NOTTINGHAM TRENT UNIVERSITY

RED SQUIRREL IN THE BRITISH ISLES: APPROACHES TO CONSERVATION MANAGEMENT AND GREY SQUIRREL CONTROL

By

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Paper 1

PINE MARTEN RECOVERY: A TOOL FOR BIOLOGICAL INVASIVE SPECIES CONTROL AND RED SQUIRREL CONSERVATION? A LITERATURE REVIEW

By

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ABSTRACT:

Since the 19th century, red squirrels (*Sciurus vulgaris*) across the British Isles have gradually been displaced, through direct resource competition with the invasive grey squirrel (*Sciurus carolinensis*), and associated squirrelpox virus transmission. Significant declines have led to only isolated remanent populations remaining. Despite various conservation efforts and legislation for the control of grey squirrel, red squirrels still only inhabit a small portion of their original native range. However, there is emerging evidence that the recovery of a native predator, the pine marten (*Martes martes*), is suppressing grey squirrel populations in various regions. Dietary analysis has evidenced pine marten predation on grey squirrel, population declines have been attributed to pine marten presence, perhaps due to naïve anti-predatory behaviours, though ranging behaviour changes have been observed in some study areas, and mathematical models indicate changes in disease-mediated competition. Utilising PRISMA protocols, this literature review collates and examines the current research on this subject. Proposing this form of biological invasive species control as a viable component, to a carefully considered multi-faceted approach to red squirrel conservation.

1. Introduction

Sciurus vulgaris (red squirrel) face a multitude of threats across the British Isles, with the introduction of the invasive non-native species (INNS), *Sciurus carolinensis* (grey squirrel) being a key driver of population decline. Grey squirrels were introduced in the late 19th and early 20th centuries (Gurnell *et al*, 2004). The first recorded instance being in 1876, at Henbury Park in Cheshire, England (Shorten, 1957). Part of a misguided effort to enhance the aesthetics of the landscape by introducing a novel, non-native species. This event marked the beginning of subsequent introductions (Gurnell *et al*, 2004), resulting in the geographical expansion of this INNS, and the replacement of the native red squirrel (Chantry *et al*, 2014).

Grey squirrels possess broad dietary overlap with their native counterparts, with an adept ability to digest high polyphenols in tree seeds, such as oak (*Quercus spp*.) acorns (Kenward *et al*, 1993). Providing the advantage of access to this key food source earlier than red squirrel can utilise. Subsequently, grey squirrels have rapidly replaced red squirrels in deciduous woodlands, where they reach higher densities (Slade *et al*, 2020).

Grey squirrels are also generally larger than red squirrels (Bryce *et al*, 2001). This size difference provides them with advantages, such as a greater capacity for storing body fat, particularly advantageous during periods of food scarcity (Gurnell *et al*, 2004). They are more aggressive, exhibiting this behaviour toward red squirrels more readily than their own species (Santicchia *et al*, 2022). These competitive advantages have significantly contributed to displacement and population decline observed in red squirrel across the British Isles.

The spread of squirrelpox virus (SQPV) has had a devastating effect on red squirrels (Chantrey *et al*, 2014). While greys function as an immune reservoir, displaying no pathological symptoms, when SQPV is transmitted to the native red squirrel, the disease has fatal consequences (Chantrey *et al*, 2019; Everest *et al*, 2021). The virus causes lesions on the skin, mouth, and feet, and affected red squirrels may experience lethargy and an overall health decline (Everest *et al*, 2021). The mortality rate for untreated infected red squirrels is typically one hundred percent (Atkin *et al*, 2010). Regions where grey squirrels test positive for SQPV antibodies, exhibit a decline of red squirrel populations twenty times higher than those where SQPV is not present (Rushton *et al*, 2006). Illustrating the scale at which this virus decimates native populations.

Habitat loss and anthropogenic pressures have also played a role in the overall decline of red squirrel in the British Isles (Lurz, 2014). They do occur throughout Britain, with around two thirds of the population found in northern Scotland (Slade *et al*, 2021a). The rest remain within isolated areas, with poor habitat connectivity, areas of coniferous forestry plantations or islands where greys have failed to invade (Nie *et al*, 2023). As such, it is imperative to design and implement carefully considered conservation management strategies, as poor execution or in complete absence of, these remnant populations could realistically very rapidly decline. Thus, strategising a collaborative, multi-faceted approach and exploring novel methods will be vital in the successful conservation of this iconic species.

Another British mammal which has experienced significant population decline in recent history, is the European Pine Marten (*Martes martes*). Once common throughout Britain (Maroo & Yalden 2000), in the 19th and early 20th centuries, their population faced significant declines in both numbers and distribution. Primarily due to intense predator control efforts (Langley & Yalden 1977), plus extensive habitat loss and fragmentation of woodlands (Ridding *et al*, 2020). By the onset of the 20th century, pine martens had become all but extinct in England and Wales (Sainsbury *et al*, 2019). The remaining populations were mainly confined to the north-west highlands of Scotland, with remnants in the uplands of England and Wales (Langley & Yalden 1977). Although afforestation and legal protection led to successful recovery and expansion of the pine marten population in Scotland since the 1980s, the same positive trend did not extend to other regions in Britain (Sainsbury *et al*, 2019).

In Wales, the pine marten population was considered functionally extinct (Sainsbury *et al*, 2019). Following feasibility studies (MacPherson *et al*, 2014), pine martens were sourced under license from Scotland and translocated to Wales between 2015 and 2017 (McNichol *et al*, 2020a). Released in north Ceredigion, long term monitoring using various methods, confirmed successful breeding annually, from 2016 to present, with the population dispersing beyond the initial release area (McNichol *et al*, 2020a). Following this success, subsequent translocations occurred in Britain, with the hopes that these populations would establish themselves further, eventually connecting (MacPherson *et al*, 2021).

Whilst reestablishment of pine marten in Britain is in its relative infancy, current monitoring indicates clear successes in their recovery (McNichol *et al*, 2020a). In recent years, there have been observations and emerging research, suggesting that the reintroduction of this native predator has also been beneficial to red squirrel conservation (Slade *et al,* 2023). Not only in applications of successful mammal recovery, but also in the role pine marten play in predator-prey dynamics (O'Meara *et al,* 2014), with both the native red squirrel, and the invasive grey squirrel.

As a natural predator of red squirrel, one might assume they would pose a serious risk to any kind of species recovery effort. These concerns played a significant role in site selection for initial pine marten reintroductions (MacPherson *et al*, 2014). However, research indicates that the presence of this mustelid has been beneficial to red squirrel conservation (Twining *et al*, 2022a), through the predation on the grey squirrel, for which the pine marten is a novel predator.

Instances where these three mammals co-occur at a given location are minimal (Bamber *et al*, 2020). Once grey squirrel arrive, red squirrel typically rapidly decline (Rushton *et al*, 2006). Additionally, while the INNS is well established, red squirrel no longer are (Lurz *et al*, 2014), and while in recovery, pine marten populations are not what they once were (MacPherson *et al*, 2021).

However, as native species recovery has occurred, more interactions between these animals have been observed (Twining *et al*, 2022a).

This paper aims to comprehensively review the existing literature regarding the impact of pine marten presence on both the native red squirrel and their invasive counterparts. It will delve into research findings and explore the outcomes associated with the successful reestablishment of pine martens. If the recovery of a native predator can contribute to the conservation of another iconic species, it is imperative that the mechanisms behind this are understood and appropriately applied.

2. Methods

2.1. Literature Search

In November 2023, a literature review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) protocols (Page *et al*, 2021)). Systematic reviews using strict methodologies were first implemented in the field of medicine, aiming to overcome selection and interpretation biases more likely to occur in traditional literature reviews (Haddaway et al. 2015). Recently, this has gained prominence within conservation biology, thanks to the associated benefits of improved transparency and objectivity, and informing evidence-based conservation management decisions (Cook et al. 2013).

A literature search was conducted on Google Scholar, Scopus, and Web of Science, with the following search terms: (‘red squirrel*’ OR ‘Sciurus vulgaris’) AND ('Martes martes' OR 'Pine Marten*') AND ('Grey squirrel*' OR 'Sciurus carolinensis') AND ('invasive' OR 'alien') AND ('control' OR 'management'). All the results listed on Scopus and Web of Science were collected. Only the first 200 articles of Google Scholar were collected, following the threshold used in other reviews (Fingland *et al*, 2022) after this, results become less relevant. No search parameters regarding year of publication were included. Articles were excluded if they were not peer-reviewed or available in English.

2.2. Inclusion Criteria

Remaining articles were screened with the following criteria: the title, abstract or key words included at least 'red squirrel' or '*Sciurus vulgaris*' or 'grey squirrel' or '*Sciurus carolinensis'* and 'pine marten' or '*Martes martes*', and that the study explored the role of pine marten as a predator of native and or non-native squirrels.

Articles that passed this initial screening were then assessed in full for the following: study identified grey squirrel as an INNS (i.e. European study) and included empirical research relating to pine marten presence on the behaviour or abundance of red or grey squirrel. Only key findings from the final collated data set were reported on.

3. Results

The initial literature search returned 189 articles once duplicates were removed. During the screening assessment 171 were excluded in total, resulting in a final dataset of 18 articles (Fig. 1). These were published between 2014 and 2023 (Fig. 2), with the majority conducted in the UK and Republic of Ireland (n = 17), and one in Finland.

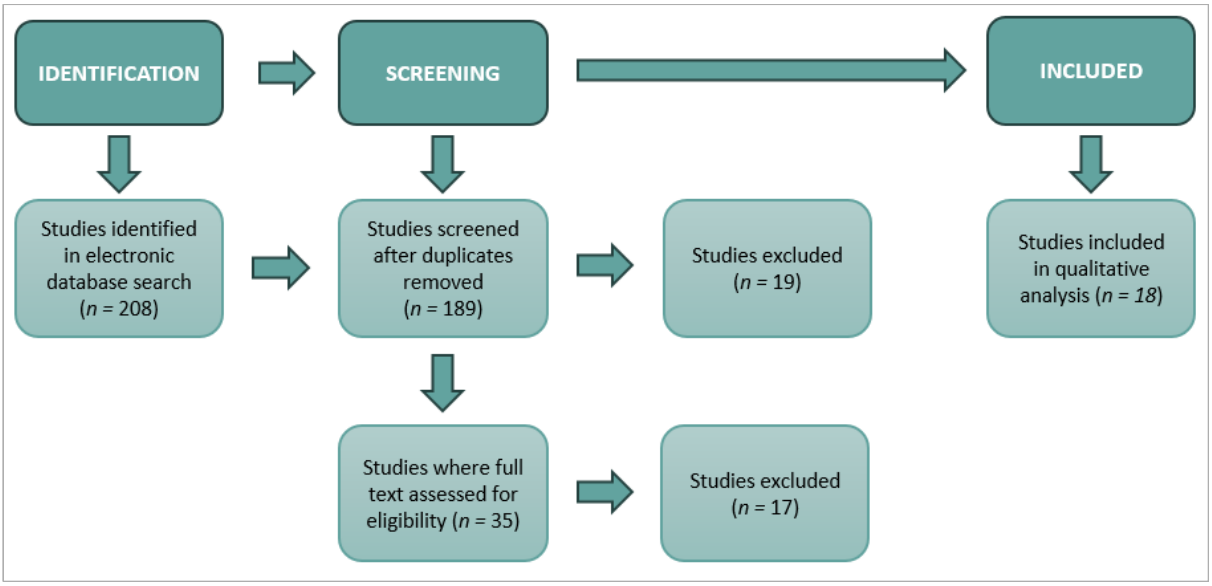

Figure 1. PRISMA literature search and screening flow diagram of studies included and excluded from analysis.

