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Conservation management within strongholds in the face of disease-mediated invasions: red and grey squirrels as a case study

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Summary

1. There is increasing evidence that disease-mediated invasions are widespread across a range of vertebrate, invertebrate and plant systems. We therefore need a better understanding of the role of disease in managing conservation threats due to introduced and invasive species.

2. Here, we develop a general theoretical model framework to assess the impact of disease-mediated invasion on the viability of conserving native species through refuges taking into account explicit spatial and stochastic processes.

3. The model techniques are applied to the well-documented red and grey squirrel conservation system in the UK as a case study.

4. By combining general and specific modelling approaches, we are able to make management predictions while also gaining an understanding of the processes that underlie population outcomes leading to more robust conservation practice.

5. Model results indicate that in the absence of control of the invading species, native populations are driven to extinction both in the absence of disease (through competition) and more rapidly when the disease is included (through competition and disease processes).

6. When control is applied to reduce the abundance of the invading species, there is a threshold in the level of control, above which the invading population can be prevented from establishing and the native species can be protected.

7. Highly virulent infections – squirrelpox in red squirrels – lead to periodic outbreaks of disease in the native population due to continual invasion attempts from the disease-carrying invader. Infections with low virulence may become established at endemic levels in native populations. Therefore, an important finding is that the disease can spread through the native species even when the invading species is prevented from establishing.

8. The benefits of increased density may be countered by an increased risk of disease outbreaks. Therefore, a critical message is that there is a correlation between native density (and therefore habitat quality) and the impact of disease 'harmful' to native species.

9. Control of the invading species to prevent it establishing in strongholds can protect the native species from exclusion, but may not protect it from disease outbreaks.

10. *Synthesis and applications*. Disease outbreaks in the absence of the invading species can result in significant population crashes and therefore represents a serious threat because it contributes to the risk of population extinction by suppressing the size of the population making it more vulnerable to extinction through stochastic processes.

Introduction

Introductions and invasions of exotic organisms continue to cause significant damage to native communities (Kolar & Lodge 2001). The rate at which human activity is introducing species into new habitats is increasing (Prenter *et al*. 2004), and furthermore, changes in climate are likely to lead to significant and potentially rapid shifts in species ranges (Rosenzweig *et al*. 2007). As a consequence, the invasion and establishment of non-native species are

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recognized as a major international threat to native biodiversity (Rosenzweig *et al.* 2007; Ruddock *et al.* 2007). Understanding the processes that determine successful invasion is therefore a key challenge for ecological theory to underpin conservation efforts and thereby sustain ecosystems and species communities (Sutherland *et al.* 2006). Clearly there are likely to be many factors that determine the success and rate of spread of invasive species, but it is now recognized that shared infectious disease is often a key determinant of invasive success (Daszak, Cunningham & Hyatt 2000; Prenter *et al.* 2004; Strauss, White & Boots 2012). We therefore need a better theoretical understanding of the role of disease in managing conservation threats due to introduced and invasive species.

Typically, when the role of parasites in invasions is discussed, it is through their absence because parasites along with predators and herbivores are key components of the 'enemy release hypothesis', where the non-indigenous species gains an advantage because it arrives in a new habitat without its natural enemies (for reviews see Wolfe 2002; Torchin & Mitchell 2004). However, invasive organisms may also introduce diseases that can infect native competitors leading to a 'disease-mediated invasion' (Strauss, White & Boots 2012). There is increasing evidence across a range of vertebrate, invertebrate and plant systems that such disease-mediated invasions are important determinants of invasion success (Prenter *et al.* 2004; Strauss, White & Boots 2012). Well-known examples include (i) crayfish plague introduced with the invasive signal crayfish which has been responsible for mass mortality in British white-clawed crayfish populations and poses a threat to native crayfish species in Europe and Asia (Gherardi & Holdich 1999); (ii) the replacement of the pedunculate oak in the UK by the introduced Turkey oak due to the impact of the knopper gall wasp (Hails & Crawley 1991); and (iii) the expansion of the white-tailed deer in North America into territories occupied by moose and caribou which has been aided by macroparasitic meningeal worms carried by the white-tailed deer and which proved lethal to the other species (Oates, Sterner & Boyd 2000). A recent review (Strauss, White & Boots 2012) makes the case that disease-mediated invasions are a widespread phenomenon in both plants and animals and may be particularly important in explaining the replacement of native animals with phylogenetically similar exotic species.

A key example of a disease-mediated native replacement is the invasion of grey squirrels *Sciurus carolinensis* into the UK. Since its introduction into the UK in Victorian times, the grey squirrel has replaced the native red squirrel *Sciurus vulgaris* throughout most of England and Wales, and in parts of Scotland and Ireland (Bryce 1997; O'Teangana *et al.* 2000; Gurnell *et al.* 2004). There are now only particular regions in which the red squirrel survives, and maintaining these populations is a major conservation priority (Ruddock *et al.* 2007; Parrott *et al.* 2009). There is strong evidence both that grey squirrels are superior competitors in habitats dominated by large-seeded deciduous tree species (Gurnell *et al.* 2004) and that a shared virus, squirrelpox (SQPV), plays a critical role in red squirrel replacement. The virus infects but is relatively avirulent to the greys yet is highly virulent to reds (Tompkins *et al.* 2002; McInnes *et al.* 2006). Mathematical models suggest that while competition alone can lead to the replacement of reds by greys, the inclusion of SQPV infection is required to match the rapid replacement of red populations observed in the field (Tompkins, White & Boots 2003). Importantly, the models (Tompkins, White & Boots 2003) show that the density of infected red squirrels is predicted to be low, despite the marked effect on the population dynamics. This emphasizes that low visibility of a disease does not imply low importance of the disease and highlights the difficulty in observing infection by virulent infectious diseases in the field. In the case of SQPV, it took over 50 years from the first observation of disease outbreaks in red squirrels (Middleton 1930) to the identification of the virus (Scott, Keymer & Labram 1981) and a further 14 years before its impact on red survival was suggested (Sainsbury & Gurnell 1995); culminating with the current view that SQPV is unequivocally linked to the replacement of red squirrels and a key threat to the species survival in the UK (Bosch & Lurz 2012).

