat for each coypu status class (never established/ extinct, present, no data, or eradicated) as defined by Carter and Leonard (2002) for each model of the five models.

models agreed on suitable/ unsuitable classification 92% of the time. The GLM targeted model predicted a much greater amount of suitable habitat across the southern USA than the GLM country model (Fig. 2b). The ensemble of all five models for the USA revealed that the addition of the correlative models to the ecophysiological based models resulted in a more restricted distribution of agreement (Fig. 2c). Model agreement on habitat suitability was more confined to east Texas, Louisiana, Mississippi, and Alabama, mainly following the restricted distribution of the GLM country model. On the Pacific coast, model agreement was restricted to areas in Washington, Oregon and the northern half of California.
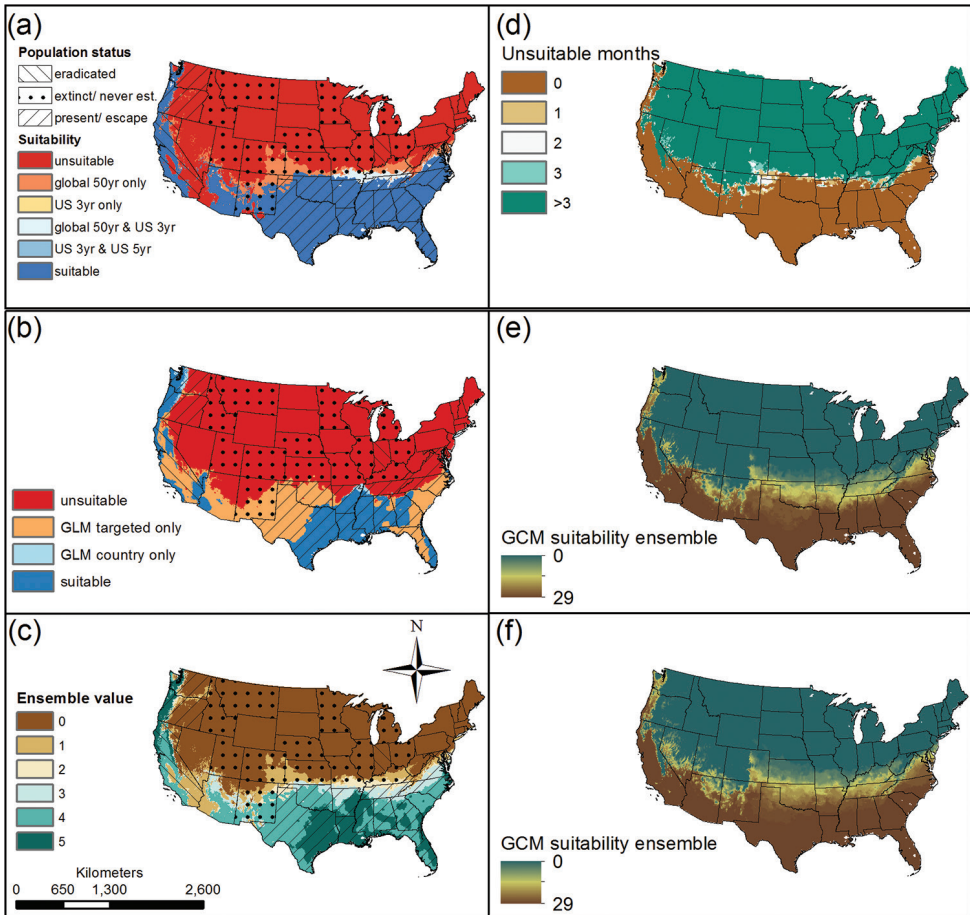
Figure 2. Model predictions for *Myocastor coypus* [Molina, 1782] for **a** an ensemble of US 3yr, US 5yr, and global 50yr **b** an ensemble of GLM country and GLM targeted **c** an ensemble of all five models **d** number of months classified as unsuitable using the Maurer observed climate data for 2001 to 2010 **e** the number of GCMs defining each pixel as suitable (ensemble of the 29 binary downscaled GCMs using the Maurer dataset as the reference) **f** ensemble of the 31 downscaled GCMs average from 2040 to 2050. All maps are overlaid with USA state population status according to Carter and Leonard (2002). Unsuitable habitat is defined as areas where no models predicted the area as suitable, while suitable areas are defined according to which model(s) predicted suitable habitat. Maps are in Albers Equal Area projection.

Global assessment

At the global scale, global 50yr, GLM targeted, and GLM country models had varying levels of performance when compared to country level classification by Carter and Leonard (2002; Table 2). For country level comparison, all models had high sensitivity values (>0.85) and low specificity values (<0.25) with the number of countries correctly classified > 70% (Table 2). When comparing global model performance to

independent coypu locations, sensitivity values decreased to 0.73 for all models and specificity values and percent correctly classified increased (Table 2).

An ensemble of the three global models had high agreement in coypu suitability for regions with established invasions (Fig. 3), although all three models only agreed on classification as suitable or unsuitable across 59% of the globe. The GLM targeted and global 50yr models were more similar with 81% agreement between predictions. The GLM targeted model predicted much more of the earth's surface as suitable (59%) compared to the other models (GLM country = 32%; global 50yr = 45%). All models predicted suitable coypu habitat in Western Europe and portions of the USA where coypu populations are known to exist – and where occurrence data were available to fit the models. At least a portion of all countries reporting coypu as native were predicted as suitable by all models. Evaluation of the models in the native range is difficult, however, as range maps that do exist do not provide information on how they were derived, and the rigor with which they were created is questionable.

The GLM country model predicted the least amount of tropical areas as suitable, with the GLM targeted model and the global 50yr model being more similar. However, many of these areas had novel environmental conditions. In dry areas such as North Africa, however, the global 50yr model did not predict suitable habitat due to the added arid region mask. The GLM country model excluded some of these dry areas, while the GLM targeted model included almost all of them.

Ecophysiological based versus Correlative

Model evaluations from HUC coypu density for the northwestern USA show very little difference between ecophysiological based and correlative models (Table 1). The same was true for evaluations at the state level. The greatest difference was for the 'no data' category where the GLM country model predicted suitable habitat in only ten states while the other models predicted suitable habitat in three to six states (Fig. 1).

Table 2. Evaluation metrics for global extent models. Evaluation metrics include percent correctly classified, sensitivity and specificity for global models of coypu (*Myocastor coypus* [Molina, 1782]) habitat suitability (global 50yr: ecophysiological based model based using average monthly temperature for 1950 to 2000, generalized linear model [GLM] country: GLM model using coypu presence locations and random background locations from countries containing coypu locations, and GLM targeted: GLM model using coypu presence locations and targeted background consisting of muskrat locations), evaluated using country level classification according to Carter and Leonard (2002; 'country' columns) and independent coypu location data ('independent' columns).

