Biosecurity for conservation translocations: Fisher's estuarine moth (*Gortyna borelii lunata*), short-haired bumblebee *(Bombus subterraneus),* pool frog (*Pelophylax lessonae*) and cirl bunting *(Emberiza cirlus)* translocations as case studies

Abstract:

Exposure to parasites in conservation translocations increases the risks to recipient and translocated populations from disease, and therefore there has been interest in implementing biosecurity methods.   Using four case examples we described how biosecurity was applied in practical translocation scenarios prior to and during a translocation and also post-release. We implemented biosecurity, including quarantine barriers, at specific points in the translocation pathway where hazards, identified by the disease risk analysis, had the potential to induce disease.   Evidence that biosecurity protected translocated and recipient populations, included an absence of mortality associated with high risk non-native parasites, a reduction in mortality associated with endemic parasites, the absence of high risk pathogenic parasites, or associated diseases, at the destination; and the apparent absence of diseases in closely related species at the destination site.   The biosecurity protocols did not alter the level or duration of translocated species confinement and therefore probably did not act as a stressor. There is a monetary cost involved in biosecurity but the epidemiological evidence suggests that conservation translocation managers should carefully consider its use. Breakdowns in quarantine have occurred in human hospitals despite considerable investment and training for health professionals, and we therefore judge that there is a need for training in the objectives and maintenance of quarantine barriers in conservation translocations. Biosecurity protocols for conservation translocations should be continually updated in response to findings from disease risk analysis and post-release disease surveillance and we recommend further studies to evaluate their effectiveness.

3620 words

Introduction

Conservation translocations, defined as the intentional movement and release of a living organism where the primary objective is improving the conservation status of the target species, and/or to restore natural ecosystem functions or processes (IUCN 2013), are known to increase the risks to recipient and translocated populations from disease because of the increased probability of contact between hosts and novel parasites (Davidson and Nettles, 1992), stressor effects on animals as a result of the move to a new environment (Dickens et al., 2010) and exposure to parasites or non-infectious disease agents during transit or in the destination environment (Kock et al., 2010). When novel parasite – host interactions occur as a consequence of translocation the impact on populations can be severe, as illustrated by the squirrelpox viral disease outbreak in the UK (Sainsbury et al., 2008). Free-living wild animals are known to have been exposed to novel parasites as a consequence of conservation translocations, for example *Batrachochytrium dendrobatidis* was introduced into the free-living Mallorcan midwife toad (*Alytes muletensis)* population with animals from a captive-breeding facility where there were no quarantine barriers between species from different geographical locations (Walker et al., 2008). Likewise stressor associated infectious disease in conservation translocations has led to severe disease outbreaks, for example with cirl buntings (*Emberiza cirlus*) (McGill et al., 2010).

Given that a component of the increased risk from disease in translocations stems from exposure to parasites (defined as viruses, bacteria, fungi, protozoa, helminths and ectoparasites) there has been considerable interest in setting up biosecurity protocols when undertaking translocations (Kirkwood and Sainsbury, 1997; Kock et al., 2010; Woodford and Kock, 1991) but the details of specific protocols have not been published. Ballou (1993) noted the importance of quarantine prior to reintroduction to reduce the risk from disease and advocated the removal of animals from planned reintroduction if quarantine facilities were absent. Woodford (2000) described the principles of quarantine and screening protocols prior to translocation, and set out recommended screening tests and methods for different taxa, but did not specifically explain how a quarantine barrier should be set up to achieve the aims.

In this paper we describe four cases where biosecurity has been set up by the Disease Risk Analysis and Health Surveillance Project (DRAHS), a collaboration between the Zoological Society of London and Natural England, for the purpose of reducing the risk from disease in translocation for conservation purposes. The four cases were translocations of the cirl bunting (*Emberiza cirlus*), Fisher's estuarine moth (*Gortyna borelii lunata*), short-haired bumblebee (*Bombus subterraneus*), and pool frog (*Pelophylax lessonae*) and they illustrate how the principles of biosecurity can be applied in practical translocation scenarios, not only prior to translocation but also during a translocation and post-release. For most translocations considered by DRAHS a disease risk analysis (DRA) has been undertaken (Sainsbury and Vaughan-Higgins, 2012), followed by the writing of a disease risk management and post-release surveillance protocol (DRM PRHS) in which detailed biosecurity recommendations are made. This protocol is then used to monitor the effects of translocation on translocated and sympatric species at the destination. Disease risk management (DRM) of hazards identified in the DRA potentially includes: (i) biosecurity including quarantine, (ii) preventive medication (iii) screening for parasites (iv) vaccination, and (v) therapeutic elimination of parasites but in this paper we describe only biosecurity, through quarantine, measures in detail.