Models have played an important role in highlighting the importance of the disease, but they can also be used to help determine conservation management strategies in the face of disease-mediated invasions. A key conservation strategy to protect endangered native populations is the establishment of refuges or strongholds. These tend to be legally protected areas that contain particular habitat types and/or management that favour the threatened species when faced with threats that encompass environmental and land-use change, habitat loss and degradation, disturbance, poaching, alien species and climate change (e.g. Demeke, Renfree & Short 2012; Laurance *et al.* 2012). Well-known examples include bamboo forests for mouse deer *Moschiola indica* in India (Ramesh *et al.* 2013), sanctuaries for elephants *Loxodonta africana africana* in Ethiopia (Demeke, Renfree & Short 2012) or strongholds that offer protection for endangered primates such as the drill *Mandrillus leucophaeus* in Africa (Morgan *et al.* 2013). As refuges play a key role in the protection of endangered species from invasion and the impact of disease-mediated invasion is increasingly recognized as a significant threat to native biodiversity (Strauss, White & Boots 2012), it is important to assess the potential and limitations of the role of refuges or strongholds in the conservation of threatened native species.

In this study, we develop a theoretical model framework to assess the impact of disease-mediated invasion on the viability of conserving native species in refuges. The underlying model system combines classical frameworks for modelling species interactions and disease transmission, and the results therefore provide critical information on the impacts of preventative strategies and their likely

consequences on alien–native–disease systems in general. We examine in detail the well-documented red–grey squirrel conservation system in the UK as a case study. Refuges (strongholds) have been employed in the UK to conserve red squirrel populations. These strongholds are local forest regions that are large enough to sustain viable red squirrel populations over the long term and in which habitat composition and management offers native red squirrels a competitive advantage over greys. Strongholds may be isolated from surrounding (grey squirrel) populations (through poor connectivity or poor adjoining habitat), or grey squirrel density may be controlled through trapping and removal (Anon 2012). To date, there are 18 strongholds in Scotland and 17 in the North of England managed by government, non-government and charitable organizations (such as the Forestry Commission, Scottish Natural Heritage and the Wildlife Trust). In addition, five red squirrel preferred areas (RSPAs) were identified in Northern Ireland, and management for red squirrels in 'Focal Areas' is being implemented in Wales (see Scottish strongholds: http://www.forestry.gov.uk/pdf/FCSStrongholdsGuidance.pdf/$FILE/FCSStrongholdsGuidance.pdf; see also overall UK strategy for all regions: www.forestry.gov.uk/fr/ukrsg). The general model framework was parameterized to focus on the specific case study of the UK squirrel system. This allowed an assessment of the effectiveness of management in terms of refuge habitat characteristics and grey squirrel control on the outcome of red squirrel population survival. The model framework was able to take into account the, often subtle, interactions between individual species population dynamics, competition, the infectious disease and grey squirrel control in strongholds. Overall, our models give us insight into management within strongholds in the face of disease-mediated invasions and can provide predictions of the impact of specific control strategies in the UK red squirrel refuges.

The red–grey squirrel system represents a general framework for understanding the role of disease-mediated invasion when the disease is highly virulent to the native species. The general framework can also be modified to consider alternative disease characteristics, and we consider a parasite that has low virulence in the native species. By considering different host densities in the refuge, we alter the long-term reproductive ratio of the disease (R_0) for both the high- and low-virulence cases and therefore capture a wide range of host and parasite scenarios. In particular, we contrast the impact of an acute highly virulent parasite, such as SQPV, with that of a chronic low-virulence parasite.

Materials and methods

Our previous models on the impact of disease-mediated invasion have developed a framework that combines the classical deterministic approaches in population ecology of competition modelling and disease modelling to understand the role of competition and shared disease in the replacement of red squirrels by greys (Tompkins, White & Boots 2003). The benefit of this modelling approach is that it provides a framework from which to understand the influential mechanisms that arise between interacting populations, but the disadvantage is that they cannot accurately assess the risk of invasion (as they do not include the chance of extinction or disease fade-out at low density which may arise due to stochasticity). In reality, invasions occur from low numbers, and hence, many attempts may fail before establishment and successful invasion. Stochasticity has been included in rule-based, spatially explicit models of the UK squirrel system (Rushton *et al.* 2000, 2006). While these models have predictive power, it is difficult to isolate the key drivers of the population dynamics due to the complicated choice of rules and large number of parameters. The framework developed in this study falls between the deterministic and rule-based approaches and will represent the stochastic nature of invasive spread and success, but also allow the key mechanisms that drive the dynamics to be understood. As such, we can make relevant predictions while also gaining insight into the processes that underlie the outcomes.

The model system represents the dynamics of native and invasive species and disease in a landscape of connected patches (see Fig. 1, where we assume the stronghold is composed of a refuge and buffer zone). Within each patch, the dynamics are represented by a stochastic version of the model of competition and disease developed by Tompkins, White & Boots (2003). Below we outline the model for the specific red–grey–SQPV system and later discuss how the results apply more broadly to disease-mediated invasive systems.

The deterministic model of Tompkins, White and Boots (2003) where the dynamics of susceptible and infected red (S_R and I_R) and grey (S_G and I_G) squirrels and recovered (immune) greys (R_G) are represented by the following equations.

$$\begin{aligned}
\frac{dS_G}{dt} &= (a_G - q_G(H_G + c_R H_R))H_G - bS_G - \beta S_G(I_G + I_R)\\
\frac{dI_G}{dt} &= \beta S_G(I_G + I_R) - bI_G - \gamma_G I_G\\
\frac{dR_G}{dt} &= \gamma_G I_G - bR_G\\
\frac{dS_R}{dt} &= (a_R - q_R(H_R + c_G H_G))H_R - bS_R + \gamma_R I_R - \beta S_R(I_G + I_R)\\
\frac{dI_R}{dt} &= \beta S_R(I_G + I_R) - bI_R - \alpha I_R - \gamma_R I_R
\end{aligned} \qquad \text{eqn 1}$$

Here, $H_G = S_G + I_G + R_G$ and $H_R = S_R + I_R$ representing the total grey and red squirrel populations in each patch,

