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Coimisean na Coilltearachd Alba

Report

Living with pox project: Forest management for areas affected by squirrelpox virus

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Executive Summary

I. The Project: The native red squirrel (*Sciurus vulgaris* L) is under threat as a result of the expansion of the introduced North American grey squirrel (*S. carolinensis*). Scotland has a special responsibility for red squirrels in a UK context as an estimated 75% of the remaining UK populations are thought to live there. Recent research and modelling work (White and Lurz 2014, White et al. 2014) focussed on red squirrel strongholds and large scale linked populations throughout Scotland and has provided insights and valuable lessons on how to manage red squirrel populations and to potentially "live" with the virus in southern Scotland where the virus is present. "Living with pox" in this context refers to all areas supporting red squirrel populations in southern Scotland which are adjacent or sympatric with grey squirrel populations that are a reservoir for squirrelpox virus (SQPV).

II. Aims: The aim of this tender is to deliver advice and guidance for the management of red squirrel populations exposed to squirrelpox virus. Specifically, the project has:

- Reviewed published literature, unpublished information and drawn on the recognised expertise of project staff to review the evidence of population recovery of red squirrels following a squirrelpox virus outbreak.
- Recommended science-led land management measures to manage the disease and promote red squirrel population survival.
- Explored the potential for red squirrel populations to persist based on current knowledge of squirrelpox virus epidemiology.

III. Key findings - Review: Reports on the role of disease in local red squirrel population declines date back to the 1930s. Although grey squirrels were at the time suggested as a potential source and carrier of the then unknown disease, it took until the 1980s to identify the virus and another 20 years to painstakingly collect sufficient genetic, serology, experimental infection and field data to clearly demonstrate the role of squirrelpox virus in the decline of red squirrels.

Evidence suggests that the virus arrived with some of the grey squirrels that were introduced from the US to the UK in the 19th century. Grey squirrels can act as a reservoir for the virus and can spread the disease. The virus does not cause any obvious disease in grey squirrels but causes very high mortality in native red squirrels (approx. 80%). In regions where the virus is present, the combination of 'disease and competition for resources' has led to greatly increased red squirrel population declines (red

replacement by greys can be 17-25 times faster when the disease is present) and local extinctions.

There are still uncertainties with respect to the precise mechanisms of transmission but current research findings suggest multiple potential routes for transmission:

a) **Skin abrasions**: Experimental infections of red and grey squirrels have confirmed that transmission and subsequent successful infection can occur via abrasions in the skin.
b) **Ectoparasites**: Transmission via an arthropod vector such as fleas in the drey remains a possibility, although this would occur through external transport of virus particles on fleas rather than fleas being an actual host for replicating virus.
c) **Environmental contamination**: A role for transmission via environmental contamination has been suggested and that virus left in the environment may remain live for at least a month, with poorest virus survival in wet conditions.
d) **Oral route**: Combined genetic, histopathology and immunohistochemistry testing has recently shown the presence of replicating virus in the tonsil-like structures of red squirrels suggesting that there is potentially also an oral route to infection and transmission**.**

IV. Key findings - 'Living with pox': SQPV appears to readily spread and remain endemic (although with fluctuating levels of prevalence) in grey squirrel populations. Where red squirrel populations are sympatric to greys, SQPV can spread from greys to reds. SQPV infection in a red population is then likely to lead to a local epidemic that results in high mortality in reds. It is however important to note that SQPV is not supported within red-only populations and the disease will fade out and SQPV is therefore not considered to spread extensively through red-only populations at the landscape scale. Provided greys are either unable to establish (e.g. due to unsuitable habitat) or prevented from establishing (e.g. through control) and competing locally with reds, red populations can return to pre-infection levels following an outbreak.

Return to pre-infection levels is not reliant on dispersal from nearby red squirrel populations (although this will occur), but will happen mainly from local residents that a) were not exposed to the disease; and b) from the few local individuals that survived the disease. It is important to stress that a local outbreak is not a reflection of poor conservation strategy and is not necessarily catastrophic for reds. It may be an inevitable consequence of red populations beings adjacent to disease carrying greys.

The likelihood of an SQPV epidemic is strongly linked to local population density, with pox unlikely to reach epidemic levels in populations with low density (e.g spruce dominated habitats with densities of typically 1-2 red squirrels per 10 hectares). Although outbreaks are more likely in high density populations, higher densities are also better for overall red squirrel population viability as low density populations have a high risk of extinction through stochastic events (seed crop failures, severe winters etc.).

The current strategy of large, diverse conifer forests as red squirrel strongholds is therefore a sound approach as these areas can support red squirrels with minimal management input and the risk of widespread disease outbreaks are low due to relatively low squirrel densities.

It is also important to note that dispersing individuals are not the key cause of SQPV spread. Disease is spread by two processes: 1) disease is passed on from individual to individual through an interlinked resident population with individuals undertaking core range movement only; 2) dispersing infected animals move from one population to another. In large continuous forest blocks, of high density, SQPV spread is rapid and driven by process 1. When there are significant gaps in forest coverage, or extensive low density areas, SPQV spread relies on long-distance dispersal of an infected individual (process 2). In most cases rapid spread in 'stationary' populations (process 1) is the main driver.

V. Recommendations Potential management advice with respect to living with pox fall into 4 main areas:

1) Grey squirrel control - evidence from a range of studies indicates that grey squirrel control, when carried out along dispersal corridors leading to vulnerable and or stronghold populations, can reduce the risk of disease transmission; and if carried out systematically and over prolonged periods it can reduce levels of seroprevalence in grey squirrels. More crucially, as part of the wider conservation strategy, grey squirrel control in and around vulnerable red squirrel populations will help reduce competitive impacts of grey squirrels and enable red squirrel populations to return to pre-infection levels following an outbreak of SQPV.

