UNIVERSITY OF WINCHESTER

Stereotypical Pacing in Captive Red Squirrels (*Sciurus vulgaris*) – Possible Aetiology

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The Thesis has been completed as a requirement

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Abstract

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British red squirrels (*Sciurus vulgaris*) are listed as endangered on the IUCN Red List due to the invasive grey squirrel (*Sciurus carolinensis*), introduced to the UK in 1876 which outcompete red squirrels for resources. Grey squirrels also carry squirrel poxvirus (SPQV), harmless to the hosts but fatal to red squirrels and the cause of localised extinction. Urgent conservation efforts, including captive breeding and release programmes, are required to prevent extinction of the wild population by 2040. However, stereotypical pacing is prolific in the captive population, starting post weaning and often continuing into adulthood, and although it is recognised as a sign of poor welfare little is understood as to why it occurs. The aim of this study is to observe the onset of stereotypical behaviour in captive individuals and how this behaviour develops over time, through different seasons and in different environments and to use keeper replies to a behavioural survey on the wider British captive red squirrel population to compare and corroborate observed data to determine the major predictors and possible aetiology of stereotypical behaviour in post-weaned, captive red squirrels. Two studies comprise this research project. The first, involved 500 hours of observations of the breeding enclosure and walkthroughs at Wildwood Escot (Devon) and Wildwood Kent captive red squirrels (n=16) over 18 months. The results showed significant differences between wild red squirrels and pacing red squirrels' behaviour ($p<=0.001$) and a strong negative correlation ($r=-0.6$) between feeding and stereotypical behaviour. There was also an absolute difference in the presentation of stereotypical behaviour before and after alterations to the breeding enclosure (addition of privacy boards, leaf pile substrate and hoarding containers). Study two involved sending a survey to eight zoos involved in captive breeding programmes, analysis of these completed surveys showed that stereotypical pacing is linked with dispersal needs in post weaned juveniles and foraging and hoarding stress in older squirrels. In conclusion, moving juveniles between 10-14 weeks old, increasing hoarding potential, offering seasonal diets, increasing enclosure complexity, and providing cover could potentially reduce stereotypical behaviour in captive red squirrels and improve welfare.

Key Words: Red Squirrel, Stereotypical Behaviour, Pacing, Hoarding, Dispersal.

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1. Introduction and Literature Review

1.1 Red Squirrel Ecology and Natural History

It is important to understand the ecology of Red Squirrels (*Sciurus vulgaris*) and how they interact with both living and non-living factors in their environments as this informs us what they need to survive and thrive and therefore informs the captive management of the species. Understanding the natural habitat, home range, food, and other resource selection and why and how their behaviour has developed allows zoos to understand the type of enclosure, resources, social group, substrate, diet, and other resources required to keep the species in captivity. Studying the ecology of animals and their natural history also highlights different changes in ecosystems that has or could affect the future survival of the species. Red squirrels are rodents, belonging to the order *Rodentia*, suborder *Sciurognathi* and grouped into the family *Sciuridae* (Bosch and Lurz, 2012), a collective group of arboreal, territorial rodents. The British red squirrel (*Sciurus vulgaris Linnaneus*) evolved in Britain during the last ice age, over 10,000 years ago (Mathews et al., 2018). There are 17 sub species of *Sciurus vulgaris* due to interbreeding across Europe, *Sciurus vulgaris vulgaris, Sciurus vulgaris varius* and *Sciurus vulgaris fuscoater* are the closest relatives to the British red squirrel (Holm, 2010).

There are approximately 140,000 wild British red squirrels, 80% of which are found in the Highlands of Scotland, with other small populations in Northern England, Ireland, Wales, and the Isle of Wight (RSS, 2020). Red squirrels live an average of five to six years in the wild, but up to eight years in captivity, where food supply is constant and there is protection from disease, predation, and inter-species competition (Gutziller and Riffel, 2008). Red squirrels are not sexually dimorphic, males and females are of a similar size, between 200 and 400 grams (Holm, 2010). Both sexes can be identified by their characteristic reddish fur, though different colour morphs are reported in different areas, with the lighter coloured squirrels being found in deciduous woodlands and darker red squirrels found in conifer forests (Bosh and Lurz, 2012). They have ear tufts which moult in summer, long slender bodies, and a characteristic long fluffy tail. The tail not only acts as a counterbalance, but as it has a vascular bundle underneath with a counter current heat exchange, when wrapped around the body it keeps the animal warm in winter and cool in summer (Holm, 2010; Bosch and Lurz, 2012). The tail is also a means of communication, particularly used in territorial displays (Holm, 2010). Red squirrels have a double-jointed ankle that can turn 180 degrees, which, together with long claws, allows them to run head-first down trees, a clever adaptation as it allows them to see any potential ground dwelling predators such as red foxes (*Vulpes vulpes*) and wildcats (*Felis sivlestris*) (Petty et al., 2003; Holm, 2010).

British red squirrels live in both broadleaf and coniferous forests (Wauters et al., 2011) and spend 80% of their time in the canopy where they are protected from both aerial and ground dwelling predators. They are solitary and territorial as is typical for the taxonomic group, selecting home ranges that have high food availability and good quality habitat of typically two - ten hectares with a core area of about one hectare (Gurnell, 1983; Wildlife-on-line, 2020). Males tend to have larger home ranges overlapping with several different female territories to maximise breeding potential, whilst females maintain stable ranges, the quality of the range dependent on social status, with dominant females establishing smaller home ranges but with higher quality resources than subordinate females (Holm, 2010). Most juveniles settle only 0.320-1.5km from their natal home range and mothers may bequeath part of their territory to their young (Wauters et al*.,* 2011; Siracusa et al., 2017). Red squirrels protect the core area of their territory with rattles, a territorial vocalisation, to reduce potentially costly physical conflicts with their neighbours (Shonfield et al., 2012; Siracusa et al., 2017) and scent marking by rubbing the ground and trees with scent glands found in their cheeks and hind quarters (Raynaud and Dobson, 2011; Ferkin et al., 2013). Due to intra- and inter-specific food competition, maintaining a territory is particularly important for individual survival.

Red squirrels are diurnal and bimodal, they are highly active both in the morning and again in the afternoon (Krauze-Gryz et al., 2016). Active time is spent mostly foraging, between 54%-85% of total daily activity (Wauters et al., 1992). Red squirrels have evolved to be omnivorous; their diet being seasonal. In autumn seeds from Spruce (*Picea*), Lodgepole pine (*Pinus contorta*), Larch (*Larix*), Hazel (*Corylus*), Beech (*Fagus*) and Horse Chestnut (*Aesculus hippocastanum*) trees form the vast majority of dietary intake (Salmoaso et al., 2009; Bosch and Lurz, 2012). In December fungi found naturally dried on tree branches makes up to 85% of their diet (Krauze-Gryz et al., 2016), while buds and shoots are consumed from the end of winter to early summer, providing 'extra' energy intake ready to condition the animals for breeding in the spring. During long daylight periods, red squirrel energy demands are sustained by the consumption of fruits and berries, the main dietary components from summer through to autumn (Holm, 2010). Red squirrels also eat insects, larvae, birds' eggs and even chicks, as supplementary protein and fat sources and pregnant females will maintain high calcium demands (to sustain pregnancy and lactation) by gnawing on shed deer antlers (Bosch and Lurz, 2012). Red squirrels' cache large quantities of food, particularly in autumn as they do not hibernate so require enough stored food supplies to survive winter (Edelman et al., 2012). Caching is therefore an important behaviour that further extends the time red squirrels spend foraging in the autumn months.

Red squirrels build dreys, ball-shaped nests, built into a fork of a tree, made of twigs, and lined with dry leaves, grass, moss and lichen for thermoregulation (Selonen et al., 2014). This is used for sleep and rearing young (Holm, 2010). Red squirrels build several dreys in their territory and regularly move between them to gain access to different resources and prevent parasitic overload (Edelman et al., 2009). Red squirrels are promiscuous; hence females allow different males to mate with them (Lane and Boutin, 2018), and consequently litters can comprise of up to 83% offspring with different fathers (Bonanno and Albrecht, 2009). Females become sexually mature at approximately 11 months of age, dependent on size and are only in oestrus twice a year (Holm, 2010). Mating chases normally start in January with the first litter of an average of three - four kittens being born in early spring, after a gestation of 38 days (Larsen and Boutin, 1994; Garven, 2018). Some squirrels can have two litters per year, but this is dependent on resources and individual health. The second litter are typically born in June-July (Humphries and Boutin, 2000; Holm, 2010). Red squirrel kittens emerge from the natal nest around six weeks of age and are fully weaned when they reach 110-115g, around 10 weeks of age (Bonanno and Albrecht, 2009). British red squirrels are the only native mammal to evolve in this niche in the UK. There morphology, physiology and behavioural ecology mean they are perfectly adapted to inhabit broadleaf, coniferous, and woodland ecosystems and should therefore occur throughout the whole of the UK wherever such habitats exist.

1.2 The Decline of the Red Squirrel

British red squirrel numbers have declined by 60% over the past three generations and 90% over the last 20 years (Battersby, 2005; Mathews et al., 2018). This decline initially started in 15th and 16th centuries due to deforestation, as timber was in high demand and by the 18th century the red squirrel was almost extinct in Scotland (Bosch and Lurz, 2012). The red squirrel population in England expanded briefly at the start of the 19th century as trees were planted to replace ancient woodland, however by the end of the century their numbers started to decline due to deforestation and forest fragmentation as forests were converted for agricultural use (Wauters et al., 2010; Robinson, 2020). Red squirrels were also culled as they were considered a timber pest (they damage trees by bark stripping); the Highland Squirrel Club destroyed 100,000 red squirrels between 1903 and 1946 (Rotherham and Boardman, 2006).

However, the biggest threat to their existence was (and continues to be) the introduction of the grey squirrel (*Sciurus carolinensis*) in 1876 when Mr T. U. Brocklehurst translocated a pair of North American grey squirrels into Henbury Park in North England (Holm, 2010; Bosch and Lurz, 2012).

This was unlikely an isolated event and it is estimated hundreds of individuals were released throughout the UK from USA and Canada between 1876 - 1931 (Bosch and Lurz, 2012). Grey squirrels thrive in the same habitats as red squirrels and are much larger than their red evolutionary cousins, outcompeting them for food, as not only do they eat more than red squirrels they can also break down polyphenols in tree seeds allowing them to eat unripen fruit and nuts, something red squirrels are unable to do (Bamber et. al., 2020). Grey squirrels also bark strip to a much greater degree than red squirrels (Shuttleworth et al., 2012; Nichols et al., 2016) so that they can eat sap from young trees in particular oak, beech, and hornbeam, destroying the growth of the tree and decimating whole forests where red squirrels used to live (Holm, 2010). This costs the UK economy approximately £14m/year via losses to the forestry industry (Mountford, 2006; Robinson, 2020). Due to the destruction caused by grey squirrel's huge ancient woodlands have been replaced with Sitka spruce, a more economically valuable timber species, however because Sitka spruce cone production is considerably variable this habitat has displaced red squirrels further (Holm, 2010).

The single biggest threat posed by grey squirrels to red squirrel populations is that they are a reservoir host for squirrel poxvirus (SQPV) which is harmless to grey squirrels but fatal to red squirrels. Approximately 65% (Forestry Commission, 2020) of grey squirrels carry SQPV (Wibbelt et al., 2017) and it is believed to spread amongst animals via body fluids, for example by infected feeders or shared parasites (Bosch and Lurz, 2012), and through contact with infected lesions in highly populated areas, causing ulcers and lesions to appear around the feet, genitalia, eyes and mouth, preventing the animal from foraging and feeding and causing death within two weeks (Northern Red Squirrel, 2020). This gives the invading species an advantage over the native species (White et al., 2016). SPQV transmission increases red squirrel decline 25 times higher than competition stress alone (Holm, 2010) and can kill 80% of a localised population (Fingland, 2020). Due to the pressure from the grey squirrel, the red squirrel population is set to reduce by a further 50% over the next three generations with the real threat of becoming extinct in the wild by 2040, they are now listed as endangered and urgent conservation required under the IUCN Red List (Kubasiewicz et al., 2018; IUCN, 2020).

1.3 Attempts to Increase Red Squirrel Numbers

There are several ongoing projects to attempt to reduce grey squirrel numbers and simultaneously increase the red squirrel population. Red squirrels are protected under Section 5 of the Wildlife and Countryside Act 1981 which makes it illegal to kill a red squirrel or destroy its habitat (Gov.uk, 2020).

However, there are loopholes in this law; private landowners do not have to seek a licence from NatureScot to carry out tree felling on private land even if red squirrels are dwelling or breeding on their land and if a tree is considered unsafe or if the cost of the delay to any projects is over £50K, tree felling can continue even if red squirrels are breeding or raising young there (Kortland - Red squirrel conference 24/9/20). The Act also allows for the management of grey squirrels which includes the strategic removal (culling) of grey squirrel populations and with the introduction of the EU Regulation on Invasive Alien Species Order 2019, it is now illegal to release any rehabilitated grey squirrels into the UK environment (IUCN, 2020). This is a very controversial Act, whilst red squirrels epitomise a conservation icon, studies have shown that people enjoy seeing and feeding grey squirrels, as this is often the only squirrel they see and offers them a positive link with nature; whilst 82% of people studied understood and sympathised with the red squirrel plight, 64% of them were against culling grey squirrels (Rotherham, Boardman, 2006). However, following Brexit, legal protective status of wildlife is set to be reviewed and red squirrels could lose their special status to benefit property developers and infrastructure projects, (DEFRA, 2021).

Following research by the UK Red Squirrel Accord, complimented by the Animal and Plant Health Agency, and being funded by DEFRA and the UK Government from January 2021, an immuno-contraceptive has been developed over the past 3 years which has had a 90% success rate in breeding infertility in female grey squirrels (RSS, 2020). Immunocontraception involves administering a drug orally through food that induces an adaptive immune response in an animal and causes infertility. Rhodamine B is being used as a marker which is consumed in the bait and causes the grey squirrel’s hair to become fluorescent under UV light. Research has found that in as little as four days grey squirrels in a wood the size of 18 hectares can be effectively given the contraception, which would last over a year and be re-administered every summer. Over the next two years studies will be continuing into management methods, including the best bait and hopper to use so that non-target animals do not access the contraception, such as using traps with heavy doors that only the grey squirrel is able to push open, and the smaller red squirrel is unable to access (UKRSA, 2020).

In parallel with this the Roslin Institute and European Squirrel Initiative are partaking in a seven-year study using a genetic control strategy to complement existing control methods which relies on genetic engineering through genome editing to isolate the gene which produces only males in grey squirrels. Normally animals inherit sex chromosomes from both their parents but genetic engineering changes inheritance and deletes or modifies genes using transgenes.

This will cause female infertility and lead to a preponderance of males, reducing the grey squirrel population. This study has proved successful so far resulting in a reduction in grey squirrel numbers and fragmented populations (evidence of localised extinction) on Anglesey (Signorile and Shuttleworth, 2016). DNA sequencing is also being studied in the hope to understand the genetic bases for immunity of SQPV in grey squirrels, which could potentially be copied and inserted into the genomes of the red squirrel, providing immunity to the disease. This could be used in captive breeding programmes and introductions or translocations (Fingland, 2020). However, both these genetic-based studies would take several generations to successfully reduce the grey squirrel population and as red squirrel extinction in the wild is predicted to occur by 2040 other methods of controlling grey squirrel numbers need to be researched and current control methods continued.

There has been a great deal of research into developing a SQPV vaccine to be administered to red squirrels, but although a number of vaccines were in development in the 2000's and a live vaccine from Moredun Institute showed high efficacy, delivery of a vaccine was estimated at approximately £90,000 per year (English Heritage, 2009) and as the vaccine resulted in severe side effects in test animals, no further research has been completed since 2013 (UKRSA, 2020).

