Published by Associazione Teriologica Italiana
Hystrix, the Italian Journal of Mammalogy
Volume 26 (2): 69–75, 2015
OPEN ACCESS
Available online at:
`http://www.italian-journal-of-mammalogy.it/article/view/11166/pdf`
doi:10.4404/hystrix-26.2-11166

Research Article

Assessing causes and significance of red squirrel (*Sciurus vulgaris*) mortality during regional population restoration: An applied conservation perspective

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Keywords:
Red squirrel
Sciurus vulgaris
mortality
adenovirus
Wales

Article history:
Received: 21 February 2015
Accepted: 28 August 2015

Acknowledgements
The authors are indebted to the estates and individuals who reported and collected red squirrel carcasses for pathological study. The Animal and Plant Health Agency "Diseases of Wildlife Scheme" analyses were undertaken as part of a national disease surveillance programme, funded by DEFRA. We also gratefully acknowledge the assistance of Mark Stidworthy and Andrew Greenwood of International Zoo Veterinary Group, Anna Meredith of Edinburgh University (Dick) Veterinary School and Dr. Colin McInnes of Moredun Institute. Zoological Society of Wales generously provided financial assistance during the initial captive and release phases of the red squirrel reintroduction including for post mortem investigations. Finally, we are indebted to Peter Litherland, Nick Jackson MBE and Dr. Matt Hayward of Bangor University for the provision of pathological sample storage facilities, and to both Luc Wauters and an anonymous reviewer for their useful comments on the draft manuscript.

Abstract

Red squirrel (*Sciurus vulgaris*) mortality was monitored opportunistically during the period 2004 to 2013 on the island of Anglesey in North Wales. Road traffic proved a most significant cause of death (48%) mirroring the findings of earlier United Kingdom (UK) studies. Red squirrels were also found to have died from a range of pathological infections and disease previously unrecorded in Wales. These data have increased our knowledge on the national distribution of such causal factors. The study found male red squirrels were less likely to have an adenovirus infection than females and that animals dying from disease had a lower body mass than those associated with a traumatic death. No red squirrels were found with squirrelpox infection or antibodies to this virus which reinforces earlier findings from Anglesey that intensive grey squirrel (*Sciurus carolinensis*) culling reduced the prevalence of this infection within sympatric populations. Finally we highlight the potential intra and inter-specific infection risk presented by supplemental feeding.

Introduction

Red squirrel (*Sciurus vulgaris*) populations naturally fluctuate in relation to weather conditions and food availability (Gurnell, 1987; Lurz et al., 1995; Wauters et al., 2008; Selonen et al., 2015). They are directly affected by woodland fragmentation (Andrén and Lemnell, 1992; Verbeylen et al., 2009), habitat loss (Rocha et al., 2014), changes to forest tree species composition (Lurz et al., 1998; Shuttleworth et al., 2012) and wider management practices that alter stand structure (Flaherty et al., 2012). Red squirrel population dynamics and life history processes such as dispersal (emigration, immigration), body condition of individuals, reproduction and rates of mortality are therefore influenced by stand or forest characteristics and composition. In addition, research (e.g. Rushton et al., 2006; LaRose et al., 2010) has shown that disease can play a critical part in regional red squirrel population decline.

Historical anecdotal reports of pathogenic coccidiosis, and what was described as 'mange', coincided with local population declines in Britain (Shorten, 1954). In the absence of post mortem data and stored tissue samples, the true extent of the involvement of such diseases at that time will remain unclear. However, with advancements in infection surveillance techniques involving Transmission Electron Microscopy (TEM), Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA), scientific research has more recently been able to focus upon quantifying the scale and impact of a range of pathological infections on native red squirrel population dynamics. British studies have identified squirrelpox virus (SQPV) (Sainsbury et al., 2008) and emerging diseases including adenovirus (Everest et al., 2014), fatal exudative dermatitis associated with *Staphylococcus aureus* (Simpson et al., 2013a) and *Mycobacterium lepromatosis* (Meredith et al., 2014) as important within surveillance regimes.

It is noteworthy that the number of UK studies on the causes of mortality still remains quite limited, and to date these have relied exclusively upon opportunistic and sporadic sampling of carcasses presented for post mortem investigation in England, Scotland and the Channel Islands (Keymer, 1983; LaRose et al., 2010; Duff et al., 2010; Simpson et al., 2013b). There are no studies from Wales. In their recent review, Meredith and Romeo (2015) highlighted various important bacterial and parasitic infections and found that with an opportunistic and sporadic surveillance approach, road traffic accidents, predation and SQPV accounted for the majority of all deaths where a cause of death could be ascertained.