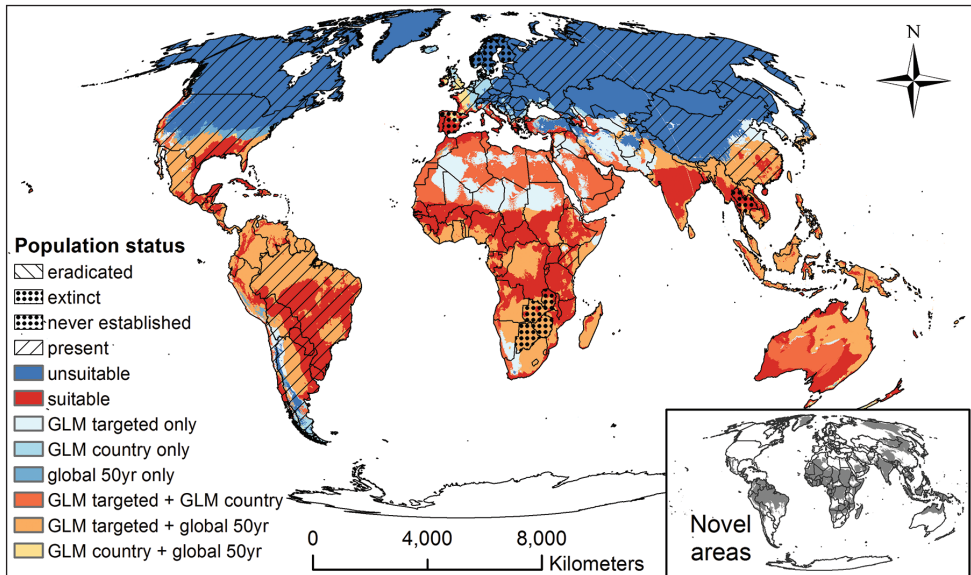|	Global 50yr		GLM country		GLM targeted	
	Country	Independent	Country	Independent	Country	Independent
Percent correctly classified	0.7	0.76	0.76	0.76	0.67	0.68
Sensitivity	0.85	0.73	0.97	0.73	0.82	0.73
Specificity	0.25	1	0.17	1	0.25	0.33

Figure 3. Global predictions of habitat suitability for coypu (*Myocastor coypus* [Molina, 1782]). Ensemble predictions using three models at the global scale including an ecophysiological based model based on average monthly climate data from WorldClim, a correlative model using country background and a correlative model using a taxonomically targeted background approach. Maps are in Mollweide projection.

For the global models, predictions between the correlative models and the ecophysiological based model were again very similar for both country level evaluations and independent location evaluations.

Potential future distribution

There is substantial variation in potential future climate from year to year as well as between GCMs and RCPs (Fig. 4). RCPs do not vary greatly until after mid-century. Comparing predictions of suitability between the modeled climate for current conditions (2006-2016) and the observed climate on which it is based (Maurer 2001-2010) revealed some discrepancies, with a mean disagreement in predictions (over or under prediction) of 6.1% of the area of USA (range of 3.2 to 10.6%). The Maurer dataset defined 34% of the USA as suitable (Fig. 2d). Extremes among the GCMs ranged from giss-e2-r-cc predicting 27% to canesm2 predicting 40% of the USA as suitable during the 2006-2016 period, though both had higher than average levels of disagreement (7.4% and 10.6%, respectively). The average amount of suitable area (32.4%) was comparable to the Maurer reference dataset. The range was similar for 2040 to 2050, with a minimum of 27.5% (ACCESS1-0), a maximum of 44.1% (CSIRO-MK3-6.0) and an average of 35.5%. Only three of the 29 GCMs predicted a decrease in suitable habitat (-3.7%, -0.2%, and -0.2%) and the maximum predicted increase in suitable

habitat was 9.4% by HADGEM2-AO. The average increase in suitable habitat was an additional 3.1% of the USA. There was more discrepancy among GCM predictions for the eastern USA than the western USA (Fig. 2e and f).

Discussion

Despite the fact that our ecophysiological based model is relatively simplistic and is based on physiological data from one location, it showed overall agreement with current knowledge of coypu distribution in local regions (e.g., the Pacific Northwest of the USA) and globally. For endotherms, prolonged exposure to thermal stress can decrease fitness and our relatively coarse temporal scale of monthly climate data accounts for extended periods of potentially stressful cold temperatures. There are also likely microclimatic factors that influence coypu distribution at local scales, especially in arid regions where there may be narrow suitable habitat along riparian areas. These results concur with previous research that winter temperatures may limit coypu distribution, at least in the invaded range (Gosling et al. 1983, Doncaster and Micol 1990).

While minimum temperature thresholds have been identified for coypu, thermal tolerance at high temperature has not been studied. This tolerance could be another limiting factor in locations such as the Amazon and portions of Africa where the models did not match known distributions. Examining tropical climate designation using WorldClim climate data to hypothesize an upper thermal limit matched well with known coypu distribution (Fig. 5 and Suppl. material 1: Figure 2). In South America the northern native range boundary has been described as -23 degrees latitude which matches the southern boundary of the tropics (Fig. 5a). Despite their widespread introduction globally, the sole successful establishment in the equatorial region is Lake Naivasha, Africa (Fig. 5b; Carter and Leonard 2002). Lake Naivasha is equatorial (latitude 0°46'S), but its climate, according to WorldClim average monthly temperature data (Hijmans et al. 2005), does not meet the Koppen climate criteria for tropical climatic designation (each month's average temperature >= 18 C; Peel et al. 2007). For our area of interest, the USA, this tolerance is likely not a factor with the exception of the southern tip of Florida, where coypu are absent (Fig. 5c). Location data in the Non-indigenous Aquatic Species database, which is the best source of location data for the southeastern USA, indicates coypu are found throughout Florida north of the tropical designation area (Suppl. material 1: Figure 2 and Fig. 5c). Thus, observational data support the hypothesis of an upper thermal limit, but physiological studies are required to further evaluate this hypothesis.

