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Research Article

Are nest boxes a useful tool in regional red squirrel conservation programs?

Craig Shuttleworth[a,*], Pia Schuchert[b]

[a] *Honorary Visiting Research Fellow, School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd, United Kingdom*
[b] *School of Biology, Newcastle University, Ridley Building 2, Newcastle upon Tyne NE1 7RU, United Kingdom*

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Abstract

We examined temporal (annual & seasonal) patterns of nest box occupancy by red squirrels in a coniferous habitat in Great Britain. The effects of woodland habitat variation, and competition from nesting Great tits were investigated over a nine year period using basic linear models with a binomial error structure. Box use varied seasonally with peaks in the summer and autumn and was positively associated with the abundance of larch. 60 nest boxes yielded nine red squirrel carcasses, and although a small number, the material would be useful in genetic and viral infection studies. Nest boxes were not favoured by breeding female red squirrels and only three litters were found. We highlight the value and limitation of nest boxes as a tool in the applied conservation of red squirrels, and make recommendations for future areas of research on this topic.

Introduction

Red squirrels (*Sciurus vulgaris*) typically nest within a drey; a compact woven structure constructed of twigs, leaves, moss, dry grass and stripped bark which is built in the forest canopy (Shorten, 1954; Holm, 1987; Wauters and Dhondt, 1988; Bosch and Lurz, 2012). The species will also nest within tree cavities (Shorten, 1962) and larger sized boxes erected for birds (Petty et al., 1994; Juškaitis, 1999). For example, red squirrels have occasionally been found using owl nest boxes in commercial coniferous plantations in Great Britain (Petty, 1992; Shuttleworth and Shaw, 2001). However, it is difficult to draw conclusions about the value of bird nest boxes to red squirrels from these types of limited observational studies as; box designs may not have been optimal, the squirrels may have faced competition from roosting/nesting owls, and boxes may have been erected in unsuitable or suboptimal locations in the forest.

A range of European arboreal mammals are known to use nest boxes (Morris et al., 1990; Juškaitis, 2000; Kryštufek et al., 2003; Madikiza et al., 2009), and mammal box studies have not only helped advance scientific understanding of population ecology (Bright et al., 2006; Selonen et al., 2007), but in many instances have proven to be a resource which has benefited populations such as *Graphiurus murinus* (Madikiza et al., 2009) and *Sciurus carolinensis* (Barkalow and Soots, 1965).

In a red data book for British mammals, Morris (1993) highlighted the need to evaluate the use of nest boxes by red squirrels as one of a raft of pressing research questions which would evolve red squirrel conservation in Great Britain. Since this publication, Shuttleworth (1999, 2001) presented some preliminary and quantified assessments of box use in England, but it is notable that the population studied was of an unusually high density and also contained animals which had access to supplemental feeding (Shuttleworth, 1997). Consequently it is uncertain how representative these findings actually are of more typical red squirrel populations. In their European review, Bosch and Lurz (2012) also present some additional examples of red squirrel nest box studies. However, although useful, these were short-term observations reflecting the general paucity of long-term study data.

In 2001, red squirrel nest boxes were erected in the 244 hectare Mynydd Llwydiarth forest on the 720 km^2 island of Anglesey, North Wales. The Sitka spruce *Picea sitchensis* and pine *Pinus* spp. dominated plantation contained an isolated remnant red squirrel population with a spring density of 0.5 adults per hectare (Shuttleworth et al., 2012). This is a density typical for commercial coniferous habitats with a significant pine component present (see Lurz et al. 1995). The population had historically been sympatric with grey squirrels (Shuttleworth, 2003). However, as part of an island wide eradication program these local grey squirrels had been culled, and although a wider population remained elsewhere on the island, this was progressively fragmented and eventually almost completely removed (see Schuchert et al. 2014).

Nest boxes were erected with the aims of determining population persistence and providing data on the spatial distribution of nesting and therefore squirrel activity. Shuttleworth (1999) had recovered squirrel carcasses from boxes in woodland in England, and it was envisaged that any carcasses found in Mynydd Llwydiarth would be subjected to veterinary pathology examination including diagnostic tests for the presence of pathogenic disease (including Squirrelpox virus which is an asymptomatic infection in grey squirrels, see Bruemmer et al. 2010;

*Corresponding author
Email address: craig.shuttleworth@rsst.org.uk (Craig Shuttleworth)