SQPV is almost always a sub-clinical infection in the grey squirrel (*S. carolinensis*) but is, with a few exceptions, fatal to their native European congener (Carroll et al., 2009; Chantrey et al., 2014; Collins

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doi:10.4404/hystrix-26.2-11166

et al., 2014). In red squirrels, squirrelpox disease can cause significant mortality and predictive modelling has illuminated how this can accelerate the rate at which grey squirrel populations can otherwise replace red squirrels through resource competition alone (Rushton et al., 2006). The cumulative result of intensive squirrelpox research has led to the development of specific national guidance on measures to reduce potential inter or intra-specific infection rates such as regular disinfection of red squirrel supplemental feeding sites and preventing grey squirrel access to them (McInnes et al., 2013).

However, the ability to develop proactive and reactive disease contingency planning is often complicated for other infections because there is a paucity of scientific research and hence their regional significance remains very much unclear. In the absence of mortality surveillance, or where surveillance is limited only to areas or habitats where recovery of bodies is easy, applied conservation projects will likely assume that the main local disease threats and frequency of causes of red squirrel mortality will simply reflect those reported in the published literature, irrespective of geographical and habitat variations which may be evident.

Disease and infection are especially important in the context of IUCN guidelines (IUCN, 2013), which make health and mortality monitoring an integral part of trans-location studies and crucially require disease risk to be quantified in the development phase of project proposals. Assessments of the extent to which (re)-introduced and establishing red squirrel populations are experiencing disease must subsequently be undertaken and identification of underlying causes made. It is also increasingly recognised that populations with a small effective size in particular can be susceptible to extirpation by infectious disease (see Smith et al., 2008).

In this paper we examine patterns and causes of red squirrel mortality on the 710 km^2 North Wales island of Anglesey in the period 2004 to 2013 following a series of trans-locations (see Shuttleworth et al., 2008, 2009). These led to a progressive geographical increase in the spatial distribution and abundance of the regional population (see Schuchert et al., 2014). In 2002 the population was estimated to be around 95 individuals (Shuttleworth, 2003) and this increased to 700 by 2014 (Halliwell et al., 2015). We broadly interpret the presented mortality data in the context of these temporal changes.

The aims of this paper are to: 1) Quantify the relative frequency of causes of mortality in Welsh red squirrel populations on Anglesey following conservation trans-locations (IUCN, 2013); 2) Investigate whether variation in the frequency of red squirrels exploiting supplemental foods in gardens, and changes in the abundance of red squirrel nest boxes may have affected the retrieval of red squirrels that had died from different causes; 3) Interpret causes of mortality in red squirrels found at supplemental feeding sites and within nest boxes in an attempt to better understand the potential of such management intervention to affect inter-specific infection.

Materials and methods

Red squirrel nest boxes were present and monitored in two coniferous forest areas during the first four years of the study 2006 to 2009. In Mynydd Llwydiarth forest 60 nest boxes were widely distributed through the forest (Shuttleworth and Schuchert, 2014), whilst in Newborough forest 45 were present and associated with a re-introduction site (Shuttleworth et al., 2009). Boxes were checked quarterly except for 15 in Newborough which were inspected only once or twice per year. After 2009, these nest boxes were checked sporadically. A small number of nest boxes were also found in gardens and broadleaved woodlands from 2006 onwards but inspection was *ad hoc*. Supplemental feeding stations, either wooden feeders or garden bird tables, were known to be present in woodlands and gardens throughout the study but quantifying their geographical abundance and intensity of different feeding regimes was not possible. In 2014 there were around 150 feeding stations recorded (C.M. Shuttleworth unpublished data). Squirrels found dead close to, beneath, or within/upon a garden feeding station were reported to Red Squirrels Trust Wales, a local conservation group, and bodies were collected for scientific research.

Cases of recorded mortality in wild red squirrels on Anglesey in the period January 2006 to December 2013 were collated. These encompassed carcasses recovered, but not presented for post mortem examination, as well as those presented for examination, which therefore had associated gross post mortem and histological reports. There were four instances where a body from a reported road death was not recovered, but material was subsequently collected by a third party and was catalogued in our database. Veterinary investigations were initially carried out by International Zoo Veterinary Group (IZVG) pathologists and after 2009 carcasses were examined within the Animal and Plant Health Agency (APHA) Diseases of Wildlife Scheme (DoWS).