The baseline dataset (PRISM or WorldClim) and time frame used (3 year, 5 year, 10 year) made a difference in the predictions of current suitable habitat. Climate is not in equilibrium (Fig. 4), and therefore we expect the edges of distributions to be dynamic. An unusually cold winter could negatively affect coypu populations in otherwise suitable areas, which could be re-inhabited later when temperatures are again
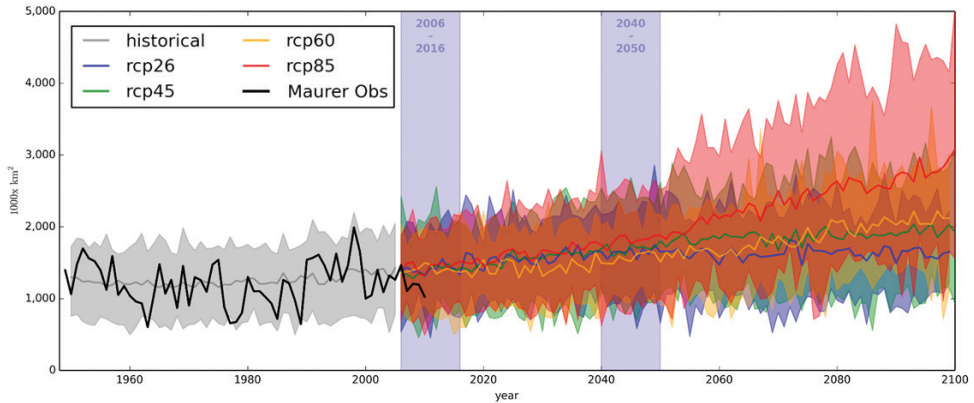
Figure 4. Amount of suitable habitat for coypu by year starting in 1950 and extending to 2100. Amount of suitable habitat is defined as thousands of km² within the continental USA without any months where average minimum temperature was <0 °C while average maximum temperature was also <5 °C. The solid black line from 1950 to 2013 is the Maurer observed dataset, the historical data is the 12 General Circulation Models (GCMs) calibrated between 1950 and 2013 using the Maurer dataset, and the projected climate by the GCMs with the average amount of predicted suitable habitat (solid line) and variation in predicted suitable habitat (solid colored area) for the four different representative concentration pathways (RCPs) describing possible climate futures by the GCMs. The solid vertical bars indicate the time periods for which we created geographic maps of predicted suitable habitat.

favorable. The US 5yr model contained less suitable habitat along the northern border of predicted suitable habitat in the USA due to colder winter temperatures in 2003 to 2004 compared to 2005 to 2007. For Delaware, Carter and Leonard (2002) based their classification of 'present' on reports from 2000. Coypu were not detected in Delaware between 2002 and 2009, but they were again found in 2010. While there are relatively large differences in GCM predictions, the models generally agree that there will be more suitable coypu habitat in the future. Examining a suite of GCMs highlighted the uncertainty that exists in future climate projections. The areas of suitable habitat highlighted by the models, especially those on the edge of the current known range, could be used for early detection of the spread of coypu populations for management purposes.

The model comparisons also are consistent with other studies that produced both ecophysiological based and correlative models (e.g., Martínez et al. 2014). The ecophysiological based models predict more suitable habitat than the correlative models. This pattern is expected as correlative models may capture factors not included in ecophysiological based models such as biotic interactions and unknown physiological limits. Movement restrictions and the biotic and abiotic environment define where a species occurs (Soberon and Peterson 2005). For invasive species, such as the coypu, understanding physiological limits of a species is desirable because constraints on native range distribution imposed by movement restrictions or biotic interactions may
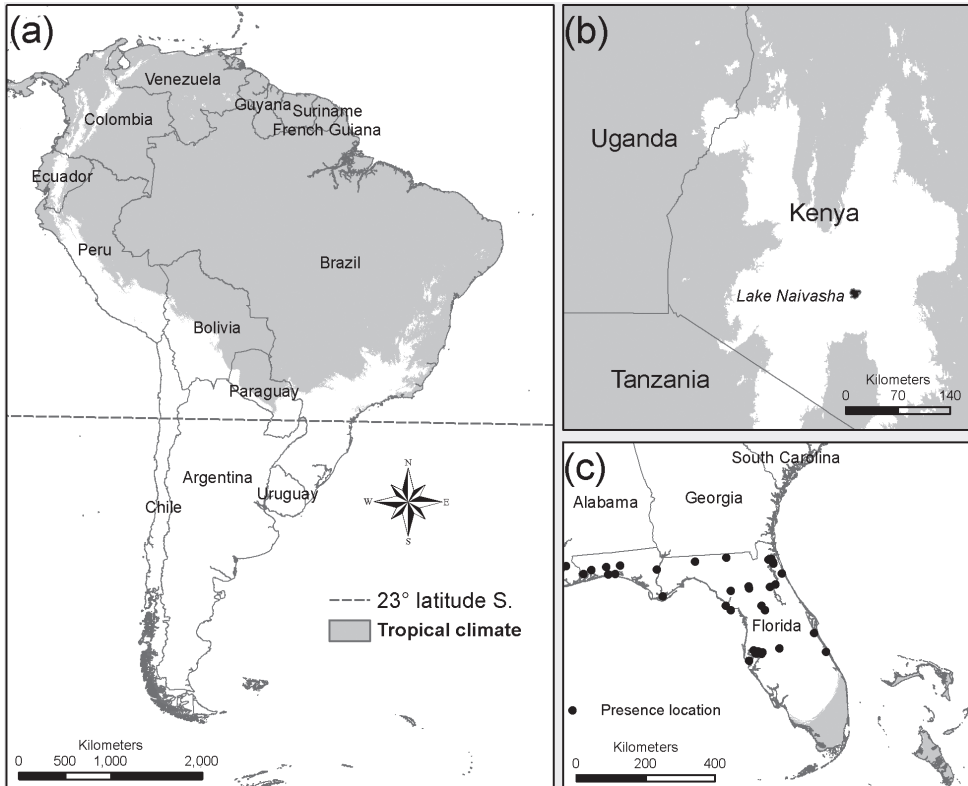
Figure 5. Tropical areas in relation to coypu presence. Areas defined as tropical are shown in **a** South America where coypu are native south of -23° latitude **b** Kenya where coypu have only been reported around Lake Naivasha, and **c** Florida, USA where coypu have not been reported in the southern part. Maps are in Mollweide projection.

not be expected to remain in the invaded range. Having information on physiological tolerances of a species to climate may better define potential distributions beyond a species' native range (Jiménez-Valverde et al. 2011). Additionally, information on the global distribution of invasive species that are widely distributed can assist development of hypotheses about physiological tolerances. These direct linkages to environmental conditions are needed for predicting species' distributions to novel locations or times (Jiménez-Valverde et al. 2011).

Future research could incorporate additional factors into the ecophysiological based model, such as an upper thermal limit. For the correlative model, obtaining more locations from the native range may improve model performance. We know there was particularly poor coverage in our observation data for this region. Finer temporal resolution of global climate data may improve all global models, as 50 year averages do not capture the extremes that may be important for species with distributions limited by thermoregulatory processes.