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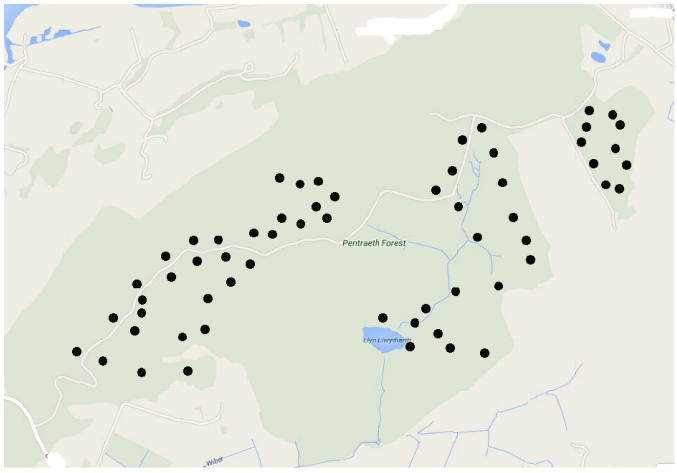

Figure 1 – Location of nest boxes in Mynydd Llwydiarth.

Carroll et al. 2009) and allow forest managers to follow reactive disease protocols.

It was also anticipated that boxes might provide data on litter sizes and parturition dates to complement short-term mark-recapture studies. These showed 58% (n=19) and 70% (n=40) of adult females were reproductively active when caught in the period March to August of 2001 and 2002 respectively (Shuttleworth et al., 2002) . With prioritised and proactive control of grey squirrels within adjacent woodlands seeking to prevent immigration into this and other forests containing red squirrels, trapping studies in Mynydd Llwydiarth were often infrequent and the boxes were employed as the main population monitoring tool.

Given the small and isolated nature of the historical remnant population in North Wales (Shuttleworth, 2003), a need to identify the geographical distribution of active nest sites and to indirectly monitor squirrel presence within forest blocks would clearly have been useful in 2001. However, in the absence of parallel weather data, seed crop data and mark-recapture population assessments, it is not possible from this study to draw detailed conclusions about the range of factors which influence nest box use by red squirrels. No data were available on nest box orientation for example, or the tree species onto which individual boxes were fixed, nor were boxes spaced regularly. As a consequence, the study does not lend itself to replication. However, in the absence of alternative empirical long-term studies, there is an opportunity here to critically examine the value of boxes in the context of an applied conservation tool.

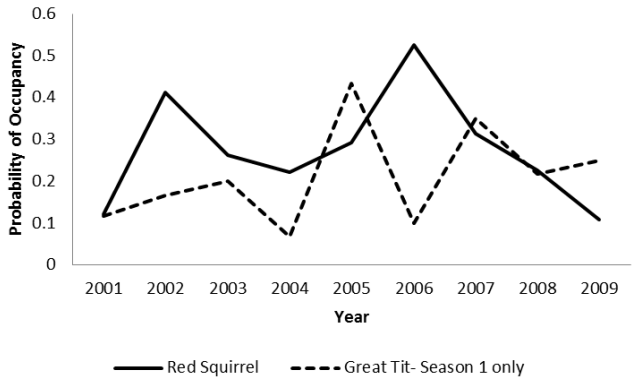

Figure 2 – Occupancy by red squirrels and great tits. Probability of occupancy of a nest box by red squirrels is for the whole year, while the probability of occupation by great tits is for spring (Season 1) only.

Methods

Nest boxes were constructed of commercially available exterior grade five-layer ply-board. Each had internal dimensions of 28 × 24 × 28 cm (maximum height) with a slope from the rear (28 cm) to front box face (24 cm). A small entrance hole (7 cm diameter) was cut in the top corner on the right hand side of each box. When the box was mounted on a tree trunk this hole was adjacent to the trunk in order to facilitate movement by squirrels into and out of the box. Boxes were fixed at heights of 5-6 metres and held in position using two 8 cm threaded coach-screws inserted top and bottom of a 10 cm wide ply back board which protruded 15 cm from the box. Each box was initially filled with hay to provide material within which squirrels could nest. Inspection of the box interior was carried out by removing the lid which was held in place by two 2.5 cm long galvanised steel self-tapping screws.

Sixty nest boxes were erected within the Mynydd Llwydiarth plantation in February 2001 (Fig. 1) and the contents inspected at the end of each season: winter (February), spring (May), summer (August) and autumn (November). The boxes were dispersed through selected stands at a density of approximately one box every 1-2 hectares. All forest stands selected were planted in the period 1951-1967 (Shuttleworth et al., 2012) and were of mature timber crops. Although no data were available on stand characteristics within a radius of each individual box, data were available for larger scale forest compartments within which groups of boxes were erected, and it was therefore possible to use these data to investigate any broad habitat influence upon box use. Each nest box remained at its location throughout the period for which data are available.