Sheehy and Lawton (2014) found evidence that when the pine marten (*Martes, martes*), a once extinct native species, was reintroduced to North Scotland and West Wales, grey squirrels reduced and red squirrel numbers increased (Shuttleworth et al., 2020). Although red squirrels are predated upon by pine martens, red squirrels only make up 0.1-2.4% of the pine marten's diet compared to 15.6% of grey squirrels (Sheehy and Lawton, 2019). Pine martens and red squirrels have evolved together over many centuries so red squirrels have learnt how best to escape them, whereas grey squirrels have not yet learnt this survival skill (Sheehy et al., 2014). Grey squirrels are larger than red squirrels so cannot run as quickly and spend 80% of their time on the ground unable to access the small branches at the top of the canopy that red squirrels can reach, leaving them more vulnerable to predation (Kenward and Tonkin, 1986; Sheehy and Lawton, 2014). However, whilst this is considered a more ethical way of decreasing grey squirrel numbers, only Northern Ireland have recorded considerable success with a 33% increase in pine marten and red squirrel populations and an associated decrease in grey squirrel numbers. This may be because of a reduced human population and the landscape being more favourable for pine martens. Socioeconomic factors also need to be considered regarding pine marten introduction/reintroduction which include poultry loss, game shooting and human-wildlife conflict, such as rifling through bins and living in attics, and damage to property (Stinson, 2020 RSS Conference).

1.4 Captive Breeding and Release Programmes

Alongside these different methods to attempt to reduce grey squirrel numbers, there are also a number of zoos involved in captive breeding and release programmes, and translocating squirrels between different locations, as releasing red squirrels into the wild not only helps to increase their numbers but also helps maintain habitat biodiversity and increases environmental heterogeneity (Zhang, 2003; Gippoli and Amori, 2007; Fraser, 2008).

Trees for Life (2020) are currently involved in translocating red squirrels from strongholds in the Northeast of Scotland to ten previously inhabited forests in the northwest highlands; red squirrels are unable to naturally disperse in fragmented forests, as red squirrels travel through the canopy to avoid predation on open ground (Gurnell et al., 2002). Seven new populations have successfully been translocated and numbers are rapidly increasing in these forests as it is thought that wild squirrels have developed homing behaviour and immediately move away from the release site to more resourceful areas and therefore increase their chance of survival, supporting the theory on wild-to-wild translocation (Wauters et al., 1997). Although the IUCN (2010) states that translocation conservation is a better means to increase endangered populations to former historical ranges as captive stress is avoided, other programmes have not been as successful, for example only 33% males and 50% females survived when translocated from the wild to an Antwerp Park (Wauters et al., 1997) as wild red squirrels have not adapted to capture and transport stress.

Captive breeding and release programmes such as those in this study are now considered an acceptable conservation tool under the IUCN/SSC Reintroduction Specialist Group and zoos play the major role in managing and conserving threatened species and increasing populations through these programmes (Forder, 2006; Marinatha et al., 2019). Whilst there are significant benefits to using captive breeding and release programmes to boost wild populations of red squirrels, including controlling breeding and genetic variability via stud books and the ability to select the animals for release with the greatest chance of survival (Sheehy et al., 2018; IUCN, 2019), captive animals set for release are at a disadvantage as captivity does not allow animals to get used to predation or traffic on busy roads (Tenhumberg et. al., 2004). Captive individuals therefore require training to survive in the wild, including how to avoid predation.

Pathogen transfer and minimising disease spread is another important consideration for captive reintroductions (Tenhumberg et al., 2004; IUCN, 2010). Adenovirus (ADV) is a pathogenic intestinal disease which lays dormant in red squirrels and is triggered by stress.

Associated mortality levels of 75% have been observed and ADV was spread from the captive population to the wild population in 1997 following a reintroduction project involving the translocation of captive red squirrels from Cumbria to Suffolk (Nichols et al., 2017; Shuttleworth, 2020). Parasitic infection, whilst treated for in captivity is now rife in wild red squirrels, and not only affects the individual's fitness but host individuals have been found less attractive to mating partners (Raveh et al., 2011), and infestation effect survival of juveniles when parasitic overload occurs (Patterson et al., 2015).

However, the biggest problem with captivity is it is linked with both physiological and behavioural problems and the emergence of stereotypical behaviour, particularly in the form of pacing in red squirrels, due to limited space, inappropriate housing, conflict with conspecifics, husbandry procedures and human interactions (Morgan and Tomborg, 2007). There are an estimated 85 million individual captive animals worldwide (Dellaire et al., 2012; Jones et al., 2012) and 40% of them are rodents (Gippolit and Amori, 2007), more than 10% of which display abnormal behaviour (Querioz and Young, 2018).

In the UK there are currently 10 zoological collections involved in captive red squirrel breeding programmes (Belfast Zoo, The British Wildlife Centre, Longleat Zoo, Paradise Park in Cornwall, Peak Wildlife Park, Pensthorpe Conservation Trust, Walton Zoo, Welsh Mountain Zoo, Wildwood Escot, Wildwood Kent) (Walton Zoo, 2019; BIAZA, 2018; WMZ, 2019) and stereotypical behaviour is reported as being prolific in these captive red squirrels (anecdotally reported by keepers on forums and meetings e.g., the ABWAK Red Squirrel Captive Breeding Workshop, 2019). Due to the prevalence of stereotypical behaviour, there is concern that animals who are due to be released may have lost natural behaviour patterns and instead developed captivity-induced behaviours that are maladaptive (Mason et al., 2007).

1.5 Why Stereotypical Behaviour Emerges

Stereotypical behaviour can be defined as repetitive, unvarying behaviour, which has no obvious function and is not seen in the wild, but which may enable animals to cope in environments that do not meet their appetitive, consummative, or behavioural needs (Broom and Fraser, 2007). Stereotypical behaviour is therefore considered a welfare concern and in red squirrels normally starting in juveniles, post-weaning and takes the form of linear or figure of eight pacing, somersaulting, weaving and bouncing (Welsh Mountain Zoo, 2019).

In this study locomotor stereotypical behaviour took the form of linear and figure of eight pacing and therefore stereotypical behaviour is described as pacing behaviour throughout this thesis.

When red squirrels spend so much time on stereotypical pacing, it reduces the amount of time they spend in other wild-type and species-specific behaviour which is important for them to not only survive but to thrive and can reduce their welfare state. However, it is difficult to successfully evaluate animal welfare given species and individual's needs (Binding, 2020). The former Farm Animal Welfare Council's Five Freedoms of Animal Welfare offered a foundation on how to define welfare standards focussing on minimising distress and negative experiences in animals, including freedom from hunger and thirst, discomfort, pain, injury and disease and freedom to express normal behaviour. However, the new 2020 Five Domains Model sets out the indicators for animal welfare, including nutrition, health, environment, and behaviour and concentrates on the management of animals to provide positive experiences, taking note of positive mental states and quality of life (Mellor et al. 2013; EAZA, 2020).

Animals are sentient beings (Spedding, 2000) and zookeepers have an ethical responsibility to ensure that all unnecessary suffering is significantly reduced and ideally eliminated (Salas et al., 2018). Abnormal behaviour is a welfare measure (the more abnormal behaviour shown, the poorer welfare is) related to psychological well-being and affective state expression. It is directly linked with suffering, as suffering includes negative experiences such as fear, pain, hunger and boredom, and the inability to perform behaviours that animals are highly motivated to do (Dawkins, 2003). However, the cause for the emergence and the maintenance of this behaviour in rodents, including captive red squirrels remains understudied.

1.5.1 Stereotypical Behaviour as a Coping Mechanism

Mason (2010) states stereotypy is a coping mechanism and disputes therefore it is functionless, as repetitive movement can result in reinforcement which serves to maintain the behaviour, the performance of the behaviour itself being intrinsically rewarding. The lack of stereotypical behaviour does not necessarily mean better welfare, in fact, although wild- caught mice (*Mus musculus)* did not pace they were more fearful than pacing captive-bred mice and had higher levels of corticosterone ('stress-hormone'). Patton et al. (2013) similarly concluded that stereotypical behaviour does not necessarily mean animal welfare is compromised as animals that stereotypically pace often cope better (in general) than their non-pacing counterparts.

Balcombe (2006) disputes this coping theory as corticosterone levels did not decline in male mice that stereotypically chewed their cage bars and Wurbel et al. (1998) states that if stereotypical behaviour did relieve stress, animals blocked from cage biting would rebound by gnawing more when they were allowed to stereotype again, but this was not the case. Cooper and Nicol (1991) found that prevention of a coping mechanism should result in an increased tendency to perform a different stereotypy to compensate, but mice became more inactive, so wire biting is therefore thought to be strongly dependent on external causal factors and not a coping mechanism.

These studies support findings of Wurbel et al. (1998) that stereotypies are therefore not coping mechanisms but an indicator of poor welfare arising from prevention of highly motivated behaviour, stress, fear, boredom, or brain damage. Additionally, even if an animal develops stereotypy as a coping mechanism, they are still having to abnormally cope with an environment in a maladaptive way. Mason et al. (2007) states that there should be zero tolerance of abnormal behaviour performance, however Rose et al. (2017) argues that it is not always possible to eradicate stereotypies, simply removing ways to display stereotypical behaviour can lead to depressed animals unable to display any behaviour. Nevison et al. (1999) found male mice prevented from gnawing for 10 days had chronic stress levels, therefore rather than trying to eliminate stereotypical behaviour it is better to attempt to understand what causes this behaviour to emerge (Lierop, 2010), yet research is very limited (Morgan and Tomborg, 2007) and contradictory explanations of stereotypy persist in the literature. For the purpose of this thesis, the performance of stereotypical behaviour was considered a welfare concern.

1.5.2 Enclosure Size and Design and Visitor Interaction

A great deal of research supports the findings that stereotypical behaviour in captive animals is linked to enclosure design because of associated stressors such as lighting, noise, odours, restricted space, including retreat space, loss of ability to display species specific behaviour and human interaction (Morgan and Tomborg, 2007; Bryan et al., 2017). Stereotypical behaviour is not only a concern for animal welfare (Fernandez et al., 2009; Tanaka and Soga, 2020) but findings suggest that visitors are less likely to visit or support zoos where they have witnessed animals displaying stereotypical behaviour such as pacing; visitors believe this behaviour is linked with lower levels of animal care and the presence of this behaviour is also found to reduce the educational value of the enclosure (Ballantyne et al., 2018; EAZA, 2016).

Shepherdson et al. (1998) and Choo et al. (2011) state that visitors prefer to visit natural looking enclosures, appearing similar to that found in the wild, as this encourages animals to display natural behaviour and is linked to breeding success. However, these environments can also make it harder to see the animals, and visitors remain longer at exhibits where animals are visible (Davy, 2007). Bitgood et al. (1988) found that not seeing the animals reduced visitor's empathy with the animal's conservation status. Harrison et al. (2017) suggests natural environments encourage natural behaviour including breeding success by promoting the expression of species-specific behaviour such as foraging, mate-seeking and predator avoidance, whereas an unnatural, barren environment decreases behavioural repertoire and increases abnormal behaviour moving away from the wild-type activity budget, which has implications for those animals that are bred for wild reintroduction.

Visitors enjoy being near animals with walkthrough enclosures and specific keeper-led animal interactions becoming increasingly popular, but animals are not able to move away from visitors, and this proximity can course stress and the emergence of stereotypical behaviour (Tamborski et al., 2009; Fernandez et al., 2009). Carder and Semple (2008) found with increased visitors, animals decreased social behaviour and increased antagonistic behaviour and aggression. Although Hosey (2005) found that animals can find visitors enriching and Jones et al. (2011) suggests visitors in a walkthrough had a positive effect on decreasing aggression in animals. Both these studies concentrated on primates and lemurs and not rodents, who are typically smaller and more vulnerable to predation and these interactions need to be ethically evaluated to ensure animal welfare, educational needs and conservation outcomes are being met (De Mori et al., 2019).

Davy (2010) suggests that the impact of visitors is dependent on the age and noise levels of visitors, and this is supported by recent research by Woolway and Goodenough (2020) on red squirrel behaviour at the walkthrough enclosure at Wildwood in Escot. Their findings suggest that stress was not necessarily linked with the number of visitors but the types of visitors, with red squirrels increasing locomotion and decreasing feeding when surrounded by noisy visitors, particularly children. Adult visitors reported increased enjoyment levels in the walkthrough due to higher encounter rates with red squirrels (Woolway and Goodenough, 2020). This interaction with visitors is probably directly linked to the fact that keepers use positive reinforcement when conducting their 11am talks, by offering high value food rewards to red squirrels who approach both the keepers and visitors. These findings could highlight that positive animal keeper relationships allows animals to choose whether to interact with members of the public, improving their welfare and visitor experience.

However, Hosey (2005) suggest that this type of operant training is not natural behaviour and is linked to food soliciting behaviour, which could be detrimental to animals to be released into the wild (Mun et al., 2016).

In contrast Haigh et al. (2017) found that red squirrels in managed parks altered their daily activity patterns to avoid opening times at the park and were more nervous and only visible 15% of occasions when humans were around, with highest levels of faecal cortisol metabolites being recorded in areas most densely visited by humans. However, Conomy et al. (1998) and Bjordal (2015) states that animals quickly become habituated to regular human interaction as predictable stimulus stops representing a threat. Bjordal (2015) research suggests red squirrels use optimal escape theory and reduce their flight response to human disturbance, however this study took place in late summer where foraging and caching may be more important than human disturbance, and findings by Uchida et al. (2015) and Rose et al. (2020) suggest that seasonal changes affected animal behaviour more than the presence of keepers or visitors.

Querioz and Young (2018) argue enclosure design and proximity to people affects different animals in different ways. The findings suggest that herbivores are more negatively impacted by visitors than carnivores, probably because carnivores are predators, and more aggressive and less threatened by visitors; and diurnal species are more affected than nocturnal animals. They also found arboreal animals living in closed habitats were more negatively affected than terrestrial animals in open habitats, suggesting that diurnal, herbivorous, arboreal animals such as red squirrels, living in closed habitats are more impacted by zoo visitors and therefore more likely to develop stereotypies.

Cage size also influences welfare and behavioural expression of captive mammals. Balcombe (2006) found rats (*Rattus*) in large pens had better body condition and were friendlier and less fearful than rats in small cages, whilst Valuska and Mench (2013) found large cages reduced aggression in rodents because resources such as wheels, could be spread evenly around the environment, allowing better access opportunity for any given individual. Callard and Price (2000) state that enclosure size is not necessarily the main contributing factor for emergence of stereotypical behaviour. Height is considered more important to arboreal habitats for species such as red squirrels. Red squirrels have bred successfully in enclosures as small as 2.7 metres^2 with a height of 2.2 metres (Shuttleworth et al., 2015). However, the Welsh Mountain Zoo (2019) states a minimum of 3 metre height is needed for red squirrels to conduct mating chases and build dreys to safely rear young.

In the wild red squirrels live in home ranges of 1.3 animals per hectare (Holm, 2010) and can easily travel six - nine miles a day (Deeney, 2019) with a home-range of between two and ten hectares, approximately 14 acres, compared to voles and gerbils who range over less than an acre and even rats that live in a range of 1.87 hectares (Wildlife online, 2020). Thus, it could be assumed that meeting red squirrels' behavioural needs in small enclosures is extremely difficult and raises welfare concerns. However, comparing home range in the wild to that in captivity does not necessarily simply mean poorer welfare, as in captivity food and other resources are provided in abundance. So, small spaces, with increased resource access could be a key to improving welfare to prevent stereotypies developing. Casey (2019) suggests large cages can actually increase stress and abnormal behaviour in prey species, and environments that offer natural foraging behaviour is more important than cage size.

Rose et al. (2020) also state that complexity is more important than size and complex environments increase normal behaviour and reduce stereotypical behaviour as environmental enrichment enhances physical or social surroundings to stimulate the brain (Mason et al., 2007). Cooper et al. (1996) found housing complexity reduced stereotypical behaviour in bank voles (*Arvicola amphibious*) and adding a carboard tube to a mouse enclosure reduced wire gnawing by 40% (Chapman et al., 1998). Conversely, Akrea et al. (2011) found adding enrichment increased aggression in rodents as fights broke out over limited and valued resources Brandao (2011) reports that although small cages induced stress in hamsters (*Cricetinae*), using vertical fragmentation of the cage and adding microenvironments offering more opportunity to forage, reduced stress behaviours. However, most research on rodent enclosure size are conducted in laboratories, where animal behaviour can be affected by several unnatural variables, such as artificial lighting, being kept in isolation and handling and experimental stress, so is not always comparable to the experience of animals kept in zoos; as Callard et al. (2000) state that, incorrect social groupings and repeated husbandry such as enclosure cleaning and handling increased heart rate and stress levels in laboratory rats.