Conclusions

Overall, the national and global models for suitable coypu habitat performed well. By utilizing two different approaches (correlative and ecophysiological based) that produced similar projected distributions, we have more confidence in our results than we would using a single method. With these models we can now predict where coypu are likely to invade given climatic changes and regional hydrologic networks. These predictions can help focus early detection efforts by identifying areas to monitor for and potentially eradicate nacent coypu populations. Furthermore, the models can provide specific information about which areas might be invaded based on recent weather trends and hydrologic pathways. This is important because it has been demonstrated that the costs of early intervention with respect to a coypu invasion are much less than the costs of the damage they do and control efforts once their populations become established (Bertolino and Viterbi 2010; Panzacchi et al. 2007). Although our ecophysiological based model was rather simplistic and did not require a lot of detailed information about coypu, it still proved useful, especially in conjunction with correlative models. Using combined techniques, even with a simplistic ecophysiological based model such as we used here, could be useful in modeling potential distributions of invasive species now and in the future.

Acknowledgements

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Supplementary material 1

Supplementary figures and table

Authors: Catherine S. Jarnevich, Nicholas E. Young, Trevor R. Sheffels, Jacoby Carter, Mark D. Sytsma, Colin Talbert

Data type: Adobe PDF file

Explanation note: Supporting information including global location data used to create models (Supplementary figure 1), global distribution of tropical environments (Supplementary figure 2), and Global circulation model climate data used for forecasts of *Myocastor coypus* distributions (Supplementary table 1).

RESEARCH ARTICLE

Mind the gap – context dependency in invasive species impacts: a case study of the ascidian *Ciona robusta*

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Abstract

In the face of increasing invasions and limited resources, appropriate management of invasive species requires prioritisation of species for management action. This process often relies on knowledge of species specific impacts. However, as studies explicitly measuring impact of marine alien species are rare, prioritisation of management actions is often based on studies from outside the geographic area of interest. Further, few impact studies account for context dependency (e.g. seasonal variability or distinct environmental regimes), raising the question of how transferrable knowledge about the impact of a species is between invaded ranges. This study addressed this question by using the widespread invasive solitary ascidian *Ciona robusta* as a case study for assessing impacts across two invaded regions: South Africa and California, USA. We replicated a previously conducted experiment from California that showed that *C. robusta* depresses local species richness in San Francisco Bay. Our South African experiment showed no effect of *C. robusta* on species richness, the Shannon-Weiner diversity index or community composition, despite experiments being carried out over two years and at two depths. While these results may reflect strong density dependency in the impact of *C. robusta*, they serve to highlight context dependency in invasive species impacts. This suggests that until studies of impact in marine systems become common place, context dependency should be explicitly addressed as a source of uncertainty during the prioritisation of species for management action.

Keywords

Ciona robusta, community structure, fouling, impacts, management, species richness

Introduction

The increasing rate at which alien species are being spread across the globe is well acknowledged (Wonham and Carlton 2005, Ruiz et al. 2011) and resulting invasions are recognised as an important driver of global change (Pysek and Richardson 2010, Simberloff et al. 2013, Blackburn et al. 2014). In response to this, increasing focus has been placed on quantifying impacts associated with invasions (Blackburn et al. 2014) with this body of work strengthening our knowledge about how impacts of invasions manifest at the genetic, individual, population, community and ecosystem level (Parker et al. 1999). Despite general consensus on what constitutes a biological impact by an alien species (i.e. a significant change (increase or decrease) of an ecological property or process, regardless of perceived value to humans (Pysek et al. 2012)), invasion biology still faces the challenge of comparing impacts among invasions (Hulme et al. 2013), a critical step in prioritising appropriate management actions. To this end, recent work has offered a system for classifying alien species based on the magnitude of their ecological impacts (Blackburn et al. 2014, Hawkins et al. 2015). However, this progressive framework remains reliant on the primary studies that document impact, but such studies are surprisingly seldom undertaken for marine alien species (Simberloff et al. 2013, Ojaveer et al. 2015a, Ojaveer et al. 2015b, Alexander et al. 2016). Further, many existing impact studies tend to be unevenly distributed among geographic regions, different taxa and study systems (Pysek and Richardson 2010). Most studies have focused on invasive species (Pysek et al. 2008), leaving the impacts of many other species unquantified, especially in regions where they have not transitioned from alien to invasive status (*sensu* Blackburn et al. 2011). Whilst these gaps in knowledge are understandable as they reflect an uneven distribution of specialist researchers, research funds and research impetus, they hinder appropriate and effective management of invasions (Ojaveer et al. 2015a).

Such management challenges can be particularly relevant in developing nations. For instance, 89 non-indigenous marine species are known from South Africa (Robinson et al. 2016), but impacts have been considered for only 16% of these (Alexander et al. in 2016). Under such circumstances prioritisation of management actions is unavoidably based on studies of impact that have taken place elsewhere, despite a strong call for the application of the precautionary principle under such circumstances (Ojaveer et al. 2015a). This raises the question of how transferrable knowledge about the impact of a species is between invaded ranges.

Due to their prevalence in fouling communities, and the reported ecological or economic impacts of some species, ascidians are often a focal group in marine invasion studies (e.g. Herborg et al. 2009, Rius and Shenkar 2012, Cordell et al. 2013). The *Ciona* complex of solitary ascidians is one such group that has received much attention in the invasion biology literature (see Therriault and Herborg 2008). Recent genetic and morphological studies have, however, recognised that the species nominally referred to as *Ciona intestinalis* in the literature, in fact constitutes two species *Ciona robusta* (also referred to as Ciona Type A) and *C. intestinalis* (also referred to as Ciona

Type B) (Zhan et al. 2010, Sato et al. 2012, Brunetti et al. 2015). Despite a present lack of clarity around the native range of *C. robusta*, it is known from the Mediterranean Sea, the English Channel, Japan, the west coast of north America, the south east coast of Australia, as well as from South Africa and New Zealand and is thought to be native in the Mediterranean Sea and the English Channel (Zhan et al. 2010, Rius et al. 2016). Due to its limited larval dispersal capabilities and the sessile nature of its adult phase, *C. robusta* (referred to as *C. intestinalis*) is thought to have been translocated primarily via hull fouling (Lambert and Lambert 2003) and aquaculture transfers (Castilla et al. 2005), with intra-regional spread closely associated with recreational boating (McDonald 2004). Despite its large introduced range, the ecological impacts of *C. robusta* have only been considered in San Francisco Bay, California, where it was found to reduce diversity of sessile communities (Blum et al. 2007). Although economic impacts on shellfish farms are widely reported for *C. intestinalis* (Lesser at el. 1992, Tan et al. 2002, Carver et al. 2003, Braithwaite and McEvoy 2004), such impacts by *C. robusta* have received less attention (but see Robinson et al. 2005, Rius et al. 2011).