2. Supplementary feeding - would in effect counteract other measures at reducing disease spread. Where grey control would reduce squirrel density and the risk of disease transmission, supplementary feeding would potentially increase squirrel numbers and more importantly create points of contact and significantly increase the risk of disease transmission. We therefore recommend that there should be information highlighting the potential for disease transmission and advice on cleaning/sterilising feeders (including bird feeders) to reduce the risk of SQPV and other disease spread.

3. Managing dispersal links - an overall goal of actively managing links or corridors would be to provide internal linkage between discrete local woodland blocks with red squirrels but restrict them to the outside (i.e. limit the number of corridors from which greys can invade). Such corridors may take the form of hedge, tree rows, or streamside plantings, and should consist of neutral shrub and tree species providing cover but not permanent habitat for resident squirrels. This would increase local population viability by providing dispersal links but reduce the risk of SQPV spread. Given the restricted tree

and shrub species diversity advocated for these dispersal links and potential other conservation objectives when planning corridors, this approach may be most suitable in upland areas where the recommended species are part of the natural vegetation community.

4. Disease management and therefore management of SQPV will require monitoring. This may take the form of passive surveillance that asks the public to report sightings of diseased animals and send individuals for post-mortem analysis, or involve more active measures such as for example systematic sampling for antibodies to SQPV, if resources allow.

VI. Knowledge gaps - current gaps involve questions on:

- Data on relative SQPV transmission rates in red and grey squirrels.
- Is there an optimum density at which to manage priority areas for red squirrel conservation (PARCS) and stronghold populations that reduces the risk of disease outbreaks without increasing extinction risks through stochastic events? In other words, are extensive low density populations the answer?
- To what extent will SQPV affect the balance between red and grey squirrels along the Highland line should it spread there?
- How will pine martens in some regions influence SQPV dynamics in red and grey squirrels?

1. Introduction

1.1. Project staff

Project partners: Prof Andy White, Mathematics, Heriot-Watt University
Dr Peter Lurz, Ecological Consultant
Prof Mike Boots, Biosciences, University of Exeter
Dr Colin McInnes, Moredun Research Institute
Prof Anna Meredith, Veterinary Studies, University of Edinburgh

Project leaders: Prof Andy White and Dr Peter Lurz will lead this project

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1.2. Background

The native red squirrel is under threat as a result of the expansion of the introduced North American grey squirrel. Scotland has a special responsibility for red squirrels in a UK context as an estimated 75% of the remaining UK populations are thought to live in Scotland.

The red squirrel has already disappeared from a large part of its former Scottish range in central and south-eastern Scotland. Replacement of red squirrels by grey squirrels takes the form of disease-mediated competition and decline is significantly faster in areas where grey squirrels act as carriers of squirrelpox virus (SQPV). The virus is currently present in some parts of southern Scotland and efforts to stem its spread in the south of Scotland managed to slow but not prevent SQPV spread. Experience from northern England has shown that red squirrels can persist in the presence of grey squirrels (and the virus) in the wider landscape (although commonly this relies on grey control to protect vulnerable red populations). Recent research and modelling work, by Heriot-Watt University, focussed on red squirrel strongholds and large scale linked populations throughout Scotland (White et al. 2014, White and Lurz 2014) and has provided insights and valuable lessons on how to manage red squirrel populations and to potentially "live" with the virus in affected areas in southern Scotland where the virus has currently spread. These findings explain recent observations in the field in red squirrel strongholds that are sympatric to grey populations with SQPV in Northern England (Chantrey et al. 2014).

1.3. Project aims

The overall purpose of this tender was to provide an assessment based on published evidence and research data and to deliver advice and guidance for the management of red squirrel populations exposed to squirrelpox virus.

Specifically, the project has:

- Reviewed published literature, unpublished information and drawn on the recognised expertise of project staff to review the evidence of population recovery of red squirrels following a squirrelpox virus outbreak. In particular we will consolidate evidence from current literature which suggests that:
    - SQPV is likely to spread extensively through Southern Scotland and with a real risk of spreading to Central Scotland and beyond (White and Lurz 2014).
    - SQPV may spread into red only populations adjacent to infected greys, but the disease is predicted to burn-out and the red population can return to pre-infection levels, since not all local individuals will become infected (White et al. 2014, Chantrey et al 2014).
    - SQPV is not predicted to spread extensively through red only populations and infection is likely to be limited to periodic outbreaks (White et al. 2014, White and Lurz 2014).
- Recommended science-led land management measures to manage the disease and promote red squirrel population survival.
- Explored the potential for red squirrel populations to persist based on current knowledge of squirrelpox virus epidemiology.

1.4. Proposed approach and method statement

The project team carried out a review of published and unpublished literature, monitoring data (e.g. sightings records, antibody blood testing results) and research (fieldwork and modelling predictions), and consult with known experts in the field to assess the epidemiology of SPQV in red squirrels including evidence of disease burn-out, and the population dynamics of reds following an SQPV outbreak. Based on the findings of this review and drawing on the unique expertise and experience of the project team with respect to squirrelpox virus disease and serology (A. Meredith; C. McInnes), squirrel ecology and conservation (P. Lurz) and predicting disease spread and its impact on population dynamics through mathematical modelling approaches (A. White; M. Boots), we recommend and discuss potential land management measures (including implementation time scales) to help minimise the impacts of SQPV on red squirrel populations in Scotland.

2. Review

2.1 History of squirrelpox virus (SQPV) spread in the UK

Squirrelpox virus (SQPV) has had a dramatic but largely hidden role in the early decline of red squirrels in the UK (Tables 1, 2). Whilst Middleton (1930) reported local disease outbreaks with high mortality in red squirrels, the role of disease in the decline of red squirrels only slowly emerged from work by Edwards (1962), Vizoso (1968, 1970) and Keymer (1974) who examined diseased individuals from Shropshire, Thetford Forest and Norfolk. Edwards (1962) had described myxomatosis-like symptoms in diseased red squirrels and there appeared to be a similarity between the descriptions of clinical signs (e.g. lesions on the eyelids and ears, ulcerations in the mouth etc.) between cases in Shropshire and East Anglia and the descriptions by Middleton (1930) suggesting the involvement of a common agent. It took until the early 1980s, however, to identify a virus that was the likely cause of the disease (Scott et al. 1981) and a further 3 years to isolate it in cell culture (Sands et al. 1984).