In a previous study by the Author (Adams-Wright et al., 2015) on the effect of three different enrichments on stereotypical behaviour (a swinging tube, a puzzle feeder and a swinging willow ball with food inside) it was found that whilst feeding enrichment significantly reduced stereotypical behaviour in captive red squirrels, a swinging tube enrichment caused stereotypical behaviour to increase as it was felt this intended enrichment was aversive to the animals causing them to move away from the tube and actively avoid interacting with it.

Interestingly the puzzle feeder was the preferred enrichment of the female red squirrel and reduced pacing from 18%-10% of observed time. The swinging food enrichment decreased stereotypical behaviour in the male red squirrel from 34%-27% of observed time probably because squirrels naturally forage upside down using their hind limbs for suspension (Holms, 2010) so this is fulfilling an appetitive and behavioural need. However conversely on the introduction of the willow ball feeder, female red squirrels' stereotypical pacing increased to 45% of total observed time as the male become territorial over the willow ball and attempted to prevent the female from accessing food by aggressively chasing her away (Adams-Wright et al., 2015).

Therefore, enrichment is not necessarily an easy answer to tackling stereotypical behaviour as intended enrichment can increase agonistic behaviour or simply reduce stereotypical behaviour by taking up all the animal's time (Vickery, 2003; Mason 2007). It is more important to find a way to ascertain the underlining motivation for the emergence of stereotypical behaviour to find the best way to treat and hopefully eradicate the stereotypy.

1.5.3 Thwarted Attempts to Perform Highly Motivated Behaviour

Enrichment only reduces stereotypical behaviour and increases species specific behaviour if it facilitates highly motivated behaviour; being defined as intrinsic behaviour which an animal is highly motivated to preform even in the absence of an appropriate trigger, such as foraging or caching in red squirrels, and the inability to partake in these behaviours negatively impacts on animal welfare, with stereotypical behaviour more likely to increase (Morgan and Tromborg, 2007). Welfare and behavioural needs are extrinsically linked, as a behavioural motivation means the animal has a need to perform that behaviour and that need is related to welfare; if the animal is not able to perform the behaviour this would likely cause poor welfare and vice versa. Therefore, the most important way to improve welfare is by tackling the underlying frustrations and causes for the emergence of abnormal behaviour (Mason and Latham, 2010).

Understanding an animal's motivation is not always easy (Chapman et al., 1998) without being anthropomorphic (Lewis and Hurst, 2004). However, whilst it may be considered that linking human emotions to animals is not an appropriate or scientific way to ascertain the welfare of an animal (EAZA, 2020), animals are sentient beings, with emotions, uniquely expressed by individuals (Spedding, 2000). Red squirrels have been made an iconic animal, not only because of the publics love for cute furry animals but also through cartoon creations such as Beatrix Potter's Squirrel Nutkin and the Tufty Fluffytail from the Tufty club.

These have enabled humans to have more empathy for red squirrels and therefore be more interested in the not only the conservation of the species in the wild but also the welfare of captive animals (Bitgood et al., 1988). For example, the observer witnessed several visitors commenting on the pacing of Radish and telling their children the animal was 'unhappy' or 'sad' which motivated an empathetic response in their children. Whilst anthropomorphism is often contradicted, on balance it can also be a valuable way to engage people in the plight of animals both in both the wild and in captivity.

The same motivation may trigger different behavioural strategies in animals, for example the motivation for chickens (*Gallus gallus domesticus*) to eat or to dust bathe are associated with an increase in exploratory and scratching behaviour (Wurbel et al., 1996). Similarly, pacing in red squirrels could be linked to the motivation to escape, dispersal needs or hoarding stress or a combination of several factors. Uchida et al. (2016) states the best way to find out an animal's motivation is to understand their in-situ behaviour and activity budgets in the wild, as this would indicate which behaviour is most important to an animal to meet survival and fitness needs (also suggested by Wauters et al., 1992). However, Veasey et al. (1996) states the use of wild activity budgets as a comparison to captive behaviour is not an appropriate evaluation tool for the wellbeing of zoo animal. Schonecker (2014) stating application of consumer-demand theory motivational tests are more important to work out the amount an animal is willing to "pay" to gain a positive resource or escape a threat, for example whilst complexity and novelty of enrichment in mice cages had no effect on stereotypical behaviour, mice worked harder to gain nesting material as nesting behaviour is a highly motivated behaviour in mice (Gross et al., 2011). This theory has its criticisms as motivation can only be tested given current choices in the experiment and these might not be the same as in an animal's enclosure. Dawkins, (2003) found that laboratory rats were willing to push open heavy doors in a maze to access company, although the method used did not consider that older rats may not have the strength to push open the heaviest doors.

Dawkins (2003) states a better way to ascertain motivation is to give animals choice using preference testing to ascertain what motivates animals and give them control over how to spend their time as lack of control is considered one of the greatest stressors to captive animals (Callard and Price, 2000; Baumans et al., 2010; Rose et al., 2017). However, animals can make poor choices that affect their long-term health and welfare, for example, given a choice a red squirrel will often choose a peanut over other food options, which does highlight foraging motivational needs, however these are highly fatty foods with little long term nutritional value (Holm, 2010).

Mason et al. (2003) state that simply being managed causes stereotypical behaviour and is a displacement activity and not related to motivation, for example gnawing is normal behaviour in rodents to wear down their teeth or cut twigs for nesting material but juveniles gnawing on bars is linked to premature weaning (Wurbel et al, (2006).

1.5.4 Motivation to Perform Foraging, Feeding and Caching Behaviour

As red squirrels spend so much time in the wild foraging, it is considered a highly motivated behaviour, seasonally driven and appetitive, which they find rewarding and are motivated to perform in all environments (Holm, 2010; Manteca Red Vilanova, 2020). The reason for the large amount of time spent stereotypically pacing in red squirrels could be linked to the inability to fulfil this highly motivated and rewarding behaviour to an appropriate extent. Red squirrel diet and behaviour is driven by seasonal changes throughout the year, however in captivity their diet mainly consists of commercial parrot mix, fruit and vegetables delivered in the same way each day (Welsh Mountain Zoo, 2019). Although research has shown that captive squirrels released into the Glenarm Castle Estate did not seem to understand how to reach berries or nuts from the end of branches (Shuttleworth et al., 2015), seasonal feeding or presenting food similar to that found in the wild is not always common practice in captivity.

Caching (the hiding of food items) is a hoarding behaviour considered to be a compulsion in red squirrels that is stimulated by seasonal changes and availability of resources and is vital for individual survival and fitness (Jansen et al., 2012). It involves excavating a shallow pit, placing food item/s into the pit, and covering the food over with soil and leaves ready to be excavated in the winter when food supplies are low (Steele et al., 2008).

Hoarding is a behaviour considered a primary motivation in red squirrels and stress hormones and daily energy expenditure is highest during hoarding season (Fletcher et al., 2012). Hoarding is a complex and stressful behaviour as whenever a red squirrel finds a food item, they not only have to decide whether to eat or cache it but be wary of predation and also of pilfering conspecifics that may be watching (Dally et al., 2006; Steele et al., 2011). Red squirrels only retrieve about 74% of their cache, with up to 26% being either pilfered or lost due to decay or natural disaster, such as flooding over the winter season (Gerhardt 2005; Steel et al., 2008). Red squirrels suffer from hoarding stress in captivity which could contribute to increased stereotypical behaviour.

Red squirrels do not like to cache in the presence of others (Lucas and Zielinski, 1998) and will cease, reduce, or delay caching behaviour in the presence of potential thieves, caching in shady sites away from onlookers, they turn their backs to stop conspecifics seeing their cache (Dally et al., 2014; Alpern et al., 2019). However, in captivity, red squirrels are often housed in breeding pairs and juveniles can stay with their parent's far beyond what is normal in the wild, making it hard to secure the privacy needed to hoard. Additionally, there is also the stress of human interactions from both visitors and husbandry procedures by staff members.

There are single caches, where red squirrels bury one object at a time or multiple caches where several items are buried often on top of each other and deceptive caching. Deceptive behaviour is considered a higher order set of adaptive behavioural responses which occurs more in the presence of conspecifics and humans less than twenty metres away (Jansen et al., 2012). Caching behaviour differs amongst individuals, with dominant animals caching more when they are kept in a group, but submissive animals suppressing this behaviour when housed with dominant animals. Juveniles disperse earlier when living with aggressive cachers which could explain why juveniles start pacing after being weaned (Steele et al., 2008). When squirrels are housed at lower densities, they have more choice of how and what to forage and do not have to be concerned about pilfering, but the higher the density of conspecifics the more they must consider how and where to hide food (Alpern et al., 2012). The way red squirrels are housed appears to increase hoarding stress and could explain why stereotypical pacing emerges and increases in the presence of other animals and humans.

Caching behaviour is still not truly understood. Emsems et al. (2012) states red squirrels use "central place foraging theory" by increasing or decreasing their foraging areas based on resources, conspecifics, and predation risks. Hurley and Robertson (1986) state red squirrels use the "optimal density model" to cache food, burying food in low densities up to 10 metres away from source, whereas Alpern et al. (2019) states squirrels have limited digging energy so have to decide between placing nuts deeply hidden in one place or scattering in shallow depths, "optimal foraging theory". Makowska and Kramer (2007) states squirrels use "theoretical models of vigilance" which means they choose different types of food dependent on the presence of predators, for example eating easy to handle items of food such as sunflower seeds and moving valuable items such as hazelnuts to a safer area away from predation before handling and caching. Esposita et al. (2010) state squirrels use "optimal foraging strategies" where the forager caching strategies are dependent purely on the availability of food. However, Vander (1993) states "optimal burial depth" is more important, with a 10-30mm hole being the optimal depth and 20mm having the greatest chance of food being successfully retrieved and less food stolen.

Most squirrels bury their caches between 5-20mm depth as it takes a lot of energy to continue burying items and caches must be made quickly when housed with many conspecifics. Zong et al. (2014) found the availability of suitable hoarding microhabitats changes hoarding patterns, as red squirrels prefer fallen leaves and moss as hoarding substrate, finding organic litter helps retain cone moisture, and red squirrels select for different hoarding habitats when caching food (Jianzhang et al., 2006).

In the wild, females were thought to hoard more than males probably due to reproductive needs in the female (Jansen et al 2012). Conversely, Archibald et al. (2013) found that female hoards were smaller as they cannot recover quick enough after their second litter to both restore their fat stores from lactation and hoard enough for winter. They clip more, chew through pinecones to reach the seeds in the centre, than males but hoard less and eat and steal more from male caches and Jenkins (2011) states that although males hoard more, they are less flexible in the way they hoard compared to females. Rong et al. (2013) states hoarding is an adaptive strategy and relies on behavioural plasticity and Huang et al. (2011) found when resources are low or whole cache pilferage occurs, rodents increase hoarding and consumption, however Luo et al. (2014) contradicts these findings and states hoarding is a fixed response as after even entire whole caches are lost red squirrels continued to cache in the same manner. However, he methods used in Rong's (2013) study mean findings are questionable as activity budgets were not devised for individual squirrels so trade-offs between consumption and hoarding behaviour had to be presumed.

Schmidt and Ostfeld (2008) found red squirrels' false cache and dig up caches and recache food items elsewhere (Steel et al., 2008). Recaching is not a well understood behaviour, but it appears red squirrels are involved in a great deal of caching management, ensuring adequate food supplies for winter (Bartlow et al., 2018). Research shows that red squirrels can only remember where individual caches are for 20-30 days, so recaching is important for cache management (Hirsch et al., 2013).

It is also unknown exactly how red squirrels know how to retrieve their caches, Wang et al. (2018) states they use olfaction to find caches, whereas Benhamou (1996) found they rely on visual cues, such as trees and bushes and good spatial memory. Hirsch et al. (2013) found red squirrels' cache along trails and patrol their territory as 'memory enhancement hypothesis'. However, Brodin (2010) states scatter hoarding is a conspicuous behaviour and successful retrieval is purely dependent on the hippocampus, part of the brain critical for learning and spatial memory and which serves to enhance survival (Johnson et al 2010).

Foraging in the wild is a complex behaviour (Hurly and Robertson, 1990) and red squirrels can spend up to 80% of their active time on the search for, consumption or caching of food (Wauters et al., 1996). It may not be fully understood how they forage but they spend a large proportion of their daily activity in this behaviour so it can be assumed it is an important behaviour – the more individuals do something, the more time they spend doing it the more important the behaviour is to the individual and potentially the species. However, in captivity, food is simply delivered at certain times and is often different from a wild-type diet, being higher in protein, different in texture and lower in fibre with decreased handling time, offering restricted foraging opportunities (Callard et al., 2000). Feeding in captivity is also predictable which is shown to produce anticipatory stress and an increase in repetitive locomotive activity (Sandhua et al., 2020). Increasing food complexity has been shown to reduce baseline stress and stereotypical behaviour in rodents (Malmkvist et al., 2013). Therefore, unfulfilled foraging motivation, hoarding stress, cage size, conspecifics and human interaction could all contribute to stereotypical behaviour (Maslak et al., 2013).

1.5.5 Post-weaned Stereotypical Behaviour, Natal Dispersal and Escape

A potential reason for the emergence of stereotypical pacing in post-weaned juveniles is an inability to disperse from their parents. Dispersal is achieved when a young animal leaves its natal territory, relocates, and settles in a territory of their own. This is a key behaviour for all red squirrels, but little is known about the intrinsic and extrinsic cues that lead to the need to disperse (Wauters et al., 2010; Franc et al., 2012). Juvenile red squirrels in the wild emerge from the nest around 40-50 days (six- seven weeks) of age and are fully weaned around ten weeks of age, at which stage, they start making short roundtrip explorations near their natal territory (Haughland and Larsen, 2004) and normally settle in a new territory by 90 days of age (Price and Boutin, 1999).

Yao et al. (2019) states juveniles will mostly have dispersed by autumn as they need to locate a territory with high resources to be able to increase body fat and start caching food in readiness for winter; juvenile squirrels reach sexual maturity at nine months and will start breeding the following year. However, some captive red squirrels, are kept with their parents until they are 22 weeks old even though husbandry guidance states young should be moved at 12 weeks to stop inbreeding (Boonstra and McColl, 2000). Conversely, the guidance notes written by the Welsh Mountain Zoo (2019) states that juveniles can be kept as a family group for up to 12 months, which does not appear to fit with the dispersal patterns observed in their wild counterparts.

As males become sexually mature, they become more aggressive (Boonstra and McColl, 2000). Red squirrels rattle 50% more when housed in higher density areas of 2.2-3.2 squirrels/ hectare and this is reduced to 20% when 0.4-1.2 squirrels are housed/hectare (Shonfield et al., 2012). Dispersal is thought to be caused by resident adults' behaviour towards juveniles and the need to disperse increases with conspecific aggression and higher number of siblings (Wauters et al., 2011). Mothers are known to change their hoarding strategy to stop their young depleting their hoard and this also encourages juveniles to disperse (Larsen and Boutin, 1994; Kerr et al., 2007). Up to 90% of wild red squirrels disperse between 21 – 4000 metres and 50% of those settle just 320 metres away from natal home (Wauters et al., 2011). However, Larsen and Boutin (1994) suggests that juveniles that settle further from natal home ranges survive better, but this study was carried out on red squirrels living near the edge of a forest who then dispersed into mature forests with increased resources.

Wauters et al. (2011) state there is no proof that males disperse further than females however as females raise young alone and defend their territories, males have larger but more overlapping home ranges with dominance hierarchy between other males in the area which needs to be constructed and maintained. Some studies suggest it is the mother that moves away and not the young. Price and Boutin (2012) found that older mothers who live in good territories and have finished their breeding life bequeath some or all of their territory to their juveniles. If mothers are younger and live in a poor territory, they may disperse in search of better one. By bequeathing some of their territory to their young, this reduces aggressive interaction on the borders of the home range by 25%.