Previously referred to as *C. intestinalis*, *C. robusta* has been known from South Africa for more than 50 years (Millar 1955), where it occurs extensively in harbours along almost the entire coastline (Rius et al. 2014). Despite this there has been no consideration of its ecological consequences. As such, an opportunity exists to use this species as a case study to consider the transferability of information on ecological impacts of alien species between two different invaded ranges, in this case between the west coast of North America and South Africa. We did this by repeating the experiments of Blum et al. (2007) so as to gain a measure of ecological impact by *C. robusta* in South African waters. Specifically, we assessed the effect of this ascidian on sessile community structure and species richness at two depths, over two years. This enabled an assessment of the spatial and temporal variability associated with the impacts of *C. robusta* across distinct biogeographical regions.

Methods

This study took place at two locations, Yacht Port Marina (33°01'36"S; 17°57'40"E) in Saldanha Bay on the South African west coast and Gordons Bay Yacht Club (34°09'52"S; 18°51'42"E) in False Bay on the south coast (Figure 1). These sites were chosen as they are both sheltered from wave action and currents by breakwaters that protect moored yachts from rough sea conditions. A pilot study that included sites with greater water movement revealed that *Ciona robusta* preferentially settled in sheltered conditions and thus more exposed sites were excluded. While these are both enclosed yacht basins, they differ in that they occur in distinct ecoregions, Saldanha Bay in the cool nutrient rich Southern Benguela Ecoregion and False Bay in the warmer less productive Agulhas Ecoregion (Sink et al. 2012).

During the austral winter of 2012 and 2014, 18 experimental arrays were deployed in Saldanha Bay and False Bay. This season was chosen as this is when the peak settle-
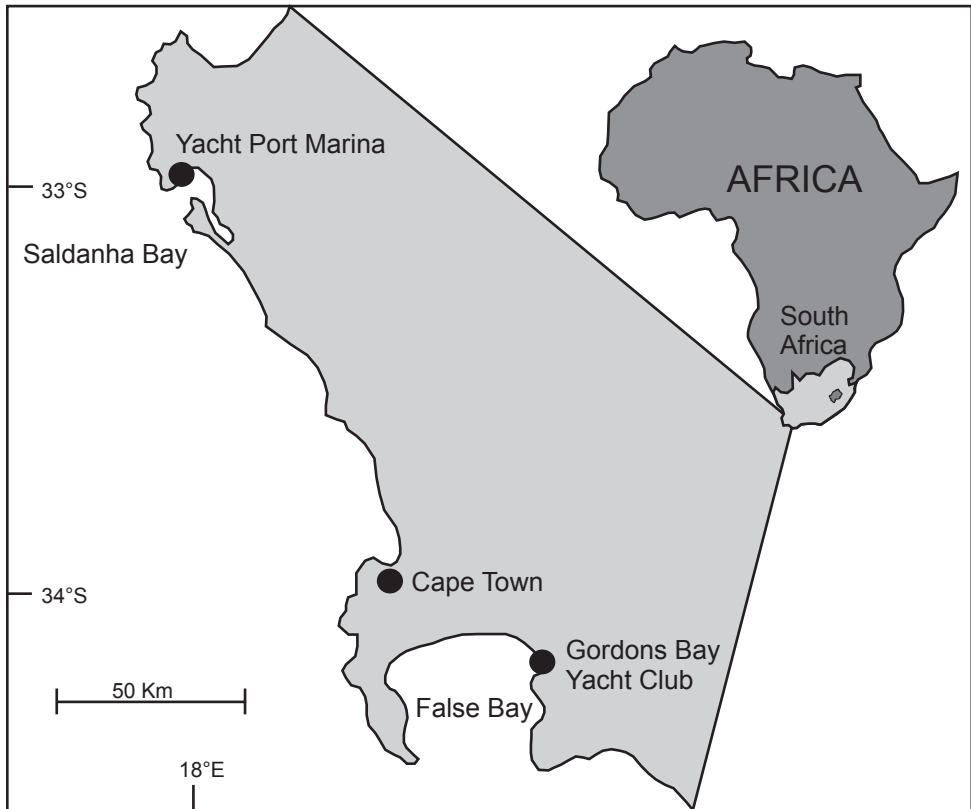
Figure 1. Sites along the South African coast where the ecological impacts of *Ciona robusta* were quantified.

ment of *C. robusta* occurs in this region (Millar 1955). Despite adult *C. robusta* being present in False Bay in pre-experiment surveys, no settlement occurred in either experimental year forcing this site to be excluded from all statistical analyses. Each array hung vertically in the water column and consisted of two PVC panels, one at 1m depth (i.e. shallow plates) and another at 3m (i.e. deep plates). Panels were opaque in colour, 0.25 cm thick and offered a settlement area of 20 × 20 cm. This size was chosen to align with the 'large' panels used by Blum et al. (2007). Prior to deployment the sanded plates were soaked in sea water for two weeks in order to leach chemicals that may have affected fouling. The arrays were randomly allocated to one of three treatments: (1) *Ciona* removal (*n*=6), where all *C. robusta* were removed from the plates by hand at two weekly intervals; (2) the treatment control (*n*=6), where plates were removed from the water for the same length of time as the treatment plates but without removing *C. robusta* to control for treatment artefacts; and (3) the control (*n*=6), where plates were left undisturbed for the duration of the study. Thus, each array had a shallow and deep plate, providing *n*=6 for each treatment at each depth. This sample size allowed for 0.91 power to detect the effect size recorded by Blum et al (2007), which is above the level of 0.8 advocated by Cohen (1977). The individuals removed from the *Ciona* removal treatment were counted, and

wet weighed to the nearest gram and compared among years (2 levels: 2012 and 2014) and depths (2 levels: shallow and deep) in a GLM, with quasipoisson error distribution to account for overdispersion. Plates were deployed for 16 weeks after which they were removed from the water, photographed and preserved in Formalin. Back in the laboratory, percentage cover was measured using 25-point counts generated by the random placement of a 5 by 5 grid on the photographs taken in the field. In addition, biota from each plate were identified to species level and weighed to the nearest 0.1 gram.