Here we define biosecurity as the implementation of management methods to reduce the probability of negative effects on ecosystems from biological organisms (including parasites) which break ecological or geographical barriers and build up in the environment of translocated animals. The purpose of biosecurity in translocations monitored by DRAHS has been to reduce the risk from disease from parasites and we have targeted it at hazards, of several different categories (source, destination, carrier, transport and population; Sainsbury and Vaughan-Higgins 2012), at the location in the translocation pathway at which they act. Using the hazard identification system proposed by Sainsbury and Vaughan-Higgins, (2012) we have systematically identified the points in the translocation pathway at which biosecurity was assessed as paramount to prevent (i) transfer of parasites across barriers and (ii) build-up of parasites in the environment during the translocation. Building on the OIE (2010) definition, we define quarantine as an area where wild animals are maintained in isolation,

through the use of a barrier, with no direct or indirect contact with animals on the other side of the barrier, to reduce the likelihood of transmission of parasites across the barrier while the animals are undergoing observation and, if appropriate, screening, testing and / or treatment prior to, during or after translocation.

Case histories

The cirl bunting is rare and range restricted in England, and bred only in south Devon until a reintroduction , as defined by IUCN (2013), to Cornwall was carried out between 2006 and 2011. The reintroduction pathway involved the annual capture of 75-80 free-living cirl bunting chicks from the remnant Devon population and captive-rearing for reintroduction as fledglings into Cornwall, England (Figure 1). A DRA, undertaken prior to the first translocation, predicted that the greatest risk from disease to the project stemmed from the plan to rear the buntings from six day-old chicks to approximately 35 day old fledglings in a zoological collection. The DRA advised that the captive rearing facility should be sited distant from the zoological collection to reduce the risk of disease from four groups of non-native (alien) parasites (source hazards: namely poxviruses, *Borrelia* spp, *Mycoplasma* spp and *Chlamydia* spp) potentially present in the zoo and, as a result, a new captive-rearing facility was set up at a remote location close to the destination reintroduction site behind a quarantine barrier (McGill et al., 2005; Fountain et al. 2016). Aviculture staff were managed by the zoo but based at the remote location.   All fieldworkers, avicultural, and veterinary staff in contact with cirl buntings were dedicated to the programme and did not handle non-native bird species or visit zoos or other non-native bird collections, to reduce the possibility of non-native pathogens infecting cirl buntings.

Further disease risk management measures included maintaining a quarantine barrier at all stages of transport and rearing because the buntings were held in close proximity to each other (a maximum of 40 buntings were present in the rearing facility at one time) and therefore a build-up in parasites in the environment was predicted (McGill et al 2006) and a concomitant disease outbreak had occurred in a trial reintroduction (McGill et al 2010). Since translocation acts as a stressor on animals leading to immunosuppression (Dickens et

al., 2010), a build up in parasites in the cirl bunting's environment was believed to have precipitated disease in the trial reintroduction. For example, all transport vehicles used to collect chicks from Devon and transport them to Cornwall were clean and not previously used for the transport of birds. Chicks were transported in disposable cardboard or polystyrene boxes which were discarded after single use. In the rearing facility, each brooder, canary cage, and pre-release aviary was an individually quarantined rearing unit, for example each brooder was placed on a separate table with its own labeled equipment and tools. Staff washed their hands in F10 disinfectant and changed into dedicated clothing (aprons) for each quarantined rearing unit. A change of boots and use of a disinfectant foot dip was mandatory on entry and exit of the brooder facility, the canary cage facility, and for each individual pre-release aviary. All working surfaces were cleaned daily with F10 disinfectant. All waste at every stage of rearing was kept separate and disposed of using clinical waste protocols.