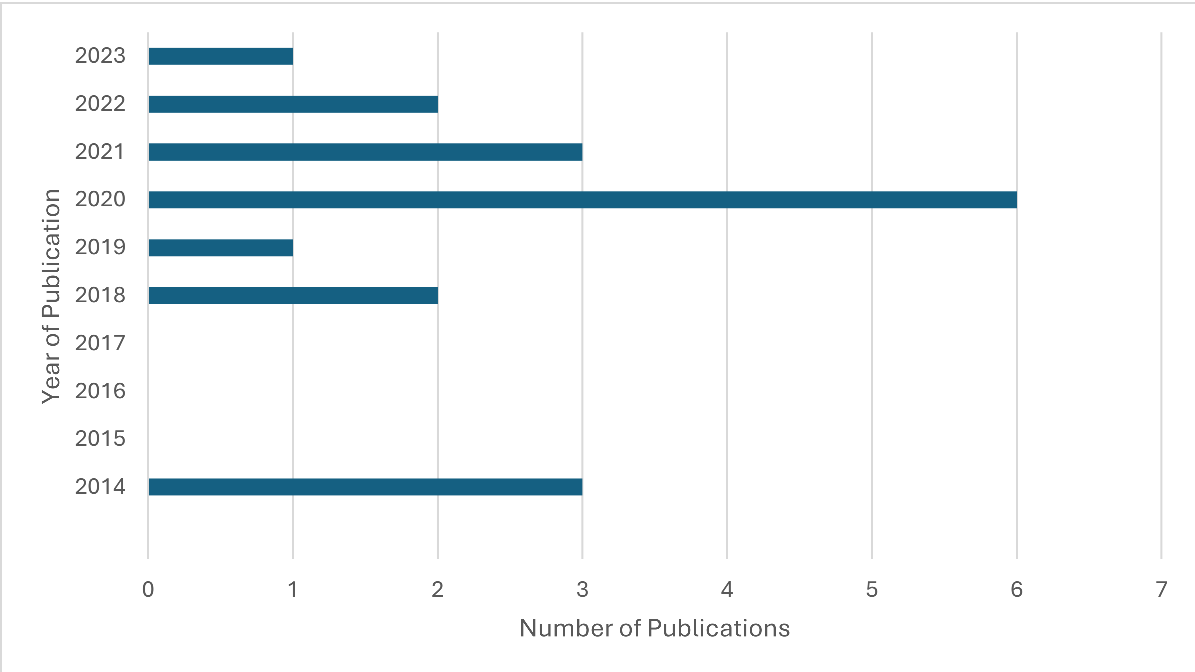

Figure 2. Number of studies included in final dataset (n = 18), published each year from 2014 to 2023.

4. Discussion

The 18 studies identified for this review were categorised into five topics for qualitative analysis. Here the literature is reviewed for evidence relating to pine marten recovery as a potential tool for red squirrel conservation and grey squirrel control.

4.1. Pine Marten Dietary Analysis

The literature search found that the earliest presented evidence of pine marten predation on the invasive grey squirrel was observed in Ireland (Sheehy et al 2014a). Although earlier anecdotal observations have been reported (Caryl, 2008), this was the first scientifically robust evidence. A key aim of the study was to quantify the frequency of occurrence of small mammals in the diet of pine marten within the study regions, with an emphasis on identifying red and grey squirrel presence through scat analysis, using molecular and macro analysis. Collected scats were confirmed as pine marten using a ZR Genomic DNA II Kit™. Collection sites were classified as having either red squirrel or grey squirrel present. Although a greater number of scats were collected from red squirrel sites (329) versus grey squirrel sites (32), grey squirrels (15.6 %) were significantly ($p < 0.05$, Fisher exact) more frequently detected than red squirrels (2.5%) as prey items. Both species were identified in pine marten scat analysis at low frequencies throughout 9 months of the year, most commonly in spring and early summer. While confirming the presence of grey squirrel in the diet of pine marten, this result favouring grey squirrel over red should be interpreted cautiously. Small sample sizes may skew the representation of prey items in dietary analysis (Trites and Joy, 2005).

A subsequent study (O'Meara *et al*, 2014) in Ireland using real-time PCR found red squirrel was detected as a low frequency prey item. However, found no evidence of grey squirrel in pine marten scat samples, despite areas of potential overlap between the species. By including a control group of captive pine marten, fed with grey squirrel, this study demonstrated that grey squirrel can only be detected one to two days after consumption, leaving a short opportunity to detect a species that occurs at low frequencies within pine marten diet (Sheehy *et al*, 2014a; O'Meara *et al*, 2014).

These discrepancies indicate real-time PCR may have more limitations in terms of detection of prey in predator scat analysis, but also illustrate the need for suitable sample sizes to provide more reliable conclusions.

A more recent study (Twining *et al,* 2020a) with a somewhat larger sample size was able to demonstrate seasonal variation in pine marten predation. Pine marten scats contained grey squirrel DNA during the spring and summer seasons, with no indications of presence in autumn or winter. Whilst, red squirrels were identified during spring, summer, autumn, and similarly absent during winter. Grey squirrel occurrence in scats showed no significant difference between spring and summer, and red squirrels between spring, summer, or autumn. Grey squirrels were proportionally more frequent than red squirrels in summer ($p = 0.034$), but no significant difference was found in spring. Frequency of occurrence of grey squirrel remains in pine marten scats were similar to Sheehy *et al* (2014) findings, whilst the sample here is considerably larger ($n = 155$). The seasonality analysis of Twining *et al* (2020a) suggests that pine martens may prefer grey squirrels whilst juveniles are more abundant and restricted to dreys.

4.2. Novel Predator, Naïve Prey

Although further research is needed, if grey squirrel do occur more frequently in the diet of pine marten, the causation of this should be examined. This topic was addressed among multiple studies of the literature search. Perhaps red squirrels' lower population density (0.3–1.5 per ha) compared to grey squirrels (2–16 per ha) is a factor here (Twining *et al,* 2020a). Additionally, their lighter physique, and arboreal foraging habits make them less accessible as prey, than the more ground-dwelling grey squirrel (Sheehy *et al,* 2018). In their native north American range, grey squirrel do not encounter arboreal predators like pine marten (Slade *et al,* 2023). Perhaps this naïve invasive prey exhibits ineffective responses to this novel native predator.

Sheehy *et al* (2018) undertook a study in Scotland and Northen England, investigating impact of predation exposure on red and grey squirrel by pine marten, in regions of varying pine marten establishment. Combining spatial capture–recapture to estimate pine marten density, and squirrel occupancy data utilising feeding stations, hair, and camera traps. Considerable behavioural differences were observed. Where red squirrels were still present, they were less likely to visit feeding stations in areas of high pine marten connectivity. Whereas, where grey squirrels were present, they were more likely to use feeders in sites with high pine marten connectivity. Suggesting a naïve response to a predator with which they have not co-evolved. Occupancy modelling demonstrated that where native predator recovery is more advanced, red squirrel now occur at a greater portion of the landscape than non-native grey squirrels. A tentative interpretation being that over time, pine martens suppress grey squirrel numbers even in their preferred habitats of broadleaf woodland. Holding significant implications for red squirrel conservation management.

It is important to consider the degree of variation that may occur in differing landscapes, and while predicative modelling is a useful tool, it cannot feasibly consider all variation between regions.

An experimental study in Ireland (Twining *et al,* 2020b) using camera traps and feeders at 20 sites, squirrels were observed before and after the application of pine marten scent to the feeders. Red squirrels reduced visitation by up to 92% for 48 hours after applying pine marten scent, displaying decreased feeding (12%) and increased vigilance. Grey squirrels, in contrast, showed a 36% increase in visitation, with minimal changes in feeding and vigilance. The results suggesting grey squirrels lack predator recognition and exhibit a bolder response to a novel scent, whereas native red squirrels exhibit effective anti-predator behaviours. While this boldness may have provided grey squirrel a competitive advantage over native reds in past, perhaps in the presence of a recovering native predator, could have significant impacts on their populations.

However, an observational study in North Wales (McNichol *et al,* 2020b) explored the effects of recently translocated pine marten, on GPS collared grey squirrels. In contrast to predictions of diminished ranging, squirrel range size and daily distance travelled was greater, by male but not female grey squirrels, with increased exposure to pine marten. Suggesting they identified pine martens as a threat, responding by modifying their behaviour. Such changes could be permanent or plastic (McNichol *et al* 2020b). Should such responses evolve to permanent anti-predator behaviour, the effect of pine marten as a biological control of grey squirrel would be diminished (Slade *et al,* 2022). As a short-term study, long-term population level changes were not observed, meaning this could be a case of temporary behavioural change, responding to novel stimuli (newly translocated pine marten) which would eventually return to 'normal'.

Based on the reviewed literature, there is certainly evidence to support the mechanism of invasive prey naivety to a novel predator in grey squirrel. How long lasting these effects might be however, will likely vary between specific populations and landscapes, and longer-term studies might provide further insight.

4.3. Squirrel Population Changes

Since there is evidence of pine marten predation on grey squirrel and a level of prey naivety demonstrated within the research, it is unsurprising that several studies report grey squirrel decline across their non-native range, with a positive effect for red squirrels.

Sheehy *et al* (2014b) were the first to report notable population decline of grey squirrel in the Irish midlands. Public animal sighting data revealed that grey squirrel sightings accounted for just 8% of animal sightings, extremely low for a species which is considered much less elusive than their native counterparts. Conversely, red squirrel and pine marten were widespread, accounting for

46% of sightings. Hair tube surveys delivered alongside this study corroborated this evidence, finding grey squirrel undetectable at any level. Survey respondents within the study area for more than two decades reported red squirrel had begun to recolonise from the late 1990s. Whilst this anecdotal information should be considered carefully, this co-occurred with the recovery of pine marten. And is the first recorded occurrence of grey squirrel decline without active intervention or management. However, this decline can only be considered as regional, with grey squirrels persisting in eastern study areas (Sheehy *et al* 2014b). Surmising that, failure of grey squirrel to establish in western Ireland may have been related to barriers in the landscape and persistent historic pine marten populations (Sheehy *et al* 2014b). Therefore, subsequent pine marten recovery could be responsible for grey squirrel decline in the midlands, and persistent eastern populations due to the lack predator presence. The implications of these findings cannot be understated, but more robust evidence is certainly needed.

A subsequent study (Flaherty and Lawton, 2019) across Ireland corroborated these findings, citing landscape barriers were difficult to cross but not impossible for grey squirrel, evidencing some presence but failure of establishment in the west. Attributing this to pine marten presence and lack of suitable habitat and fragmentation. Documenting further evidence of grey squirrel population crash in an area of long establishment in the Irish midlands, through live trapping, hair tube and public sightings.