For analytical purposes, we partitioned deaths and causes of mortality into one of six broad categories:

1. Road traffic deaths;
2. Deaths associated with domestic pets, drowning in water butts or trap deaths;
3. Respiratory disease, congestion of lungs and poor body condition;
4. Enteric disease, infections and coccidiosis;
5. Cases where autolysis precluded determination of cause of death;
6. Bodies not presented for post mortem and which were not associated with road traffic (including remains of an animal consumed by a stoat).

Where an animal could equally have been classified within one of two of the six different categories, we allocated it to a category based on the most significant clinical observation in the post mortem report. In a case where an animal was confirmed positive for adenovirus by TEM but had no clinically-significant enteric pathology noted, this was classified within the enteric disease category.

Where data were available, we transcribed the following: Post Mortem Identification Number, Season, Year, Sex, Body mass, Age (adult or juvenile based upon skeletal size), pathologist's determination on Cause of Death, location of body (nest box, beneath or within a red squirrel wooden supplemental feeder, on the ground) and the adenovirus infection status. The presence of adenovirus infection was confirmed via PCR and or TEM tests (see Everest et al., 2010, 2012 for methodologies). Sample sizes were relatively small and hence for analytical purposes we looked at the frequency of infection *per se*, and hence we did not differentiate between sub-clinical and clinically-significant cases. Data from ELISAs on SQPV antibody concentrations in blood sera were also gathered from histological records.

Unfortunately no data on local red squirrel population densities, weather patterns, or annual abundance of seed crops were available. There was a progressive increase in red squirrel distribution as grey squirrels were eradicated from the island (Schuchert et al., 2014). The expansion of red squirrel distribution was assessed by calculating the cumulative area (in hectares) of woodland fragments known to be occupied in a particular year based upon live trapping or parallel sighting records (see Shuttleworth et al., 2015). The progressive increase in forest occupancy will have reflected an increase in red squirrel abundance.

A generalized linear model (GLM) was used to evaluate if the cause of death, our response variable, was related to any of the examined predictors: Season, Year, Sex, Body mass, Age, location of body and an identified disease. Years were grouped in three factors (1, 2 and 3) of three years each. The response variable was set as binomial: it scored 0 if the cause of death was accidental (traffic or human-related), otherwise it scored 1 if the cause of death was any form of disease, using a logit link distribution. Samples were excluded from the model if the cause of death was not determined, so a data sub-set of 106 squirrels was used. A second analysis assessed if any of the predictors was related to the absence (0) or presence (1) of the adenovirus in a squirrel. The model was fitted with the sub-set of data containing information on the adenovirus presence (n=65). Multiple logistic regression was used to model the effects of both continuous and categorical predictor variables on our binary response variables with a logit link function. Deviance (G^2) was used to check if the model assumptions (lack of fit, over dispersion) were met. Models were simplified and only predictors explaining significant amount of variance were included in the final

Table 1 – Causes of mortality[1] 2006 to 2013.

Year	All Mortality	Road Deaths	Poor condition/ Resp/ Disease/ undetermined cause from PM	Enteric/Coccidia	Decay Precludes Examination[1]	Drowning, domestic pets, traps
2006	6	0	3	0	3	0
2007	10	0	1	4	5	0
2008	7	1	1	1	4	0
2009	14	5	2	3	3	1
2010	14	10	1	1	2	0
2011	10	8	0	0	0	2
2012	28	13	2	5	6	2
2013	30	20	5	3	1	1

[1] Excludes one case of stoat predation/scavenging, where fresh partial remains were found in a nest box.

model. Chi-square statistics were used for the calculation of the significance of the factors in the models. Models were tested against each other with a χ^2 test. Linearity between the log odds ratio of the cause of death and the body mass was confirmed with a component residual plot using the car library. The statistical software package R 3.1.1 (R Core Team, 2013) was used for the analysis.

Results

Causes of mortality on Anglesey

A total of 119 mortality cases were recorded in the period 2006 to 2013 of which 74 (64%) were presented for post mortem examination (Tab. 1, Fig. 1). Of the 45 bodies not presented for pathology, advanced autolysis precluded submission of 18. A single predation or scavenging of a carcass was also recorded. This meant a total of 19 animals for which no cause of death was known. The remaining 26 cases comprised road traffic deaths where recovered carcasses were either completely crushed or fragmented by road traffic, or it was too dangerous to recover whole or partial bodies.