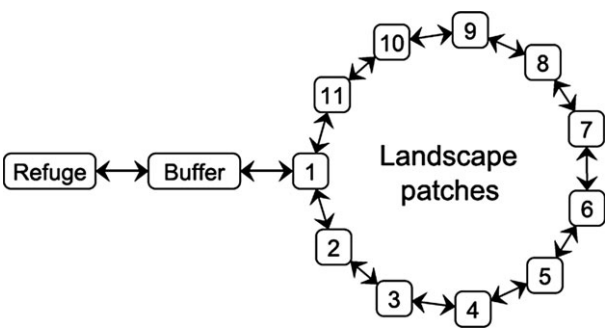

Fig. 1. Landscape patches are linked to neighbours in a ring and these are linked to a buffer zone and refuge (which together form the stronghold). The stochastic model, eqns (2) and (3) represents the dynamics in each patch. Dispersal occurs to neighbouring patches as indicated by the arrows.

respectively. In Tompkins, White and Boots (2003), the two species have the same rate of adult mortality (b) but different rates of maximum reproduction (a_G, a_R) and different carrying capacities (K_G, K_R) which lead to susceptibilities to crowding (q_G, q_R) (since $q = (a - b/K)$. The competitive effect of grey squirrels on red squirrels is c_G, while that of red squirrels on grey squirrels is c_R. Squirrelpox virus is transmitted at the rate β both within and between each squirrel species with infected reds dying due to the disease at rate α, and infected greys recovering at rate γ_G (with reds recovering at rate γ_R when we consider general disease characteristics; $\gamma_R = 0$ for SQPV). To generate the stochastic model, the rates in the deterministic model are converted to probabilities of events that account for changes in individual abundance within each patch (Renshaw 1991). The probabilities, P, of each event are determined as follows:

Birth of Grey to S_G	$P(S_G \rightarrow S_G + 1)$	$: [(a_G - q_G(H_G + c_R H_R))H_G]/R$	
Natural Death of S_G	$P(S_G \rightarrow S_G - 1)$	$: [bS_G]/R$	
Infection of Grey	$P(S_G \rightarrow S_G - 1, I_G \rightarrow I_G + 1)$	$: [\beta S_G(I_G + I_R)]/R$	
Natural Death of I_G	$P(I_G \rightarrow I_G - 1)$	$: [bI_G]/R$	
Recovery of Grey	$P(I_G \rightarrow I_G - 1, R_G \rightarrow R_G + 1)$	$: [\gamma_G I_G]/R$	
Natural Death of R_G	$P(R_G \rightarrow R_G - 1)$	$: [bR_G]/R$	eqn 2
Birth of Red to S_R	$P(S_R \rightarrow S_R + 1)$	$: [(a_R - q_R(H_R + c_G H_G))H_R]/R$	
Natural Death of S_R	$P(S_G \rightarrow S_G - 1)$	$: [bS_R]/R$	
Infection of Red	$P(S_R \rightarrow S_R - 1, I_R \rightarrow I_R + 1)$	$: [\beta S_R(I_G + I_R)]/R$	
Natural/Diseased Death of I_R	$P(I_R \rightarrow I_R - 1)$	$: [(b + \alpha)I_G]/R$	
Recovery of Red	$P(I_R \rightarrow I_R - 1, S_R \rightarrow S_R + 1)$	$: [\gamma_R I_R]/R$	

In addition, there are probabilities of individuals of each class moving to neighbouring patches. The probability of leaving the current patch for class S_G is

$$P(S_G \rightarrow S_G - 1) \quad : [mS_G F(H_G, H_R)]/R \qquad \text{eqn 3}$$

here m is the long distance dispersal rate and $F(H_G, H_R) = (H_G + c_R H_R)^2/(K_G)^2$ to represent saturation dispersal (Rushton *et al.* 2000) which reflects the situation in which squirrels are less likely to disperse when the absolute density in the grid patch is below the carrying capacity and more likely to disperse when above it. (Similar terms are used to represent dispersal in other classes.) In eqns (2) and (3), $R = \sum$ [rates] (the sum of the terms in square brackets) and therefore transforms the rates to probabilities. We additionally specify the dispersal rate between the landscape and the buffer (Fig. 1) as $p_m m$ where p_m is the proportional connectivity (such that when $p_m = 0$, the stronghold is isolated from the main landscape and when $p_m = 1$ the stronghold is connected with the same strength as the landscape patches). When an individual leaves a patch, it enters a neighbouring habitable patch (see Fig. 1). We use the best estimate parameter set determined for the UK squirrel system as outlined in Tompkins, White and Boots (2003) and additionally define $m = b$ to reflect that on average an individual is likely to undergo long distance dispersal to a neighbouring patch once in its lifetime. These parameter values would lead to the replacement of red squirrels by greys in the absence of disease (through competition) but more rapid replacement when the disease is also included, see Tompkins, White & Boots (2003). The parameters are also representative of a general parasite type that has high virulence in the native species. In addition, we consider alternative parasite types in which the native species suffers low virulence ($\alpha = 2$) and in which R_0 is kept constant (by setting $\gamma_R = 24$) to allow direct comparison between the high- and low-virulence scenarios. (A low-virulence parasite without recovery greatly increases R_0 and is considered in the Supporting Information.)

The time between events is an exponentially distributed random variable and can be determined as $T_{event} = -\ln(\sigma)/R$ where σ is a random number drawn from a uniform distribution between 0 and 1 (see Renshaw 1991). The events are incremented at random with the associated probabilities updated due to changes in population density after each event. Individual simulations can be undertaken using a Gillespie algorithm and provide information of the behaviour in a single realization. Monte Carlo methods can be used to generate multiple simulations to assess the average behaviour and variability across realizations.

The spatial set-up is shown in Fig. 1. Here, the ring of linked patches (landscape patches) is intended to represent the dynamics of the grey squirrel population that has invaded and replaced the native reds (as is the case throughout much of England and Wales). From one of these patches, there is a connection to the buffer zone that surrounds a refuge which is assumed to initially contain red squirrel populations. Management strategies are employed in the buffer and refuge. This set-up approximates the situation in strongholds where the protected red populations are surrounded by established grey populations (such as many of the current strongholds in England; Parrott *et al.* 2009).