Another Northern Ireland study (Twining *et al,* 2021), utilising a citizen science camera trapping project, also found notable impact of pine marten presence on the decline of the invasive grey squirrel, positively influencing native red squirrels. However, they note recovery of the pine marten alone may not lead to the complete eradication of grey squirrel, resulting instead in fugitive coexistence due to the presence of refugia in urban areas.

Observational evidence, gathered through a well-structured survey with a high sampling resolution across diverse habitats, indicated pine martens as woodland specialists in Ireland

(Twining *et al,* 2021). Both coniferous plantations and broadleaf and mixed forests positively influence pine marten occupancy, with broadleaf habitats exhibiting a stronger positive effect. But reveals the strong avoidance of human settlements by pine marten, emphasising the role of habitat in shaping their distribution (Twining *et al,* 2021). Grey squirrels show a preference for urban areas and broadleaf habitats, while red squirrels are positively correlated with coniferous plantations (Twining *et al,* 2021). The findings support the idea that grey squirrels outcompete red squirrels in deciduous and mixed woodlands, leading to the latter being largely confined to large coniferous plantations (Lurz *et al*, 2014.

Bamber *et al* (2020) cites similar findings in North Wales, predicting a future scenario where, despite the recovery of pine marten populations, grey squirrels may persist in urban areas, acting as potential sources for reinforcement in the broader landscape. The limitations on pine marten influence, constrained by the lack of suitable habitat and their avoidance of urban areas, suggest that complete control of grey squirrels may require additional measures in human-populated areas.

In a grey squirrel absent system in Finland, pine marten presence has not been associated with red squirrel decline through predation (Turkia *et al,* 2020). Whilst pine marten recovery may provide grey squirrel control as an ecosystem service, it is not a fix-all solution. Grey squirrel trapping and traditional management approaches will still be required in most cases. Thus, it should be explored as a multi-faceted approach for red squirrel and broader ecosystem conservation (Bamber *et al,* 2020; Twining *et al,* 2021). While the evidence of positive outcomes for red squirrel populations and the wider ecosystem is certainly compelling, the generalist nature of pine marten diet must also be considered. In the absence of preferred prey, e.g. woodmouse, vole (Sheehy *et al,* 2014a; Turkia *et al,* 2020), pine marten may predate on nesting birds (Turkia *et al,* 2020), which could prove problematic in some areas where such species could be in decline.

4.4. Disease-Mediated Competition

The key drivers of replacement of native red squirrel with invasive grey squirrel are resource and disease-mediated competition. If pine marten can drive grey squirrel decline as evidenced in the literature, naturally resource competition will decrease. Also presented, evidence on potential shifts in the transmission of SQPV, in a system where a native predator is reintroduced.

Roberts and Heesterbeek (2021) present a comprehensive model examining the interaction of red and grey squirrels, SQPV, and pine martens. The study identifies an amplification effect of SQPV transmission at higher grey squirrel density. Contrary to expectations, increased grey squirrel density correlates with heightened SQPV transmission rates, challenging the hypothesis that a higher density of grey squirrels would dilute SQPV transmission. Slade *et al*. (2021; 2023) build on this, also using mathematical modelling to suggest that pine marten predation on grey squirrels contributes to a reduction of SQPV in these populations. This is vital when considering the fatal consequences of SQPV transmission to red squirrels. An earlier study by Travaglia et al (2020) supports the likelihood of the system evolving towards conditions favouring red squirrels in the presence of pine martens, but the persistence of SQPV remains endemic. Achieving complete eradication of grey squirrels through pine marten predation seems unlikely.

It is important to note that these are model-generated predictions, and their reliability is contingent on the accuracy of underlying assumptions. Real-world ecological processes are inherently complex and unpredictable. Whilst models provide valuable insights, further empirical research is needed to validate these predictions.

4.5. Attitudes to Biological Invasive Species Control

Based on the literature search, it seems viable that pine marten can contribute to a level of red squirrel conservation through INNS control. However, it is also important to consider the attitudes toward this, especially when navigating members of the public, who consider the grey squirrel a part of perceived native wildlife, without an understanding of the effects on native species and habitats.

A study (Dunn *et al,* 2018) on public attitudes toward squirrel management revealed humaneness as crucial factor of grey squirrel control, with 25% supporting the idea of nature taking its course (no control), and 36% opposing it. Humaneness emerged as the most pivotal factor in both support and opposition to control methods. Effectiveness being another significant factor, influencing 56% of respondents in favor of proven efficacy. Non-lethal methods, such as contraception and habitat control, were widely accepted, with biological control, specifically pine marten, ranking as the most acceptable lethal option, surpassing commonly used methods like live capture and shooting. This is a positive outcome, but it is also important to consider the wider implications of predator recovery, which can present its own challenges within public perceptions (MacPherson *et al*, 2014).

5. Conclusions

Reviewing the literature, it is interesting to consider what the incursion of grey squirrel across the British Isles may have looked like, had pine marten populations not been so depleted. It seems top-down ecosystem effects, and the potential recovery of native predators is not only intrinsically valuable in of itself, but also as a tool for INNS control and potential recovery of other suppressed native wildlife. However, as a relatively recent area of research, it is important to note the number of regions of study are limited. Pine marten recovery may contribute to grey squirrel control, but as part of a wider strategy and evaluated on a case-by-case basis, where different ecological dynamics are at play. As pine marten populations continue to establish, there is growing scope for research. Priority should be given to investigating effects on SQPV transmission, while longer-term studies will offer valuable insights into sustained effects of pine marten recovery on red squirrel conservation and the wider ecosystem.

6. References

Atkin J. W., Radford A. D., Coyne K. P., Stavisky J., Chantrey J., 2010. Detection of squirrel poxvirus by nested and real-time PCR from red (Sciurus vulgaris) and grey (Sciurus carolinensis) squirrels. *BMC veterinary research*, 6(1), p.33. Available at: http://dx.doi.org/10.1186/1746-6148-6-33.

Bamber J. A., Shuttleworth C. M., Hayward M. W., Everest D. J., 2020. Reinstating trophic cascades as an applied conservation tool to protect forest ecosystems from invasive grey squirrels (Sciurus carolinensis). *Food webs*, 25(e00164), p.e00164. Available at: http://dx.doi.org/10.1016/j.fooweb.2020.e00164.

Bandyopadhayay K., Ashby S. S., Warren G., Koprowski J. L., Baker P., 2022. Simulating a ‘landscape of fear’: Eastern grey squirrels (Sciurus carolinensis) reduce proportional feeding duration under exposure to native and invasive predator cues. *Research Square*. Available at: http://dx.doi.org/10.21203/rs.3.rs-2211518/v1.

Bryce J. M., Speakman J. R., Johnson P. J., Macdonald D. W., 2001. Competition between Eurasian red and introduced Eastern grey squirrels: the energetic significance of body-mass differences. *Proceedings. Biological sciences*, 268(1477), pp.1731–1736. Available at: http://dx.doi.org/10.1098/rspb.2001.1700.

Caryl, F. M., 2008. Pine marten diet and habitat use within a managed coniferous forest. *University of Sterling*. [Online Thesis] Available at: https://dspace.stir.ac.uk/bitstream/1893/2261/1/Fiona%20Mae%20Caryl.%20Pine%20Marten%20Diet%20%26%20Habitat%20Use%20Within%20A%20Managed%20Coniferous%20Forest.pdf.

Chantrey J., Dale T. D., Read J. M., White S., Whitfield F., Jones D., McInnes C. J., Begon M., 2014. European red squirrel population dynamics driven by squirrelpox at a gray squirrel invasion interface. *Ecology and evolution*, 4(19), pp.3788–3799. Available at: http://dx.doi.org/10.1002/ece3.1216.

Chantrey J., Dale T., Jones D., Begon M., Fenton A., 2019. The drivers of squirrelpox virus dynamics in its grey squirrel reservoir host. *Epidemics*, 28(100352), p.100352. Available at: http://dx.doi.org/10.1016/j.epidem.2019.100352.

Cook C. N., Possingham H. P., Fuller R. A., 2013. Contribution of systematic reviews to management decisions: Systematic reviews. *Conservation biology: the journal of the Society for Conservation Biology*, 27(5), pp.902–915. Available at: http://dx.doi.org/10.1111/cobi.12114.

Dunn M., Marzano M., Forster J., Gill R. M., 2018. Public attitudes towards “pest” management: Perceptions on squirrel management strategies in the UK. *Biological conservation*, 222, pp.52–63. Available at: http://dx.doi.org/10.1016/j.biocon.2018.03.020.

Everest D., Floyd T., Holmes P., Duff P., Man C., Dunnett E., Locke R., Savage L., Sutcliffe S., Sapsford B., Shuttleworth C., 2021. Disease monitoring and surveillance: case studies in the applied conservation of fragmented red squirrel (Sciurus vulgaris) populations in England and Wales. *Mammalian biology*, 101(6), pp.1079–1088. Available at: http://dx.doi.org/10.1007/s42991-021-00157-8.

Fingland K. Ward S. J., Bates A. J., Bremner-Harrison S., 2022. A systematic review into the suitability of urban refugia for the Eurasian red squirrel Sciurus vulgaris. *Mammal Review*, 52(1), pp.26–38.

Flaherty M., Lawton, C., 2019. The regional demise of a non-native invasive species: the decline of grey squirrels in Ireland. *Biological invasions*, 21(7), pp.2401–2416. Available at: http://dx.doi.org/10.1007/s10530-019-01987-x.

Gurnell J., Wauters L. A., Lurz P. W., Tosi G., 2004. Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. *The journal of animal ecology*, 73(1), pp.26–35. Available at: http://dx.doi.org/10.1111/j.1365-2656.2004.00791.x.

Haddaway N. R., Woodcock P., Macura B., Collins A., 2015. Making literature reviews more reliable through application of lessons from systematic reviews: Making Literature Reviews More Reliable. *Conservation biology: the journal of the Society for Conservation Biology*, 29(6), pp.1596–1605. Available at: http://dx.doi.org/10.1111/cobi.12541.

Kenward R.E., Holm, J.L., 1993. On the replacement of the red squirrel in Britain: a phytotoxic explanation. *Proceedings. Biological sciences*, 251(1332), pp.187–194. Available at: http://dx.doi.org/10.1098/rspb.1993.0028.

Langley P.J.W., Yalden D.W., 1977. The decline of the rarer carnivores in Great Britain during the nineteenth century. *Mammal review*, 7(3–4), pp.95–116. Available at: http://dx.doi.org/10.1111/j.1365-2907.1977.tb00363.x.

Lurz P. W. W., 2014. Changing 'red to grey': alien species introductions to Britain and the displacement and loss of native wildlife from our landscapes. In: *Displaced Heritage: Responses to Disaster, Trauma, and Loss*. Suffolk: Boydell Press, 2014, pp. 265–272.

MacPherson J., Croose E., Bavin D., O'Mahony D., Somper J. P., Butriss N., 2014. *Feasibility Assessment for Reinforcing Pine Marten Numbers in England and Wales* [online report]. Available at: https://www.vwt.org.uk/wp-content/uploads/2015/04/Feasibility-Assessment-for-Reinforcing-Pine-Martens-in-England-and-Wales.pdf.