Balcombe (2006) found there is evidence that thwarting this dispersal behaviour increases both attempts to escape and stereotypical pacing by juveniles, particularly if they are housed with aggressive cage mates. Female rodents that attempt to escape have a higher cortisone level than females who do not try and escape, and rats who attempted to escape aggressive conspecifics spent most of their time in stereotypical behaviour stretching up against the walls of their enclosure. Laboratory mice preferred to bite the bars that were potential exits (Nevison et al., 1999), and similarly, red squirrels may pace by entry doors, as from time to time these doors do open which reinforces their pacing behaviour (anecdotal report from keepers). However, stereotypical pacing may not necessarily be linked to escape and dispersal but a need to investigate the external environment (Nevison et al., 1999). Catlard et al. (2000) found arousal or fear from extraneous stimulation such as visitors and zookeepers evoked escape responses in young rodents. Rodents are predisposed to explore external stimuli in the immediate environment as they are driven to investigate airborne odour cues or potential predators (Morgan and Tromborg, 2007).

This raises plasma levels of corticosterone, increases blood pressure, and stimulates changes in anxiety like behaviour in rats (Catlard et al., 2000; Lewis and Hurst, 2004). Bar-biting does not necessarily mean an aversion to the cage but an attempt to investigate cues from outside (Wurbel, 2006) as Perspex cages have been found to reduce this stereotypical behaviour by reducing outside odours (Balcombe, 2006).

Stereotypical pacing begins in most rodents' post weaning, this behaviour in caged rodents is often linked to abrupt weaning procedures (Finnegan et al., 2020) but it is also the age when rodents naturally start exploring their surroundings which is part of normal juvenile behaviour (Catlard et al., 2000; Meers and Odberg, 2005). Stereotypical behaviour is thought to originate from these source behavioural patterns, natural behaviour that graduates into stereotypies, known as "Gradualism Hypothesis" (Wurbel and Stauffacher, 1997; Chapman et al., 1998; Mason et al., 1999). Evidence of this can be found in Catlard et al. (2000) research which found that stereotypical backflipping develops in post weaned rats who, as pups are often very active, jumping and crawling on walls and this play behaviour becomes maladapted. Similarly, red squirrels protect their territories by aggressive chases and vocalisation and patrolling the edges of their territories and cache reserves, (Larsen and Boutin 1994) therefore pacing in captive red squirrels could be maladapted territorial behaviour. Evidence is found in Dantzer et al. (2011) when captive rodents investigated holes in netting by patrolling near the netting looking for possible escape routes and stereotypical pacing developed from this predominant behavioural reaction. Morgan and Tromborg (2007) also found stereotypical pacing accidently arose from increased autonomic activity and increases in metabolism encouraging the fight of flight response which increases locomotion and increased locomotion is linked to increased pacing.

1.5.6 Learnt Behaviour Vs Inherited Behaviour – Nature-Nurture Debate

Behaviour can be passed onto offspring via inheritance and/or through social/observational learning which is a process that offers an opportunity to learn from others (Jones et al., 2008; Mendoza and Schultz, 2013). Weigl and Hanson (1980) research illustrates that squirrels learn very quickly from their peers by observing them and learn factors that aid survival quicker than other behaviours. Therefore, it could be debated that if juveniles have stereotypical parents (parents that perform stereotypical behaviour), they could learn this behaviour through observational learning.

However, although learning and environment can induce stereotypical behaviour, Jones et al. (2008) found offspring are five times more likely to pace if both their parents have stereotypies and three times more likely to pace if sired by stereotypical fathers than those without stereotypical parents, suggesting a strong genetic component to stereotypical behaviour (Lierop and Pillay, 2008). Jones et. al., (2008) found mice only paced if their mother paced but Schoenecker and Heller (2000) found offspring were seven times more likely to pace even if only one parent displayed stereotypies.

Catlard et al. (2000) states that when siblings were housed with non-pacing foster parents even in different cages and different family groups, they formed a similar amount of stereotypical pacing to each other suggesting a genetic link and not a social learning link. Schwaibold and Pillay (2001) found similar results in human studies, however their findings suggested that although fostered offspring do pace to the same degree as their parents, they did not necessarily adopt the same stereotypical behaviour, indeed mothers of ASD (autism spectrum disorder) children have specific antibodies reactive to foetal brain proteins which are not found in mothers of children without autism, suggesting that autistic-like behaviour similar to stereotypical pacing is inherited (Camachoa et al., 2014).

Jawaid and Mansuy (2019) state that animal behaviour is influenced not only by life experiences and environmental exposures but also determined by changes in gene expression passed through generations at a molecular level through germline RNA (ribonucleic acid). Studies in rodents show that abnormal behaviour, altered sociability and behavioural despair develops in offspring of parents that experience stress and trauma. Further evidence of these findings illustrates those children and even grandchildren of the Cambodian and Rwandan genocide survivors show increased psychopathology and increased hyper-arousal and anxiety, depression, and neurodevelopmental delays, and emotional deficits have been linked to offspring of adults suffering from PTSD due to the Serbian-Bosnian conflict. The reader recognises that these studies are based on humans however, all animals, including humans inherit traits not only through their DNA but also through RNA and other molecules such as proteins and metabolites ('epigenetic markers') through both Intergenerational (passed to offspring from their parents) and/or Transgenerational epigenetic inheritance (passed between generations other than parent-to-offspring, e.g., grandparent to grandchild). Intergenerational inheritance has been found in rodents, as fear can be passed from parent to offspring, as transgenerational epigenetic inheritance such as stress in adult mice has been shown to cross three generations (Jones et al., 2010).

Problems with trauma impact on hippocampus memory and synaptic plasticity up to third or even fourth generation in rodents and brain plasticity is important to prevent stereotypical behaviour from developing (Kolb et al., 2010; Jawaid and Mansuy 2019). Jones et al. (2010) state genetic inheritance has an enduring effect on the organisation of the CNS and thus the animal's behaviour, psychological and physiological responses to their environment and their coping ability. McFarlane et al. (2014) states that stereotypies are inherited in striped mice and increases in prevalence over generations. Whilst positively enhancing fitness in captive conditions, this may be detrimental for survival in the wild. Mason et al. (2007) found selecting against stereotypies in poultry enabled farmers to select for animals who appear less frustrated in captivity. This research is still in its infancy, but evidence is growing that life experiences leads to behavioural phenotypes being transmitted across generations.

1.5.7 Individual Behaviour and Personality

Cooper et al. (1996) state the main contributing factor for the emergence of stereotypical behaviour is how individuals react, their age and personality. With different behaviours, including problem-solving performance such as foraging, being connected to personality dependent differences (Zandberg et al., 2017). Powell (1997) found there are individual differences in the development of stereotypies and Cooper et al. (1996) found rodents can react to enrichment 'actively' or 'passively'. Active responders increased locomotion, and as previously stated increased locomotion can lead to increased stereotypical pacing. Dantzer et al., (2010) states stereotypical behaviour is also linked to the shy/bold axis. Active rodents seem to be more aggressive, less docile, and bolder whereas neophobic shyer animals are much slower to interact with environmental enrichment, reducing its effect on stereotypical behaviour. Patton et al. (2014) found that bold and shy animals used an enrichment wheel differently, with bolder animals using it as enrichment and shyer animals using it to re-direct behaviour. Callard and Price (2000) state that being adaptable is more important than being shy or bold as the ability to adapt is a necessary trait for survival in the wild and Mason et al. (2010) found animals that cannot adapt in the wild often also suffer more in captivity but states attributes such as boldness, behavioural flexibility and being non-migratory as the three key characteristics predicting adaptability to captivity. Being bold rather than fearful may be the reason why some individuals do better than others in captivity.

Cooper et al. (1996) and Meers and Odberg (2005) found how individuals react to thwarted behaviour or reduced complexity in environment is linked to age. Gibbs (2010) explains that the younger brain is more responsive to current environmental experience and if stereotypical animals are moved to a more complex environment under six months of age, their stereotypical behaviour is reduced however if they are moved after this age the stereotypies persist. This is similar to findings by Tilly et al. (2010) study on voles, and an explanation for this could be that older mice find enrichment unrewarding and even aversive as they get used to the sameness and routine like autistic, neophobic, or anhedonia human patients which are all conditions that escalate with age. This research is important as red squirrels are rodents and therefore the success of adding more complexity to their environment to reduce pacing could also be affected by age.

Jones et al. (2011) states birth origin may be as important as age when it comes to developing stereotypical behaviour. This research found that although wild mice were more fearful than captive bred mice and have higher level corticosterone when put into captivity, the stereotypical behaviour was lower in adult wild-caught than juveniles with the adults appearing to be more protected from stereotypies than juveniles, possibly because wild-born adults have a larger behavioural repertoire to protect them from stereotypies developing – they have developed appropriate (adaptive) coping strategies in the wild. Similarly, Catlard et al. (2000) states rodents, including red squirrels born in breeding enclosures that have developed post weaning stereotypies continue to do so even if moved to bigger enclosure whereas adult rodents born in large enclosures that have never paced and moved to barren enclosures appear to be protected from preforming stereotypical behaviour. This is evidence that once established stereotypies are hard to eliminate (Coleman and Maier 2010) and relevant to current research where in order to control breeding, red squirrels breed in smaller breeding cages where stereotypical behaviour starts to emerge in juveniles.

It might therefore be safe to suggest that as there is a possible individual basis for the emergence and maintenance of stereotypical behaviour, this behaviour cannot always be studied at a group level but to be effective needs to be considered and treated at an individual level (Dalliare et al., 2006).

1.6 Why Stereotypical Behaviour Persists

1.6.1 Stereotypical Behaviour causes Damage to the Brain

Wurbel (2001) states that once established stereotypical behaviour can persist even if the animal is moved to a condition where stereotypies would not normally develop (emancipation). The basal ganglia is a richly interconnected neural network involved in the adaptive control of behaviour responsible for behavioural flexibility and stereotypical behaviour can arise due to changes in areas of the forebrain especially the neural pathways between the cortex and the basal ganglia (Pochon et al., 2001).

Changes to dorsal basal ganglia occur over time and being reared in barren environments can damage the basal ganglia and lead to a loss of plasticity, preservation of the behaviour (Mason and Latham, 2010) and reduced behavioural flexibility (the continuation of an activity without an appropriate stimuli). This does not occur in naturalistic captive environments which meets the motivational needs of rodents (Jones et al., 2011). Jawaid and Mansuy (2019) showed that exposing male mice to two weeks of environmental enrichment increased long term protection and memory in offspring. Exposing them to four weeks of enrichment induced resistance to depression and emergence of stereotypies in young, presumably because exposure to enriched environments caused learning and increased neural plasticity.

Wurbel (2001) found different housing conditions change functions in the brain and Gross et al. (2012) state rodents kept in barren cages develop stereotypical behaviour due to pathological dysfunction in the basal ganglia circuit, which stimulates or inhibits the cortex and performance of motor behaviour and many functions like voluntary motor control, learning, habits, and cognition, emotional functions and decision making (Graybiel, 2008; Mostard, 2011).

Mason et al (2007) observed that rearing young in barren environments (which fail to meet behavioural needs) affects the development of the central nervous system (CNS) resulting in stereotypies persisting past infancy. CNS disfunction interferes with breeding success and increases neglect of offspring thus reducing reintroduction success. This prevents the animal being able to behaviourally adjust to the captive environment leading to expression of abnormal behaviour (Mostard, 2011). CNS damage is hard to reverse and can make environmental change stressful, older animals preferring the status quo even if welfare is compromised (Hendershott et al., 2016).

Complex environments improve brain plasticity encouraging play within rodents and the release of dopamine which is shown to increase the weight of the cerebral cortical and occipital cortexes, improving cognition and memory (Balcombe, 2006; Griva et al., 2017), increasing synaptic plasticity, neurogenesis and decreasing reactivity to stressful stimuli and stereotypical behaviour (Gabriela et al., 2020). Exposure to prolonged stress affect the hippocampus which effects learning tasks, reduces cognitive decline and increases anxiety (Mostard, 2011; Gabriela et al., 2020; Chao et al., 2020). Hippocampus plasticity is mediated by serotonin in pre-frontal cortex, stress can affect serotonin levels and neurogenesis, which is important for cache retrieval. Although enrichment is known to increase neurogenesis in the basal ganglia and hippocampus, and protect the CNS, animals must be motivated to perform the task or engage with the enrichment for such effects to be realised (Klaus and Amrein, 2012). Yao et al. (2019) state seasonal brain plasticity contributes to physiological and behavioural processes. Food hoarding is linked to breeding, the hypothalamus releases gonadotrophin releasing hormones and that causes the pituitary gland to release follicle stimulating hormones which in females travels to the ovaries to cause ovulation. In a seasonal breeder this will be regulated by the action of melatonin from the pineal.

Grey squirrels have a longer-term memory and higher problem-solving ability than red squirrels and are 22% more accurate at locating caches (Chow et al., 2017). If stereotypical behaviour effects the brain, certain behaviour such as problem solving, persistence, behavioural plasticity, breeding, and exploration could have an impact on red squirrel fitness and survival (Jacobs, 2010; Chow et al, 2018). Chronic unpredictable mild stress is known to cause depression and cognitive deficits in humans and rodents (Shen et al., 2019) therefore it could be presumed that the impact on red squirrel behaviour would be similar, and welfare would be equally compromised. However, although there is much research on the effect of stress on cognitive behaviour and the impact it has on the emergence and preservation of stereotypical behaviour in elephants and bears little is known about red squirrels (Hopper, 2017).

1.6.2 Addiction Preserves Stereotypical Pacing

Stereotypical behaviour activates the dopamine system mediated by endogenous opioids and endorphin release in the hypothalamus, meaning animals can become addicted to these opioids and quickly get stuck in these appetitive sequences, preserving the stereotypies (Alosi et al., 1995). Loijens et al. (2002) states the circulating concentration of opioids is 50% higher in stereotypic pacers, suggesting the performance of stereotypies exerts a mitigating effect on environmentally induced stress, providing a physiological basis for the concept of stereotypies as a coping strategy.

Amir and Brown (1980) found rats with infected feet licked them more, increasing the release of opioids and endorphins in the hypothalamus helping the animals to cope with the pain, a behaviour that was preserved even after the infection was cured (Zavie et al., 1980). This positive feedback loop causes increasingly more endorphin release and the development of an addiction to the behaviour and may explain why stereotypical behaviour is harder to eliminate in older animals, as the behaviour leads to reward and becomes self-enrichment (Wurbel, 2001). Gabriela et al. (2020) supports these findings as young bank voles given opioids, reduced stereotypical behaviour (Nakamura et al, 2014), however, it had no effect on the performance of stereotypies in adult animals (Loene et al., 1993). Meers and Odberg (2005) argue against this as voles given opiates before weaning at 20 days did not decrease this stereotypical behaviour. However, giving SSRI (Selective Serotonin Reuptake Inhibitor) treatment their stereotypies did decrease, probably because serotonin is one of the transmitters involved in the neurobiochemical mechanisms of abnormal behaviour in bank voles, a characteristic which is shared by OCD human adults (Meers and Odgberg 2005) and could be linked with obsessive like behaviour such as stereotypical pacing in red squirrels.

Mason et al (2007) offers an alternative explanation, stating abnormal behaviour is not an addiction but a habit, similar to egg rolling in geese (Tinbergen, 1951). This is typically called a Fixed Action Pattern (FAP) - a stimulus produces a reflex-like behavioural response. Van Lierop (2005) found that rats in a maze disturbed half-way through their progress, do not carry on through the maze but went back to the beginning and start again, suggesting the behaviour must be performed in a fixed, linear, pattern. However, it is now recognised that genetics and environment act as the stimulus for behaviour in animals. Animals have flexible control over the behaviour they display as long as they are kept in appropriate environments and the notion that behaviour are simply fixed action patterns is typically now considered over-simplistic as both nature and nurture determine behavioural expression

1.7 Conclusion

Captive breeding is considered an important conservation tool for the preservation of the British red squirrel population (IUCN, 2019). However, as stereotypical behaviour appears to be prolific in the species and is difficult to eradicate, breeding in captivity is a real ethical concern (Shuttleworth et al., 2015). Zoos have a moral obligation to provide good welfare to animals in their care, but as illustrated in this literature review there is a real gap in knowledge as to why stereotypical behaviour emerges in the captive population and how to prevent or reduce it.