Results

BASELINE DYNAMICS IN THE LANDSCAPE AND STRONGHOLD

By considering high and low carrying capacity in the refuge and buffer zone, we reflect the impact of variation in habitat composition of different strongholds (Parrott *et al.* 2009) and assess the influence of population density on disease-mediated invasion. The refuge and buffer zone were initialized with red squirrels at their carrying capacity (in the absence of disease). The landscape cells were initialized with grey squirrels at their carrying capacity (when considering competition mediated invasion) or, when considering competition and disease-mediated invasion, at the endemic equilibrium values determined from eqn (1) and assuming an SQPV prevalence of 74% (as in Tompkins, White & Boots 2003). Test runs using 30

model simulations when there is no connection between the landscape and buffer zone ($p_m = 0$) indicate that populations remain close to these initial conditions with some fluctuation due to the stochastic nature of the simulations (Figs S1–S3, Supporting Information).

DYNAMICS IN THE STRONGHOLD IN THE ABSENCE OF CONTROL

When there is a connection between the buffer zone and the landscape cells, grey squirrels can disperse into the buffer (and subsequently the refuge), compete with reds and potentially transmit the infection. This leads to the exclusion of red squirrels from the refuge with the dynamics and rate of replacement of reds dependent on the density in the stronghold, the level of connection between the buffer and the landscape and whether the disease is present.

When both the buffer and refuge have high carrying capacities, the impact of disease is marked and reduces the time to exclusion of reds (compared with the action of competition alone) at all levels of connection between the stronghold and landscape (Fig. 2a). Dispersal of infectious greys from the landscape can trigger an epidemic in the red population in the buffer with subsequent dispersal of infectious reds leading to an epidemic in the refuge (Fig. 2b,c). Thus, the initial decrease in red abundance is due to a disease epidemic in reds that occurs predominantly in the absence of grey invaders. This reduction in the red population reduces the competitive pressure on greys, allowing them to increase in number, and subsequent disease outbreaks in reds and greys lead to further crashes in the red population and their eventual exclusion. In the absence of disease, the replacement process is significantly slower as the action of competitive replacement does not result in dramatic crashes in red abundance (Fig. 2d,e). The time to exclusion increases as the connection strength to the stronghold decreases (Fig. 2a). In particular, when the connection is low, the chance of initial invasion is rare and it takes time before an invasion is successful. This explains the increased variability in replacement time at low levels of connectivity, which is particularly pronounced for disease-mediated replacement because epidemics in the stronghold rely on rare dispersal events of infected individuals from the surrounding landscape. Typical time-series plots highlighting the variability in exclusion times are shown in Figs S4–S6 (Supporting Information).

When carrying capacity is high in the refuge but low in the buffer zone, the difference between disease-mediated

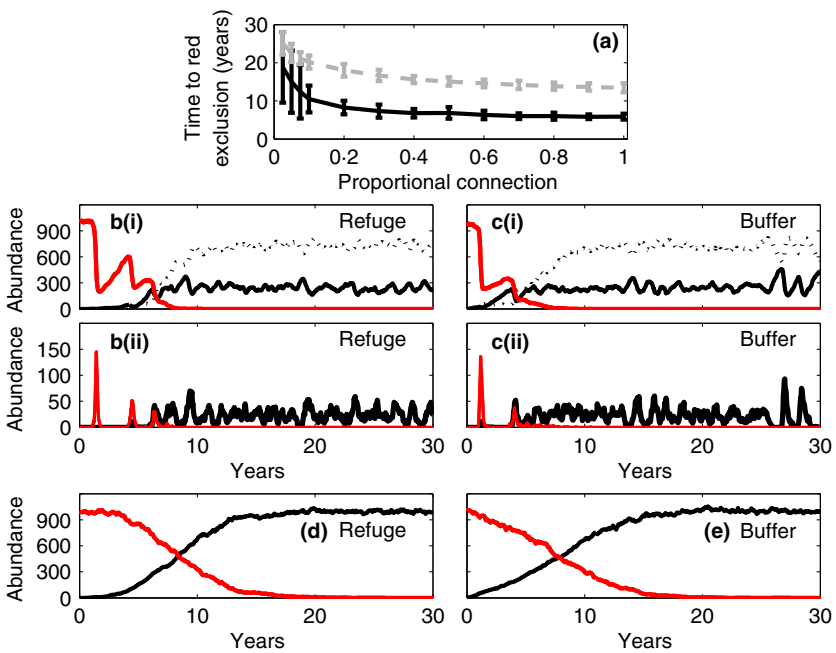

Fig. 2. Time to exclusion and population time series for parameters that represent squirrelpox (high virulence) when the carrying capacity for red and grey squirrels is high (1000) in the refuge and buffer zone. In (a) the time to exclusion of red squirrels in the refuge is plotted against the proportional connection between the landscape and buffer zone. The solid line represents the mean time to extinction (from 30 simulations, with 95% confidence intervals) when the disease is at endemic levels in the landscape patches and the dashed grey line when the disease is absent. Time series plots of population abundance (with proportional connection, $p_m = 0{\cdot}2$) showing disease-mediated replacement in (b) the refuge and (c) the buffer zone are shown for (i) susceptible reds (red line), susceptible greys (black line) and recovered greys (dotted line) and (ii) infected reds (red line) and infected greys (black line). Time series plots for competition (only) replacement are shown in (d) the refuge and (e) the buffer zone for red squirrels (red line) and greys (black line). Parameters are as in Tompkins, White & Boots (2003) which are: $a_G = 1{\cdot}2$, $a_R = 1{\cdot}0$, $b = m = 0{\cdot}4$, $\alpha = 26$, $\gamma_G = 13$, $\gamma_R = 0$, $\beta = 0{\cdot}056$ the carrying capacity in the refuge and buffer are stated above and in the landscape, $K = 1000$.

and competition-only replacement times can still be observed, but is less marked (Fig. 3a). Here, the density of reds in the buffer zone is insufficient to cause a disease outbreak until the density of greys has increased through competitive replacement. Once an epidemic occurs in the buffer zone, infection can spread to the refuge causing a crash in red population abundance and accelerated replacement of reds (Fig. 3b,c). In some simulations, disease outbreaks fail to establish in the refuge and the replacement process exhibits a similar pattern to that in the absence of disease (Fig. 3d,e).