MacPherson, J., Wright P., 2021. *Long-term strategic recovery plan for pine martens in Britain* [online report]. Available at: https://www.vwt.org.uk/wp-content/uploads/2021/07/Pine-Marten-Recovery-Plan-VWT-10June2021.pdf.

Maroo, S., Yalden, D. W., 2000. The Mesolithic mammal fauna of Great Britain. *Mammal review*, 30(3–4), pp.243–248. Available at: http://dx.doi.org/10.1046/j.1365-2907.2000.00073.x.

McNicol C. M., Bavin D., Bearhop S, Bridges J., Croose E., Gill R., Goodwin C. E., Lewis J., MacPherson J., Padfield D., Schofield H., Silk M. J., Tomlinson A. J., McDonald R. A., 2020a. Postrelease movement and habitat selection of translocated pine martens *Martes martes*. *Ecology and evolution*, 10(11), pp.5106–5118. Available at: https://www.vwt.org.uk/wp-content/uploads/2021/01/McNicol-Postrelease-movement-and-habitat-selection-of-translocated-pine-martens.pdf.

McNicol C. M., Bavin D., Bearhop S., Ferryman M., Gill R., Goodwin C. E., MacPherson J., Silk M. J., McDonald R. A., 2020b. Translocated native pine martens Martes martes alter short-term space use by invasive non-native grey squirrels Sciurus carolinensis. *The journal of applied ecology*, 57(5), pp.903–913. Available at: http://dx.doi.org/10.1111/1365-2664.13598.

Nie P., Yang R., Feng J., 2023. Future Range Dynamics Suggest Increasing Threats of Grey Squirrels (Sciurus carolinensis) against Red Squirrels (Sciurus vulgaris) in Europe: A Perspective on Climatic Suitability. *Europe: A Perspective on Climatic Suitability. Forests*, 14(6).

O'Meara D. B., Sheehy E., Turner P. D., Omahony D., Harrington A. P., Denman H., Lawton C., Macpherson J., Reilly C., 2014. Non-invasive multi-species monitoring: real-time PCR detection of small mammal and squirrel prey DNA in pine marten (Martes martes) scats. *Acta Theriologica*, 59, pp.111–117.

Page M. J., McKenzie J. E., Bossuyt P. M., Boutron I., Hoffmann T. C., Mulrow C. D., Shamseer L., Tetzlaff J. M., Akl E. A., Brennan S. E., Chou R., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ (Clinical research ed.)*, 372, p.n71. Available at: http://dx.doi.org/10.1136/bmj.n71.

Ridding L. E., Watson S. C., Newton A. C., Rowland C. S., Bullock J. M., 2020. Ongoing, but slowing, habitat loss in a rural landscape over 85 years. *Landscape Ecology*, 35(2), pp.257-273. Available at: http://dx.doi.org/10.1007/s10980-019-00944-2.

Roberts M. G., Heesterbeek J. A. P., 2021. Infection dynamics in ecosystems: on the interaction between red and grey squirrels, pox virus, pine martens and trees. *Journal of the Royal Society, Interface*, 18(183), p.20210551. Available at: http://dx.doi.org/10.1098/rsif.2021.0551.

Rushton S. P., Lurz P. W., Gurnell J., Nettleton P., Bruemmer C., Shirley M. D., Sainsbury A. W., 2006. Disease threats posed by alien species: the role of a poxvirus in the decline of the native red squirrel in Britain. *Epidemiology and infection*, 134(3), pp.521–533. Available at: http://dx.doi.org/10.1017/S0950268805005303.

Sainsbury K. A., Shore R. F., Schofield H., Croose E., Campbell R. D., Mcdonald R. A., 2019. Recent history, current status, conservation and management of native mammalian carnivore species in Great Britain. *Mammal review*, 49(2), pp.171–188. Available at: http://dx.doi.org/10.1111/mam.12150.

Santicchia F., Wauters L. A., Tranquillo C., Villa F., Dantzer B., Palme R., Preatoni D., Martinoli A., 2022. Invasive alien species as an environmental stressor and its effects on coping style in a native competitor, the Eurasian red squirrel. *Hormones and behavior*, 140(105127), p.105127. Available at: http://dx.doi.org/10.1016/j.yhbeh.2022.105127.

Sheehy E., Lawton, C., 2014b. Population crash in an invasive species following the recovery of a native predator: the case of the American grey squirrel and the European pine marten in Ireland. *Biodiversity and conservation*, 23(3), pp.753–774. Available at: http://dx.doi.org/10.1007/s10531-014-0632-7.

Sheehy E., O'Meara D. B., O'Reilly C., Smart A., Lawton C., 2014a. A non-invasive approach to determining pine marten abundance and predation. *European journal of wildlife research*, 60(2), pp.223–236. Available at: http://dx.doi.org/10.1007/s10344-013-0771-2.

Sheehy E., Sutherland C., O'Reilly C., Lambin X., 2018. The enemy of my enemy is my friend: native pine marten recovery reverses the decline of the red squirrel by suppressing grey squirrel populations. *Proceedings. Biological sciences*, 285(1874), p.20172603. Available at: http://dx.doi.org/10.1098/rspb.2017.2603.

Shorten M., 1957. Squirrels in England, Wales and Scotland, 1955. *The journal of animal ecology*, 26(2), p.287. Available at: http://dx.doi.org/10.2307/1748.

Slade A., White A., Kortland K., Lurz P. W., 2021a. Natural strongholds for red squirrel conservation in Scotland. *Nature Conservation*, 43, pp.93–108. Available at: http://dx.doi.org/10.3897/natureconservation.43.62864.

Slade A., White A., Kortland K., Lurz P.W., 2020. An assessment of long-term forest management policy options for red squirrel conservation in Scotland. *Hystrix, The Italian Journal of Mammalogy*, *31*(2).

Slade A., White A., Lurz P. W., Shuttleworth C., Lambin X., 2022. A temporal refuge from predation can change the outcome of prey species competition. *Oikos (Copenhagen, Denmark)*, 2022(9). Available at: http://dx.doi.org/10.1111/oik.08565.

Slade A., White A., Lurz P. W., Shuttleworth C., Tosh D. G., Twining J. P., 2023. Indirect effects of pine marten recovery result in benefits to native prey through suppression of an invasive species and a shared pathogen. *Ecological modelling*, 476(110216), p.110216. Available at: http://dx.doi.org/10.1016/j.ecolmodel.2022.110216.

Slade A., White, A., Lurz, P., 2021b. The Impact of Pine Marten Predation on Red Squirrel Conservation. *Red Squirrel Trust Wales*. [online report]. Available at: https://www.macs.hw.ac.uk/~awhite/PM_Wales.pdf.

Trites A.W., Joy R., 2005. Dietary analysis from fecal samples: How many scats are enough? *Journal of mammalogy*, 86(4), pp.704–712. Available at: http://dx.doi.org/10.1644/1545-1542(2005)086[0704:daffsh]2.0.co;2.

Turkia T., Jousimo J., Tiainen J., Helle P., Rintala J., Hokkanen T., Valkama J., Selonen V., 2020. Large-scale spatial synchrony in red squirrel populations driven by a bottom-up effect. *Oecologia*, 192(2), pp.425–437. Available at: http://dx.doi.org/10.1007/s00442-019-04589-5.

Twining J. P., Lawton C., White A., Sheehy E., Hobson K., Montgomery W. I., Lambin X., 2022a. Restoring vertebrate predator populations can provide landscape-scale biological control of established invasive vertebrates: Insights from pine marten recovery in Europe. *Global change biology*, 28(18), pp.5368–5384. Available at: http://dx.doi.org/10.1111/gcb.16236.

Twining J. P., Montgomery W. I., Tosh D. G., 2020a. The dynamics of pine marten predation on red and grey squirrels. *Mammalian biology*, 100(3), pp.285–293. Available at: http://dx.doi.org/10.1007/s42991-020-00031-z.

Twining J. P., Montgomery W. I., Tosh D. G., 2021. Declining invasive grey squirrel populations may persist in refugia as native predator recovery reverses squirrel species replacement. *The journal of applied ecology*, 58(2), pp.248–260. Available at: http://dx.doi.org/10.1111/1365-2664.13660.

Twining J.P., Montgomery I., Price W., Kunc H. P., Tosh D. G., 2020b. Native and invasive squirrels show different behavioural responses to scent of a shared native predator. *Royal Society open science*, 7(2), p.191841. Available at: http://dx.doi.org/10.1098/rsos.191841.

Twining, J.P., Sutherland C., Reid N., Tosh D. G., 2022b. Habitat mediates coevolved but not novel species interactions. *Proceedings. Biological sciences*, 289(1966), p.20212338. Available at: http://dx.doi.org/10.1098/rspb.2021.2338.

Paper 2

RED SQUIRREL IN MID WALES: A REVIEW OF LONG-TERM MONITORING DATA AND RECOMMENDATIONS FOR FUTURE CONSERVATION MANAGEMENT

By

ALICE S. CHAPMAN

ABSTRACT:

Once widespread in Wales, red squirrel (*Sciurus vulgaris)* are now limited to just three fragmented populations here. Driven by the incursion of the invasive grey squirrel (*Sciurus carolinensis*), through resource competition and the introduction of the fatal squirrelpox virus. The Wildlife Trust of South and West Wales have managed a population in Mid Wales for over two decades. Here the long-term monitoring data from camera and live trapping is collated and spatially analysed using density heatmaps in GIS software. The changes in detection frequency of red and grey squirrel, and pine marten (*Martes martes*) are analysed over the last five years, to attempt to identify drivers of shifting population dynamics. Due to issues with missing data, while this study was unable to define specific observed changes with confidence, it provides a starting point for the development of improved monitoring methods. These are explored and a preliminary plan for future monitoring and research is outlined, with a focus on standardising data collection and handling procedures.

1. Introduction

Sciurus vulgaris, (red squirrel), were once widespread across Wales but like most of the British Isles, populations have declined significantly, resulting in localised extinctions (Lurz, 2014). They are classed as endangered in England and Wales and near threatened in Scotland (UK Squirrel Accord, 2023). Although a combination of drivers can be attributed to the decline of this species, the introduction and rapid dispersal of the invasive of *Sciurus carolinensis* (grey squirrel) during the 19^{th} and 20^{th} century, has been the primary culprit in diminishing red squirrel populations (Gurnell *et al,* 2004; Chantry *et al,* 2014).

Grey squirrels live at higher population densities, dominating food resources through competitive exclusion, causing weight loss, reducing winter survival rates, increasing stress, and leading to poor reproductive success in red squirrels (Bryce *et al,* 2001; Gurnell *et al,* 2004). Grey squirrels also introduced squirrelpox virus (SQPV), while they act as asymptomatic carriers, it proves fatal when transmitted to red squirrels (Chantrey *et al,* 2014).

The red squirrel is one of our most iconic, native small mammals. They provide key ecosystem services in coniferous forests, helping to disperse tree seeds and fungi (UK Squirrel Accord, 2023). Without active, targeted conservation strategies, it is likely the remaining populations will be lost. It is imperative that every effort is made to prevent this. Ongoing pressure on ecosystems and inaction in Wales, make it one of the most nature depleted countries on Earth (State of Nature Report, 2023).