There also seems to be conflicting ideas regarding the best ways to house red squirrels, including their husbandry, feeding regimes and social groupings. Literature on red squirrel behaviour in captivity remains sparse, hence why a number of rodent studies has been used as a comparison, being of the same genus as the red squirrel.

There is a lack of funding from the UK government for red squirrel conservation, particularly for breeding and release programmes, coupled with this is a lack of public awareness of the red squirrel's plight and a decrease in the number of appropriate release sites and suitable captive animals for release. Red squirrels may have lost their wild-type behaviour, replacing them with stereotypical/abnormal type behaviour, like the typical captive rodent model taking all these factors into account the continuation of captive breeding programmes must be in question.

1.8 Rationale for Research

This thesis investigates stereotypy in captive red squirrels using a mixed method approach. A breeding pair of captive red squirrels housed in a breeding enclosure at Wildwood in Escot were observed, as were their litters over 16 months (18-month period). Keepers reported that the six-year-old male of the pair (Radish) had stereotypically paced since 10-week-old, and the female (Autumn) paced infrequently during seasonal hoarding.

Keepers further reported that all juveniles from the pair's previous litters have developed stereotypical pacing behaviour (Twiglet and Cherry) once they had weaned and, reportedly, the offspring continued to pace even when moved into a walkthrough enclosure (a larger, more complex enclosure, separate from the natal enclosure and parental squirrels). This study involved observing two different litters from the same parents (2019 litter being Douglas, Thistle and Blossom and 2020 litter being Kitten 1, 2 and 3), to record when pacing first starts and attempt to ascertain how and why stereotypical behaviour manifests, how seasonal changes impacted on behaviour and whether the zoos addition of ply-boards to create a solid perimeter and increased hoarding opportunities reduced this behaviour in both the adults and juveniles. The 2019 litter juveniles were also observed once they moved from their breeding enclosure to walkthrough enclosures, Thistle and Blossom to Wildwood in Escot and Douglas to Wildwood in Kent to see if their behaviour changed and to observe the impact the juvenile's presence had on behaviour of other squirrels already resident in the walkthrough enclosures, (Fern, Twiglet, Cherry and Bracken in Escot and Basil, Shep, Lucky and Smokey in Kent).

In addition, a keeper survey was performed using a questionnaire, requesting information regarding when stereotypical behaviour emerges in captive juveniles, how it manifests, and any actions taken to eliminate it. The questionnaire was sent to the ten breeding zoos (eight responded) in the UK to ascertain their different experiences and knowledge regarding the emergence and preservation of stereotypical behaviour in their red squirrels. Mellor et al. (2015) states zoos tend to focus on species management rather than individuals, however factors that improve welfare for some do not benefit everyone (Shepherdson et al., 1998) and as scientists, researchers and biologists are now focussing on the welfare of individual animals, this study focused on both groups and individuals.

While keepers anecdotally report stereotypical pacing is a problem in red squirrels, there is very little research on how and why stereotypies emerge in post-weaned red squirrel juveniles and why this behaviour is not only maintained but persists into adulthood, even if the adults are moved to larger enclosures. When stereotypical behaviour becomes a large part of an animals' behaviour repertoire it contributes to an inappropriate time budget as they spend so much time on abnormal behaviour and time spent on important behaviour such as foraging, caching and mating is greatly reduced, causing a reduction or even loss of species-specific behaviour important for survival in the wild. It is important to know if this impacts on whether captive reared red squirrels could be successfully released into the wild and would be able to survive as they are part of a breeding and reintroduction programme. The study could also increase knowledge on the welfare of squirrels and potentially positively impact on how they are managed in captivity and reduce stereotypical behaviour in the captive species.

1.10 Hypotheses

Multiple hypotheses were simultaneously tested in this study. Hypotheses are not relevant for some points as there is very limited data on captive red squirrel behaviour on which to build a theory. When possible, the following hypotheses were deduced:

Null Hypothesis 1 (Captive vs Wild)

There will be no difference in the types of behaviour recorded between red squirrels observed in captivity and wild red squirrel behaviour noted in the literature. In addition, it is predicted that there will be no significant differences in the time spent performing social, feeding, locomotion, grooming, drey building and inactivity (calculated as %total behaviour) in wild red squirrels (using averaged data from the literature) and captive red squirrels (directly observed).

Alternate Hypothesis 1 (Captive vs Wild)

There will be changes in the types of behaviour recorded between red squirrels observed in captivity and wild red squirrel behaviour noted in the literature. In addition to these changes, it is predicted that there will be significant differences in the time spent performing social, feeding, locomotion, grooming, drey building and inactivity (calculated as %total behaviour) in wild red squirrels (using averaged data from the literature) and captive red squirrels (directly observed).

Null Hypothesis 2 (Feeding/SB)

Red squirrel feeding behaviour (% total behaviour) and stereotypical behaviour (% total behaviour) will not be significantly negatively correlated.

Alternate Hypothesis 2 (Feeding/SB)

Red squirrel feeding behaviour (% total behaviour) and stereotypical behaviour (% total behaviour) will be significantly negatively correlated.

Null Hypothesis 3 (Anticipatory)

Red squirrel stereotypical pacing (% total behaviour) will not significantly increase prior to feeding times compared to directly post feeding times.

Alternate Hypothesis 3 (Anticipatory)

Red squirrel stereotypical pacing (% total behaviour) will significantly increase prior to feeding times compared to directly post feeding times.

Null Hypothesis 4 (Juvenile Move)

Juvenile red squirrels will not stereotypically pace (% total behaviour) significantly less when moved to a large, complex walkthrough enclosure compared to when housed in a small, less complex breeding enclosure.

Alternative Hypothesis 4 (Juvenile Move)

Juvenile red squirrels will stereotypically pace (% total behaviour) significantly less when moved to a large, complex walkthrough enclosure compared to when housed in a small, less complex breeding enclosure.

Null Hypothesis 5 (Alterations)

The stereotypical pacing behaviour (%total behaviour) of a red squirrel breeding pair and their juvenile offspring will not differ significantly before, compared to after enclosure alterations (privacy boards added, substrate increased by adding leaf piles and hoarding potential increased by 10% using hoarding containers).

Alternate Hypothesis 5 (Alterations)

The stereotypical pacing behaviour (%total behaviour) of a red squirrel breeding pair and their juvenile offspring will differ significantly before compared to after enclosure alterations (privacy boards added, substrate increased by adding leaf piles and hoarding potential increased by 10% using hoarding containers).

Null Hypothesis 6 (2019 vs 2020 Litter)

Stereotypical pacing (% total behaviour) will not be significantly less in the litter born in 2020 post alterations to the breeding enclosure at Wildwood Escot (addition of privacy boards, hoarding pots and leaf pile substrate) compared with the litter born in 2019 prior to breeding enclosure alterations.

Alternate Hypothesis 6 (2019 vs 2020 Litter)

Stereotypical pacing (% total behaviour) will be significantly less in the litter born in 2020 post alterations to the breeding enclosure at Wildwood Escot (addition of privacy boards, hoarding pots and leaf pile substrate) compared with the litter born in 2019 prior to breeding enclosure alterations.

The independent variables were the different individuals, social group size, enclosure type and site and the uncontrollable variables such as visitors, noises, were noted on marking sheets. Each check sheet detailed the date, time, weather, location and temperature and other notes.

2. Methodology

Ethics Statement from Winchester University (Appendix I)

This study was granted ethical approval by the University of Winchester Ethics Committee (HWB_REC_20_17_Adams-Wright). Please see Appendix I for approved ethics form.

2.1 Study Part A – Behavioural Observations

2.1.1 Behavioural Observations – Context

In April 2019 the author of this thesis attended the "Turning the Tide Conference on Red Squirrel Conservation" held at the University of Exeter. This afforded the author opportunity to meet with Kerry Church, Senior Keeper at Wildwood Escot and discuss a research project regarding the development of stereotypical pacing in their post weaned red squirrel juveniles, how this behaviour manifests and becomes entrenched into the adult behavioural repertoire and any means to attempt to reduce or preferably eliminate this abnormal behaviour. The study adopts a positivist approach as it involves gathering primary data using quantitative analysis to answer the predicted theory as denoted in the hypotheses. As data collection was deemed to be non-intrusive and once a risk assessment was completed with Wildwood it was agreed that the researcher could gather primary data for this study (See Appendix I and II for Risk Assessment, Research/Ethical Forms and approval emails from Wildwood Escot and Wildwood Kent). Subsequently, and as data collection progressed, the author applied to become an MPhil research student to further develop this study. Hence, data collection commenced before the start date of the MPhil. Once the researcher started Winchester University a triage form was completed with the help of the Faculty Ethics Representative. It was deemed that as observational study is non-invasive research, and therefore does not involve direct contact with animals, and that all changes to the enclosure were modified by the zoo for husbandry-, not research- purposes, only Faculty level ethical clearance (Form 3) was needed (see Appendix I for ethical approval letter).

2.1.2 Subjects

Captive red squirrels were observed in one of two sites, Wildwood Escot (Devon) or Wildwood Kent. (Permission given to share findings and signed form in Appendix IV). Sixteen individual red squirrels were observed; a summary of each red squirrel's characteristics including date of birth, when they moved to Wildwood, where they were housed and where observations took place is in Table 1.

Table 1 – Details of individuals in the study (n-16), including sex, date of birth, place of birth, current location and where observation took place

Name	Sex	Date of Birth	Place of Birth	Location Observed	Enclosure observed
Autumn	F	May 2015	British Wildlife Centre	Wildwood Escot (2018)	Breeding Enclosure
Radish	M	April 2016	Wildwood Escot (Breeding Enclosure)	Wildwood Escot	Breeding Enclosure
Fern	F	July 2012	Wildwood Escot (Walkthrough)	Wildwood Escot	Walkthrough Enclosure
Bracken	F	June 2014	Wildwood Escot (Walkthrough)	Wildwood Escot	Walkthrough Enclosure
Twiglet	F	May 2018	Wildwood Escot (Breeding Enclosure)	Wildwood Escot	Walkthrough Enclosure (From September 2018)
Cherry	F	May 2018	Wildwood Escot (Breeding Enclosure)	Wildwood Escot	Walkthrough Enclosure (From September 2018)
Blossom	F	May 2019	Wildwood Escot (Breeding Enclosure)	Wildwood Escot	Breeding Enclosure then Walkthrough from Sept 2019
Thistle	F	May 2019	Wildwood Escot (Breeding Enclosure)	Wildwood Escot	Breeding Enclosure, then Walkthrough Sept 2019
Douglas	M	May 2019	Wildwood Escot (Breeding Enclosure)	Wildwood Escot then Wildwood Kent (09/19)	Breeding Enclosure at Escot and Walkthrough Kent fromSept '19
Basil	M	June 2012	Pensthorpe Zoo	Wildwood Kent (2019)	Walkthrough Enclosure
Smokey	M	March 2018	Private Breeder Via Welsh Mountain Zoo	Wildwood Kent (2019)	Walkthrough Enclosure
Lucky	M	March 2017	Private Breeder Via Welsh Mountain Zoo	Wildwood Kent (2019)	Walkthrough Enclosure
Shep	M	April 2018	Wildwood Kent (Breeding Enclosure)	Wildwood Kent	Breeding Enclosure then Walkthrough (Oct'19)
Kitten 1	M	April 2020	Wildwood Escot (Breeding Enclosure)	Wildwood Escot	Breeding Enclosure
Kitten 2	M	April 2020	Wildwood Escot (Breeding Enclosure)	Wildwood Escot	Breeding Enclosure
Kitten 3	M	April 2020	Wildwood Escot (Breeding Enclosure)	Wildwood Escot	Breeding Enclosure

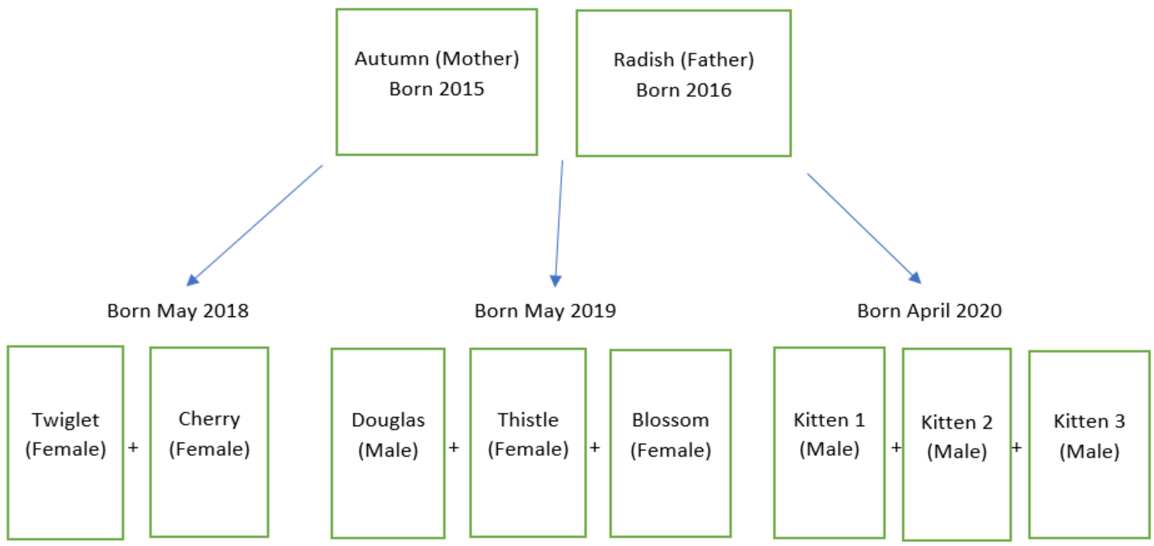


Figure 1 – Family Tree for Autumn and Radish litters (breeding pair housed in a breeding enclosure at Wildwood Escot (Devon). 2018 litter including Twiglet and Cherry, 2019 litter including Douglas, Thistle and Blossom and 2020 litter including Kitten 1, 2 and 3.

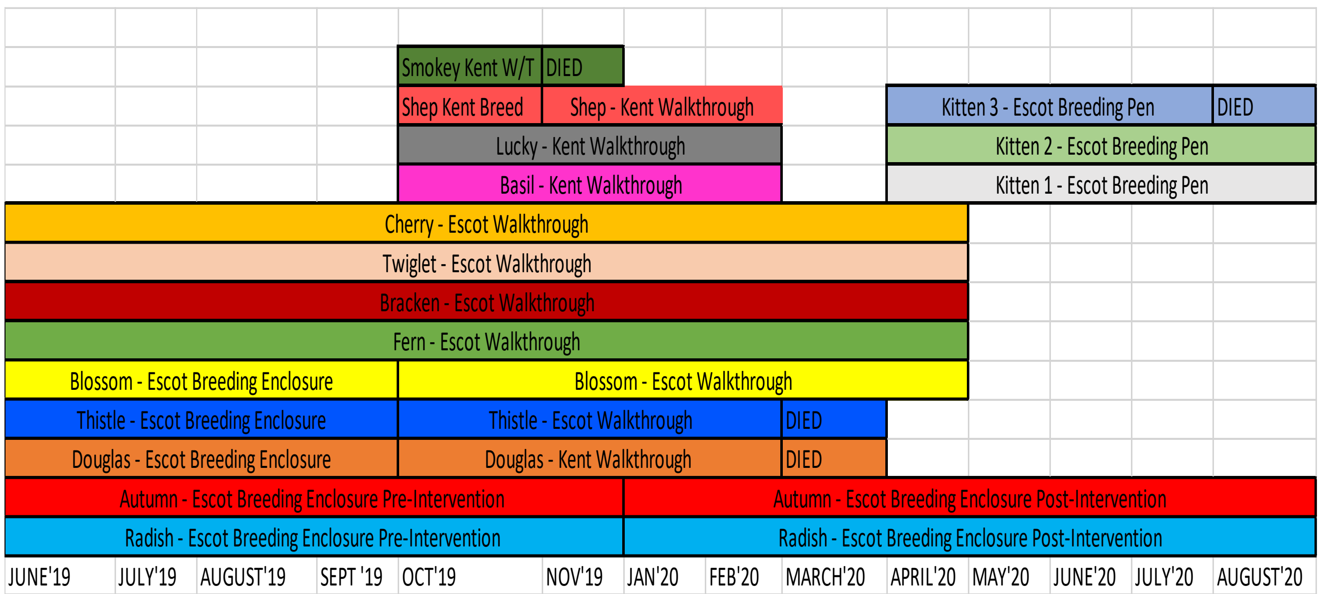


Figure 2 – Illustration of when individual squirrels were observed (n-16) at the Breeding Enclosure and Walkthrough at Wildwood Escot (Devon) and Wildwood Kent between June 2019 and end of August 2020. Shep Kent Breed (Indicates Shep in the Breeding Enclosure for one weekend of observations)

A family tree showing how each squirrel is related to each other is presented in Figure 1. Autumn and Radish, the main breeding pair, produced eight offspring that were observed in this study. The other six individuals also observed in this study were not related to Autumn and Radish, being single males housed at Wildwood in Kent (Basil, Lucky, Smokey and Shep) and two females from previous litters housed in Wildwood Escot (Fern and Bracken). During the observation time, between June 2019-August 2020, each individual had a varied rearing history and were typically moved between enclosures and/or groups at least once (Figure 2).