Initial identification based on external virus morphology as visualized by electron microscopy suggested a parapoxvirus, as particles were similar to the parapoxvirus that causes 'orf' in sheep (Scott et al. 1981). However, subsequent analyses comparing viral DNA sequences with those of other poxviruses indicated that the squirrel virus represented a previously unknown member of the *Chordopoxviridae* and Thomas et al. (2003) suggested the now familiar abbreviation of "SQPV" (results later confirmed by McInnes et al. 2006; for a detailed analysis of genome see Darby et al. 2014).

Table 1 Squirrelpox virus (formerly known as parapoxvirus) characteristics. Image courtesy of D. Everest, Animal and Plant Health Agency.

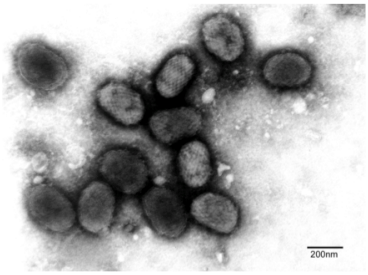	**Virus type**	Poxvirus (Chordopoxviridae)
	Origin	Considered to have arrived with greys from North America
	Impact on red squirrels	Causes very high morbidity and mortality
	Impact on grey squirrels	No apparent signs of disease
	Role of grey squirrels	Greys act as a reservoir for the virus and can spread the disease

Table 2. Brief time line illustrating the growing knowledge about SQPV and its role in the decline of red squirrels

Year	Comment	Reference
1930	Many outbreaks of disease since 1900 with high mortality in red squirrels – cause unknown.	Middleton 1930
1962-1974	Examinations of diseased red squirrels in Shropshire, and East Anglia. Findings suggest a common responsible agent.	Edwards 1962, Keymer 1974, Vizoso 1968
1981	Initial identification as a parapoxvirus based on external virus morphology.	Scott et al. 1981
1984	Isolation of a poxvirus in cell culture from a red squirrel.	Sands et al. 1984
1985	Reds decline in East Anglia due to a viral disease. Role of grey squirrels uncertain.	Reynolds 1985
1995-2000	Mounting evidence of the threat posed by SQPV to red squirrel populations and the role of grey squirrels in its northward spread in England.	Sainsbury & Gurnell 1995; Rushton et al. 2000; Sainsbury et al. 2000
2001	Experimental infection confirms that SQPV has deleterious effect in red and no apparent effect in grey squirrels.	Tompkins et al. 2002
2003	SQPV plays crucial role in red decline.	Tompkins et al. 2003
2006-2008	Reds decline 17-25 times faster in areas with SQPV. SQPV in reds spatially linked to grey squirrel presence.	Rushton et al. 2006 Sainsbury et al. 2008
2007	First known SQPV case in Southern Scotland.	McInnes et al. 2009

The presence of squirrelpox is linked to the presence of grey squirrels and it is highly probable that squirrelpox virus arrived with some of the introduced grey squirrels in the UK. The fact that grey squirrels in Wisconsin, USA have tested positive for antibodies to SQPV (McInnes et al. 2006) supports this claim. McInnes et al. (in press) go even further and now argue that "*It is almost certain that the virus has co-evolved with the grey squirrel on the American continent to a stage where it no longer caused obvious disease in that species...It was only when the virus was introduced to a species of squirrel, the European red squirrel, that had never encountered it before that the disease, which we now refer to as squirrelpox, emerged*."

There were multiple introductions of grey squirrels to the UK in the late 19th and early 20th century. Middleton (1930) reports six separate introductions from the USA, one from Canada, and one from South Africa (where grey squirrels had also been introduced), as well as 25 known translocations from established populations in England to other parts of the UK. Recent analyses of genetic variation of grey squirrel populations in the UK have shown that the large number of human-mediated releases of

grey squirrels have in fact been a crucial ingredient to its successful range expansion (Signorile 2013).

Middleton as early as 1930 noted that: "*It is possible that grey squirrels may act as a carrier of a disease which is fatal to red squirrels but non-pathogenic to the grey species*..." However, he questioned this as the pattern of disease outbreaks seemed not to be linked to centres of introductions for grey squirrels in the UK. It is now apparent that only some of the introduced grey squirrels probably carried the virus (explaining its absence from Scotland) and disease spread would have occurred between red and grey squirrels and then spread locally within red squirrel-only populations.

The role of SQPV in the red squirrel decline observed in large parts of the UK was initially overlooked. Red replacement by greys was at first viewed mainly in terms of potential competitive interactions, mitigated through differences in reproductive success, social structure (i.e. densities in different habitat types), or competitive advantages involving, for example, differences in the exploitation of acorn seed crops (e.g. McKinnon 1978; Gurnell 1987, Kenward & Holm 1989) (although it was also suggested that grey squirrels could occupy areas left vacant following disease outbreaks in reds). However, in the mid to late 1990s evidence of the threat and role of disease began to emerge (Warns 1995, Sainsbury & Gurnell 1995, Sainsbury & Ward 1996, Rushton et al. 2000) and the virus began to be seen as an important potential factor in the disappearance of red and the success of grey squirrels in the UK (Tompkins et al. 2003). Post-mortem studies on causes of mortality in red squirrels list SQPV and associated secondary infections as a prime mortality factor in red squirrels in both England and South Scotland (Sainsbury 2008, Duff et al 2010a, LaRose et al. 2010)[1].

2.2 Key studies and milestones in understanding the role of SQPV

By the late 1990s the potential role of SQPV in the replacement of reds by greys was gaining acceptance and led to an increased research effort to understand the impact of SQPV:

Sainsbury et al (2000);

- blood sampling of hundreds of grey squirrels in different populations across the UK indicated over 60% of grey squirrels tested were positive for antibodies to SQPV.
- authors postulate that grey squirrels act as a reservoir host for the virus.