Following the arrival of grey squirrels in mid Wales in the late 1950s, the red squirrel population here retreated to the upper Tywi valley, occupying an extensive coniferous forestry plantation in the Cambrian Mountains (WTSWW, 2017). The area is bordered by extensive open upland habitat, offering partial refuge across an area of over 15,000 hectares (WTSWW, 2017). This is one of only three red squirrel populations remaining in the entirety of Wales (Slade *et al,* 2023).

Since its inception in 2002, the Mid Wales Red Squirrel Partnership (MWRSP) has worked to conserve and monitor this remnant isolated population of red squirrel (WTSWW, 2017). MWRSP also actively works to mitigate the impact of grey squirrels in and around the site, while promoting sympathetic forest management (WTSWW, 2017). Since 2014, the Wildlife Trust of South and West Wales (WTSWW) has led most of the work undertaken in the focal area of the project.

A limited genetics study at the site found this population as particularly significant for Wales, in that two of the five haplotypes (genetic markers) identified had never been previously recorded outside of Ireland, and the other three, while recorded in Wales, had never been identified in the same locality (WTSWW, 2017). There were however issues with obtaining enough data to assess the overall genetic diversity of the population.

Additionally, pine marten (*Martes martes*) reintroductions were undertaken by the Vincent Wildlife Trust (VWT) around 20km from the focal area (McNicol *et al,* 2020a) and they have since dispersed and have been recorded within the site for several years. This is significant as emerging research has associated the recovery of this native predator as a form of biological control of grey squirrel (Sheehy *et al,* 2018).

Other long-term monitoring of the study area has consisted of primarily opportunistic and exploratory data collection, through camera trapping, live trapping, and public reported sightings, rather than a structured sampling design. Here the methods utilised so far by WTSWW are reviewed, with the aim of establishing if these have been successful in providing indicative data on red squirrel population estimates and dynamics. Sighting and trapping data of pine marten and grey squirrel will also be included in analysis, considering the impacts of a native predator and invasive competitor.

Results of the reviewed data will contribute to exploring and developing new methodologies to improve long term monitoring for this population and identify gaps in knowledge for future research.

2. Methods

2.1. Study Area

All data included in analysis collected within the focal area (Fig. 1) of the MWRSP in Tywi Forest, Mid Wales.

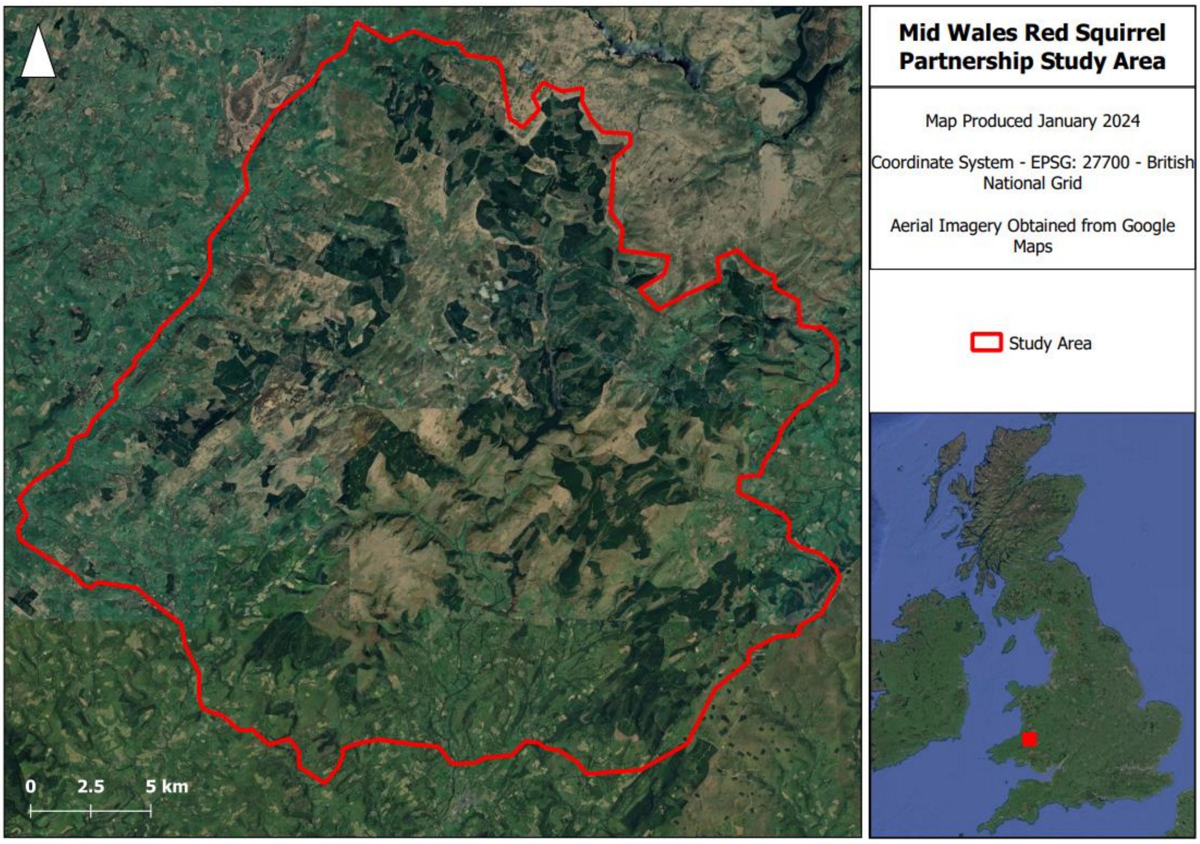

Figure 1. Map of Mid Wales Red Squirrel Partnership Study Area.

2.2. WTSWW Data Collection

2.2.1. Camera Trapping

Camera trapping carried out by combination of WTSWW staff and volunteers, with locations informed by on the ground staff through local knowledge and scoping surveys (identifying feeding signs etc). Various camera traps used, with both video footage and photographs collected. No standardised set-up of cameras, varying on a case-by-case basis.

Camera trap footage is reviewed by WTSWW staff and volunteers, no analysis software utilised. Data compiled in series of Microsoft Excel Spreadsheets and returned to WTSWW staff. No standardised reporting method.

2.2.2. Live Trapping – Grey Squirrel

Live trapping of grey squirrel carried out by WTSWW staff and trained volunteers, through trap loan scheme, as part of grey squirrel management plan. All trapping carried out by WTSWW staff recorded, intermittent reporting from volunteers. Traps baited and left unset initially to habituate squirrels before being set. Any grey squirrels caught are humanely dispatched, bycatch are released.

2.2.3. Live Trapping – Red Squirrel

Live trapping of red squirrel carried out by WTSWW staff under appropriate species licences. This has been undertaken for variety of purposes, including genetic study, individual identification, and health checks. Traps baited and left unset initially to habituate squirrels before being set. All trapping recorded, red squirrels and other bycatch are released, grey squirrel dispatched.

2.3. Collation of Secondary Data

Secondary data provided by WTSWW across multiple Microsoft Office Excel spreadsheets. Due to the variety of formats and reporting styles of staff and volunteers, a master spreadsheet was created to pull all data into a standardised format. Target species of red squirrel, grey squirrel and pine marten were pulled from the various datasets into this format.

Large quantities of data were omitted, due to improper recording, for example where date ranges and number of clips were provided, rather than specific dates, times, and number of individuals. There was also inconsistent recording in terms of age and sex, with the majority of records lacking this information. All included data provided location in either grid reference or latitude and longitude, date, species, and number of individuals.

Although records from 2015 to 2023 were originally included in the collated data, earlier records would be omitted from spatial and quantitative analysis, due to small datasets and inconsistent recording periods during this time. As such the final dataset would cover a five-year period, between 2019 to 2023.

There are more records from this monitoring period that cannot be included in this study due to access and data management issues at this time. As such, this must be considered in the analysis.

2.4. Quantitative Analysis

Due to lack of absence data, options for meaningful statistical analysis were lacking. As such quantitative analysis was undertaken in Microsoft Office Excel. Where changes in species sightings between years were calculated using calculative functions.

2.5. Spatial Analysis

All usable data were converted to CSV files and point data into QGIS version 3.4.1 (Madeira). Frequency of species occurrence were explored and displayed by location and used to create a density heatmap for each year of the included dataset. Allowing species density changes to be spatially compared. Aerial imagery obtained from Google Maps.

3. Results

Table 1. Calculated percentage change in frequency of species records from camera trap and live trapping data.

Year	Percentage Change in Sightings from Previous Year		
	Red Squirrel	Grey Squirrel	Pine Marten
2019-2020	+1778.57%	+2362.79%	+250%
2020-2021	-71.74%	-82.44%	+177.92%
2021-2022	-43.95%	-71.51%	+52.82%
2022-2023	-60.80%	+16.98%	-44.65%

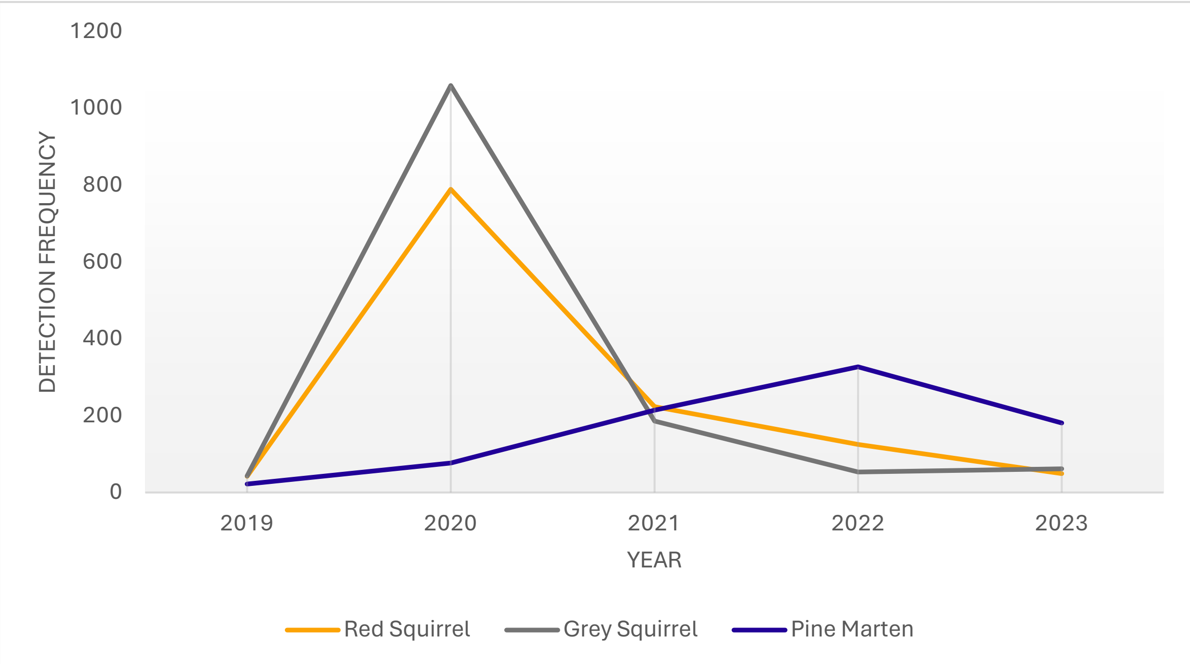

Figure 2. Line graph displaying frequency of species detections from camera and live trapping data. 3.2. Spatial Analysis