When the refuge and buffer zone have low carrying capacities, then there is no significant difference between replacement times with and without the disease (Fig. 4a). This is because the disease does not cause an epidemic in red populations in either the low-density refuge or buffer (Fig. 4b,c), and so replacement occurs through competition processes only (see Fig. 4d,e). The time to exclusion is reduced for all levels of connectivity compared with the high-density scenario (compare Figs 2a and 4a) simply because it takes less time to out-compete an initially less abundant population.

When the chronic (low virulence) parasite type is considered, the replacement times when compared to the equivalent high-virulence scenarios are increased (Fig. 5). Disease outbreaks still occur, and the disease can persist in the native population. However, the reduced mortality from the disease means that the overall reduction in native abundance is less marked, and so replacement times lie between those reported for the highly virulent parasite and the competition-only scenarios. A parasite type with low virulence and no recovery has a greatly increased R_0, and here disease outbreaks lead to all native species becoming infected (Fig. S7, Supporting Information). This disease type markedly reduces native abundance, and so the disease leads to a significant reduction in replacement times.

GENERALITY OF RESULTS IN THE ABSENCE OF CONTROL

We have undertaken a range of simulations with different levels of high and low densities in the stronghold and different densities in the landscape along with variations in the disease and life history parameters and find results that are qualitatively similar to those reported above under the assumption that the invader is a better competitor and the disease is more harmful to the native species. When this assumption is suitably relaxed, the coexistence of native and invader or the exclusion of the invader is possible, and so the threat to the native species is reduced or removed and so is not a focus of this study (but see

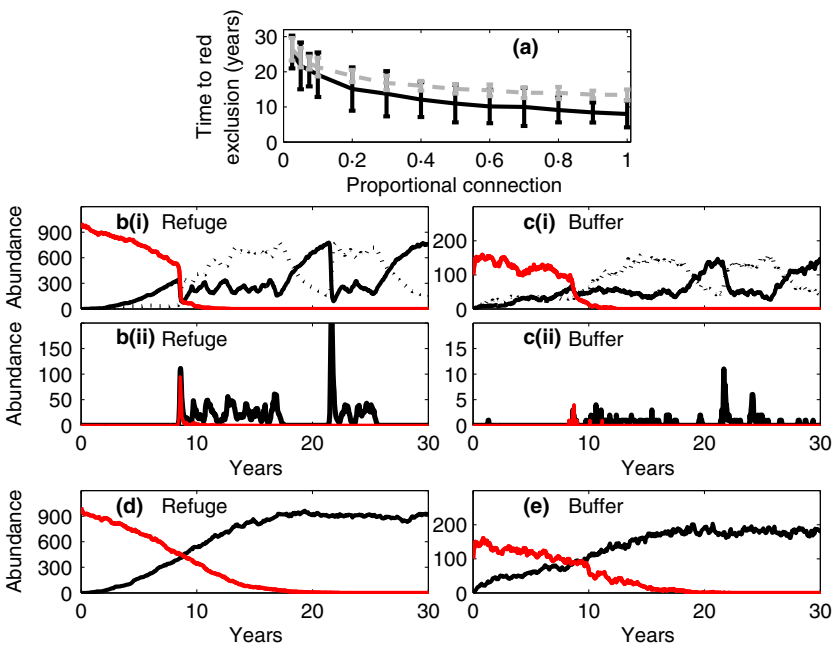

Fig. 3. Time to exclusion and population time series for parameters that represent squirrelpox (high virulence) when the carrying capacity for red and grey squirrels is high (1000) in the refuge and low (100) in the buffer zone. In (a) the time to exclusion of red squirrels in the refuge is plotted against the proportional connection between the landscape and buffer zone. The solid line represents the mean time to extinction (from 30 simulations, with 95% confidence intervals) when the disease is at endemic levels in the landscape patches and the dashed grey line when the disease is absent. Time series plots of population abundance (with proportional connection, $p_m = 0{\cdot}2$) showing disease-mediated replacement in (b) the refuge and (c) the buffer zone are shown for (i) susceptible reds (red line), susceptible greys (black line) and recovered greys (dotted line) and (ii) infected reds (red line) and infected greys (black line). Time series plots for competition (only) replacement are shown in (d) the refuge and (e) the buffer zone for red squirrels (red line) and greys (black line). Other parameters are as in Fig. 2.