Tompkins et al (2002);

- an experimental infection of grey and captive-bred red squirrels demonstrates that SQPV is pathogenic in red squirrels while having no detectable effect in greys.
- research findings show that spillover of SQPV from greys to reds can cause local epidemics in red squirrel populations which are likely to fade out due to high levels of mortality.

[1] Whilst antibodies to SQPV in grey squirrels in Ireland have been detected since sampling began in 1997 (McInnes et al. 2013); the first confirmed death resulting from SQPV infection in a red squirrel was not recorded until 2011 from both Northern Ireland (County Down) and Hollywood, Co, Wicklow, Ireland respectively (Naulty et al. 2013).

- the experiment suggests that some red squirrels can potentially survive the disease.

Tompkins et al. (2003);

- mathematical modelling work shows that SQPV plays a crucial role in rapid decline and replacement of red squirrels even though the prevalence of infection in red populations is low.

Rushton et al. (2006);

- a spatial comparison of rates of decline (area of red squirrel disappearance over time) based on sightings data in areas with SQPV (Norfolk, Cumbria) and without the virus (Scotland, Italy) shows that in areas with the virus replacement of red by grey squirrels is 17-25 times faster.

Sainsbury et al 2008;

- a GIS analysis of SQPV outbreaks in red squirrels illustrates that the disease only occurred in areas of England also inhabited by seropositive grey squirrels, and as the grey squirrel range expanded SQPV occurred in these new habitats.
- authors suggest that SQPV will significantly affect red squirrel populations in Scotland over the next 25 years.
- post-mortem examinations of red squirrels in the north of England provided the first evidence that eight juvenile and sub-adult red squirrels had survived SQPV infection in the wild, confirming that some red squirrels survive the disease.

McInnes et al. 2009;

- First grey squirrels positive for antibodies to SQPV found in South Scotland in 2005. First detected outbreak of SQPV in red squirrels near Lockerbie in 2007.

2.3. Clinical signs - what does SQPV infection look like in red squirrels?

Visible early clinical signs often include ocular discharge and a swelling of the eyelids, later lips become swollen and the inside of the mouth can be sore and ulcerated (Edwards 1962, Keymer 1983). As the disease progresses, ulcers and bloody crusts develop around the eyes, mouth, and face, which can be very severe and lead to complete closure of the eyes, and also on the body, legs and feet. These lesions can then become infected with bacteria and lead to severe debility, including the inability to see and feed properly. Clinical signs of SQPV infection have been described by Sainsbury (2008) as "*an exudative erythematous dermatitis, which was in some cases secondarily infected with bacteria (Staphylococcus aureus). Some lesions became ulcerated and were covered with haemorrhagic crusts and were usually located around the face, ventral skin surfaces of the body, medial skin surfaces of the legs and on the toes*" (See also Fig. 1). Red squirrels start to show signs of being infected within the first 10-15 days and the course of the disease is 10 days on average (Carroll et al. 2009), mortality rates have been estimated at 84% or higher (Edwards 1962, Sainsbury 2008, Chantrey et al. 2014).

Figure 1. Red squirrel showing signs typical of SQPV infection.

2.4. Current range of SQPV and seropositive grey squirrels in Scotland

Blood sampling of grey squirrels in 2005 and afterwards in populations across South Scotland showed that 47-100 % of animals tested positive for antibodies to SQPV, indicating that they have been or are infected with the virus. (Table 3; White & Lurz 2014). Figure 2 illustrates the early spread of SQPV in South Scotland, and Table 3 shows examples of the often high levels of seroprevalence (percentage of animals that carry antibodies to SQPV in their blood) in populations in South Scotland between 2005-2012.

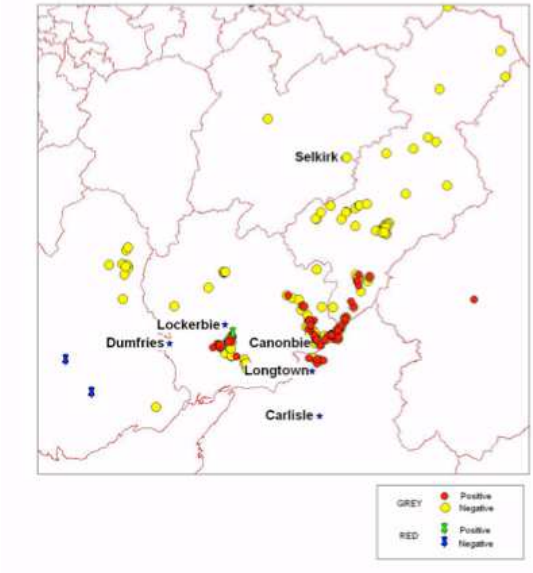

Figure 2. Early spread of SQPV up to July 2007 based on blood sampling in grey squirrels and outbreaks of SQPV in reds in south Scotland

Table 3. Selected blood sampling data to illustrate the seroprevalence range of greys in the South of Scotland between 2005 - 2012 (data courtesy of SWT).

Location	Date	Seroprevalence (%)	Number tested
Newcastleton	Jun-05	100	5
Hollowellbogs, Canonbie	Jun-06	75	8
Newcastleton	Jul-06	60	5
Rowanburn, Canonbie	Feb-07	100	6
Wauchope	Apr-07	0	26
Langfauld Wd, Langholm	Apr-08	100	5
Glencartholm, Canonbie	May-08	92.3	13
Kettleholm	Jun-08	71.4	7
Kinmount Policies	Jul-08	57.1	14
Luce, Brydekirk	Feb-09	100	7
Mallyford, Thornhill	Apr-10	100	4
Langholm	May-10	83.3	6
Dunglass Wd, Coldstream	Mar-11	82	11
Mauchline (Kilmarnock)	Sep-11	15	20
Wauchope	Mar-12	83.3	6
Wauchope	Jun-12	46.6	15
Wauchope	Aug-12	25	8

It is worth noting that values of seroprevalence can be significantly higher than the average 60% reported by Sainsbury et al. (2000) and reach up to 100%. Values can also fall, as in the case of Wauchope (Table 3), and this is most likely a reflection of local control efforts and their success (SSRS project and FCS control efforts). Figure 3 shows the situation from 2012 onwards until September 2014 and indicates the pattern of continued SQPV spread across the region, with the first cases of seropositive animals near Edinburgh and Glasgow and, worryingly, south of Stirling by 2014.