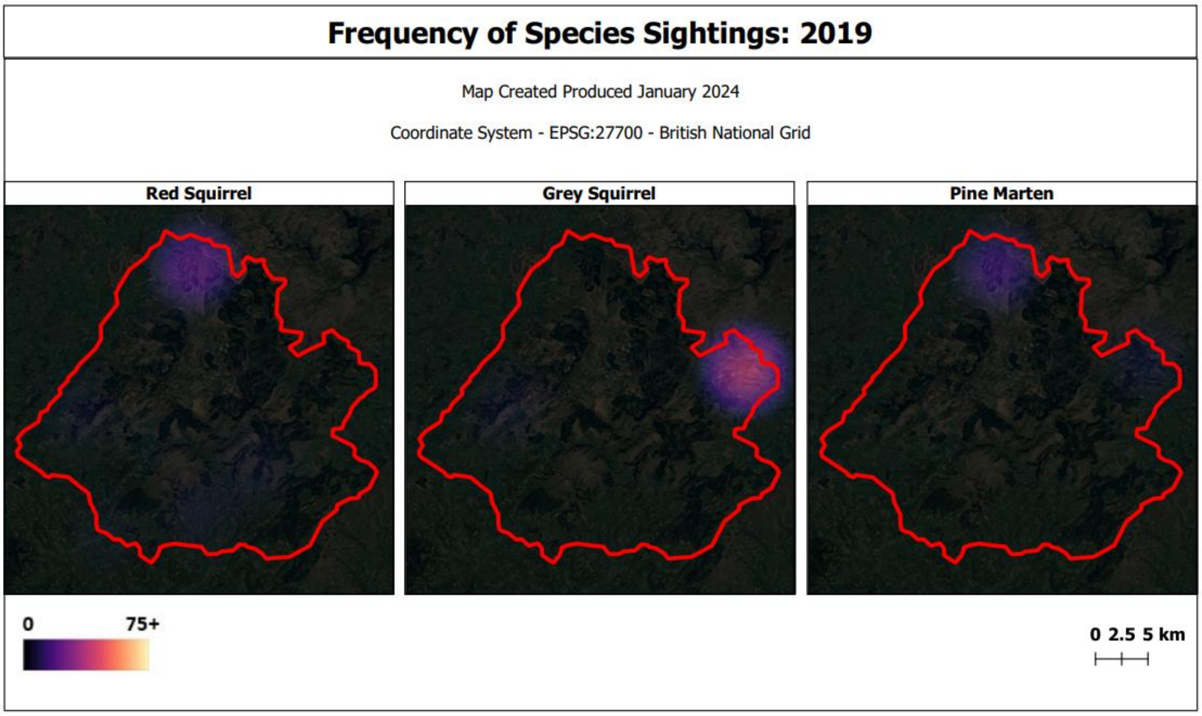

Figure 3. Heatmap displaying frequency of species sightings from collated 2019 survey data.

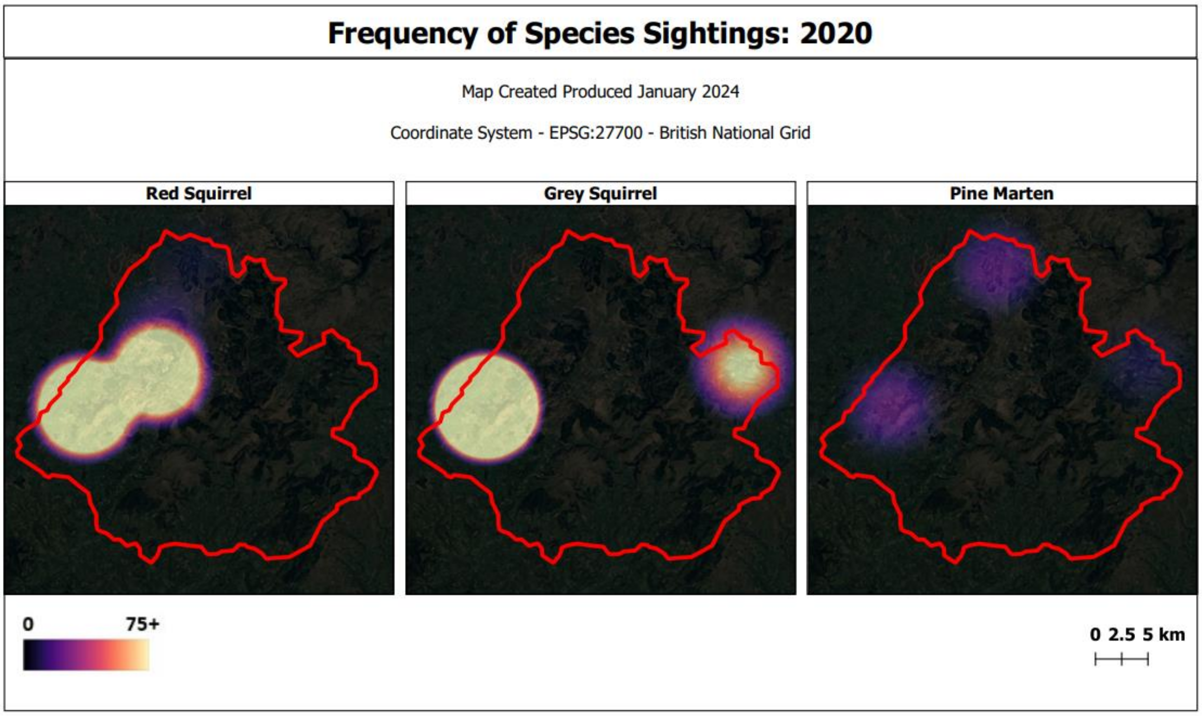

Figure 4. Heatmap displaying frequency of species sightings from collated 2020 survey data.

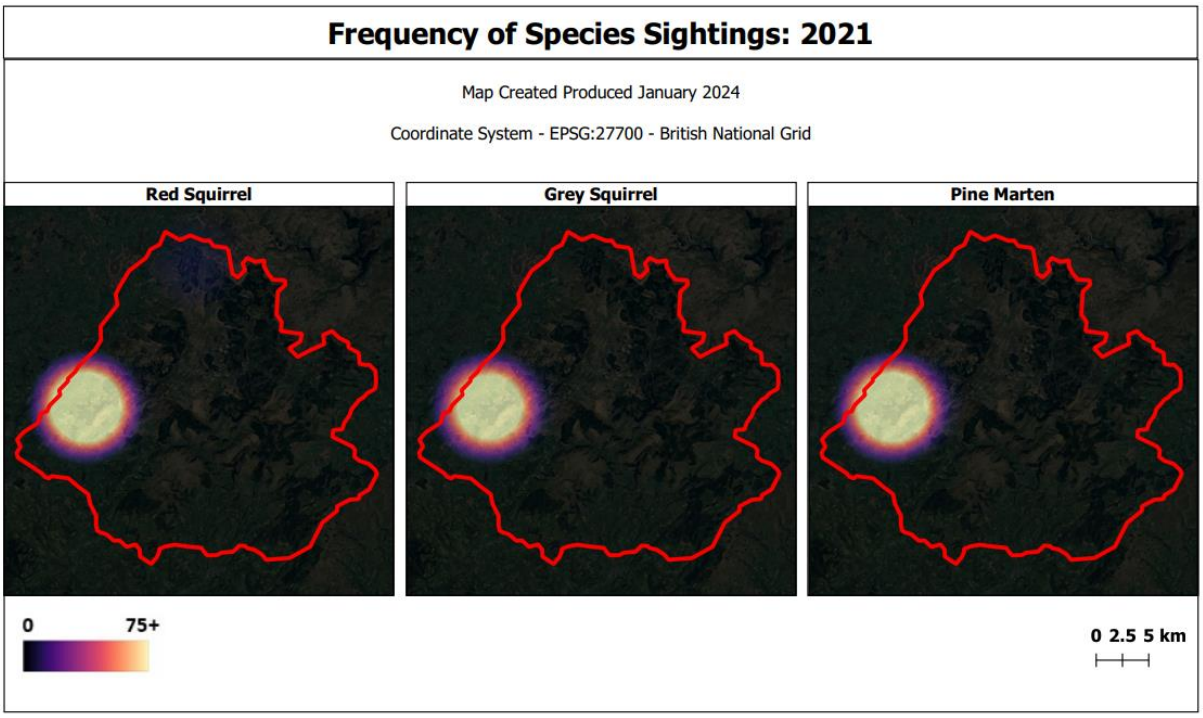

Figure 5. Heatmap displaying frequency of species sightings from collated 2021 survey data.

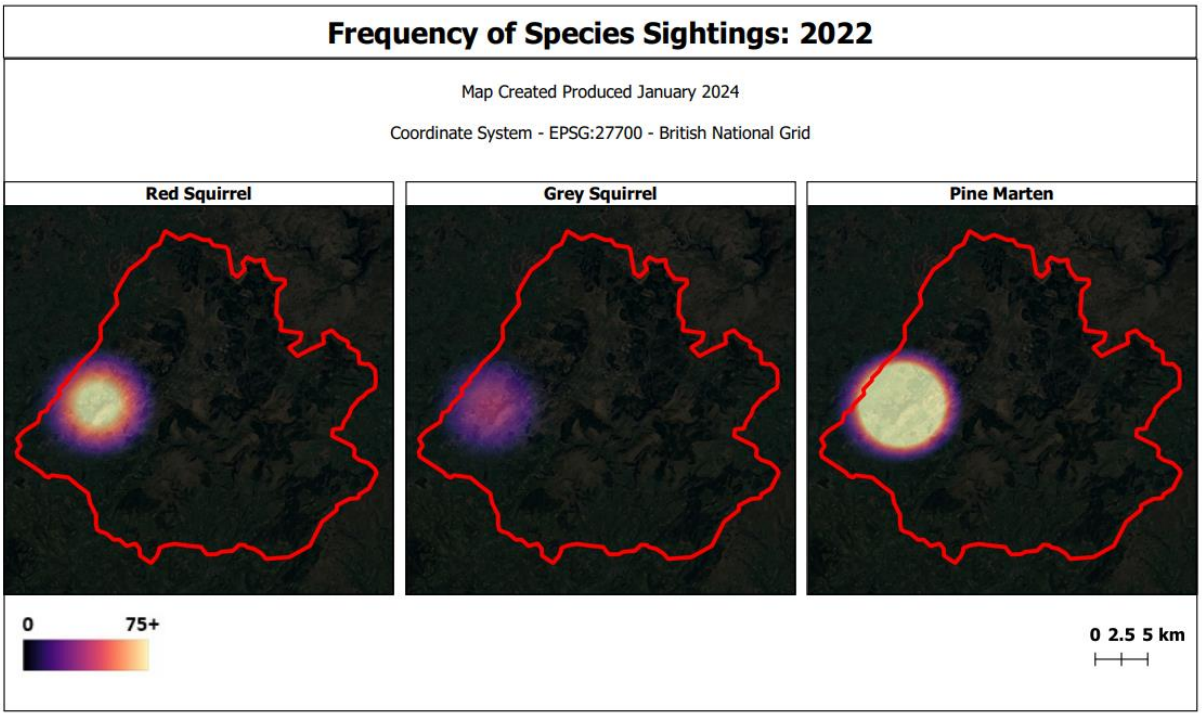

Figure 6. Heatmap displaying frequency of species sightings from collated 2022 survey data.