During each box inspection a fresh 8 cm long piece of double sided carpet tape was placed inside the box just below the entrance hole. This sticky material trapped red squirrel hairs and these helped demonstrate box use. In addition to hair evidence, notes were made on any nest structure encountered (e.g. central cavities formed within hay or hollows evident on the surface of the hay), and of any obvious material that had been added to the box. The remains of dead animals were recorded as were the presence of pre-weaned litters. The presence of honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.) were recorded along with nesting attempts by Great tits (*Parus major*) (following Shuttleworth, 2001). Great tit nesting attempts included the presence of chicks, pre-fledged brood, the remains of dead young, cup nests constructed of moss, sheep wool and feathers and built on the hay box filling.

In order to examine temporal (annual and seasonal) patterns of box use, and how habitat variation may influence box use, we used basic linear models with a binomial error structure to determine the factors influencing the occupancy of a box by either red squirrels or great tits. We used the proportion of larch (*Larix* spp.), spruce and pine in the surrounding area of a nest box, season and year as covariates. As a predictor for great tit occupancy, in addition to earlier defined variables, we also used the number of nest boxes in the area occupied by red squirrels to determine whether the abundance of red squirrels might impact on the use of boxes by great tits. To determine whether occupancy of nest boxes was associated with spatial distance between the boxes we applied k-function using 1000 Markov Chain Monte Carlo trials to identify possible space-time clusters. Finally we extended the basic models by including spatial correlation structures that use the information of their latitude and longitude and used a semivariogram of the standardized residuals to investigate spatial correlation in the errors.

Results

Seasonal box use by red squirrels varied from 2% to a maximum of 53%, with the highest annual occupation rate in 2006 (Fig. 2) and by great tits between 10 and 43%, with the highest occupation rate in 2005 (Fig. 2).

Neither red squirrel nor great tit occupancy showed any spatial relations. Red squirrels showed a significantly higher occupation probability in summer (season two) and autumn (season three) than in the winter or spring (Tab. 1), but there was no significant annual effect on red squirrel box occupation (Fig. 2). The proportion of larch in an area

had a significant and positive impact on the occupancy of boxes by red squirrels, whilst changes in the proportion of pine and spruce were non-significant for red squirrel box use (Tab. 1). Great tits and their nesting attempts were only recorded in spring, hence only data from this season were included in the analysis of factors affecting tits. In contrast to the red squirrels, the occupancy rate of great tits increased over years and with the increase of proportion of Larch, Pine and Spruce (Tab. 2). On fifteen occasions a box had Bumble bees present (queens prospecting nest sites through to colonies) and honey bee colonies were found on 21 occasions. There was no evidence of grey squirrels occupying boxes (either direct observation of animals or indirect evidence in the form of hair).

The remains of nine dead red squirrels were discovered (five adults and four juveniles) during our study. The nine bodies were recovered in four of the nine years that boxes were monitored. Advanced autolysis meant that none of the bodies recovered were particularly suitable for post mortem examination. In two cases a veterinary pathologist was able to determine that coccidiosis and enteric disease was present. Three litters were recorded during the study. These were all spring litters observed in 2002 (2 young), 2003 (2 young) and 2007 (3 kittens).

Discussion

Red squirrels in North Wales showed a strong seasonal pattern of box use similar to that reported in previous studies (Gurnell and Pepper, 1994; Shuttleworth, 1999, 2001) with a peak probability of box occupancy in the summer and autumn months. Red squirrels occupied boxes in every season throughout the nine years of study revealing a population persistence and providing spatial nesting data which were particularly useful to project managers at a time when the population was small and vulnerable to extinction (Shuttleworth, 2003; Schuchert et al., 2014).

Project managers had anticipated that boxes would provided data on mortality, and indeed the remains of nine dead red squirrels were recovered. This averaged only a single body per annum, and advanced autolysis typically precluded post mortem examination. In the absence of these data no information relating to deaths within nest sites would have been gathered. It is also notable that in the period since 2001 there have been progressive technical advances in the use of Polymerase Chain Reaction (PCR) as a tool in identifying potentially pathogenic viral infections (e.g. see Everest et al. 2012; Romeo et al. 2014; Shuttleworth et al. 2014 for *S. vulgaris* enteric infections). This means some viral screening could now have been undertaken despite autolysis. Consequently, nest boxes now offer at least some value as a disease monitoring tool, especially in upland spruce dominated habitats where recovery of any carcasses is difficult. Interestingly, Everest et al. (2009) obtained tissue samples from a pre-weaned juvenile and parent female grey squirrel found and killed within a red squirrel nest box leading to the index identification of adenovirus infection in this species.