Of the 74 animals examined by a pathologist, 31 were defined as road deaths and six were mortality associated with human settlement (predation by domestic pets, death in a live capture squirrel trap, or drowning in water butt). There were 15 animals with respiratory conditions/disease or where death was associated with poor body condition (Fig. 2). In 17 cases, enteric disease often associated with adenovirus and or coccidiosis was recorded and in the remaining five cases, advanced decomposition and tissue autolysis precluded meaningful investigations. The number of annual recorded mortality instances increased from six in 2006 to 30 in 2013. In this period, carcasses were obtained from within nest boxes (n=19), beneath or within red squirrel feeders (n=14) and from the ground in woodland, gardens or from roads (n=86).

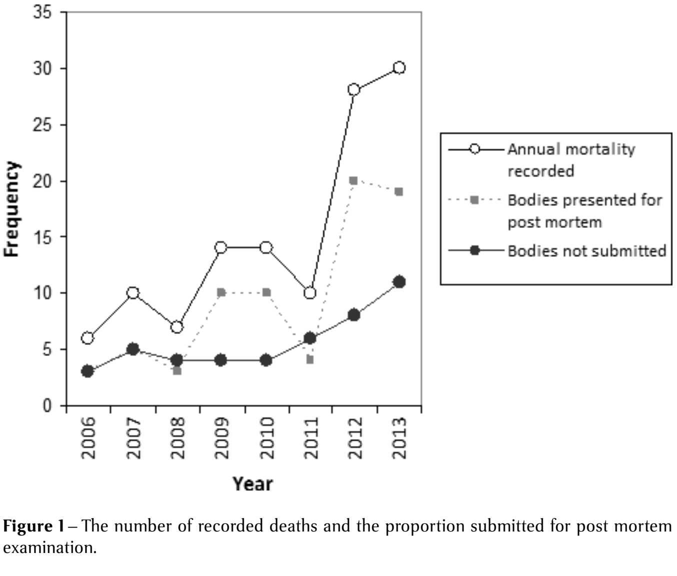

Figure 1 – The number of recorded deaths and the proportion submitted for post mortem examination.

Road traffic deaths

Road deaths represented 48% (57/119) of all recorded mortality and the frequency of traffic related mortality was observed to increase as red squirrels occupied an increasing cumulative area of mature forest habitat (a measure of squirrel abundance). (Spearman's r_s=0.98, df=6, p=0.009) (Fig. 3). No such relationship was observed between woodland occupation and the frequency with which other mortality was recorded (Spearman's r_s=0.23, df=6, p=0.58). Where an age was ascertained, 30 adults and only a single juvenile (body mass 150 g) were recovered as road traffic victims.

Other trauma

Six deaths were associated with non-road traffic trauma. Two adults (1M, 1F) were known to have been killed by domestic dogs, a juvenile male and adult female were predated by domestic or feral cats and an adult female drowned in a field water trough. Finally, an adult male was found in a live-capture trap with partial paralysis of front limbs and died later that day. Post mortem examination found faecal staining around the anus and extensive *Escherichia coli* intestinal infection.

Enteric disease

Seventeen cases where enteric disease was prominent were recorded: four adult females, one adult male and 12 juveniles (6M, 6F). Seven of these cases were found at supplemental feeders. Enteric pathology was found in a single juvenile male recovered dead from a nest box, with intussusception considered to be associated with high numbers of coccidial oocysts. The remaining nine cases were bodies recovered from within gardens or from the forest floor. PCR and TEM tests indicated that 47% (8/17) of the animals had an adenoviral infection. Of those positive cases, 50% (4/8) were confirmed from TEM screening of intestinal contents, indicating that the animals were actively secreting virus in their intestinal content and faeces.

Notable cases recorded include a prolapse through the lower abdominal wall (which could have resulted from an abdominal injury), two cases described as mucoid enteritis, with *Staphylococcus sciuri* an opportunistic pathogen cultured from the content of the small intestine in one animal (an adult female). Severe haemorrhagic enteritis was reported in an adult female, and a heavy coccidial oocyst presence and infection leading to intussusception and death were recorded in another animal. Coccidiosis was associated, or suspected as the primary cause of death in a further eight squirrels with often clinically-significant high coccidial oocyst loads (70000 to 180000) per gram of faecal material reported. From one of these cases, a juvenile male, potentially pathogenic *S. aureus* were cultured from parallel exudative dermatitis, and one juvenile female had the notable number of 800 oxyurid nematodes per gram of faecal material.

Viral infections

Squirrelpox virus

Eighteen Anglesey red squirrel cadavers were ELISA tested for SQPV antibodies as part of UK screening and all proved negative.