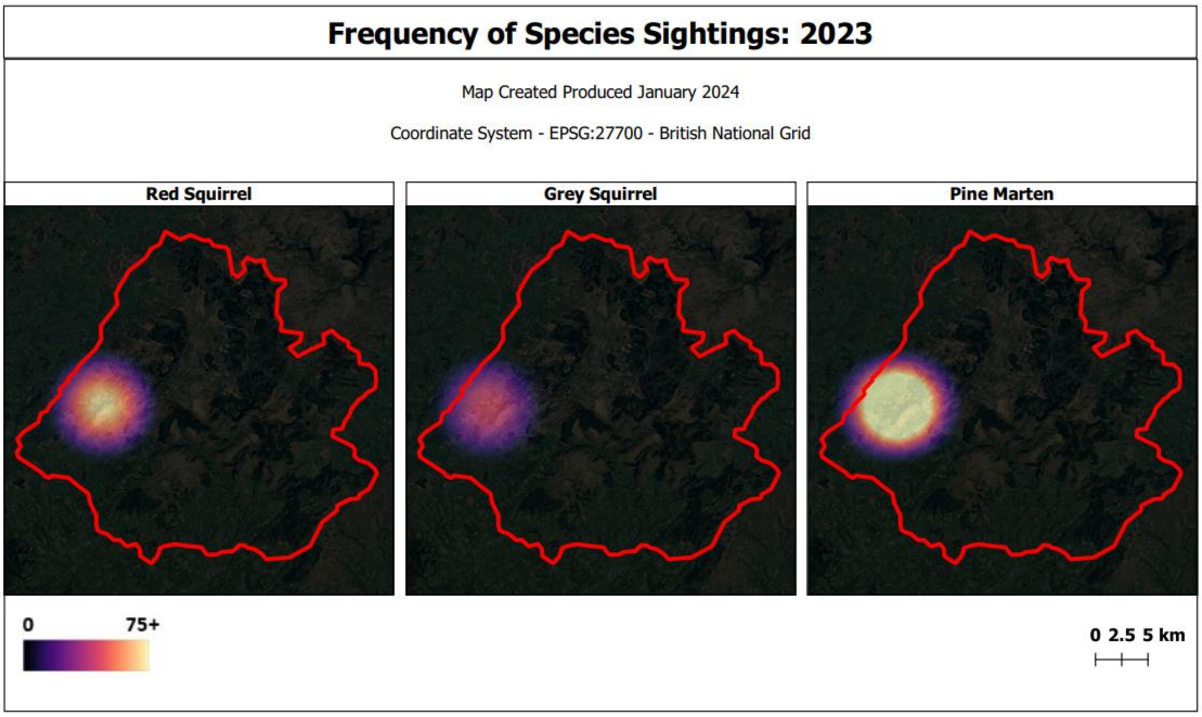

Figure 7. Heatmap displaying frequency of species sightings from collated 2023 survey data.

4. Discussion

4.1. Discussion of Results

4.1.1. 2019

From the reviewed data, a total of 42 red squirrel sightings were recorded in 2019 (Fig 2.), 41 of these from camera trapping data and 1 one from live trapping. Grey squirrels were recorded 43 times (Fig. 2), only from camera trapping data. Since grey squirrel trapping has been ongoing since the inception of MWRSP, it seems unlikely no successful culling occurred this year, and more likely reflects missing or unrecorded data. Most red squirrel sightings occurred in the north of the focal areas (Fig. 3), though there were low frequencies recorded across south and west of the site.

It appears the majority of grey squirrel incursion stemmed from the eastern side of the site (Fig. 3). This may indicate less effective control in this region and perhaps fewer landscape barriers to the study area. Pine marten sightings for this year were low, but this is unsurprising since the reintroductions occurred between 2015-2017 (McNichol *et al*, 2020) so dispersal is only just beginning at this stage. The fact that the first pine marten movements into the area occur in the north (Fig 3.) confirms it is likely from this reintroduced population and not elsewhere. Overall, the frequencies of sightings for this year are low for all species, indicating either missing data or lesser rates of camera and live trapping.

4.1.2. 2020

The sighting data from 2020 is much larger, with a total of 789 red squirrel sightings (Fig 2.), (774 from camera, 15 from live traps). This is a 1778% increase (Table 1.) in frequency of sightings from the previous year. It seems likely that the role of volunteers during the Covid-19 pandemic may have contributed to this large dataset from camera traps. The distribution of sightings shifts here, with most detections in the west of the focal area (Fig. 4), with much lower detections in the north than the previous year.

Grey squirrel detections also increase dramatically on from the previous year, with 1059 total (Fig. 2), 24 of which were live trappings (and successful culls). This is a concerning 2362% increase (Table 1.) on the previous year, though due to the comparably large data set, is a more accurate representation of grey squirrel density across the site. As well as the continuing incursion from the east, there is a very high detection rate in the west (Fig. 4).

This is concerning as red and grey squirrel do not usually co-occur for very long, with the greys out competing the reds in as little as 5 years (Atkin *et al,* 2010), due to SQPV transmission and resource competition. However, the grey squirrels tend to less frequently remain in areas of forestry plantation, occurring in the bordering broadleaf woodlands (Slade *et al,* 2020; WTSWW, 2017).

Pine marten detections also increase this year, by 250% (Table 1.), a total of 77 sightings (Fig. 2). They appear to disperse more widely across the study area (Fig. 4), including in the west where the highest red and grey squirrel densities are observed. This provides the opportunity to potentially observe density changes in the squirrels, driven by their presence.

4.1.3. 2021

Squirrel detection rates for both species drop considerably this year, red by 71% and grey by 82% (Table 1.) with 9 recorded dispatches. This coincides with a 177% increased detection rate in pine marten, which could infer direct predation on both squirrel species, or perhaps more cautious behaviour or dispersal in response to predator presence (Sheehy *et al,* 2018). This however can only be speculated, though highlights the need for investigation. All three species occur at the greatest density in the west (Fig 5.), though this may also be indicative of more active monitoring in this area due to pine marten presence. Dietary analysis studies on pine marten, indicate squirrels generally occur at low frequencies in their diet, with grey being more prevalent where both species are present (O'Meara *et al,* 2014). Additionally, they have been observed as drivers of grey squirrel population crashes in Ireland (Sheehy *et al,* 2014), while also having positive associations with red squirrel populations (Sheehy *et al,* 2018). However, studies are limited, and dynamics likely vary in each case.

4.1.4. 2022

Squirrel detection rates continue to decrease this year, red by 44% and grey by 71% (Table 1). Pine marten again increasing by 52%, with all species continuing to be detected at the greater density in the west (Fig 6). Again, if not skewed by site selection bias to this area, and even perhaps so, the presence of pine marten does appear to be having an impact on squirrels in this area, more so greys. Whether the squirrels are being heavily predated on or are simply dispersing into the buffer of the focal area, can only be speculated. However, there is one instance of camera trap data describing a pine marten with a kill, identified as 'possibly grey squirrel', which is a promising sign of this occurring. But without reviewing the clip, and ambiguous identification, this could well be a red squirrel. This is also the first year that pine marten kits are identified on camera trap, indicating they are breeding in the area. Unsurprising, given their increasingly high rate of detection.

4.1.5. 2023

Red squirrel detection again decreased from the following year, by 60% (Table 1). On the other hand, grey squirrel detection increased by 16%, while pine marten detection decreased by 44% (Table 1). This again was concentrated at the west of the site (Fig. 7), likely somewhat skewed due to staff monitoring actively undertaken here, with more reliable reporting of results than volunteer data. This does appear somewhat concerning, but when reviewing the number of red squirrel detections in 2019 at 42, compared to 49 detections in 2023 (Fig. 2), this is less stark.

4.1.6. Summary of Findings and Implications for Mid Wales Red Squirrel Populations

The greatest number of detections of both squirrel species occurred in 2020 (Fig. 2), and in 2022 for pine marten. This was also the lowest detection year for grey squirrel, which as discussed could be an indication of pine marten predation. Pine marten have been observed altering grey squirrel ranging behaviour, with males recorded travelling larger distances in the presence of this novel predator (McNicol *et al,* 2020b)). However, in another study, the use of pine marten scent on feeder stations found grey squirrels would actively visit these stations more readily than the unscented controls (Twining *et al,* 2020). It seems there is substantial variation in response to pine marten presence, warranting investigation into this dynamic in the MWRSP focal area.

While the lowest detection year for red squirrel was 2023, it is worth noting records run to mid-October, and there is almost certainly missing data due to delayed volunteer reporting. 2020 detection rates certainly appear as an outlier, likely due to greater reporting of data due to the pandemic. However, cyclic fluctuations of populations cannot be ruled out. *Sciuridae* and other rodent species commonly experience population booms and crashes, often driven by food resource availability, disease, and predation (Wauters *et al,* 2008). This was potentially a high coning year, and considering the pandemic, it is possible less disruptive forestry management was undertaken.

Ignoring the limitations of the dataset, a population increase of both squirrel species occurred in 2020, which was subsequently diminished by the reestablishment of a native predator, causing squirrel detections to return to levels more similar to those observed in 2019. It is certainly unusual, even given the limitations of the dataset, that following this, pine marten are detected at a higher rate than both squirrel species from 2021 onward (Figure 2).

4.2. Limitations

Ultimately, only speculations can be made based on the reviewed data. With the awareness that there are record gaps, likely due to under reporting from volunteers, and omitted data due to reporting quality. Absence data would be extremely valuable here and contribute to giving a greater understanding of overall occupancy. Without an understanding of the number of cameras and traps set, and monitoring start and ends points, this is difficult to achieve. Data is also almost certainly skewed in terms of density distributions, due to the areas where staff surveying is more readily undertaken.

Due to this, actual population dynamics and estimates of the three species cannot be concluded. Based on what has been observed however, it certainly highlights the need for improved methods of data recording, and areas for further research.

4.3. Moving Forward – Next Steps for Mid Wales Red Squirrel Partnership

It is clear from the presented data that current monitoring methods cannot infer detailed accurate information on current populations, other than observe density changes in areas of concentrated monitoring. It is important to consider conservation projects often have inconsistent funding streams and limited resources. Meaning that baseline monitoring might be the only feasible data that can be gathered on species of conservation concern. As such, developing standardised methodologies, proper data organisation and storage can help in providing consistent long-term data, which can be analysed to conclude population estimates and observe potential changes in population dynamics. In the event of population decline, disease outbreak etc, this data becomes an invaluable resource of information.

Presented here are proposed next steps for the continuation and improvement of the approaches to long term monitoring methods and research for the success of this project and conserving the red squirrel population in Mid Wales.

4.3.1. Long Term Monitoring – Data Recording

One of the highest priority adjustments to be made is data recording. If there is no feasibility or funding to alter current practices, this is the bare minimum which will enable vastly more valuable data analysis. Firstly, a proper record of all deployed camera traps, a catalogue specifying monitoring periods, location, and camera ID so information can be grouped by location, and any issues can be recorded (Table 2).