Species richness (i.e., total number of species) and the Shannon-Wiener diversity index (H'), which incorporates both species richness and evenness (Clarke and Warwick 1994) were used to compare diversity among treatments (3 levels; control, treatment control, Ciona removal), years (2 levels: 2012 and 2014) and depths (2 levels: shallow and deep) in a three-factor GLM, with quasipoisson error distribution. Calculations of H' were based on biomass as many fouling species are colonial, precluding counts of individuals. Using the Primer-6 software package (version 6.1.16) a PERMANOVA (version 1.0.6) was used to assess differences between fouling community assemblages among treatments, years and depths (Anderson et al. 2008). Multi-dimensional scaling (MDS) plots were used to visualise the relationships between communities while SIMPER was used to isolate the species responsible for differences in community structure. All multivariate analyses were conducted using non-standardized, fourth-root transformed biomass and cover data. The contribution made by *C. robusta* was excluded from these analyses so as to isolate its effect on the fouling community and not simply reflect its absence from treatment plates. All univariate statistics were performed in R version 3.2.0

Results

Settlement of *Ciona robusta* on experimental plates

Ciona robusta only settled on experimental plates in Saldanha Bay, despite pre-experiment surveys recording this ascidian in Gordons Bay and the presence of low densities of adults on marina infrastructure during the experiment. As such Gordons Bay was excluded from all analyses. In Saldanha Bay, both the number and biomass of individuals removed from the treatment plates were affected by 'depth' (density: $F_{1,21}=24.32$, $p<0.0001$, biomass: $F_{1,21}=24.16$, $p<0.0001$) with significantly lower abundances occurring on shallow plates (density: t=-2.16, $p<0.05$; biomass: t=-1.71, $p<0.05$) (Figure 2). There was no effect of 'year' (density: $F_{1,22}=0.53$, $p>0.05$, biomass: $F_{1,22}=1.08$, $p>0.05$) and no interaction between 'depth' and 'year'.

Impacts on diversity and community composition

In total, 58 fouling species were recorded in our study, of which 57% were only present in 2012. While a total of seven non-indigenous species were recorded, only *C. robusta*,
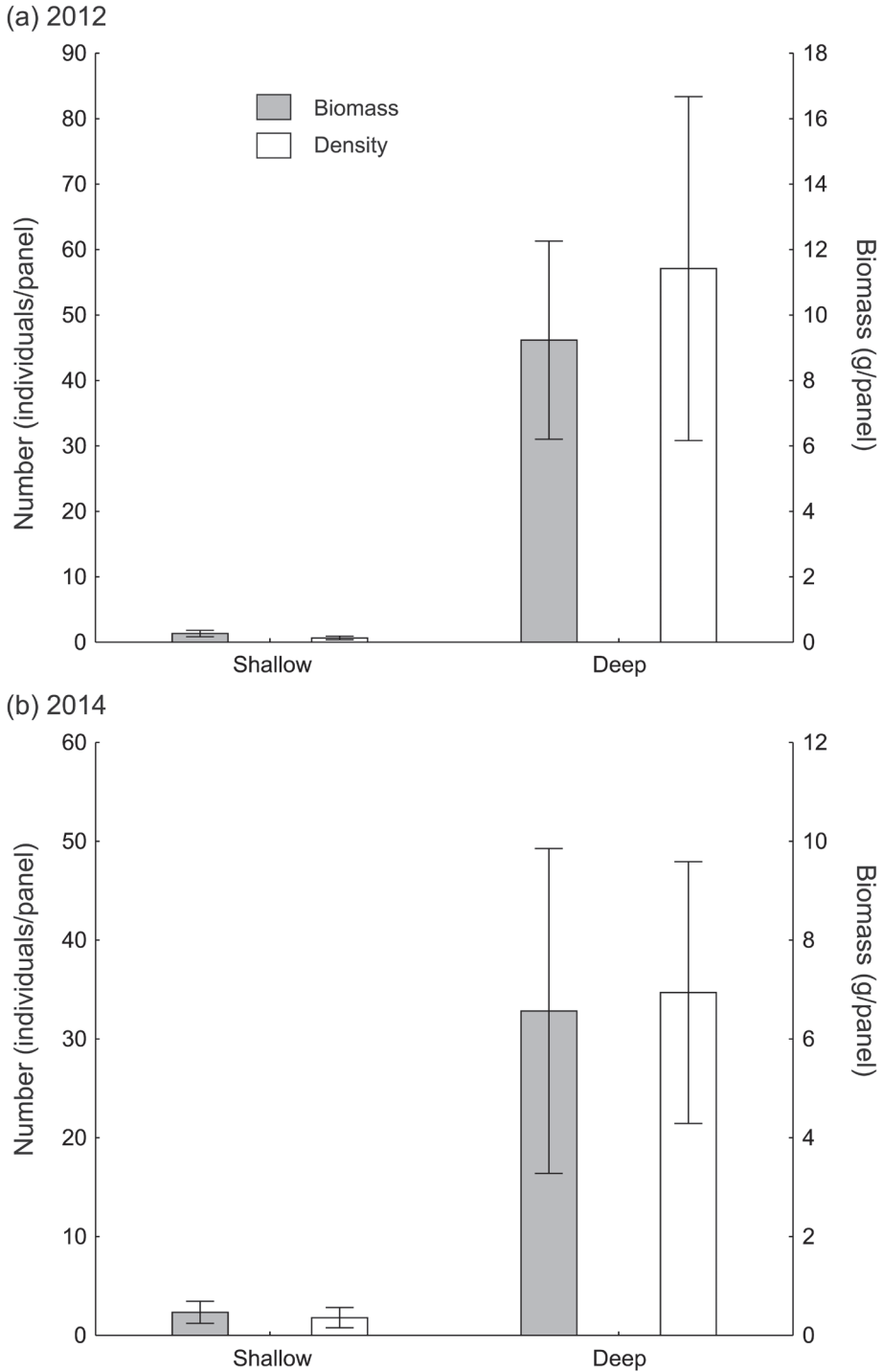
Figure 2. Abundance of *Ciona robusta* removed from treatment plates. Mean (±SE) numbers (individuals/panel) and biomass (g/panel) of *C. robusta* removed from plates in Saldanha Bay in (**a**) 2012 and (**b**) 2014.

Table 1. GLM results considering the effect of 'year', 'depth' and 'treatment' on (a) species richness and (b) the Shannon-Wiener diversity index (H'). ns = non-significant.

Factor	df Effect	Null deviance	F-ratio	*p*-value
(a) Species richness				
Year	1	142.6	103.4	*p*<0.001
Treatment	2	3.1	1.1	ns
Depth	1	6.5	3.2	ns
Year × Treatment	2	3.1	1.1	ns
Year × Depth	1	12.8	9.3	*p*<0.01
Treatment × Depth	2	0.4	0.2	ns
Year × Treatment × Depth	2	2.6	0.9	ns
(b) H'				
Year	1	5.3	71.5	*p*<0.001
Treatment	2	0.2	1.0	ns
Depth	1	1.0	2.2	ns
Year × Treatment	2	0.2	1.2	ns
Year × Depth	1	1.2	15.4	*p*<0.001
Treatment × Depth	2	0.009	0.1	ns
Year × Treatment × Depth	2	0.2	1.3	ns

the colonial bryozoan *Bugula neritina* and the lightbulb ascidian *Clavelina lepadiformis* were present in both years, while the remaining four species (the ascidian *Diplosoma listerianum*, the amphipod *Jassa marmorata*, the hydrozoan *Obelia dichotoma* and the bryozoan *Waterspora suborquata*) were present only in 2014, despite fewer species being recorded in that year. Only one species, *C. lepadiformis,* was restricted to removal treatment plates. There was a significant effect of 'year' on species richness and a significant interaction between 'year' and 'depth' (Table 1). Overall species richness was highest in 2012 (t=-3.88, *p*<0.01) with elevated richness on deep plates in 2014 driving the interaction (Figure 3a, c). There was no effect of 'treatment' on species richness. The same pattern emerged for the Shannon-Wiener diversity index (Table 1, Figure 3b, d).