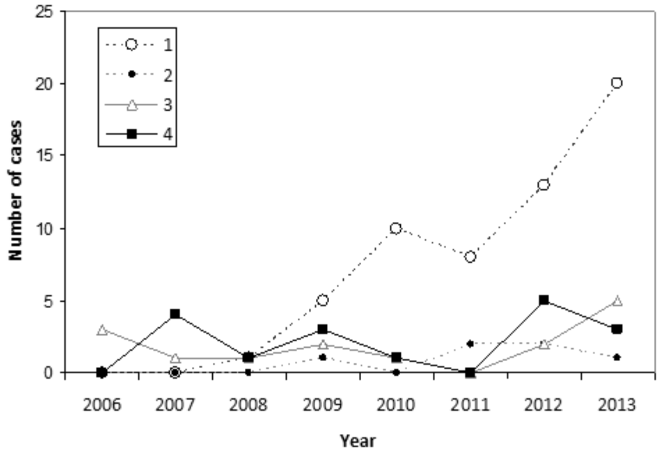

Figure 2 – Annual patterns of known mortality (Mortality categories: 1 - All road deaths, 2 - Deaths associated with domestic pets, drowning and trap mortality, 3 - Respiratory disease and poor body condition, 4 - Enteric disease, intestinal infections and coccidiosis). Data are from pathological examinations except for road deaths (n = 57) which represent 26 animals not sent for examination and 31 for which post mortem reports are available.

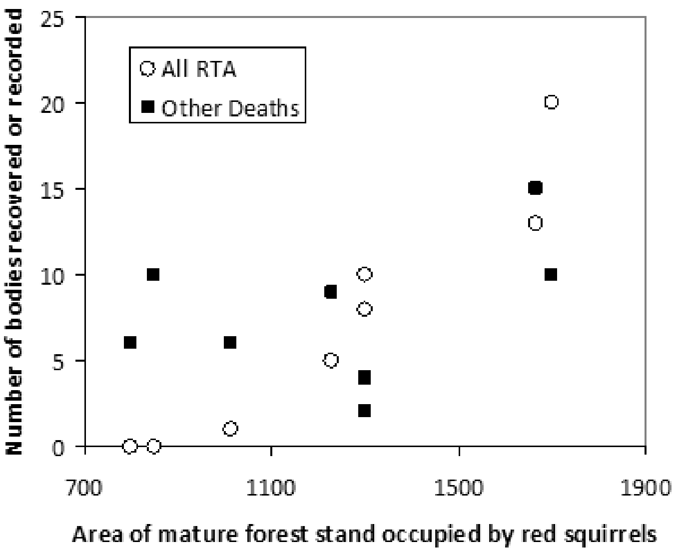

Figure 3 – Pattern of carcass recovery in relation to the extent (hectares) of mature woodland habitat known to be occupied by red squirrels.

Adenovirus

Adenovirus infection was confirmed in 38% (25/65) of carcasses tested. Those tested included an animal where tissue autolysis precluded any cause of death being established and for whom a negative test result was obtained. The prevalence of adenovirus infection in animals dying from trauma was 38% (13/34), not significantly different from the 40% positive rate (12/30) observed in other causes of mortality (Fisher's Exact Test, p=1.0). When prevalence of adenovirus infection in animals with enteric disease, diarrhoea or heavy coccidial loads (47%, 8/17) was compared with the adenovirus infection rate of 36% (17/47) in squirrels that had died from other causes, no significant difference was observed (Fisher's Exact Test p=0.56).

Causes of death associated with nest boxes and feeding stations

Feeding stations

Red squirrels were found dead within, or beneath, supplemental feeders in six of the eight years of study (Fig. 4) These consisted of four adults (3M, 1F), eight juveniles (5M, 2F: 1 unknown sex) and two squirrels for which no age or sex was documented. The sex ratio was male biased (2:1 ratio). Twelve of the 14 bodies were sent for pathological examination as in two cases autolysis precluded meaningful gross post mortem. Of these 12 animals, 27% of those tested (3/11) were found to be adenovirus positive. Notable cases include four where enteric coccidiosis loads were evident, with oocyst loads of 72800 per gram being recorded in two juvenile males. Interestingly, one of these young males also had a haemolytic *Staphylococcus* infection (cultured from skin lesion), potentially the pathogenic *S. xylosus* and *S. aureus*. A separate juvenile male case had squirrelpox-like lesions on the lower lip, on the side of the lips and on the digits. From these lesions *S. aureus* (isolate ref IS12-02915) was cultured, suggesting exudative dermatitis. An adult male was found having anaemic poor condition with associated heavy louse infestation (species not recorded on post mortem), and another adult male was found with heavy louse infestation (*Neohaematopinus sciuri*) but no other pathology.