2.1.3. Housing

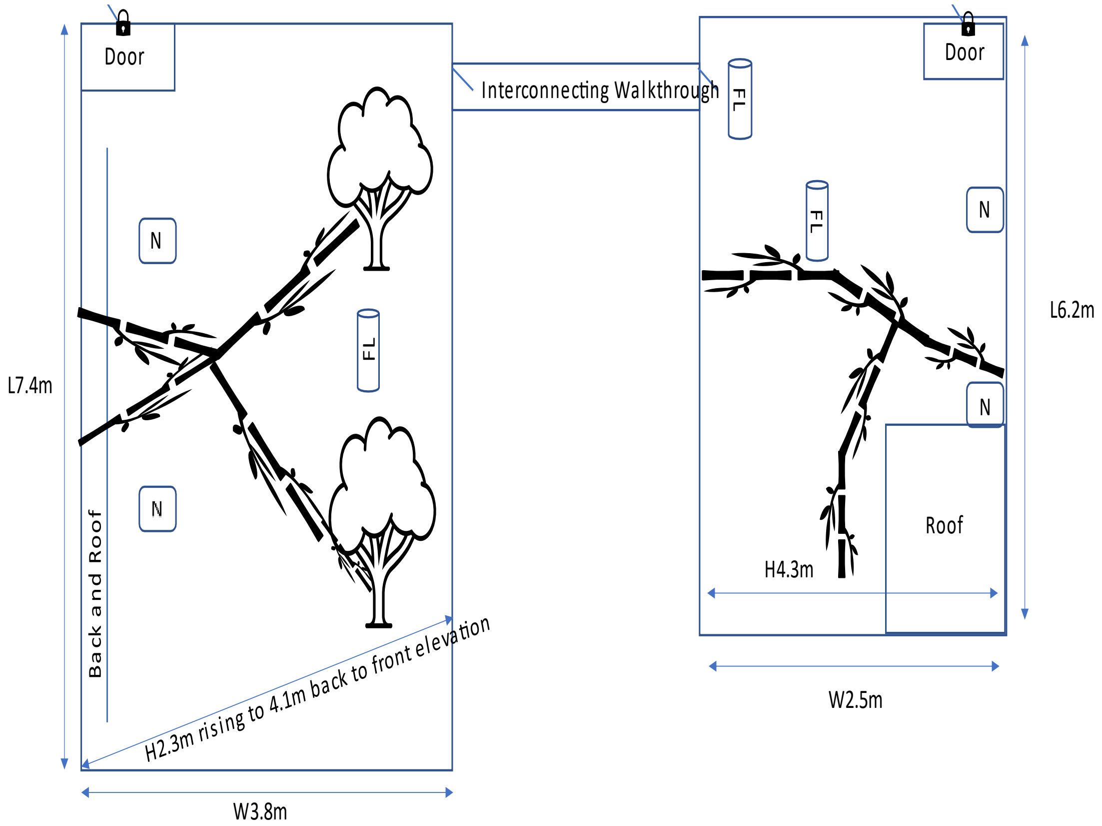
Floor plans for all enclosures are illustrated in Figure 3. The breeding enclosure at Wildwood Escot had two separate fully meshed pens either side of a pathway, joined by a tunnel which can either be shut off or opened to allow the squirrels access to both enclosure areas. The section on the left-hand side was 26.18 metres^3, 7.4 metre length, 3.8 metre width, 2.3 metre height at the back with a sloped roof to 4.1 metre hight at the front. The section on the right-hand side measured 14.64 metres^3, 6.2 metre length, 2.5 metre width, 4.3 metre height with a small roof at the far end opposite the doorway.

The walkthrough enclosure at Wildwood Escot (Devon) was approximately ¾ acre of open woodland containing deciduous and coniferous woodlands, bracken understory and various natural shrubs and bushes, surrounded by a perimeter fence comprising 1 metre of fine netting positioned upwards from the floor and solid metal sheets from the netting standing 2 metres tall all the way around the outside of the enclosure. At the very top the perimeter fence was electrified (4 strands of electric wire). There was no roof but double entry doors at the entrance and exit. A wooden boardwalk, about 1 metre from the floor runs from the entrance to the exit. A holding pen was fenced off inside the walkthrough enclosure, at the very centre to allow for soft release or to isolate individual squirrels. The holding pen had a solid roof and wire mesh perimeter, with two holes above the door for the squirrels to enter and exit from, 4.57 metres wide, 3.69 metres high.

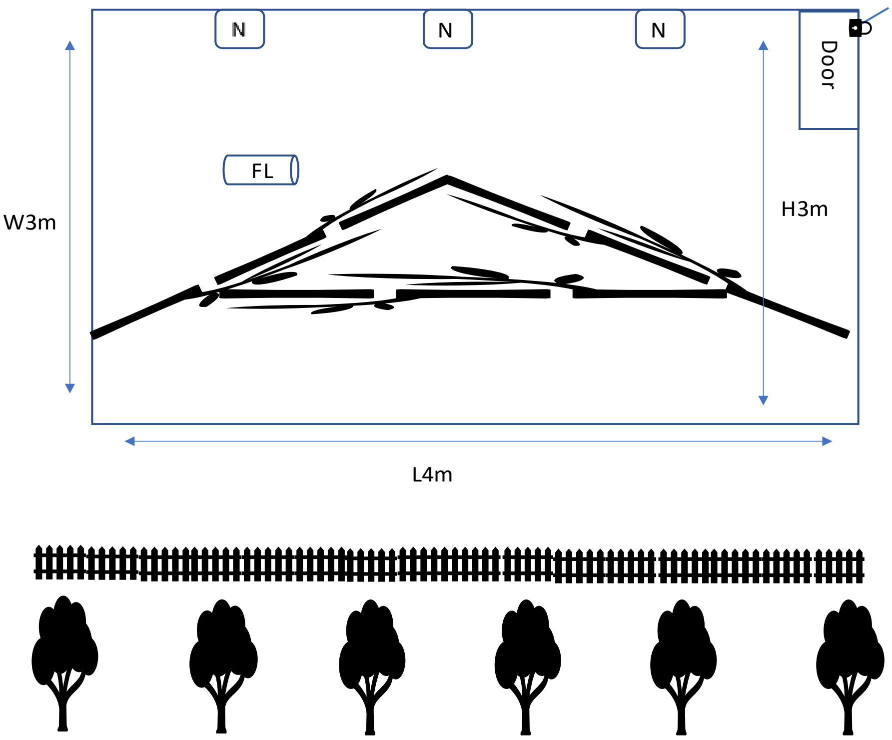
Wildwood Kent also have three breeding enclosures, one onsite, where Shep was originally housed, and two offsite. These breeding enclosures were 3 metre height by 4 metre length and 3 metre width. The author hoped to follow Shep's litter of five in September 2019 to use as a comparison for the group at Wildwood Escot, however after only one day of observations Shep killed two of his juvenile offspring and the other three were found deceased the following day. Shep was then moved to the walkthrough enclosure at Wildwood Kent.

The walkthrough enclosure at Wildwood in Kent is similar in type to the one at Wildwood Escot and housed a holding pen and boardwalk, however the metal sheets around the enclosure were much higher and run from the floor to approximately 5 metres high all the way round the enclosure. The woodland is less established with just one large oak tree and the other trees are stripped silver birch, with less natural foliage but large scrubland areas and several large log piles. There is a pond near the exit of the door and a small stream running through the enclosure, with a grate over the entrance and exit to prevent escape.

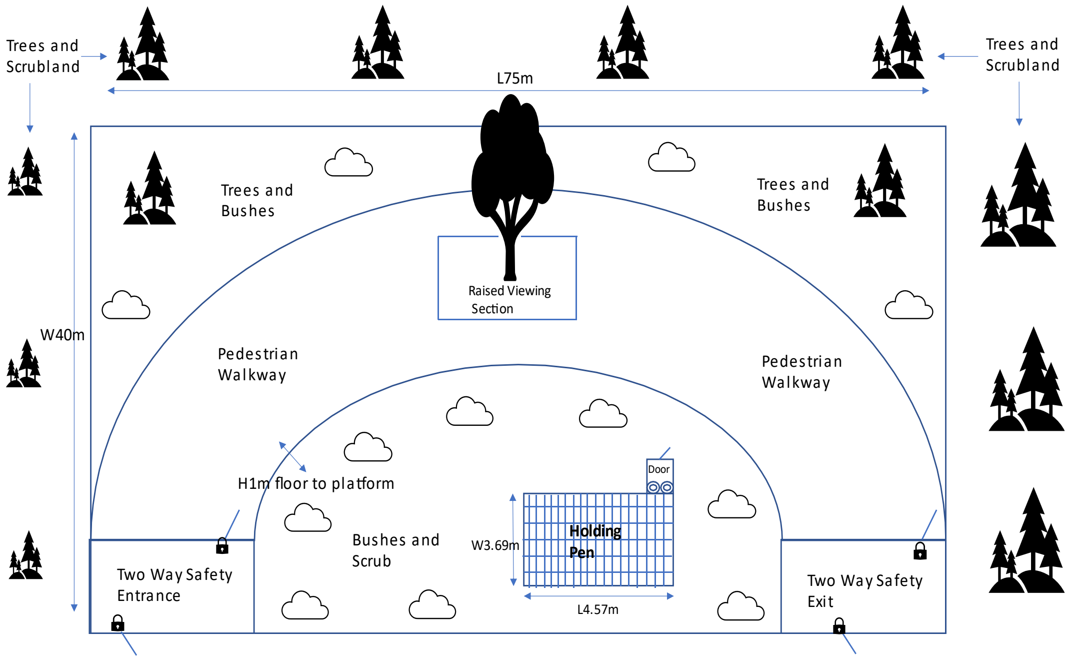
a)



b)


N
N
N
Door
FL
W3m
H3m
L4m

c)


Trees and Scrubland
Trees and Scrubland
L75m
W40m
Trees and Bushes
Trees and Bushes
Raised Viewing Section
Pedestrian Walkway
Pedestrian Walkway
H1m floor to platform
Bushes and Scrub
Door
Holding Pen
W3.69m
L4.57m
Two Way Safety Entrance
Two Way Safety Exit

d)

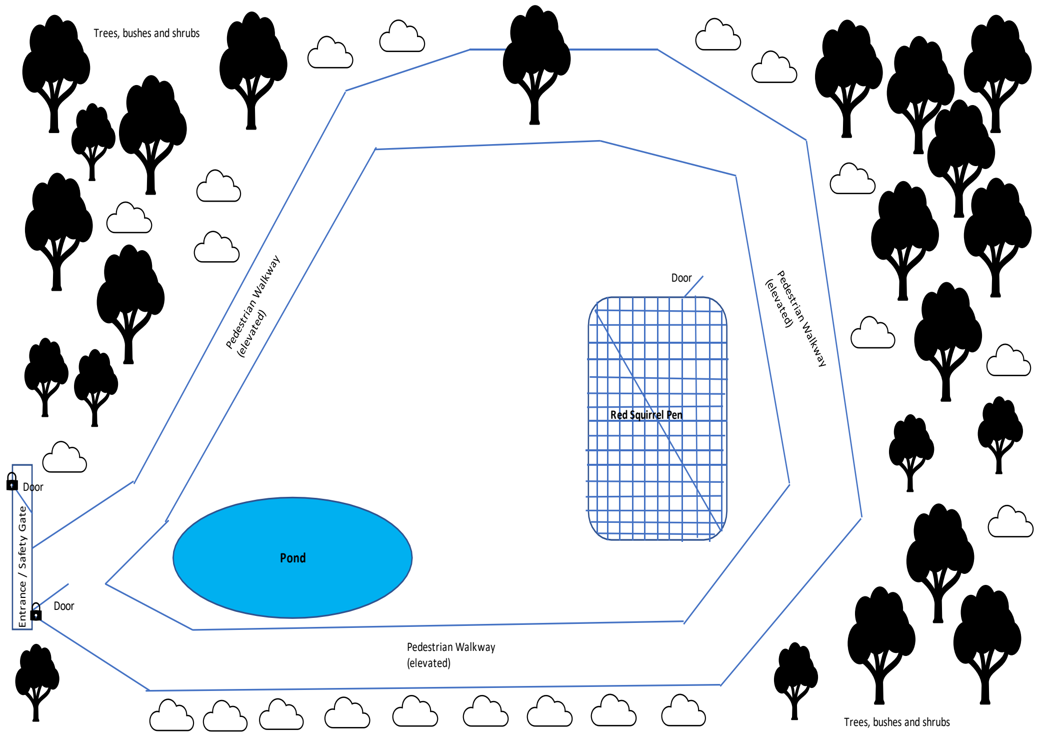


Figure 3 – Enclosure Floorplans (a) Floorplan for Breeding Enclosure in Wildwood Escot which housed Radish and Autumn and the 2019 (Douglas, Blossom, and Thistle from May 2019 until they were moved to the walkthroughs in September 2019) and 2020 litter (Kitten 1, 2 and 3 from May 2020 to Sept 2020). (b) Breeding Enclosure at Wildwood Kent, where Shep was housed in until October 2019, before he was moved after he killed his offspring. (c) Walkthrough Enclosure at Wildwood Escot which housed Fern, Bracken, Twiglet and Cherry and where Thistle and Blossom moved to in September 2019. (d) Walkthrough Enclosure at Wildwood Kent where Basil, Smokey and Lucky were housed and where Douglas and Shep moved to in October 2019 N= Nest Box, Fl -= Feeding Logs.

2.1.4. Husbandry Procedures

Husbandry procedures at both sites and in all enclosure types were similar, taking place twice a day at approximately 9am and 3pm. This involved cleaning away all unused food from feeding stations and refilling water bowls. The squirrels diet comprised mixed commercial parrot food which contain various seeds, supplemented by fresh seasonal vegetables, fruits, nuts and berries, which are placed on feeding platforms, on logs, in hanging enrichment and also scatter fed around the enclosures to encourage foraging behaviour. In the walkthrough enclosure at Wildwood Escot some hazelnuts are held back during these feeding times as the squirrels are hand fed these during a public squirrel talk at 11am each day. These husbandry procedures remained consistent during observations.

2.1.5 Materials

Key behaviours identified from four studies on wild red squirrel behaviour (Wauters and Dondt, 1987; Wauters, 1992, Bertoline et al., 2004 and Palmer and Koprowski, 2004), were used to allow comparisons to occur between wild and captive red squirrel populations. The wild-type behaviours that were identified were: social, feeding, locomotion, drey building, grooming behaviour, and inactivity.