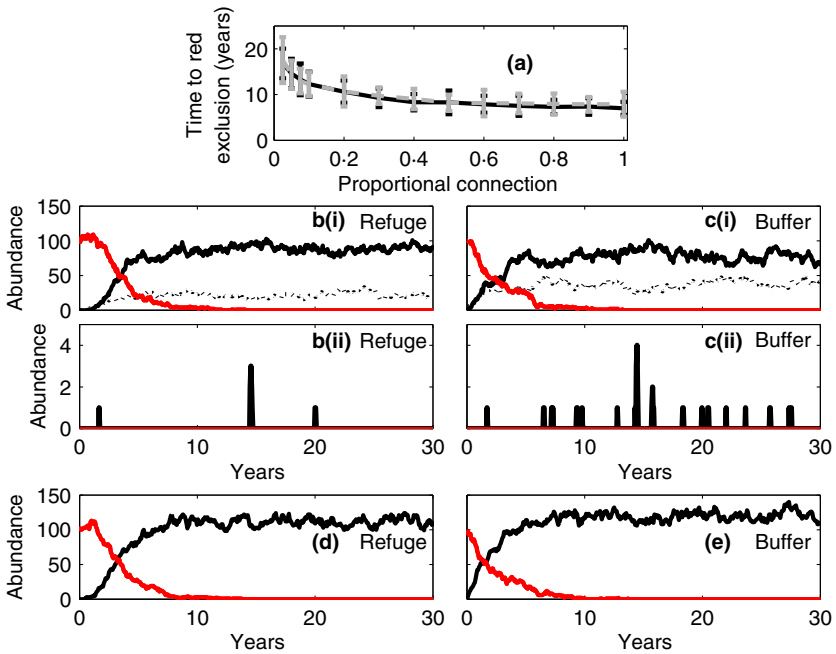

Fig. 4. Time to exclusion and population time series for parameters that represent squirrelpox (high virulence) when the carrying capacity for red and grey squirrels is low (100) in the refuge and low (100) in the buffer zone. In (a) the time to exclusion of red squirrels in the refuge is plotted against the proportional connection between the landscape and buffer zone. The solid line represents the mean time to extinction (from 30 simulations, with 95% confidence intervals) when the disease is at endemic levels in the landscape patches and the dashed grey line when the disease is absent. Time series plots of population abundance (with proportional connection, $p_m = 0{\cdot}2$) showing disease-mediated replacement in (b) the refuge and (c) the buffer zone are shown for (i) susceptible reds (red line), susceptible greys (black line) and recovered greys (dotted line) and (ii) infected reds (red line) and infected greys (black line). Time series plots for competition (only) replacement are shown in (d) the refuge and (e) the buffer zone for red squirrels (red line) and greys (black line). Other parameters are as in Fig. 2.

Bell *et al.* 2009). Both the high- and low-virulence cases illustrate that the key driver of disease-mediated replacement is for the disease to be sustained at endemic levels in the landscape and for the density in the buffer and refuge to be sufficiently high to lead to epidemics and population crashes within native populations. When the quality of the buffer is poor, it cannot support an epidemic outbreak, but rare dispersal of infected individuals through the buffer can result in epidemics in the refuge and disease-mediated invasion. When the disease cannot be supported in either the buffer or the refuge or if the disease is absent, replacement occurs through competitive mechanisms only. Replacement is then slower than when the disease has an impact.

DYNAMICS IN THE STRONGHOLD WITH GREY CONTROL

We examined the effectiveness of control of grey squirrels as a strategy to protect and maintain red squirrels in the refuge on the assumption that this may be necessary as strongholds often contain habitat suitable for greys (e.g. Slaley Forest, in Northern England, Parrott *et al.* 2009). Control was applied by removing a proportion, p_C, of greys from the refuge and buffer at regular intervals (two control periods per year at 6-month intervals). Intensive removal followed by periods of monitoring and subsequent trapping is a common field situation (for example see: www.snh.org.uk/pdfs/species/A260188.pdf). Similar findings to those reported below occur if control is applied continually at a defined rate or if control is applied whenever greys exceed a defined density. Figure 6 shows the density of reds and greys in the refuge for different levels of control in the absence of disease (Fig. 6a–c), with the disease characteristics of the SQPV (high virulence) (Fig. 6d–f) and for the low-virulence disease scenario (Fig. 6g–i). There is a threshold for the level of control above which red populations can be maintained in the refuge (which can be visualized as the lowest level of control at which red squirrel abundance is positive). This threshold level increases slightly as the connection between the stronghold and the surrounding landscape increases, that is, a higher level of control is required to prevent red extinction as the connection strength increases [compare columns (i) and (ii) in Fig. 6]. The threshold also increases as the abundance in the refuge decreases (it is more difficult to protect a low-density refuge) with the threshold minimized for a high-density refuge and low-density buffer zone. There is little difference in this threshold level for comparable parameters in the presence and absence of disease (compare Fig. 6a, d, g, etc).

The main difference between the simulations with and without disease is that when the disease is present, the

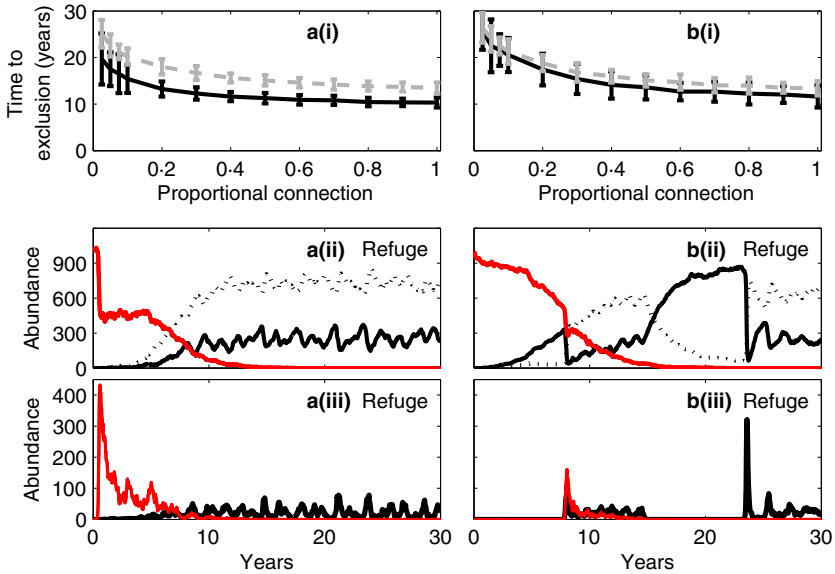

Fig. 5. Time to exclusion and population time series for parameters representing a parasite type with low virulence ($\alpha = 2$, $\gamma_R = 24$). The carrying capacity for the native and invading species is high (1000) in the refuge and in (a) high (1000) in the buffer zone and (b) low (100) in the buffer zone. In (i) the time to exclusion of native species in the refuge is plotted against the proportional connection between the landscape and buffer zone. The solid line represents the mean time to extinction (from 30 simulations, with 95% confidence intervals) when the disease is at endemic levels in the landscape patches and the dashed grey line when the disease is absent. Time series plots of population abundance (with proportional connection, $p_m = 0{\cdot}2$) showing disease-mediated replacement in the refuge are shown for (ii) susceptible natives (red line), susceptible invaders (black line) and recovered invaders (dotted line) and (iii) infected natives (red line) and infected invaders (black line). Other parameters are as in Fig. 2.

mean red abundance in the refuge is reduced and can show large variability. This occurs as a result of disease outbreaks in the red populations in the refuge (Fig. 7a–c) caused by the dispersal of infectious greys into the stronghold (that either evade control or disperse between control events) that succeed in triggering an epidemic in the red population in the buffer and refuge. For an epidemic to occur requires the abundance in the refuge to be high. In the red–grey–SQPV system, control of greys can prevent the exclusion of reds in the refuge, but may not prevent periodic outbreaks of the disease. When a disease outbreak occurs in the refuge, it dramatically reduces the population abundance (Fig. 7a,b) (because the disease is highly virulent to reds). This reduced population level