Community assemblages differed significantly among years and were affected by an interaction between 'year' and 'depth' (Table 2, Figure 4). Treatment was found to have no effect on community structure. These patterns were observed for community structure based on biomass and % cover. SIMPER on fouling biomass revealed that the 2012 community was defined primarily by *O. dichotoma,* the indigenous barnacle *Notomegabalanus algicola,* and the ascidian *Botryllus magnicoecus*. Together these species contributed 40.1% to the similarity of communities recorded in this year. In contrast, 2014 communities were primarily designated by *B. neritina* (contributing 32.3%) followed by *B. magnicoecus* (12.4%) and *Botryllus schlosseri* (12.3%). A SIMPER analysis on % cover of biota again highlighted the importance of colonial ascidians in fouling communities, with *B. magnicoecus* and *Diplosoma listerianum* contributing 42.3% to the similarity of 2012 communities, while *B. schlosseri* and *B. magnicoecus* accounted for 36.1% of similarity in 2014 assemblages.
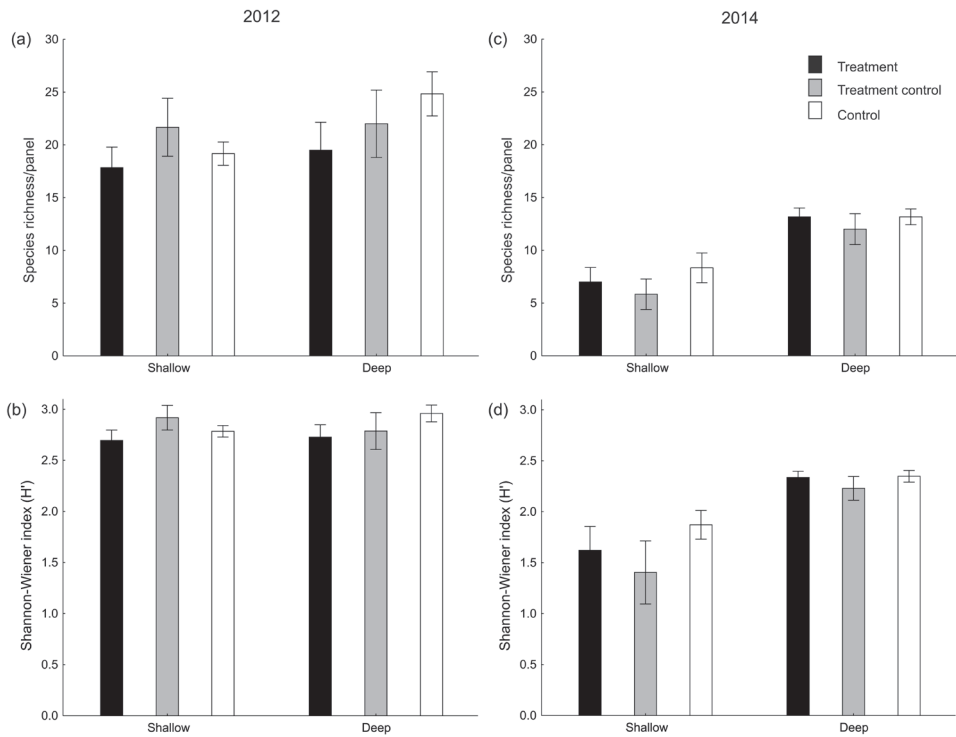
Figure 3. Changes in diversity. Mean (±SE) species richness and Shannon-Wiener index (H') recorded in 2012 (**a, b**) and 2014 (**c, d**). Both measures of diversity were significantly affected by year ($p<0.01$) and a significant interaction between year and depth ($p<0.01$).

Table 2. Test statistics for a main effects PERMANOVA considering the effect of year, treatment and depth on fouling (a) biomass and (b) % cover. ns = non-significant.