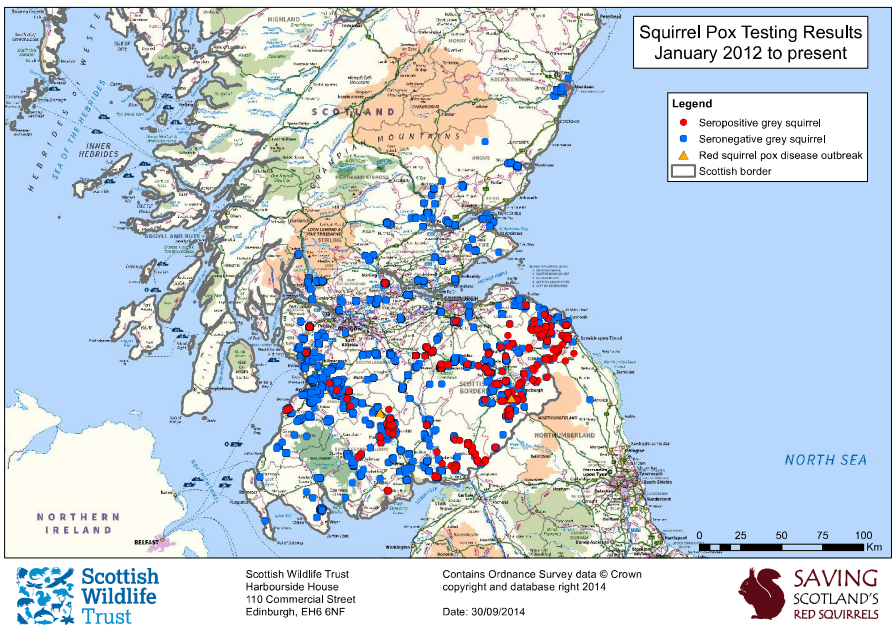

Figure 3. Blood sampling results 2012 to May 2014 indicating SQPV spread in grey squirrels up to Glasgow and potentially beyond Edinburgh (map courtesy of SWT).

White and Lurz (2014) developed a spatial, stochastic model to represent the red and grey squirrel population and SQPV dynamics for Southern Scotland. They initialised the model by assuming the absence of the disease across Southern Scotland except at Canonbie where 5 infected greys were assumed present in 2005 (thus reflecting the observed initial SQPV outbreak in Scotland). The model predicted that the disease would spread rapidly across Southern Scotland, primarily through established grey squirrel populations, but could also infect red populations that were sympatric to infected greys. They also included the impact of local grey squirrel control (as implemented under the SSRS strategy to prevent SQPV spread) and showed that while control could reduce the rate of disease spread it was unlikely to prevent SQPV spreading throughout Southern to Central Scotland. Here, while control may reduce grey density and therefore SQPV prevalence locally, the overall distribution of grey populations forms a well-connected network that allows SQPV to spread around the locally controlled region. The model predicted that SQPV could reach Central Scotland by 2014 and once there, spread rapidly through the high-density grey populations. This is in good agreement with SQPV monitoring results.

2.5. Role of grey squirrels and current understanding of transmission and spread of SQPV

Following the research by Tomkins et al. (2003), Rushton et al. (2006), Sainsbury (2008), Sainsbury et al. (2008) and Fiegna (2011) there is now compelling evidence that grey squirrels act as a reservoir host for SQPV and are a key agent in SQPV transmission and spread. In areas where the virus is present, the speed of red squirrel decline and replacement is significantly increased and the combined impacts of disease and competition have led to red squirrel extinction in most of England, Wales and large parts of southern and central Scotland. Whilst some (albeit limited) red squirrels are known to survive and recover from the disease (Sainsbury et al. 2008, Carroll et al. 2009, Chantrey et al. 2014), the virus represents a crucial threat to the remaining red squirrel populations in northern England, Scotland and Ireland. It is currently unclear if surviving red squirrels mount a successful immune response or if they survive for other reasons as 60% of red squirrels that die have antibodies to SQPV in their blood (McInnes et al. in press).

Seroprevalence in grey squirrels can be male-biased, increase with weight or testes size, vary locally, seasonally and can be affected by parasite loads, with individuals that had concurrent nematode and coccidia infections also having higher levels of antibodies to SQPV (Bruemmer et al. 2010, McGowan et al. 2014). Transmission from grey to red squirrels will therefore not only be a factor of local grey and red squirrel abundance (influencing encounter rates and probability of transmission), but also of the sex and condition of the individuals present, as well as time of year. There are a number of studies that report lag times between the observed arrival of grey squirrels and disease outbreak in red squirrels (e.g. Rushton et al. 2006, Sainsbury 2008, McInnes et al. 2009) and outbreaks of SQPV in red squirrels are therefore not just chance events linked to grey squirrel presence or abundance, but subject to a range of local factors (such as habitat type, season, condition of individuals, supplementary feeding activity which increased red-grey encounters etc.).

There are still uncertainties with respect to the precise mechanisms of transmission. Rushton et al. (2000) (see also Edwards 1962, Sainsbury 2008, Carroll et al. 2009) reviewed SQPV transmission and described several potential routes. They noted that both species use olfactory cues and communicate via scent marking behaviour. Red and grey squirrels have scent glands by the side of the mouth (Bosch & Lurz 2012) and scent marks are left through face-wiping behaviour on exposed bark of branches or roots. Small abrasions resulting from this behaviour may facilitate infection by the virus. The same is true for wounds, scratches or bites obtained in aggressive encounters (route: **skin abrasions**). In addition, transmission might occur via fleas (route: **arthropod vector)** and vector transmission is a known feature of poxviruses (e.g. myxomatosis). Grey squirrels will make use of red squirrel dreys once they colonise an area and the red squirrel fleas (*Ceratophyllus sciurorum, Tarsopsylla octodecimdentata*) and the grey squirrel flea (*Orchopeas howardii*) left within the nests could be involved in SQPV transmission. The virus may also be encountered in the form of viral particles deposited in the squirrel's habitat or the nest (route: **environmental contamination**).