Table 2. Example format for camera trap location recording.

Camera ID	Location Name	Lat	Long	Deployed	Monitoring End	Removed	Notes
4	Bryn Mawr	52.13803	-3.9511377	05/06/2022	02/07/2022	12/07/2022	SD card full, moving branch triggering. Moved to better location.

If dates where cameras are actively running are recorded, the gaps in the trapping analysis can be considered absence data. This could help develop strategies like occupancy modelling, as well as simply providing insight on where there is missing data, where trapping efforts should be prioritised.

Secondly, a standardised form (Table 3) for both staff and volunteers should be developed, with the needs of the monitoring in mind.

Table 3. Example of camera trap recording format.

Location	Date	Start Time	LAT	LONG	Grid Reference	File Number(s)	Number of Clips	Species	Sex	Life Stage	Activity	Comments
Bryn Mawr	09/11/2023	19:24			SN 667 507	23, 24, 25	3	Red Squirrel	NA	NA	Feeding	Eating pine nuts, followed by resting before moving on
Bryn Mawr	13/11/2023	07:32			SN 667 507	42	1	Pine Marten	NA	Sub Adult	NA	Walks across trail, does not stop

From observations of volunteer recording, they like to comment on what they have observed. Separating species observed, sex and age, whilst having a comments box allows the extraction of key information, while allowing volunteers to provide more comprehensive information if they so wish. This may also provide additional insight, perhaps creating opportunity for behavioural studies.

A volunteer training and engagement event would be extremely valuable here. Bringing them up to speed with WTSWW and MWRSP goals, giving explanation as to why a standardised format being brought in will contribute to their time being used more productively. All data should be stored in a central online database, to mitigate against risk of data loss and accidental omission.

A standardised set up of camera traps would also be valuable in terms of ease of collating of data and consistent reporting. Though the reality of volunteer uptake on this will likely vary. Utilising camera trapping analysis software would also be worth investigation, or design of an internal system like MammalWeb.org, for citizen science opportunities.

Where it occurs, staff live trapping data could easily be pulled into a similar format, so data can be combined. The grey squirrel trapping volunteer data also needs to be collected more regularly and combined with other monitoring data. It will highlight areas in need of more careful monitoring for red squirrel population health.

Following these practices would allow for repetition of this study, whilst giving more meaningful insights on population dynamics using further spatial analysis with the addition of occupancy modelling. Occupancy models are hierarchical statistical models designed to simultaneously estimate two key ecological processes: occupancy and detection probability (Sheehy *et al,* 2018). This is particularly useful in studies aiming to understand the spatial distribution of a species across multiple sites, while considering the realities of imperfect detection of that species across multiple surveys.

4.3.2. Population Estimates

A possible approach to gaining accurate population estimates of red squirrel in Mid Wales would be to utilise capture-recapture techniques. Employing QGIS to generate random sample points to remove selection bias (Sauvé et al, 2022), and placing camera traps at these locations across the site. Conducting surveys over multiple occasions to increase the chances of capturing individuals and to account for temporal variations. Recording detection data for each capture event, specifying the individual, location, and occasion. Using the collected data to estimate population size using statistical models, such as spatially explicit capture-recapture (SECR) models (Sheehy *et al,* 2018).

The issue presented here is with identifying recaptured animals, which can be difficult using just physical appearance. However, if there is ongoing live trapping for other monitoring purposes, ear tagging, or tail marking could be considered. Additionally with developing AI techniques, there may be scope for this to be a completely automated process in the future (Santangeli *et al,* 2022). However, the viability of such methods need to be considered within the capabilities of a conservation organisation with limited resources.

4.3.3. Genetic Analysis

Genetic studies should be high priority, to assess the long-term genetic viability of red squirrel in Mid Wales. Sheehy *et al* (2018) had considerable success collecting pine marten and squirrel hair samples using multi-species feeders, equipped with 15 × 15 × 15 cm wooden boxes, and baited with nuts and seeds. Glue strips on the lids collected hair samples, which were then collected, dried, and preserved at -20°C to maintain DNA integrity. Species identification was conducted based on colour, shape, and size using a dissection microscope (Sheehy *et al,* 2018). This study only focussed on PCR DNA amplification for pine marten, but this could be extended to red and grey squirrel, either through microsatellite or haplotype identification.

4.3.4. Population Reinforcement

Pending results of population estimating and genetic analysis, it may be necessary to consider population reinforcement. Special consideration should be given to source populations, and the associated effects this would have on the genetic composition of the Mid Wales red squirrels. Although this should be a last resort, this could also provide opportunities for public engagement and income generation.

4.3.5. Other Considerations

Other considerations should include radical approaches to engaging the wider public about invasive species control. In the case of the grey squirrel, support can be difficult to obtain when dealing with a 'fluffy' animal, that the public misguidedly view as part of British Wildlife. But greater positive engagement will naturally have positive effects.

Greater collaboration with wider conservation organisation will also be paramount, to gain wider resources for further reaching research.

5. Conclusion

While population dynamics and densities of the three species could not be accurately concluded from the provided data, this study has highlighted the gaps in knowledge and improvements that can be made. Successfully applied, this will contribute to enhancing the conservation management of red squirrel in Mid Wales.

6. References

Atkin J. W., Radford A. D., Coyne K. P., Stavisky J., Chantrey J., 2010. Detection of squirrel poxvirus by nested and real-time PCR from red (Sciurus vulgaris) and grey (Sciurus carolinensis) squirrels. *BMC veterinary research*, 6(1), p.33. Available at: http://dx.doi.org/10.1186/1746-6148-6-33.

Bryce J. M., Speakman J. R., Johnson P. J., Macdonald D. W., 2001. Competition between Eurasian red and introduced Eastern grey squirrels: the energetic significance of body-mass differences. *Proceedings. Biological sciences*, 268(1477), pp.1731–1736. Available at: http://dx.doi.org/10.1098/rspb.2001.1700.

Chantrey J., Dale T. D., Read J. M., White S., Whitfield F., Jones D., McInnes C. J., Begon M., 2014. European red squirrel population dynamics driven by squirrelpox at a gray squirrel invasion interface. *Ecology and evolution*, 4(19), pp.3788–3799. Available at: http://dx.doi.org/10.1002/ece3.1216.

Gurnell J., Wauters L. A., Lurz P. W., Tosi G., 2004. Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. *The journal of animal ecology*, 73(1), pp.26–35. Available at: http://dx.doi.org/10.1111/j.1365-2656.2004.00791.x.

Lurz P. W. W., 2014. Changing 'red to grey': alien species introductions to Britain and the displacement and loss of native wildlife from our landscapes. In: *Displaced Heritage: Responses to Disaster, Trauma, and Loss*. Suffolk: Boydell Press, 2014, pp. 265–272.

Mammalweb.org. Available at: https://www.mammalweb.org/en/ [Accessed 22 January 2024].

McNicol C. M., Bavin D., Bearhop S, Bridges J., Croose E., Gill R., Goodwin C. E., Lewis J., MacPherson J., Padfield D., Schofield H., Silk M. J., Tomlinson A. J., McDonald R. A., 2020a. Postrelease movement and habitat selection of translocated pine martens *Martes martes*. *Ecology and evolution*, 10(11), pp.5106–5118. Available at: https://www.vwt.org.uk/wp-content/uploads/2021/01/McNicol-Postrelease-movement-and-habitat-selection-of-translocated-pine-martens.pdf.

McNicol C. M., Bavin D., Bearhop S., Ferryman M., Gill R., Goodwin C. E., MacPherson J., Silk M. J., McDonald R. A., 2020b. Translocated native pine martens Martes martes alter short-term space use by invasive non-native grey squirrels Sciurus carolinensis. *The journal of applied ecology*, 57(5), pp.903–913. Available at: http://dx.doi.org/10.1111/1365-2664.13598.

O'Meara D. B., Sheehy E., Turner P. D., Omahony D., Harrington A. P., Denman H., Lawton C., Macpherson J., Reilly C., 2014. Non-invasive multi-species monitoring: real-time PCR detection of small mammal and squirrel prey DNA in pine marten (Martes martes) scats. *Acta Theriologica*, 59, pp.111–117.

Santangeli, A., Chen, Y., Boorman, M., Sales Ligero, S. and Albert García, G., 2022. Semi-automated detection of tagged animals from camera trap images using artificial intelligence. *Ibis*, *164*(4), pp.1123-1131.

Sauvé, C.C., Berentsen, A.R., Gilbert, A.T., Conan, A., Cruz-Martinez, L. and Leighton, P.A., 2022. Capture-Recapture Reveals Heterogeneity in Habitat-Specific Mongoose Densities and

Spatiotemporal Variability in Trapping Success in St. Kitts, West Indies. *Caribbean Journal of Science*, *52*(1), pp.63-81.

Sheehy E., Lawton, C., 2014. Population crash in an invasive species following the recovery of a native predator: the case of the American grey squirrel and the European pine marten in Ireland. *Biodiversity and conservation*, 23(3), pp.753–774. Available at: http://dx.doi.org/10.1007/s10531-014-0632-7.

Sheehy E., Sutherland C., O'Reilly C., Lambin X., 2018. The enemy of my enemy is my friend: native pine marten recovery reverses the decline of the red squirrel by suppressing grey squirrel populations. *Proceedings. Biological sciences*, 285(1874), p.20172603. Available at: http://dx.doi.org/10.1098/rspb.2017.2603.

Slade A., White A., Kortland K., Lurz P.W., 2020. An assessment of long-term forest management policy options for red squirrel conservation in Scotland. *Hystrix, The Italian Journal of Mammalogy*, *31*(2).

Slade A., White A., Lurz P. W., Shuttleworth C., Tosh D. G., Twining J. P., 2023. Indirect effects of pine marten recovery result in benefits to native prey through suppression of an invasive species and a shared pathogen. *Ecological modelling*, 476(110216), p.110216. Available at: http://dx.doi.org/10.1016/j.ecolmodel.2022.110216.

Twining J.P., Montgomery I., Price W., Kunc H. P., Tosh D. G., 2020b. Native and invasive squirrels show different behavioural responses to scent of a shared native predator. *Royal Society open science*, 7(2), p.191841. Available at: http://dx.doi.org/10.1098/rsos.191841.

UK Squirrel Accord, 2023. *England Red Squirrel Action Plan 2023 - 2028* [online]. Available at: https://s3-eu-west-1.amazonaws.com/media.squirrelaccord.uk/2023/01/England_Red_Squirrel_Action_Plan_2023-2028.pdf.

Wauters, L. A., Githiru, M., Bertolino, S., Molinari, A., Tosi, G. and Lens, L., 2008. Demography of alpine red squirrel populations in relation to fluctuations in seed crop size. *Ecography*, *31*(1), pp.104-114. Available at: doi.2007.0906-7590.05251.x

Wildlife Trust of South and West Wales (WTSWW), 2017. Management Plan 2017 - 2022 Mid Wales Red Squirrel Focal Site - Optimising Habitat Management for Red Squirrels. *Mid Wales Red Squirrel Partnership* [unpublished report].