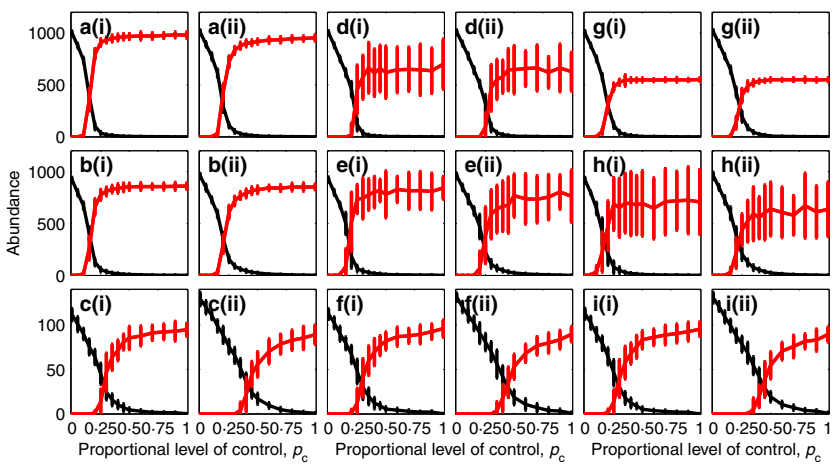

Fig. 6. The average density (with 95% confidence intervals) in the refuge after 40 years where in plots (a–c) the parasite is absent in (d–f) the parasite has the properties of squirrelpox (high virulence) ($\alpha = 26$, $\gamma_R = 0$) and in (g–i) the parasite has low virulence (alpha = 2, $\gamma_R = 24$) for red/native (red line) and grey/invading (black line) squirrels/species plotted against the proportional level of control of greys/invaders. Control is applied twice per year and removes the specified proportion of greys/invaders from the refuge and buffer zone. The proportional connection between the stronghold and the landscape is (i) $p_m = 0{\cdot}2$ and (ii) $p_m = 1$ and the carrying capacities are (a, d, g) 1000 in the refuge and buffer, (b, e, h) 1000 in the refuge and 100 in the buffer, (c, f, i) 100 in the refuge and buffer. Other parameters are as in Fig. 2.

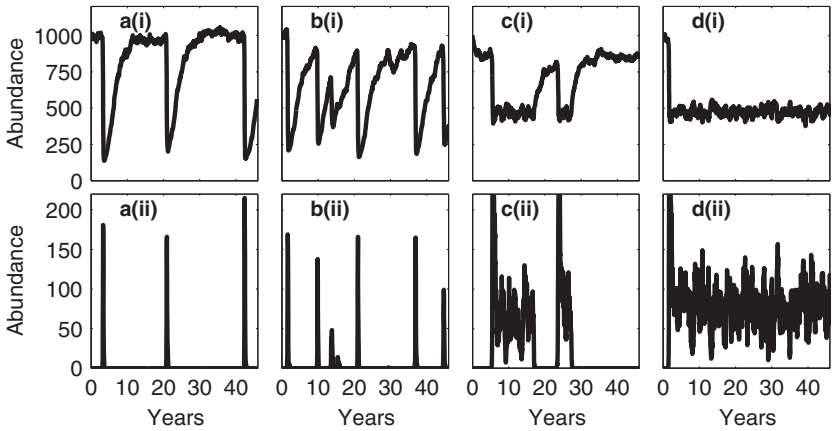

Fig. 7. Time series for the population abundance of (i) susceptible reds/natives and (ii) infected reds/natives in the refuge for a proportional control level, $p_C = 0{\cdot}5$. In (a, b) the carrying capacity in the refuge and buffer is high (1000) and the parasite has parameters representative of squirrelpox (high virulence) ($\alpha = 26$, $\gamma_R = 0$). In (a) the proportional connection $p_m = 0{\cdot}025$ and in (b) $p_m = 0{\cdot}2$. In (c, d) the parasite has low virulence ($\alpha = 2$, $\gamma_R = 24$) and the proportional connection to the buffer $p_m = 0{\cdot}2$. In (c) the carrying capacity in the refuge is high (1000) and in the buffer is low (100) and in (d) the carrying capacity in the refuge and buffer is high (1000). Other parameters are as in Fig. 2.

cannot support the disease in the long term leading to disease fade-out. The red population can then recover to pre-infection levels because the control of greys has removed or reduced the interspecific competitive pressure. Once red density reaches a sufficiently high level, the population is once more prone to a disease outbreak and therefore epidemics occur in the refuge periodically (Fig. 7a,b). When the density in the buffer zone is low or the control level is high (which acts to lower grey density in the buffer) or the connection between the stronghold and the landscape is low (compare Fig. 7a,b), disease outbreaks in the refuge are less frequent.

The results with control of the invading species highlight key differences in the outcome of disease-mediated invasion for different parasite types. In particular, while the highly virulent infection produced periodic epidemics in the native species, a low-virulence infection may remain endemic in the native population (Fig. 7d). This reduces the density and variability of native species in the refuge [Fig. 6g(i),(ii)]. Here, the lower mortality of the parasite enables it to persist for longer periods, thereby reducing the chance of disease fade-out, although for the disease to remain endemic re-infection from the reservoir landscape population is still necessary. This is highlighted by comparing Fig. 7c in which buffer is at low density and so offers a poor connection to the reservoir population, with the disease persisting over extended periods before fade-out, with Fig. 7d in which the high-density population in the buffer enables the disease to remain endemic in the refuge.

Discussion

Disease-mediated invasion is increasingly recognized as a key threat to biodiversity (Prenter *et al.* 2004; Strauss, White & Boots 2012). As such, it may have important consequences for the conservation and management of threatened native species within refuges and other biological reserves. It is therefore essential to examine the ability of conservation strongholds or refuges to maintain viable populations of native species in the context of these disease-mediated invasions. By combining strategic and specific modelling approaches, it is possible to make management predictions while understanding the processes that underlie population outcomes leading to more robust conservation practice. We discuss how our general model results provide insight into conserving native species threatened by disease-mediated invasion, drawing on observations in the field. Many of these observations relate to the red–grey–SQPV system as this has been widely studied and protection of reds in strongholds is well established, but we stress that the findings apply more generally.