<u>Current knowledge indicates the following routes:</u>

- Experimental infections of red and grey squirrels using scarification (Tompkins et al. 2002, Fiegna 2012) **have confirmed** that transmission and subsequent successful infection can occur via abrasions in the skin.

- Transmission via an arthropod vector such as fleas in the drey **remains a possibility**, although this would occur through external transport of virus particles on fleas rather than fleas being an actual host for replicating virus. Atkins et al. 2010 found comparatively high levels of viral DNA on fleas taken from both red and grey squirrels (note that the method used indicates presence of viral DNA and not live virus).

- A role for transmission via environmental contamination has **been suggested** by Chantrey et al. (2014) and virus will be shed, for example, from lesions and in scabs. Recent research by Collins et al. (2014) also indicated that viral DNA is present in faecal material, and that virus left in the environment will remain live for at least a month, with poorest virus survival in wet conditions.

- More recently, combined genetic, histopathology and immunohistochemistry testing has shown the presence of replicating virus in the tonsil-like structures of red squirrels and linked to this high concentrations of viral DNA in squirrel saliva (Fiegna pers. com.; Fiegna et al. unpublished). This suggests that there is also an **oral route to infection** and transmission with important implications for stopping supplementary feeding of red squirrels once grey squirrels arrive.

2.6. SQPV dynamics in red squirrel populations – "living with pox"

SQPV appears to readily spread and remain endemic (although with fluctuating levels of prevalence) in grey squirrel populations (Sainsbury et al. 2000, Bruemmer et al. 2010, White et. al. 2014). Where red squirrel populations are sympatric to greys SQPV can spread from greys to reds and then potentially continue to spread due to red-red transmission through red squirrel populations (Tompkins et al. 2002). SQPV prevalence in greys is likely linked to grey density but even at a low density of grey squirrels there is still a chance of disease transmission, as seen in Tollymore, Northern Ireland where grey squirrels numbers had been reduced by 90% (McInnes et al. 2013). Current understanding indicates the epidemiological dynamics are likely to exhibit the following pattern once a red population becomes infected.

- **SQPV infection in a red population is likely to lead to a local epidemic that results in high mortality in reds.** Quantitative evidence from the red squirrel stronghold in Formby, Lancashire where greys are controlled within the stronghold indicates that reds can become infected and that this leads to an epidemic that results in approximately 80% mortality (Chantry et al. 2014). Model studies support this observation and indicate that the scale of the epidemic in the red population depends on the population density. When red density is 'high' an epidemic can occur and disease induced mortality leads to a rapid crash in population abundance (White et al. 2014). The high density (2.5-4 squirrels/ha) populations in Formby, Lancashire suffered 80% mortality (Chantrey et al. 2014) and model studies focussed on the Fleet Stronghold, Dumfries and Galloway where red densities were 0.5-0.8/ha suffered a less severe epidemic with 60% mortality (White and Lurz 2014, White et al. In Press). When red density is low the disease fails to take off and few infected cases are observed (White et al. 2014). This occurred in model simulation for the Isle of Arran where red density was 0.05-0.25/ha (Macpherson et al. In press). A word of caution here is that due to a lack of field data, the model studies assume red-red transmission is the same as grey-grey transmission (and red-grey transmission). If red-red transmission were higher than grey-grey transmission then an epidemic could occur at lower red densities (Macpherson et al. In press). There is therefore currently a knowledge gap with respect to relative transmission rates and this requires further study.

- **SQPV is not supported in red only populations**. The crash in population abundance resulting from an SQPV epidemic in reds reduces the population to a level which no longer supports the disease. Specifically, the abundance of red squirrels locally becomes so low that there are not enough uninfected individuals to support sufficient disease transmission within the red only population and therefore the disease cannot be maintained. SQPV is therefore predicted to burn-out in red squirrel only populations. This is supported by field evidence (Duff et al. 2010b, Chantrey et al. 2014) and from modelling studies that focus on red strongholds (White and Lurz 2014, White et al. 2014, White et al. in press, Macpherson et al. in press). Here, it is predicted that the disease will fade-out within 2-12 months of the epidemic outbreak, with the duration depending on the severity of the epidemic (Chantrey et al. 2014, White et al. 2014, Macpherson et al. In press).

- **SQPV does not spread extensively through red–only populations.** Model studies indicate that the high rate of mortality and subsequent fade-out of SQPV in red-only populations prevents SQPV from spreading extensively through established red populations (White & Lurz 2014, Macpherson et al. in press). Outbreaks in reds are confined to local regions that are adjacent to the source of infection, such as dispersing grey squirrels or local grey squirrel populations. Disease modelling in complex landscapes by Macpherson et al. (in press) suggests that locally SQPV has the potential to spread through contiguous mature forest habitat blocks. However, SQPV spread between poorly connected forest blocks is rare, especially if there are no resident red squirrels in the corridor habitats. It is important to note that whilst SQPV will not spread extensively through red-only populations, continued grey squirrel immigration and competition will lead to new outbreaks and competition for resources and thus replacement of reds in habitats where grey squirrels can successfully establish.

- **Red populations can return to pre-infection levels following an epidemic**. Provided greys are prevented from establishing, red populations are likely to return to pre-infection densities following a SQPV epidemic. In the Formby stronghold reds returned to pre-infection levels of density approximately 3 years after the epidemic (Chantry et al. 2014) and this is also predicted in model studies (White et al 2014, White & Lurz 2014, White et al. in press, Macpherson et al. in press). SQPV can lead to red mortality of up to 80%, with the remaining red squirrels surviving simply because they avoided exposure and potentially a small number of reds survive exposure (Sainsbury et al. 2008, see also section 2.7 below). Population increase will primarily occur from these red populations, but may also occur through dispersal and thus immigration from neighbouring red populations. The rate at which red populations re-establish following an epidemic will also depend on resource quality, with populations increasing more rapidly with good seed crop availability and more slowly if there is a sequence of poor seed years.