Nest boxes

Nineteen bodies were recovered from nest boxes. Where age data were available it was observed that five were juveniles (3M, 2 undetermined sex) and three adults (1M, 2F). The majority of carcasses were collected from either Newborough or Mynydd Llwydiarth forests during seasonal box inspections in 2006 to 2009. The remaining 26%, (5/19) were recovered in two subsequent years from a small number of boxes elsewhere. Of these 19 animals, in 11 (58%) cases, advanced tissue autolysis, or scavenging/predation by stoat made carcasses unsuitable to send for post mortem examinations. In three other cases there was advanced autolysis, therefore no primary cause of death could be determined from a post mortem.

Heavy coccidial oocyst loading and infection led to intussusception and death in a juvenile male. One animal was found alive in a nest box but subsequently died with severe multifocal epithelial hyperplasia (symptoms that are consistent with squirrelpox disease). It showed secondary ulceration and bacterial colonisation of skin, conjunctiva, and tongue. The animal was negative for SQPV DNA using the PCR method. A third animal died from enteritis and both of the remaining two animals examined by a pathologist were PCR positive for adenovirus DNA but unfortunately no intestinal contents were available for testing by TEM for the virus.

In the four year period from 2006 to 2009, a total of 14 bodies were recovered from nest boxes, representing 74% (14/19) of the overall cumulative total obtained from boxes during the wider study. In the same period, 29% (4/14) of the cumulative number of bodies obtained from within or near to supplemental feeders were collected. This is a significant difference (Fisher Exact Test, p=0.015) (Fig. 4) and reflected a peak in nest box search effort.

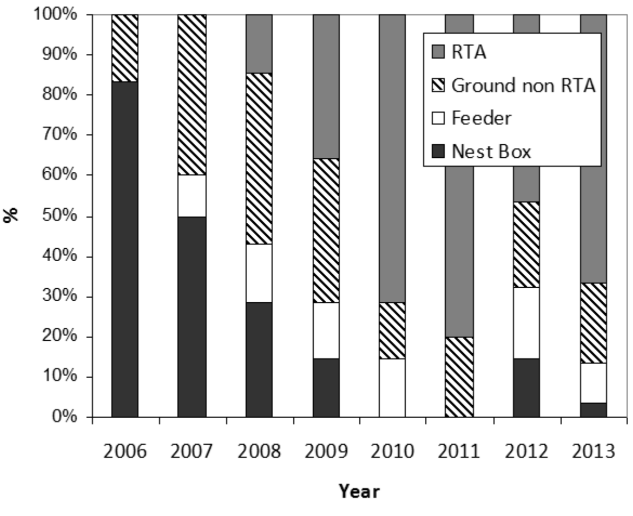

Figure 4 – Location of bodies recovered during the study including Road Traffic Deaths (RTA). The annual relative proportions are presented as percentages.

Table 2 – Simplified GLM examining the variables associated with the cause of death. The response variable (death) was binomial, accounting for accidental or pathologic causes. Likelihood ratios and *p* values using five explanatory variables are shown.

Variable	Estimate	Std.Error	z value	p-value
(Intercept)	45.55242	20.11108	2.265	0.02351*
ADENOALLP	0.82517	1.03794	0.795	0.42661
Sex M	0.6061	0.99955	0.606	0.54427
Body mass	-0.03451	0.01089	-3.17	0.0015**
Year Year2	7.80168	4.74069	1.646	0.09983•
Year Year3	23.91212	12.65686	1.889	0.05886•
WOOD	-0.03652	0.0187	-1.953	0.05077•

Assessing seasonal patterns and associations with mortality causes

The GLM model examining the variables associated with the cause of death found no annual, seasonal or area of woodland occupation significance, nor were any inter-variable interactions significant. Of the eight assessed predictors, only one, body mass, had a significant effect (*p*=0.0015) on death by accident or death by disease (Tab. 2). Death from disease was associated with lower body mass (196±64 g), in contrast to traumatic death where body mass was higher (317±55 g). This in part reflected the relative low frequency of juvenile squirrels in the trauma category. Mass and age can be correlated and indeed removing age from the model was the first step of the simplification process. This reduced the AIC from 26 to 24. Season and Location were also subsequently removed from the model, decreasing AIC. Since body mass and age are not independent, to check whether body mass effects were similar in both age-groups we created a model including as predictors age, body mass, and age×body mass. The interaction was not significant and once again the only significant factor was body mass.