Table 2 shows the amount of time wild red squirrels from each study spent in the various behaviours. This data was ultimately used to construct wild red squirrel activity budgets. Given in captivity individual animal behaviour is more visible (animals are often located close to the observer), finer-grade behaviours were defined and these categorised into the six wild-types behavioural categories (Table 3). A marking sheet was developed on which to record behaviour (see Appendix III). The ethogram also included a seventh category of behaviour 'stereotypical behaviour' which is an artefact of captivity. Stereotypical behaviour includes all types of repetitive, fixed, apparently functionless locomotor movement patterns, considered 'abnormal' in the literature, however as only linear and figure of eight pacing was observed, pacing is described throughout the Thesis when discussing stereotypical behaviour.

Table 2 - Four studies on wild red squirrel behaviour. Key behaviours being social, feeding, locomotion, drey building, grooming and inactivity (Wauters and Dondt, 1987; Wauters, 1992, Bertoline et al., 2004 and Palmer and Koprowski, 2004).

	Wauters and Dondt 1987	Wauters 1992	Bertoline et al 2004	Palmer & Koprowski 2004	Average
Social	1-2%	5%		6%	4.20%
Feeding	62-78% average 70%	54-85% Average 69%	54-63% average 56%	33%	57%
Locomotion	14-19% Average 17%	9-24% Average 17%	9-14% Average 12%	57%	26%
Drey Building	1-2% Average 1.5%	not given	not given	not given	1.5%
Grooming	2-6% Average of 4%	not given	not given	2%	3%
Inactive	2-6%	8%	4-20% average 12%	not given	8%

Table 3 - Red squirrel ethogram for key behaviours of captive red squirrels being social, feeding, locomotion, drey building, grooming and inactivity and stereotypical behaviour, including components of each key behaviour.

Behaviour	Breakdown of Behaviour	Definition
Social	Chasing	Two or more red squirrels running after each other, following the direction of the lead squirrel. The lead squirrel may turn and run after the chasing squirrels and the behaviour may involve climbing and jumping as well as running and can involve vocalisation.
	Allogrooming	One red squirrel using its front paws to pull apart and work through the fur of another red squirrel, licking or biting the body of other red squirrels to remove mites/foreign bodies or clean fur.
	Conflict	Physical altercation between two or more red squirrels, may also involve vocalisation, biting, scratching or chasing behaviour
	Play	Similar to fighting which does not involve injury. There may be some vocalisation and chasing behaviour.
	Vocalisation	Noise emitted from the throat of one squirrel and directed at one other or several other red squirrels. This may be short bursts of noise or loud repetitive noise.
	Touching	Two or more red squirrels physically in contact with each other with their bodies making contact with another.
	Scent Marking	Rubbing cheeks and mouths along different surfaces releasing hormones in glands around the mouth to communicate their territory, their cache sites or that the females are in oestrus. Red squirrels also urinate as they move and may stop and rub their cheeks on the urine.
	Feeding Young	The juvenile's mouth in contact with the mother's nipple and actively suckling.
	Mating	Dorsal-Ventral Mounting with the male on top of the female and involves pelvic thrusting
Feeding	Foraging	Actively searching for food in the enclosure using hands to filter through the foliage.
	Ingestion	Holding food in hands and actively chewing, gnawing, and digesting food
	Manipulation	Squirrels using paws to rummage through food items, sometimes picking up food to inspect or lick it but not actually ingesting the food.
	Drinking	Use of tongue to extract water from any source of water.
	Bark Stripping	Using teeth to gnaw on the bark of a tree, to remove a strip of bark to access the phloem (Girdling).

	Caching Food	Using front paws to dig a hole in the ground, place food from mouth into the hole and covering the hole, using front paws to stamp down the surface. This could also be followed by scent marking the area. This could be either true caching or false caching where the squirrel does not actually place the food in the hole but is behaving deceptively in order to prevent other conspecifics from stealing
Locomotion	Climbing	The red squirrel using front limbs to pull and back limbs to push themselves up in a vertical or horizontal position in order to move either up or down different objects.
	Running	Moving quickly from one foot to another in a forward momentum.
	Jumping	The red squirrel launches itself from one area to another with both feet off the ground at the same time.
	Walking	Moving slowly from one food to another in a forward motion.
Drey Building	Collecting foliage	Searching the ground with front paws for twigs, sorting through them by picking and dropping them. Also includes biting off small twigs from branches.
	Carrying foliage	Placing twigs and foliage into mouth to the drey sight.
	Using foliage for drey	Placing twigs, lichen and foliage into drey.
	Manipulating foliage	Using paws and mouth to move foliage, fur, lichen or twigs around in the drey. Also involves pushing the twigs into place using front and back paws.
Inactivity	Resting	The red squirrel sitting completely still, fully conscience with eyes open, but in relaxed position.
	Vigilant	Red squirrel either sitting or standing still but looking around and being watchful and observant about its surrounding. Could involve a stiffer more erect body.
	Sleeping	Red squirrel in slumber, no movement or vocalisation with eyes closed.
Other	Grooming	Holding tail in both paws and licking tip of tail. Licking paws and rubbing them over the face or ears. Parting hair on the body and removing parasites and cleaning fur.
Stereotypical Behaviour	Pacing	Repetitive, linear or figure of eight pacing along one side of the enclosure. This style of stereotypical behaviour was witnessed so pacing is used to describe all stereotypical pacing
	Stepping side to side	Repetitive behaviour, apparently functionless behaviour, stepping, hopping on alternative feet, seen in-between bouts of pacing behaviour

2.1.6 Pilot Study

Red squirrels were observed for three days between 15th-18 May 2019 in order to produce robust ethograms and devise marking sheets and ascertain the best sampling method to collect data. The pilot study also enabled the researcher to choose the best observation sight and the best times to conduct research. This study is detailed in Appendix V.

It was determined the best means to collect data was to use instantaneous scan sampling, every two minutes. Although instantaneous scan sampling did not give true durations of time spent on a particular behaviour, many empirical studies have shown that making the sample interval relatively short to the amount of time spent on a behaviour can give reasonable estimates of time budgets (Tyler 1979; Martin and Bateson, 2007). A two-minute time interval was deemed to be the most appropriate for these observations as it allowed the observer time to locate each squirrel, identify the individual and record the exact behaviour taking place. This type of sampling also allowed the observer to scan for larger amounts of behaviour, and the ability to write detailed notes on the marking sheets regarding the display of this behaviour. Identifying individuals is essential as individuals in a species do not always behave in the same species-typical way (Krebs and Davies 1987, Martin and Bateson, 2007). The research question is based on the presentation of stereotypical behaviour; however, all behaviour was recorded so that time budgets could be compared between both pacing and non-pacing squirrels and also their wild counterparts. Differences in social, non-active, consummatory or appetitive behaviour could then be accurately recorded to highlight any potential cause of/and maintenance of stereotypical behaviour.

2.1.6 Data Collection

Data collection started in June 2019 at Wildwood in Escot and all red squirrels in both the breeding enclosure and walkthrough were observed. Squirrels were observed between the hours of 7.30am-6pm dependent on observer travel times, zoo opening times, and given squirrels are diurnal and active mostly in the morning and the late afternoon). All state behaviour was recorded on the 2-minute sample point for 7 hours a day, normally starting during summer months at 8am and finishing at 4pm, with a one-hour break between 1pm and 2pm, as this was normally a quieter time of day when the squirrels were not normally active, and from 7.30am- 3pm with just a half hour break in the day during winter months.

Initially squirrels in both the breeding and the walkthrough enclosure at Wildwood Escot were observed 21 hours over three days a week, on a Tuesday, Wednesday and Thursday, and sometimes at the weekend, depending on the observers' work commitments, for June, July and August 2019. However, once the juveniles were moved to the walkthrough enclosures in Escot and Kent, and there was potentially another litter to observe in Kent, it was not possible or practical to allocate so much time to each squirrel collection. Observations were therefore reduced to 14 hours per collection over two days per month. As each individual squirrel was observed, and the observations were representative of the impression the keepers also had to the squirrels behaviour during the days of data collection (given keepers observe their animals routinely and informally daily) the researcher felt this was still a true representative of the overall individual activity budgets, In total 500 hours of observations, at Wildwood Escot and Wildwood Kent were conducted over the course of 16 months data collection (18 calendar months-between April 2019 and October 2020).

Observations were conducted on different days of the week to take into account changing visitor numbers due to school visits, school holidays and weekends and also enrichment timetables which could impact on the red squirrel behaviour. Controllable variables such as taking observations from the same place during day light hours and using the same method were standardised to increase statistical accuracy (Martin and Bateson, 2007).

The major limitation of this research was caused by the COVID-19 pandemic and two lock-downs when the observer could not travel to collect data. In the first lockdown cameras were set up in the breeding enclosure for eight weeks from 24th March 2020 to 18th May 2020. The recordings were sent to the observer on a weekly basis so that the observer could continue to collect data, however as it became clear only more visible behaviours could be noted it was decided to place marking sheets at the entrance of the breeding enclosure for the staff to complete every day during husbandry procedures. The observer worked with two members of staff who had previously conducted research on the red squirrels on how to record the behaviours to ensure everyone was identifying the behaviours in the same way. These sheets were sent to the observer every two weeks and using the information from the sheets the observer could gather as much information as possible regarding the new juveniles and adults' behaviour in the breeding enclosure. In the end only eight hours of data was collected over six weeks, so it was decided to not use this information in the statistical analysis and only use it for recording the emergence of stereotypical pacing in the 2020 litter.

2.1.8 Alterations to the Breeding Enclosure

During data collection of the red squirrels breeding enclosure several alterations to the breeding enclosure were discussed by the zoo to try and reduce the pacing displayed by Radish and potentially the litter due to be born in April 2020, as keepers speculated that the stereotypy could be caused by foraging and hoarding stress and fear of predation. These included adding 1-metre-high solid wooden boards across 1 metre length of the front and back of the old and new side of the breeding enclosure, and along the whole side of the enclosure opposite the doorway (see Appendix IV for carpenters' drawings and plates of before and after alterations). Additional substrate was added to the floor of the enclosure which included leaf piles and wood shavings, these additions increased the current substrate by 10%. The floor space and foraging and hoarding potential was also increased by 10% by adding planters filled with soil and wood chip. Food could then be hidden in these planters to increase foraging behaviour and also used to cache food.

Planters were made on the old (left) side of the enclosure by making one long planter along the back wall: 400cm length 40cm width x 6cm depth - 117cm off the ground and one small planter attached to middle beam: 77cm length x 11cm width x 5cm depth - 100cm off the ground, and to the new (right) side by adding one long planter along the back: 374cm length x 40cm width x 6cm depth - 132cm off the ground and one small planter attached to the front of the enclosure: 75cm length x 28cm width x 5cm depth - 115cm off the ground (see Appendix IV for carpenters' drawings and plates of before and after alterations)This was completed in three stages, boards put up in January 2020, substrate added in February 2020 and hoarding pots added in March 2020. Squirrels were observed and data was collected for 28 hours over four weeks in between each alteration.

2.1.9 Data Analysis

The study adopts a positivist approach gathering primary data using quantitative analysis to answer the predicted theory as denoted in the hypotheses. (Bruin, 2006; Dytham 2012). For statistical procedures, the percentage of observations of six key wild-type behaviour (social, feeding, locomotion, drey building, grooming, inactivity) and stereotypical pacing were calculated using Excel to produce activity budgets for each squirrel with the allocated behaviour being expressed as a percentage of total of observed behaviour for all observed squirrels for each month of the study (Appendix IX). These were further broken down to behaviours in each category for the breeding pair and juveniles to analyse any specific difference in each behaviour that could impact upon the presentation of stereotypical pacing.

To test Hypothesis 1 comparing wild activity budgets to captive ones, the average key behaviour (social, feeding, locomotion, grooming, drey building, inactivity plus stereotypical pacing) for all 16 observed individuals between June 2019 and August 2020 was used. and a Chi^2 goodness of fit test was preformed to compare the observed values in the data to the expected values of the wild-type behaviour to test the null hypothesis. A Mann Whitney U test was then performed on this data to highlight statistical differences in activity budgets between the walkthroughs, holding pens and breeding enclosure, and this behaviour was further analysed using a Wilcoxon matched pairs test. These tests were also run to find comparisons between the mean percentage of time red squirrels in the two walkthroughs spent in both pacing and key behaviours between June 2019 and March 2020, between any potential sex differences between males and females, and/or juvenile and adult differences in behaviour across all 16 red squirrels in all locations between June 2019 and August 2020. Individual behaviour for all 16 red squirrels in all locations, including moving between locations from June 2019 and August 2020 was recorded and the mean amount of stereotypical behaviour was calculated and a Chi^2 goodness of fit was used to test any significant difference in the distribution of pacing between individuals.

To test Hypothesis 2 The mean percentage of time spent in stereotypical pacing by all red squirrels ever housed in the breeding enclosure between June 2019 and August 2020 was split into before and after feeding and a Wilcoxon matched pairs test applied to determine if the pacing before and after feeding was statistically significant.

To test Hypothesis 3, if feeding behaviour and stereotypical pacing were negatively correlated, the mean for stereotypical behaviour before and after feeding was recorded for red squirrels who were ever housed in the breeding enclosure at Escot between June 2019 and August 2020 and a Spearman's rank correlation was used to measure the degree of association between these two variables

To test Hypothesis 4 to determine any statistical significance between the six wild-type behaviours and behaviour for Douglas, Thistle and Blossom, before and after the move from to the walkthroughs at Escot and Kent in October 2019 a Wilcoxon matched pairs test was applied to the mean behaviour for all three juveniles before the move between June - September 2019 and after the move October 2019 – March 2020. The mean behaviour of the resident squirrels in both walkthroughs in Escot and Kent from June 2019 – March 2020 were also analysed using Wilcoxon matched pairs test to highlight any changes in behaviour after the juveniles were released into the walkthrough enclosures in October 2019.

To test Hypothesis 5, the percentage of behaviour before and after the alterations in the breeding enclosure were checked to see if they were normally distributed and a nonparametric Wilcoxon matched pairs test was applied to the two paired groups to determine any statistically significant difference between the data and comparing the mean for all six wild-type behaviours against stereotypical behaviour using Spearman rank correlation which illustrated any relationships between a key behaviour and stereotypical pacing. To test Hypothesis 6, the time when pacing began in the two litters in 2019 and 2020 was recorded and the average percentage of time spent in stereotypical behaviour based on age in weeks was then compared using bar graphs

2.2 Study Part B - Survey of Squirrel keepers

Ethics Statement from Winchester University (Appendix I)

This study was granted ethical approval by the University of Winchester Ethics Committee (HWB_REC_20_17_Adams-Wright). Please see Appendix I for approved ethics form.

2.2.1 Survey Rationale

This research involved a mixed methods approach; in addition to the behavioural observations using quantitative methods the study also involved a mixed method (quantitative/qualitative) survey using a standardised questionnaire as the instrument. This mixed method approach is becoming increasingly popular as findings can reflect real-world perspectives and support quantitative data and generate broader theories regarding stereotypical behaviour in captive red squirrels (Saunders et al., 2007). By targeting a specific audience and collecting information via a survey, as opposed to simply sending out a questionnaire to random zoos, a researcher has control over the sample size and data collection so there is greater confidence that the research objectives are being met (Easterby-Smith et al., 2008). The rationale for conducting a survey with other red squirrel breeding programmes is through talking to keepers there is a significant interest in the industry about this research, as research into captive stereotypical behaviour in the species is limited. It is not practical to observe every captive squirrel in multiple breeding programmes and it would take many years to do so making any behaviour comparisons impossible. This mixed method allows the researcher to determine from the keeper’s perspective if stereotypic pacing is part of the repertoire of other captive red squirrels and helps determine if the squirrels in the research project are typical of all captive red squirrels in the UK, how widespread it is and what patterns of emergence are noted by the keepers. Keepers are best placed to comment on behaviour and welfare of their animals and a survey allows many keepers to discuss the behaviour of many individual animals.

2.2.2 Design of Survey

A pilot questionnaire was sent to Escot in September 2020 to help inform the structure of the questionnaire and this is detailed in Appendix V. Following changes made to the pilot questionnaire in September 2020, the final questionnaire included 20 questions which were either single answer questions, open-ended and closed-ended questions.