2.7. Current misconceptions about SQPV and its epidemiology and spread

Reports on SQPV in the media often contain several misconceptions with respect to the disease and how it spreads and we felt it was important to address some of these:

- *Squirrelpox virus is sometimes described as spreading through the south of Scotland, killing nearly all the red squirrels that come into contact with it within 12 days and potentially wiping out local populations.* RESPONSE: A more typical and likely pattern of disease impact are levels of up to 80% mortality, with the remaining red squirrels surviving simply because they avoid exposure and potentially a small number of reds surviving exposure (estimated by Chantrey et al. 2014 at 8.4%).
- *A stated aim of the current tender has been to recommend land management measures (including timescales) to help new pox-free red squirrel populations in Scotland spread and colonise.* RESPONSE: When red squirrel populations neighbour disease carrying grey populations there will not be a permanent pox-

free area for reds. However local red squirrel populations can expand into nearby regions in the absence of grey squirrels.

- *Recovery of red squirrel populations following an epidemic is often understood as a re-colonisation event from unaffected nearby red squirrels.* RESPONSE: Immigration will take place (provided there are reds nearby), however the most likely initial re-population is from those individuals that avoided infection and to a smaller extent those that survived it.
- *Early work on how landscape management might help pox "burn out" and then support an incoming red squirrel population afterwards suggests a scenario that is somewhat counter-intuitive to current forest management for red squirrels – namely, that it is better if the red squirrel population is less dense and the woodland has a lower carrying capacity with less habitat connectivity to the wider landscape.* RESPONSE: epidemic outbreaks are more likely in high density red populations, but on average a higher density is better for red viability. Low density populations have high risk of extinction from stochastic events (e.g. poor seed crops, severe weather) and disease. High populations have that risk only when there is an epidemic. The current strategy of large conifer forests as red squirrel strongholds is therefore a sound approach as these areas can support red squirrels with minimal management input and the risk of widespread disease outbreaks are low due to low squirrel densities.
- *SQPV wreaks its devastation more quickly amongst a less dense population, with fewer reds available to escape and take the pox with them to populations in surrounding areas.* RESPONSE: Pox is unlikely to reach epidemic levels in low-density population as indicated by modelling work on the Isle of Arran (Macpherson et al. in press).
- *Dispersing infected individuals are the key cause of disease spread.* RESPONSE: Rapid local spread in 'stationary' populations is the main driver that causes disease spread, not individual movement. In other words, there are two processes in disease spread: 1) disease is passed on from individual to individual through an interlinked resident population with individuals undertaking core range movement but without long distance dispersal of individuals, 2) dispersing infected animals move from one population to another. In large continuous forest blocks SQPV spread is rapid and driven by process (1). When there are significant gaps in forest coverage SPQV spread relies on long-distance dispersal of an infected individual (rare for red squirrels), which is driven by process (2).

3. Conclusions - Conservation Advice

The results of the current review indicate four main areas that are likely to assist with conservation management and potentially reduce disease risk:

3.1. Grey control and its impacts/benefits

Grey squirrel control as part of a wider strategy to protect red squirrels and their strongholds has a number of described impacts on SQPV transmission and spread. Whilst grey control cannot completely prevent disease outbreaks (White & Lurz 2014, White et al. 2014), it has been instrumental in protecting vulnerable red squirrel populations in the South of Scotland and Northern England. In this context it is

important to stress that an outbreak of SQPV in a red squirrel population should not be seen as a failure of this strategy. Grey control reduces the risk of disease transmission and is crucial in helping to reduce negative competitive effects from grey squirrels thus allowing red squirrel population levels to return to pre-infection levels following a disease outbreak.

- A number of studies (e.g. McInnes et al. 2013, Chantrey et al. 2014, Schuchert et al. 2014) advocate and discuss control as a tactic to reduce contact rates between red and grey squirrels and thus transmission rates.

- Schuchert et al. (2014) point out that control to reduce grey squirrel density also had an additional concomitant impact on levels of seroprevalence in grey squirrels. They report a constant decline of antibody levels as a result of trapping to very low levels between 1999-2010 on Anglesey, Wales with only 4% of grey squirrels testing positive by the end (peak of 75% in 2002). They suggest that the reduction in population density by culling greatly reduces the chance of any individual coming into contact with the virus (e.g. with an infected individual or via contact of environmental contamination).

- The authors of the current report would also argue that natural changes in grey squirrel density will lead to variation in SQPV seroprevalence. For example, grey density reductions due to poor seed crops or harsh winters are likely to lead to reductions in levels of seroprevalence.

- Experience from Wales and Northern England with respect to "living with pox" in areas where grey squirrel control was taking place to reduce grey squirrel spread, competitive effects and disease risk in and around strongholds, all indicate that no additional measures to specifically target SQPV were carried out (see Appendix I). In other words, where grey squirrel control is part of the management approach, the only additional measure taken has been disease monitoring. The latter took the form of selected blood sampling and relying on the public to report sightings of diseased animals or send in dead animals for post-mortem analysis (for example, public involvement is encouraged through poster campaigns run by local squirrel groups in North-East England).

In summary, evidence from a range of studies indicates that grey squirrel control when carried out along dispersal corridors leading to vulnerable and or stronghold populations, can reduce the risk of disease transmission; and if carried out systematically and over prolonged periods like on Anglesey reduce levels of seroprevalence in grey squirrels. More crucially, as part of the wider conservation strategy, grey squirrel control in and around vulnerable red squirrel populations will help reduce competitive impacts of grey squirrels and enable red squirrel populations to return to pre-infection levels following an outbreak of SQPV.