Adenovirus presence or absence, binary, was compared with seven categorical or continuous predictors (Sex, Age, Wood, Year, Season, Body mass and Location) in a second GLM. The simplification process reduced the variables to four (Tab. 3). However, only sex had a significant effect (*p*=0.03): less males were positive to the adenovirus (7 out of 34) than females (15 out of 28).

Discussion

Monitoring disease and causes of mortality within red squirrel populations on Anglesey has provided the first significant data for the species in Wales. In so doing, the Anglesey study has increased our knowledge on the geographical distribution of many infections in the UK, including at least one potential case of exudative dermatitis associated with *S. aureus* (see Simpson et al., 2013a). There were nevertheless significant challenges, primarily as wild animals found dead are frequently very autolysed, which often precluded confirmatory tests for coccidiosis and adenovirus disease. In some cases available funding did not allow a full suite of diagnostic tests. However given the fact that the wild red squirrel population was relatively small, these data were critical to red squirrel conservation managers as they could be used to evolve applied conservation actions. This is especially important, since disease threats to red squirrels can be location specific and findings from other European regions and habitats are of only general and limited utility. With a more limited budget available within the UK Diseases of Wildlife Scheme (DoWS), it is unlikely that such a detailed array of gross post mortem and histological tests will be undertaken in the immediate future in Wales. This economic reality means our analysis is particularly valuable. Data on the relative proportion of carcasses presented for post mortem may also prove useful in calibrating the likely scale of different mortality causes occurring.

Table 3 – Simplified GLM comparing adenovirus presence or absence, binary, with four categorical or continuous predictors (Sex, Season, Age, Year).

Variable	Estimate	Std.Error	z value	p-value
(Intercept)	2.0749	1.1704	1.773	0.0762•
Sex M	-1.3498	0.6307	-2.14	0.0323*
Season Springtime	-0.2531	0.8147	-0.311	0.756
Season Summer	-1.8869	1.0259	-1.839	0.0659•
Season Winter	-1.178	0.9751	-1.208	0.227
Age J	-0.4586	0.7045	-0.651	0.5151
Year Year2	-1.7477	0.9657	-1.81	0.0703•
Year Year3	-0.9253	0.9226	-1.003	0.3159

Challenges and value of categorising mortality causes

The lack of data on red squirrel densities within the habitats from which carcasses were recovered makes the impact of a disease or deaths upon local population difficult to assess. We also recognise that determining the primary cause of death can be challenging in cases where animals show a range of conditions. However, although these are challenges within our retrospective and opportunistic study, they are a frequent common denominator in studies of this applied conservation nature. By attempting some broad categorising of cases we have, however, had an opportunity to observe patterns and associations that smaller sample sizes would otherwise preclude. Anthropogenic causes of mortality such as road traffic collisions, predation from domestic cats and dogs and the single observed drowning in a field water trough were perhaps unsurprising given the findings of other studies (Shuttleworth, 2001; Magris and Gurnell, 2002; LaRose et al., 2010; Simpson et al., 2013b). They have an added value given the paucity of data on Welsh red squirrel populations. They have also provided a useful source of tissue for investigations into potentially sub-clinical infections (Everest et al., 2014; Romeo et al., 2014) and phylogenetic studies (Ogden and McEwing, 2011). The observed frequency of traffic mortality presented for post mortem is similar to the 43% reported in Scotland (LaRose et al., 2010), 42% in the UK populations (Simpson et al., 2013b) and 36% in Jersey (Magris and Gurnell, 2002). Adults made up 97% of Anglesey road deaths and this reflects the findings of earlier studies which have suggested that these adults range more widely than juvenile or sub-adults, resulting in them encountering and crossing roads at a higher frequency (Shuttleworth, 2001; LaRose et al., 2010). The finding also underlines the impact of traffic on native mammal populations and the need to better understand the effectiveness of mitigation measures such as rope or green bridges over roads.

The progressive increase in the number of recorded red squirrel traffic deaths from 2006 to 2013 almost certainly reflected geographical range expansion (see Schuchert et al., 2014) and the parallel increase in red squirrel abundance. It is well documented that because of their visibility, road deaths are more likely to be recorded relative to some other causes of mortality (Shuttleworth, 2001) and carcasses of road kills have been used in a wide-scale study of red squirrel macroparasites in France and Italy (Romeo et al., 2013). Consequently, in woodland habitats with a dense understory and in the absence of nest box studies, this cause of mortality will, for example, be over-represented with respect to cases of disease. Uniquely in relation to disease victims, sudden trauma mortality does allow the frequency of some sub-clinical infections to be investigated and on Anglesey such an approach illuminated adenovirus research (Everest et al., 2014).