Carcasses also present a valuable source of genetic material for research. Sotola and Garneau (2014) recommended that genetic tests on hair collected from boxes (both carcasses and double sided tape in our Welsh study) could also be used to identify individuals and help understand population size and inter-relatedness, and we echo their recommendation.

Grey squirrels readily use nest boxes (Barkalow and Soots, 1965) and therefore in Europe where sympatric grey squirrel populations are present, or the species is close to red squirrel habitats, managers must of course consider whether boxes as a potential means of detecting (and locating) greys outweighs the potential inter-specific disease risk which they might pose to red squirrel (see Everest et al. 2012; Collins et al. 2014). In this Welsh study, adjacent grey squirrel populations were exposed to intensive culling (Schuchert et al., 2014) and hence the risk of occupation of boxes within this red squirrel habitat was greatly limited.

In the absence of the congener, we did observe strong correlation between red squirrel occupancy and larch perhaps reflecting cone patterns and seed availability relative to other tree crop species. Sitka spruce sheds seed early in the winter and produces a relatively small seed (Lurz et al., 1998, 2000), and although Scots and Corsican pines generally offer good food sources, on Anglesey these species are adversely affected by pathogenic infection of red band needle blight (Brown and Webber, 2008; Shuttleworth et al., 2012) which may have reduced seed production.

Only three red squirrel litters were discovered. It might be argued that had more frequent inspections taken place then a greater number of litters might have been found. However, as weaning occurs only after 8-10 weeks then it seems unlikely that the 12 week inspection regime would have missed a significant proportion of litters. In the period 1999-2002 the percentage of adult female red squirrels breeding was 58-73% (Shuttleworth et al., 2002) and we therefore conclude that boxes as a nest site resource, were not particularly favoured by breeding adult females. It would seem that in commercial conifer plantations nest boxes do not offer a useful means of getting data on parturition dates, litter size or pre weaning survival rates.

In a study of *Graphiurus murinus*, Madikiza et al. (2009) were able to investigate variation in box occupancy by age class and sex, and had access to detailed micro-scale habitat data for each box location. In contrast we could not quantify how red squirrel occupation related to these variables or squirrel abundance. Nest cameras as used successfully by Bosch and Lurz (2013) would be revealing in this regard and also in determining how red squirrels and nesting great tits interact.

Shuttleworth (2001) provided evidence that whilst colony nesting Jackdaws preclude red squirrels from using boxes, red squirrel regularly displace great tits although to what extent predation of nests was a factor was unclear. Friesen et al. (2013) reported grey squirrels occasionally visiting Wood Thrush *Hylocichla mustelina* nests. Where eggs were present no predation took place but visits were associated with premature fledging in two of the 86 nests video-monitored. We observed a strong negative interaction between spring great tit box occupancy and the use of boxes by red squirrels. Hence, it would be useful to investigate whether red squirrel presence leads to premature fledging or nest predation and thus whether tit occupancy could be used as a novel indirect measure of red squirrel presence.

Nest boxes provide useful evidence of red squirrel persistence and nesting within forest stands, and have the potential to illuminate mortality within nest sites. They could be used as a tool in monitoring geographical expansion of populations e.g. in the Iberian peninsula (Rocha et al., 2014). The spatial data obtained in our local study reflects a more efficient return than could be achieved with the same effort allocated solely to trap based operations. However, this study has not revealed how seasonal occupation of boxes by red squirrels reflects

Table 1 – Probability of occupancy of a nest box by red squirrels.

Coefficient	Estimate	Std. Error	z-value	$p(>\|z\|)$
Intercept	-1.425	0.129	-11.065	<0.0001
Larch	0.013	0.006	2.094	0.036
Season 2	0.569	0.140	4.062	<0.001
Season 3	0.539	0.149	3.626	<0.001
Season 4	0.056	0.158	0.358	0.721

Null deviance: 2217.3 on 1882 degrees of freedom

AIC: 2196.8

Table 2 – Probability of nest box occupation by great tits in the first season..

Coefficient	Estimate	Std. Error	z-value	$p(>\|z\|)$
Intercept	-14.385	5.276	-2.726	0.0064
Year	0.110	0.042	2.623	0.009
Larch	0.138	0.060	2.279	0.023
Pine	0.112	0.048	2.349	0.019
Spruce	0.183	0.075	2.424	0.019

Null deviance : 549.27 on 529 degrees of freedom

AIC: 546.03

population abundance nor how red squirrels interact with nesting great tits in boxes. These are areas recommended for future study. Finally it cannot be overstated that our findings reflect commercial spruce dominated plantation habitat and may not reflect nest box use in more natural forests including broadleaved woodland. Additional studies focussed within these habitats would be beneficial.

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