3.2. Risks of supplementary feeding

- A number of studies explicitly warn of the dangers of supplementary feeding of red squirrels in the presence of grey squirrels (e.g. Bruemmer et al. 2010, McInnes et al. 2013). Feeding will create points of contact (both direct contact of individuals but also contact with virus on the feeders via contamination) between red and grey squirrels. This becomes particularly important when one considers the recent findings by Fiegna et al. (in prep) who found evidence for an oral route of transmission. Well-meant efforts to boost red squirrel populations via supplementary feeding will therefore be counter productive and actually significantly increase disease transmission (and mortality). Note: supplementary feeding when reds squirrels are sufficiently isolated from greys will not pose a risk of SQPV transmission. For example, supplementary feeding of reds in populations on Arran or in Highland Scotland will not increase SQPV transmission risk as the disease and greys squirrels are absent from these populations. It is also important to note that feeding stations not only promote the spread of SQPV but can facilitate the transmission of other diseases (e.g. squirrel leprosy, salmonella etc.) and the costs and benefits of supplementary feeding should be considered carefully.

In summary, supplementary feeding would in effect counteract other measures at reducing disease spread. Where grey control would reduce squirrel density and the risk of disease transmission, supplementary feeding would potentially increase squirrel numbers and more importantly create points of contact and significantly increase the risk of disease transmission. Taking note of discussions with FCS, SNH and SWT (Meeting 20.3. 2015) on this topic we agree that there should be one consistent message for the whole of Scotland. We therefore recommend that there should be information highlighting the potential for disease transmission and advice on cleaning/sterilising feeders (including bird feeders) to reduce the risk of SQPV and other disease spread.

3.3. Habitat management of dispersal links

Options for habitat management with respect to disease are limited and conservation advice is principally targeted at maintaining a self-sustaining population of red squirrels by providing a minimum diversity in conifer tree species and age classes to ensure a dependable seed food supply (Anonymous 2012). However, our review of published research indicated dispersal corridors as one potential area. Disease spread in general is likely to occur via two different processes: i) contact between resident individuals in adjoining home ranges; ii) long distance movement through a dispersing infected individual whereby the latter case will be a less frequent event (see section 2.7).

- Computer modelling work assessing SQPV spread across landscapes strongly suggests that poor quality corridors reduce the likelihood of disease spread between forest blocks. Such corridors may take the form of hedge, tree rows, or streamside plantings, which consist of neutral shrub and tree species (e.g. birch, alder, willow, rowan etc.) and therefore provide cover but not permanent habitat for squirrels. Given the rapid course of SQPV infection and the severely exhausted and incapacitated state of most affected red squirrels, these predictions assume

that individuals are unlikely to disperse long distance across the landscape. Poor dispersal corridors which do not support resident red squirrels will therefore act as breaks to disease spread and therefore SQPV.

- Managing dispersal via corridors and forest networks is probably best addressed at the forest design plan stage for future rotations. The corridors should ideally be composed of tree and shrub species that are "neutral" (see Anonymous 2012) and do not provide sufficient food resources to allow resident populations of squirrels (e.g. willows, rowan, birch, alder, hawthorn, etc.) and avoid large seeded tree species (oak, beech, hazel).

In summary, an overall goal of actively managing dispersal links would be to provide internal linkage between discrete local woodland blocks with red squirrels. This would increase local population viability by providing dispersal links but reduce the risk of SQPV spread. Given the restricted tree and shrub species diversity advocated for these dispersal links and potential other conservation objectives when planning corridors, this approach may be most suitable in upland areas where the recommended species are part of the natural vegetation community.

3.4. Monitoring - disease surveillance

Discoveries of adenovirus and rotavirus in red squirrels (e.g. Everest et al. 2010) as well as recent indications of a form of squirrel leprosy in red squirrels in Scotland (Meredith et al. 2014), all highlight the importance, and point to the necessity of disease surveillance to detect existing and emerging threats.

- Disease management and therefore management of SQPV will require monitoring. This may at its simplest take the form of passive surveillance that asks the public to report sightings of diseased animals and send individuals for post-mortem analysis, or involve more active measures such as for example systematic sampling for antibodies to SQPV, if resources allow.

4. Knowledge gaps

The current review illustrates a number of existing research or knowledge gaps. At the forefront is a lack of information on relative SQPV transmission rates for red and grey squirrels. Is transmission likely to be higher in red squirrels due to the presence of skin lesions and the virus load of such lesions? Or is it higher in grey squirrels? Despite any obvious signs of disease grey squirrel antibody rates of up to 100% indicate an effective mode of disease transmission within grey squirrels.

Whilst the mathematical modelling studies clearly show the risks to high density populations in the form of an increased risk of disease transmission, low density and thus small red squirrel populations will be at risk of extinction simply through chance events (e.g. sever winters, seed crop failures, etc). Is there an optimal red density that could be achieved through management? Or is the answer a low density but extensive area (and thus a large total red squirrel population). The proposed Galloway District

Priority Area for Red Squirrel Conservation (A. Jarrott pers. comm.) would represent an ideal case study to test the viability of an extensive low density population.

Evidence indicates that SQPV is continuing to spread from southern to central Scotland. Should this continue and SQPV spreads into northern Scotland, it will be important to assess to what extent the emergence of the disease may alter the current balance of red and grey squirrels in the Highlands. The disease may promote the spread of grey squirrels beyond their current northern limit. Also of importance in the future is an assessment of how the proposed impact of pine martens in some regions will influence SQPV dynamics in red and grey squirrels.

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Appendix I: Supplementary information

As part of the data collection for this project organisations and individuals in England and Wales were contacted for information on their experiences. Red squirrel conservation in both Wales and Northern England has involved "living with pox" as a reality of management action in these areas for a significant number of years.

Individuals and organisations contacted included Red Squirrels Northern England (N. Mason, Adam Seeward); Forestry Commission (N. Geddes, T. Dearnly) and Dr Craig Shuttleworth (Red Squirrels Trust Wales, Red Squirrel Survival Trust).