To minimize the likelihood of zoonotic infection, for example from *Campylobacter* spp, bird-dedicated areas were in quarantine from the human inhabited part of the captive-rearing facility: the barriers provided clear separation. The biosecurity protocols were communicated through a DRM protocol document, with hard copies distributed and discussions held annually at a pre-season meeting in conjunction with a site visit and updated recommendations from previous years explained. Analysis of reintroduction success reported no detected mortality associated with non-native infectious agents (Fountain et al., 2016) which the biosecurity was designed to prevent. Cirl bunting mortality was associated with a wide variety of infectious agents, causing disease secondary to apparent immunosuppression, e.g. *Isospora* spp (McGill et al., 2010*)*, and *Campylobacter* spp already harboured by the birds (native parasites and carrier hazards). The pre-release mortality rate was highest in 2007 (42.5%; n = 26/73; Fountain et al., 2016) and as a consequence quarantine and hygiene guidelines and their execution were reassessed and made more stringent where it was judged appropriate by veterinarians with expertise in wild animal management (Molenaar and Sainsbury 2008) and in the four subsequent years of rearing the mortality rate was markedly reduced (19.5%; n = 50/257) (Fountain et al., 2016).

(Insert Figure 1 here)

A similar biosecurity protocol (including quarantine barrier) was established when Fisher's estuarine moths (*Gortyna borelii lunata*) were captive bred for reinforcement, as defined by IUCN (2013). The Fisher's estuarine moth is a rare and highly threatened species, and captive breeding for release commenced to minimise the need for translocation from the small and vulnerable wild population in the north-east of Essex and in Kent, England. The dedicated captive breeding facility was sited at the perimeter of a zoological collection which housed non-native species of invertebrates (including some Lepidoptera) (Figure 2). Quarantine of the captive breeding facility was implemented to prevent infection of the moths with potentially non-native parasites harboured by the invertebrates in the zoological collection and transfer of these parasites to the wild. Quarantine included a disinfectant footbath, dedicated boots and clothing, the wearing of disposable gloves, and the use of dedicated tools. Staff caring for the moths had no contact with non-native invertebrates in the zoo and serviced the moths on arrival each day prior to servicing the zoo collection. Signage was added to denote and explain the quarantine barrier. There has been no known mortality associated with non-native infectious agents detected to date.

 (Insert Figure 2 here)

In 2012 the reintroduction (IUCN 2013) of the short-haired bumblebee *(Bombus subterraneus*) from the source environment, Sweden, to the destination environment Dungeness in England commenced (Figure 3). Habitat loss and subsequent resource depletion were the primary factors implicated in the loss of the species in the UK. Approximately one hundred queens were wild caught in April/May each year between 2012 and 2015 and transferred to a University facility in England to allow for screening of potentially non-native parasites prior to transit to the destination site. Biosecurity was implemented through quarantine to (i) prevent escape of any non-native parasites into England before and during screening because three medium risk and nine low risk source hazards were identified in the DRA, (ii) to assist in the detection of an unknown source hazard

which might cause disease in the bumblebees before release and (iii) to protect the bumblebees, and destination ecosystem, from parasites harboured by non-native invertebrates housed in the University. Quarantine measures included the disinfection of all equipment, clothing, boots and vehicle internal surfaces prior to departure from Sweden to the UK; disinfection of all equipment, clothing and surfaces in contact with bees after arrival in England; eliminating contact between staff servicing the bumblebees in England and non-native invertebrates two weeks prior to, and during, quarantine destruction of all materials in contact with the bumblebees on arrival in England; a barrier surrounding the University enclosure of bumblebees which dedicated staff crossed only if wearing boots, gloves and overalls and through a disinfectant footbath; use of dedicated labelled tools in quarantine; a locked quarantine door with appropriate signage; disposal of quarantine waste by incineration; a sealed door to prevent ingress of insects; a strip of insect trap tape (chemical- and attractant-free) placed around the internal aspect of the door and; disinfection of all surfaces, enclosures and tools at the end of quarantine, and any remaining food incinerated.

. Bumblebees which harboured four potentially non-native parasites, *Apicystis bombi*, *Crithidia bombi* , *Nosema bombi* and and *Sphaerularia bombi*, were sacrificed during quarantine (see Brown et al., 2016) and there is no evidence that these parasites have been introduced to England. No disease attributable to known and unknown source hazards or non-native parasites has been detected.