In the absence of control of the invading species, native populations are driven to extinction both in the absence of disease (through competition) and more rapidly when the disease is included (through competition and disease processes). Therefore, in regions where the invading species can out-compete the native species, control is required to prevent replacement. An important general insight is that the presence of disease does not have a significant impact on the threshold level of control required. However, while the native species can be protected from replacement, disease outbreaks may be expected due to continual invasion attempts from the disease-carrying invader. Therefore a key finding for both the high- and low-virulence parasite scenarios considered in this study is that the disease can spread through the native species even when the invading species is prevented from establishing (or at the onset of invasion), and here the results from our stochastic model framework support those of spatial deterministic frameworks (Bell *et al.* 2009).

Disease outbreaks among the native population in the stronghold are most likely when the population density is

high and can result in significant population crashes. Disease therefore represents a serious threat, since by suppressing the size of the population it is more vulnerable to extinction through stochastic factors (Woodroofe 1999). Our general predictions are supported by evidence from UK red squirrel strongholds. The high-density red populations in Formby, Merseyside and in Whinfell, Cumbria have been protected by trapping and removal of greys since the launch of strongholds in 2006 (Parrott *et al.* 2009), but have suffered repeated outbreaks of SQPV which has resulted in a marked reduction in population abundance, followed by disease fade-out and subsequent population increases. A key message, therefore, is that control of the invading species to prevent it establishing in strongholds can protect the native species from exclusion but may not protect it from disease outbreaks, and therefore such epidemics should not be seen as a failure in the control strategy (without control the native species would be rapidly replaced through disease-mediated competition). Furthermore, wildlife managers employ a number of strategies (e.g. supplemented feeding, habitat improvement) that may affect the aggregation and density of target and non-target species with the unintended consequence that parasites may also benefit (Cross *et al.* 2010). Our study highlights how the benefits of increased density may be countered by an increased risk of disease outbreaks. Therefore a critical message is that there is a correlation between native density (and therefore habitat quality) and the impact of disease 'harmful' to native species.

This study highlights some general properties for the protection of native species in strongholds. Intuitively, whenever possible strongholds should be chosen such that the native species can outcompete the invader. For example, the Fleet basin stronghold (Forestry Commission Scotland (FCS), pers. comm.) in the Galloway forest is composed predominantly of Sitka Spruce, which supports low-density red squirrel populations but not greys (although greys are supported in the surrounding better quality habitat). Here, either no or limited control is required at the stronghold margins to prevent spillover from adjacent grey populations (FCS pers. comm.). The low density of reds may also preclude widespread epidemic outbreaks (although localized infection may occur at the interface between red and grey populations). The red squirrel stronghold on the Isle of Arran and the red squirrels on the Isle of Wight are not connected to mainland populations and can therefore support high-density red populations in the long term (Lurz 2012). Nevertheless, monitoring should be undertaken and where necessary contingency plans should be invoked to control and exclude 'accidental' invasions and to limit potential disease risk. In connected strongholds where the disease-carrying invader can outcompete the native species a continual control strategy would have to be applied to prevent native replacement. Where the native species can be supported at sufficiently high-density, periodic disease outbreaks for highly virulent parasites or persistence of the disease for low-virulence parasites would be expected and may be difficult to prevent. A consequence of disease outbreaks is that it presents the opportunity for the native species to develop immunity, which cannot develop without control as the native is replaced through competition. In a few instances reds have been shown to display antibodies to SQPV implying they have survived the infection (Sainsbury *et al.* 2008). Testing in stronghold sites that exhibit disease outbreaks would provide key evidence of the potential for the development of immunity to SQPV in red squirrels.

Our study highlights key differences in the way different parasite characteristics impact native species threatened by disease-carrying invaders. Highly virulent diseases cause severe population crashes in native species that hasten their replacement by invaders. For instance, the monogenean gill fluke introduced by the Starry sturgeon to the Aral Sea decimated native bastard sturgeon (Pourkazemi 2006), and over half of the native Hawaiian bird species have gone extinct due to exposure to avain malaria and birdpox spread by introduced, domestic birds (Warner 1968; van Riper *et al.* 1986). However, the severity and short-lived nature of infection means the disease cannot be supported in native populations and fade-outs leading to population recovery to pre-infection levels can occur if the invading species is excluded [as observed for SQPV in red strongholds (Parrott *et al.* 2009)]. In contrast, our study indicates that introduced disease with low virulence (with equivalent R_0 to the high-virulence parasite), can remain endemic within native species. This may explain why squirrel Adenovirus that is less virulent than SQPV, but can cause mortality when associated with other stress factors, has been reported in natural red squirrel populations that are free from grey squirrels with the disease persisting for many years (Martínez-Jiménez *et al.* 2011; Everest *et al.* 2013). Other examples include the persistence of the trypanosome parasite *Crithidia bombi* in native honeybees which inhibits an infected individual's ability to search for nectar and can lead to death through starvation. *Crithidia bombi* is spread from commercial honeybees used in greenhouses with highest disease incidence in native species near greenhouses (Otterstatter & Thompson 2008; Meeus *et al.* 2011). Similarly, the amphibian chytrid fungus *Batrachochytrium dendrobatidis* has emerged and spread through native amphibian populations worldwide introduced by invading frog species. The virulence levels of disease is species dependent but it can persists in some native species which then facilitates the replacement of more susceptible species by resistant invaders (Akmentins & Cardozo 2010; Kilpatrick, Briggs & Daszak 2010).

Our study has highlighted the impact that disease-carrying invaders can have on replacement of native species and critically emphasised the difficulties in preventing disease outbreaks when attempting to

conserve threatened species. We suggest that modelling frameworks that can represent the stochastic nature of invasive spread and simulate disease dynamics can bridge a gap between our understanding of the risk from invading species and the consequences of conservation management on the ground (Joseph *et al*. 2013).

Data accessibility

This paper contains no new data.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Baseline results and further simulations.