| Factor	df	SS	MS	Psuedo-F	*p*-value
(a) Biomass					
Year	1	60124	60214	57.8	$p<0.0001$
Treatment	2	32785	1393	1.1	ns
Depth	1	23966	23966	4.8	ns
Year × Treatment	2	2506	1253	1.2	ns
Year × Depth	1	5032	5032	4.8	$p<0.001$
Treatment × Depth	2	2493	1246	1.9	ns
Year × Treatment × Depth	2	1291	646	0.6	ns
(b) % cover					
Year	1	49301	38454	65.3	$p<0.01$
Treatment	2	36247	1668	1.6	ns
Depth	1	26354	26354	3.2	ns
Year × Treatment	2	3803	1969	1.8	ns
Year × Depth	1	6712	5644	9.4	$p<0.05$
Treatment × Depth	2	2113	1746	2.1	ns
Year × Treatment × Depth	2	1394	452	0.3	ns
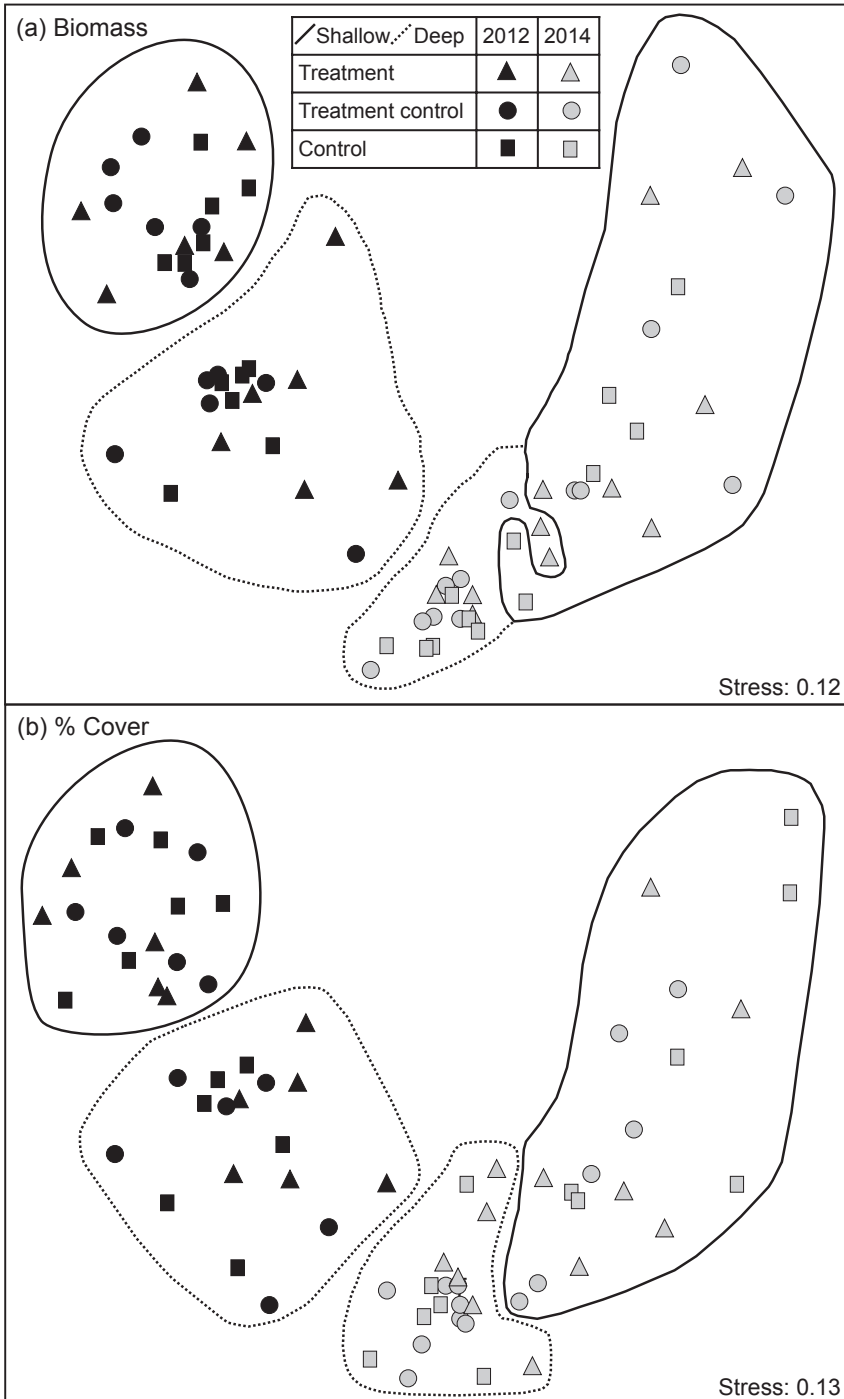
Figure 4. Community composition. Multi-dimensional scaling (MDS) plots of community assemblages formulated using fouling (**a**) biomass and (**b**) % cover of treatment, treatment control and control panels in two years at two depths.

Discussion

Alien species can have ecological, socio-economic and human health impacts in recipient regions (Mack et al. 2000, Kumschick et al. 2012, Simberloff et al. 2013). Faced with escalating rates of invasions and limited resources, managers are required to prioritise responses to species incursions (Kumschick et al. 2012), with a recent trend to do so based on the relative impacts associated with the species in question (Blackburn et al. 2014). Due to the paucity of studies directly measuring the impact of marine alien species (Ojaveer et al. 2015a), prioritisation often relies on the published literature as: (1) managers must strive for proactive and efficient management actions, an approach that inherently minimises the time available to measure impacts, and/or (2) limited resources preclude experimental assessments. Using the solitary ascidian *Ciona robusta* as a case study, we have shown differential impacts between distinct global locations where this species has been introduced. In California, *C. robusta* was shown to reduce local species richness and alter sessile community structure, whereas our work in South Africa found no ecological impacts of this species on the local fouling community.

Although previous studies have recorded dense settlement of *C. robusta* in Saldanha Bay (in 1994 an average density of more than 1000 individuals/m^2 was recorded at a depth of 3m (Rius et al. 2011)), it appears that densities may be declining through time in this area as fewer than 500 individuals/m^2 were recorded in 2010 (Rius et al. 2011) and we noted average settlement of less than 100 individuals/m^2 in 2012 and fewer than 50 individuals/m^2 in 2014. Further, when compared to much relatively higher densities reported by Blum et al. (2007), this suggests that the impacts of *C. robusta* may be density dependant, as has been recorded for other alien species (Griffen and Byers 2009). This aligns with the suggestion by Thomsen et al. (2011) that invasion impacts depend primarily on the properties associated with the alien species itself (e.g. density or species identity) and secondarily on the characteristics of native biota (e.g. indigenous community structure), resource levels (e.g. nutrient levels) and abiotic conditions (e.g. sedimentation), but remains to be empirically tested. What remains unclear, however, is what may be driving the decline of this ascidian which has been present along this coast for more than half a century (Millar 1955) and is reported from almost all South African harbours (Peters et al. 2014, Rius et al. 2014). One possible explanation relates to long-term cooling trends in water temperature along the South African west coast (Rouault et al 2010) as recruitment of this species may be closely correlated with this environmental variable as it is for *Ciona intestinalis* (Vercaemer et al. 2011). However, a decadal decline of 0.5°C and the fact that temperatures in Saldanha Bay (Smit et al. 2013) fall within the thermal range of other locations at which this species persists (Zhan et al. 2010), suggests that temperature is not the sole driver of declines in recruitment. This does, however, raise questions about recruitment trends of this ascidian along warmer sections of the South African coast and the potential impact in these regions.

Conclusion

While the impacts of alien species are often measured at different locations within a region (e.g. Dunham et al. 2002) or in different invaded ranges (e.g. Kado 2003; Sadchatheeswaran et al. 2015) and then assimilated in reviews and meta-analyses (e.g. mice on islands (Angel et al. 2009), global plant impacts (Vila et al. 2011), biological impacts of ascidians (Aldred and Clare 2014)), studies directly comparing impacts at the species level in different invaded ranges are generally conspicuous by their absence. While the theoretical framework for understanding variability in the manifestation of impacts is developing (Thomsen et al. 2011, Ricciardi et al. 2013), empirical studies are needed to support this (Thomsen et al. 2011). Although identifying potentially high risk species based on impacts reported from elsewhere remains useful, and impact quantification is obviously not practicable for every alien species in every invaded range, engaging in expensive management actions without ground truthing the applicability of reported impacts to the area of interest is also not prudent or efficient. In acknowledging the need for more quantitative studies considering the impacts of marine alien species (Wardle et al. 2011, Alexander et al. 2016) recent work has suggested that impact evaluation for data deficient marine systems should focus on the value sets that management actions seek to protect (Ojaveer et al. 2015a). While this precautionary approach aims to support management in the interim, there is a dire need for quantification of impacts to support evidence based management and provide data with which to test and develop our conceptual understanding of context dependency in invasion biology.

Acknowledgements

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