(Insert Figure 3 here)

Pool frogs (*Pelophylax lessonae*) reintroduced (IUCN 2013) to England from Sweden between 2005 and 2008, were moved directly to the destination from the airport and were released immediately (screening of source and destination populations had been carried out prior to reintroduction and informed the DRA) (McGill et al., 2004) (Figure 4). Quarantine during transport included the use of dedicated, clean tools and enclosures, not previously used for animal transport.   A quarantine barrier was implemented in the field at the destination site to (i) prevent or reduce the probability of exposure of the pool frogs to parasites (destination hazards) in England analysed as representing a high disease risk

namely ranaviruses and *Batrachochytrium dendrobatidis* and (ii) reduce the rate of spread from the release site of any unknown non-native parasites harboured by the pool frogs and / or to assist in the detection of disease associated with these non-native parasites by temporarily confining the released pool frogs.   The release ponds were surrounded by an amphibian-proof fence 0.5m high which remained in place for the first year of the project. There was controlled vehicular entry to the site and staff wore dedicated boots, overalls and gloves (which had not been used to study amphibians at other sites).   All tools, including veterinary equipment, and nets were new or disinfected and not used for any other species and stayed on site in a lockable box where possible. Any equipment or materials leaving the site was disinfected to reduce the probability that novel infectious agents introduced with pool frogs would spread. Clinical waste, including gloves, was incinerated.   Post release health surveillance on pool frogs and native amphibians (common frogs (*Rana temporaria)*, common toads (*Bufo bufo)*, great crested newts (*Triturus cristatus)* and smooth newts (*Lissotriton vulgaris*) was undertaken between 2006 to 2012. No cases of infection with ranaviruses or with *Batrachochytrium dendrobatidis,* both of which are widespread in England (Teacher et al., 2010; Smith, 2014), or disease associated with these agents, has been detected in pool frogs or native amphibians on site, and no diseases associated with non-native infectious agents, introduced with pool frogs, have been detected. The mean and range, in brackets, of pool frogs and native amphibians examined per annum between 2006 and 2012 was: mean 24 (range 1-55) pool frogs; mean 25 (range 0 -62) smooth newts; 23 (0-42) great crested newts; 9 (1-20) common frogs; 31 (29-34) common toads. Therefore the results of post-release health surveillance and screening provided no evidence that the quarantine barrier had been broken during this post-release period.

(Insert Figure 4 here)

Discussion

Here we have shown through examining four real conservation translocation cases how we have used targeted implementation of biosecurity to reduce the risk from disease at the points in the translocation pathway where specific hazards (source, carrier, destination,

population and transport), identified by the disease risk analysis, had the potential to induce disease.   While in the cirl bunting, short-haired bumblebee and Fisher's estuarine moth examples the major objective was to prevent the introduction of non-native infectious agents (source hazards) to the destination environment (from either zoological collections or natural source sites), the primary purpose in the pool frog translocation was to prevent contact with population hazards (ranaviruses and *Batrachochytrium dendrobatidis*) which could have an effect on population size at the destination. Other biosecurity objectives included (i) to reduce the potential for build-up of parasites in the environment of stressed animals at high density (cirl buntings), (ii) to minimize the likelihood of zoonotic infection (cirl buntings), (iii) to detect an unknown source hazard before release (bumblebees) or after release (pool frogs) , (iv) to reduce the rate of spread from the release site of any unknown non-native parasites harboured by the translocated animal and / or to detect disease associated with these non-native parasites (pool frogs) and (vi) to prevent contact with non-native infectious agents present in the translocation pathway (bumblebees and pool frogs).

The hazard categories described in the disease risk analysis, using the method described by Sainsbury and Vaughan-Higgins (2012), can be used to determine the best location for quarantine barriers to prevent the spread of parasite hazards to susceptible populations (either translocated animals or recipient populations). In the cirl bunting example, the DRA explained that source hazards (infectious agents in non-native birds in the zoological collection) represented the highest risk from disease to the translocation and therefore the rearing location was moved from the zoo to the release location to create a quarantine barrier between the zoo and reintroduction site.   No diseases attributable to source hazards were detected in the cirl buntings and therefore the transfer of the cirl buntings to a new location was apparently successful in preventing disease. In the bumblebee reintroduction, a barrier was set up to prevent release of source hazards from quarantine and to reduce the probability of contact with non-native invertebrates and their potential source hazards. Bumblebees were sacrificed to prevent release of source hazards and since the epidemiological sensitivity of one diagnostic test (faecal screening for *Sphaerularia bombi* infection at 14-21 days post-capture) detects 100% of infections (Vaughan-Higgins et al 2015), we know that this

biosecurity method was successful. At the pool frog reintroduction site a field quarantine barrier was established which attempted to reduce contact with destination hazards (ranaviruses and *Batrachochytrium dendrobatidis*). These destination hazards (either infection or disease) have been absent from native amphibians and pool frogs at the reintroduction site, a sign that the quarantine barrier has apparently achieved its purpose.