Infection risk at supplemental feeding hoppers

Mortality at squirrel feeders included several cases of pathogenic infections and enteric disease, in particular within juveniles, and our results show that mortality due to disease is more common (or more easily detected) in such cases. Both low body mass adults and the skeletally smaller juvenile animals are more at risk of infection resulting in disease than the rest of the population, as indicated by a non significant age×body mass interaction added to a simplified model.

Regular feeder inspection (to fill and clean hoppers) and close carcass proximity to feeders, elevated the probability of deaths being discovered, and these cases made a useful contribution to the wider data set. The presence of animals with adenovirus infection, coccidiosis (*Eimeria sciurorum*) (see Keymer, 1983) and dermatological infections at feeders highlights the intra-specific cross-infection risk that feeders pose. In the absence of radio-tracking or time-budget activity studies, it is unclear whether diseased animals were visiting supplemental feed hoppers more frequently than healthy conspecifics because their ability to forage for natural foods was impaired, or if they were visiting as frequently. It is also unclear to what extent malnourishment was directly linked to earlier infection. These are areas for future research and for the moment the discovery of diseased animals reinforces recommendations for thorough and regular disinfection of feeding stations and bird tables (Everest et al., 2014).

Squirrelpox virus

SQPV infection is clearly a major threat to red squirrel populations across Great Britain (Bruemmer et al., 2010) but on Anglesey the grey squirrel eradication programme not only reduced grey squirrel abundance, but lowered infection rates amongst surviving animals (Schuchert et al., 2014). Although historically squirrelpox had caused mortality in re-introduction studies in Conwy, North Wales (Shuttleworth et al., 2014) it remains a threat elsewhere in the UK (Chantrey et al., 2014; LaRose et al., 2010). On Anglesey, re-introductions took place after culling had decreased SQPV infection in grey squirrels and this would have lowered the risk associated with red squirrels subsequently encountering sero-positive, and potentially infectious, grey squirrels. Red squirrels were occasionally found with clinical symptoms very typical of this infection, but histological investigations revealed these to be exudative dermatitis associated with *S. aureus*, or other dermatological conditions, and no SQPV particles were ever detected in lesions by TEM (D.J. Everest unpublished data). The absence of inter-specific infection is re-inforced by the negative results of ELISA blood tests undertaken for SQPV antibodies in red squirrels on the island. In a few cases, free ranging red squirrels have been known to show an immune response without developing a pathological infection (see Shuttleworth et al., 2014).

Bruemmer et al. (2010), analysed UK data on SQPV sero-prevalence within culled grey squirrels to better understand epidemiology in relation to geographical, seasonal and associations with sex, age and body condition. As adenovirus has emerged from what at first appeared to be an infrequent and local cause of mortality (Duff et al., 2007; Everest et al., 2008; Gavier-Widen et al., 2012) to an infection of wider UK significance to red squirrel conservation, a similar approach would clearly be valuable. Our provisional finding that males are less frequently infected than females may reflect small sample size but could also hint towards inter-sex variation, perhaps patterns of intensity of nest or range use.

Surveillance of mortality

We note that when road deaths were excluded from analysis, the frequency of recorded mortality did not show a significant correlation with changes in habitat occupancy. It is noteworthy however, that more bodies were recovered from feeders in the last four years of study relative to the earlier study period when red squirrel distribution was relatively limited. This almost certainly reflects a greater surveillance effort with more animals frequenting gardens, but increased exposure to pathogens at feeding stations as a contributing factor in elevated mortality cannot be excluded (see Bruemmer et al., 2010). Understanding variation in sampling illuminates the pattern of data, for example, nest boxes were geographically limited and regular monitoring of forest nest box blocks actually ceased in 2009. Had boxes been erected within forest habitats as stands were progressively colonised by red squirrels then almost certainly a network of boxes would have revealed cases of mortality and elevated the proportion of non-road death causes.

This study has provided data on red squirrel mortality that are useful at a local, regional and national scale and there are many findings that can help underpin future research. By providing data on bodies that were not clinically examined, we have given an added dimension to the overall data-set and we would suggest that other regional projects also record such figures to identify disease as well as non-disease threats to endangered or recovering populations. Surveillance is about manpower and adequate financial resources in order to conduct meaningful clinical examinations and thereby detect both known and novel infections. An integrated and well resourced, UK-wide disease monitoring programme should therefore be a priority for all national Governments.

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Associate Editor: L.A. Wauters