On the basis of our understanding of epidemiological principles, we would expect biosecurity, largely through quarantine barriers, as illustrated above, to protect translocated and recipient populations and we have presented evidence to support this. For example an absence of mortality associated with high risk non-native parasites (cirl bunting, Fisher's estuarine moth and short-haired bumblebee projects), a reduction in mortality associated with endemic parasites (cirl bunting project), the absence of high risk pathogenic parasites (destination hazards), or associated diseases, at the destination (pool frog project); no diseases in closely related species at the destination site (pool frog project). We know of no other studies which have evaluated the ability of biosecurity to prevent transfer of infectious agents between populations in wild animal translocation scenarios. We recommend that simple biosecurity protocols are always set up for conservation translocations whatever the result of a disease risk analysis because severe disease outbreaks have occurred in association with previously unknown parasites, including the two mentioned above (squirrelpox and Bd-associated-disease), and the risk of those parasites could not have been assessed pre-translocation, a consequence of our poor understanding of wild animal parasites.

Biosecurity protocols may risk stress or injury to animals through close confinement and there is a monetary cost involved (Dickens et al., 2010, Ewen et al., 2012). All the biosecurity methods we employed were relatively simple and practical to implement with minimal cost other than staff wages Importantly, we did not change animal management methods through implementing biosecurity or quarantine: the management methods would have been used in the same way in the absence of biosecurity. We merely placed our quarantine barrier and / or biosecurity around existing management protocols; the measures did not increase the level or duration of confinement. Therefore we not believe the biosecurity or quarantine methods

had any influence on stress levels in the species translocated in these four cases. The example noted above in which Bd was introduced to the Mallorcan midwife toad population illustrates the potentially disastrous consequences of not carrying out biosecurity, and supports its use, including quarantine, in all translocations.

Yet, even when the identity of an infectious agent and the epidemiology of the disease is known, breakdowns in quarantine can occur. This has recently been illustrated in the Ebola haemorrhagic fever outbreak in West Africa. Despite considerable investment in barrier quarantine techniques, highly trained health professionals became infected and developed Ebola fever when there was contact with the external surface of quarantine suits (Chiappelli et al., 2015, WHO, 2014). Investment in biosecurity techniques for wild animal translocations has understandably not reached the level used in the control of fatal infectious human diseases.   Although the stringency of biosecurity will depend on the mode of transmission and pathogenicity of the disease agents of concern, the Ebola example suggests that training in biosecurity in wild animal translocation will be important to try to ensure it is sufficiently tight to prevent disease outbreaks, at least for highly contagious agents. Breakdowns in quarantine, such as in the Ebola fever outbreak, imply that instruction of staff in implementing biosecurity for translocation programmes is crucial. We advocate further studies to evaluate the effectiveness of biosecurity to prevent transfer of infectious agents during conservation translocations to assess whether improvements in techniques are required.

Currently, given the evidence available, we advocate the use of 'best practice' biosecurity protocols, communicated effectively through detailed workshops and written documents, and to improve on these techniques where evidence is available and costs allow. PRHS has a role to play in assessing the effectiveness of biosecurity through the detection of infectious agents which break the barrier and are introduced to destination sites and the results should be included in reviews of biosecurity protocols. Instruction in barrier techniques and communication between stakeholders including veterinarians, pathologists, parasitologists, animal care staff, ecologists and volunteers is advocated while evidence on their effectiveness is gathered. It may be possible to develop risk-based approaches where the

investment in barrier techniques is dependent on the results of a disease risk analysis, although in wild animal translocation for conservation the number of unknown parasites harboured by the many species will likely hamper such an approach.

List of figures

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Figure 3. Points in the translocation pathway where quarantine barriers were placed using the case example of the translocation of the short haired bumblebee from Sweden to the UK.

Figure 4.Points in the translocation pathway where quarantine barriers were placed, using the case example of the translocation of the pool frog from Sweden to the UK.

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