Additional questions such as ranking behaviour for how often stereotypical pacing is seen made it easier for people to answer questions and for more valid comparisons and analysis. Open-ended questions allowed respondents the ability to answer in their own words, giving detailed information about their captive red squirrels behaviour and the ability to share their expertise with the researcher. The questionnaire was split into different sections covering life history variables for each squirrel, mortality rates for births from 2017-2020, enclosure design, including husbandry procedures and types of and expression of abnormal behaviour in their animals. Other sections built on this knowledge including the keeper's opinion on why red squirrels pace, any attempts to prevent it and any success they have experienced. The final section was linked to any successful release of their red squirrels and also any barriers they perceive with breeding and release programmes.

2.2.3 How Participants Were Chosen

There are 10 zoological collections and 15 private collections involved in captive red squirrel breeding throughout the UK (Welsh Mountain Zoo, 2019). Participants were selected using two different methods. One being purposive sampling, during the 16 months of observations and attendance at the Turning the Tide Conference in Exeter in April 2019 and The ABWAK Red Squirrel Workshop at Wildwood Kent in September 2019, the observer was able to build up a network of willing contributors to answer the questionnaire.

The second way zoos were selected was using ZIMS, an information application used to manage information about animals' accessions and dispositions regarding breeding numbers to ascertain exactly which organisation housed or bred red squirrels and then emailing these directly to see if they were willing to participate in the study. This was accessed by staff at a participating zoo specifically to help the researcher distribute the questionnaire.

2.2.4 Distribution of Questionnaire

Originally the author was going to visit each individual zoo to conduct a structured interview of one hour using the questionnaire as a guide, as this method is considered one of the most useful ways to collect data, avoiding ambiguity and a means of obtaining unanticipated information (Collis and Hussey, 2003). However, because of the second lockdown in November 2020 this was not possible, therefore a second option of potentially using Microsoft Teams to conduct face to face virtual meetings was discussed with all potential respondents. However. after emailing each respondent on 11th November 2020 detailing the research and sending an information sheet and consent form to read and sign, all respondents requested to receive the questionnaire via email. The deadline for participation was given as 31st December 202 and people were sent reminder emails two weeks before the deadline if they had not yet submitted their questionnaire.

Out of 10 contacts, eight zoos completed the questionnaire (six currently involved in breeding programmes and two which have bred in the past but currently not breeding). Neither zoo, respondents or individual squirrels were identified in the results and answers to the questionnaire are in Appendix VIII.

2.2.5 Data Analysis

Data collected from this survey was analysed using a mixture of descriptive statistics, graphs, and nonparametric inferential statistics. Non-parametric statistics are used as data collected is not true measurements, but individual scores derived from keeper opinions. Different ordinal variables can be analysed at the same time and the median and or mode for these variables are used as well as frequency tables and bar charts to analyse differences and similarities between different establishments and behavioural observations. Open ended questions were analysed used semi deductive thematic analysis. Data concerning 56 red squirrels were collected, and included sex, life stage, survival rate, birth origin and percentage that paced. Respondents rated the frequency, time and bout length of pacing on a scale from 1-5, 1 being the minimum to 5 being the maximum amount and also detailed when pacing began and the type of pacing each squirrel displayed (Appendix VIII).

Respondents also gave detailed information regarding enclosure design, size, substrate, whether the enclosure had cover, climbing and drey building opportunities and how many nest boxes they had. Information on diet and feeding regimes were also given, including if the animal was seasonally fed, variety of food, presentation, and timings of feedings. Each one of these elements was then given a numerical value to allow for summary statistics and comparison between respondents (Table 4). These numbers were then applied to each individual squirrel (n=56) (Appendix VIII) and used to run a multiple logistic regression model to predict the dependent data variable (pacing) by analysing the relationship between several different independent variables (enclosure, feeding, age, sex).

Table 4 -Description of how each element of enclosure and diet were scored in order to run a logistic regression model for all red squirrels in the survey (n=56)

Size	Design						Feeding			
1 =< $100m^3$ 2=100-200 m^3 3=200-300 m^3	Substrate 1=concrete 2=leaves 3=woodchip 4=both 2&3 5=sand 6=all	Cover 1=none 2=manmade 3=natural 4=both 2&3	Climbing 1=none 2=manmade 3=natural 4=both2&3	Enrich 1=none 2=daily 3=week 4=month	No of nest box	Drey Bldng 1=no 2=yes	Season 1=no 2=yes	Variety 1=basic (parrot, nuts/ seed) 2=complex (hawthorn, browse)	No of Feed	Presentation 1=feeding stations 2=scatter 3=both 1&2

Thematic analysis was used on all open questions (Q10-Q20) to identify, analyse, and interpret patterns of meaning with qualitative data and presented in dendrograms giving specific quotes from different zoos relating to each theme, showing how each theme is derived from or a good fit for the respondents' words. A Chi^2 goodness of fit test was used to test whether respondents view on limitations for captive breeding programmes were similar with the critical p-value <0.05.

3 Results

Over the course of 16 months active data collection (18 calendar months) 16 squirrels were observed for a total of 500 hours between two breeding facilities. Daily activity was recorded to create standardised activity budgets to enable comparisons between captive squirrels and wild squirrel behaviour, and in captive squirrels examine changes in stereotypical pacing between life stages, sex, enclosure type and enclosure alterations. Statistical Package for the Social Sciences (SPSS) v26 was used for statistical data analysis.

3.1 Inferential and Descriptive Statistics from Observations

3.1.1. Hypothesis 1 - Captive vs wild red squirrel behaviour

Wild behaviour is categorised in the literature as Social, Feeding, Locomotion, Drey Building, Grooming, and Inactivity. Wild squirrels spend over 80% of their observed time feeding or in locomotion, rarely being inactive or engaging in social interaction. Captive squirrels were observed to form two distinct groups – those who performed wild-type behaviours only ('non-pacing') and those observed to stereotypically pace ('pacing'). Non-pacing captive squirrels (n=3) also spend over 80% of their time feeding or locomoting, their entire activity budget is highly comparable to wild squirrels, except that non-pacing squirrels perform double the amount of social behaviour (captive non-pacing 8.4%; wild 4.2%).

To test the effect of pacing on wild-type behaviour for non-pacing and pacing captive red squirrels, two Chi^2 goodness of fits were applied – one to the non-pacing captive individuals and one to the pacing captive individuals which used the wild-type data as expected values in both cases. The distribution of time non-pacing captive squirrels spent in Social, Feeding, Locomotion, Drey Building, Grooming and Inactivity was statistically similar to the activity budget of wild squirrels (Chi^2=10.772, N=3, df=5, p=0.056). Conversely, pacing captive squirrels (n=13) spend nearly 10% of their observed time engaged in stereotypical pacing. In addition, these squirrels spent only 31% of observed time feeding – a little under half the time wild squirrels spend feeding. Captive squirrels who paced were also more inactive compared to non-pacing squirrels (19% vs. 5%) and compared to wild squirrels (8%). The distribution of time captive pacing squirrels spent in each wild-type behavioural category was significantly different from the wild squirrel activity budget (Chi^2=32.946, N=13, df=5, p=<0.001) (Figure 4).

It needs to be noted that the wild squirrel activity budget does have large differences in the error bars particularly for both feeding and locomotive behaviour as all four studies were sampled during different times of the year and also over a range of twenty years when red squirrel numbers had started to drastically decline (Table 2). Some categories of behaviour noted in pacing and non-pacing captive red squirrels also had large error bars. This is likely a result of the squirrels being moved to difference enclosure types. For example, Basil, Lucky and Smokey were moved from a holding pen for one observation and back to the walkthrough enclosure in October 2019. Douglas, Blossom and Thistle were moved from the breeding enclosure to a holding pen after 4 months of observations (September 2019) and then released into the walkthrough in October 2019. The breeding enclosure was altered after 9 months of observations (January – March 2020) which altered Radish and Autumn’s pacing. Shep was observed for one day in the breeding enclosure and then released into the walkthrough, Twiglet and Cherry and the 2019 litter did not change location during the study. These moves and changes may have affected the results as discussed in Table 5, and individual pacing patterns are shown in Figure 5a and 5b and critically analysed at length in the Discussion.

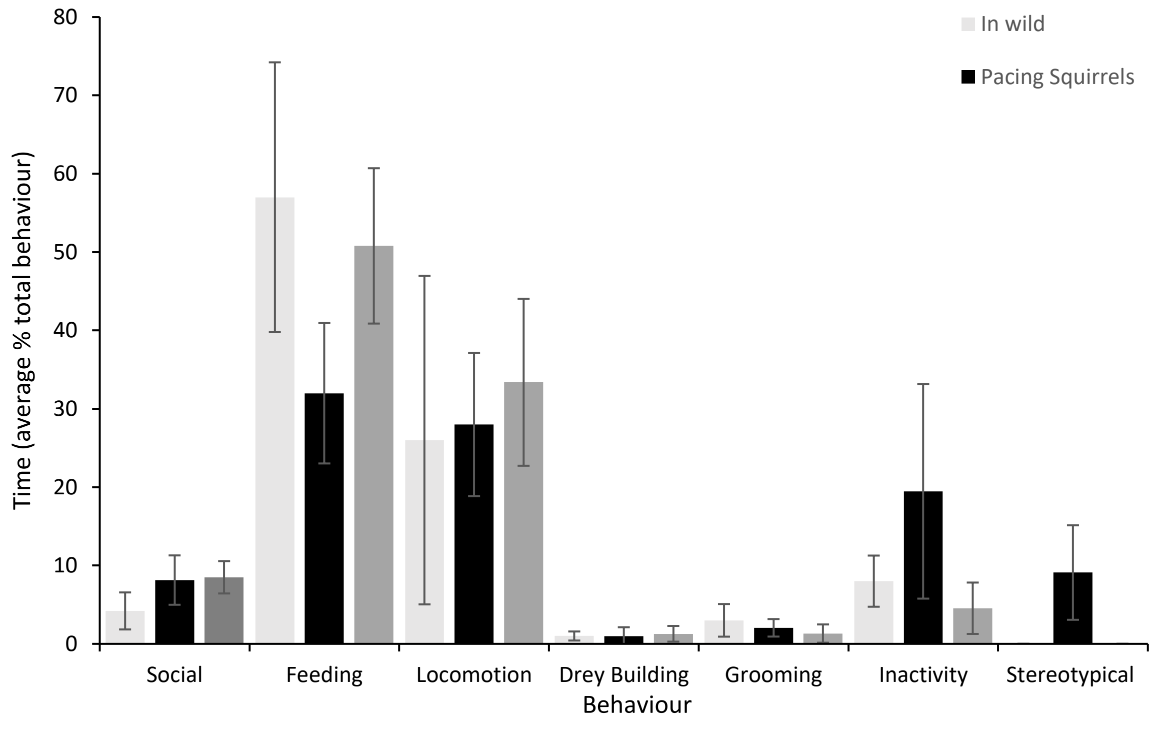


Figure 4: Wild Verses Captive Activity Budgets for Red Squirrels. Behaviour expressed as percentage of total behaviour observed. Average wild activity budget derived from four published papers on wild red squirrel behaviour (Wauters and Dondt, 1987; Wauters, 1992; Bertoline et al., 2004; Palmer and Koprowski, 2004). Captive data is averaged between all 16 individuals observed, split between those who were observed stereotypically pacing (N=13, Twiglet, Cherry, Radish, Autumn, Douglas, Thistle, Blossom, Lucky, Smokey, Shep, Kitten 1, 2 and 3) and those who did not pace (N=3, Fern, Bracken, Basil), between June 2019 and September 2020. Error bars = standard deviation.

3.1.1.1 Individual variation in pacing

Only 19% (3/16) of the observed captive squirrel population (Fern, Bracken and Basil) were never recorded pacing, either during the project or previously reported by zookeepers, with 81% (13/16) of captive red squirrels observed pacing at some point during the study or known to pace by keepers (Figure 5). To test individual differences in the average time spent pacing Chi^2 goodness of fit was used, with the distribution of pacing being significantly different between individuals ($Chi^2=71.696$, N=13, df=5, p=<0.001).

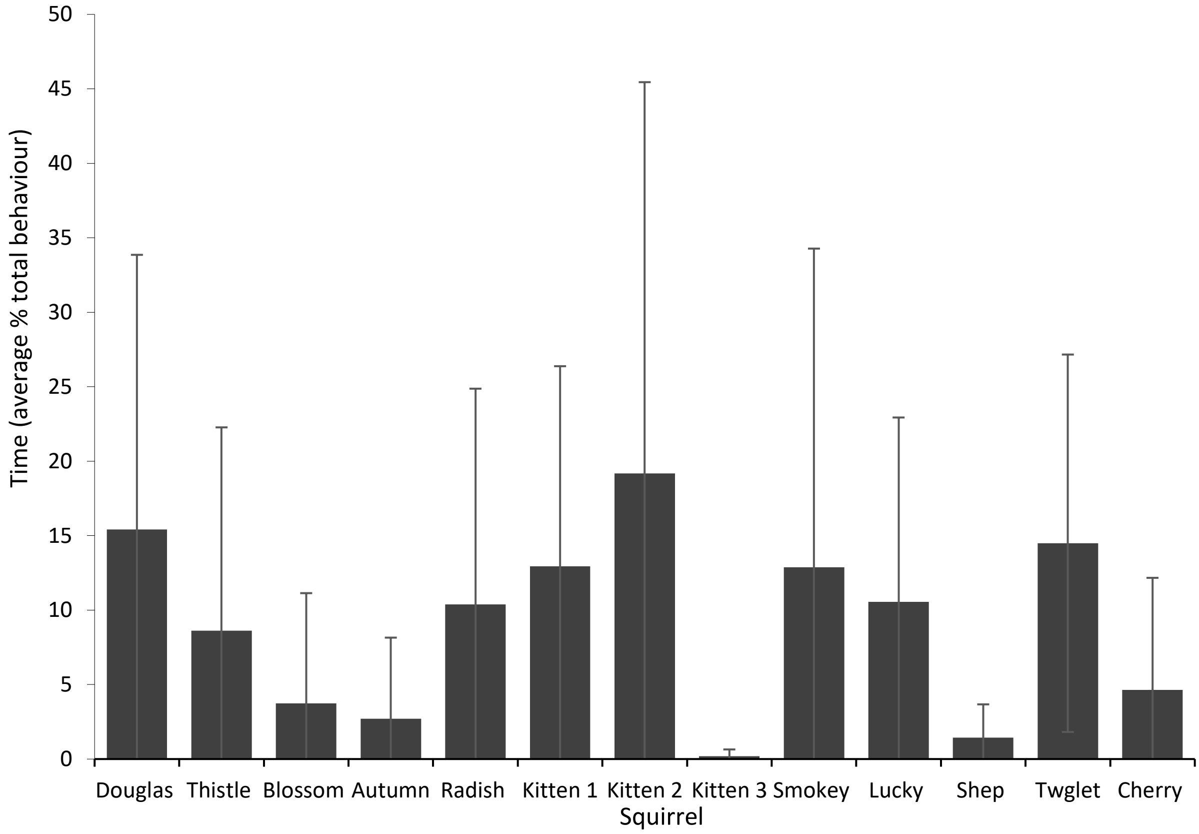


Figure 5a: Individual pacing behaviour. Comparison of the average percentage of time spent (% total behaviour) in stereotypical pacing behaviour by each observed captive red squirrel, who was known to/observed pacing, between June 2019 and September 2020 (n=13/16). Error bars = standard deviation.

Not only were significant differences in time spent stereotypically pacing observed between individuals, ranging from 1.45% to 19.17% of total time on average, but also large differences were observed within each squirrel, evidenced by the vast error bars in Figure 5a which was directly impacted by animals moving between locations and discussed above.

Figure 5b clearly shows that the greatest degree of stereotypical pacing took place in either the breeding enclosure or holding pen for most squirrels and once they were moved into the walkthroughs their pacing significantly reduced. Apart from Twiglet and Cherry who did not move from the walkthrough during the study.

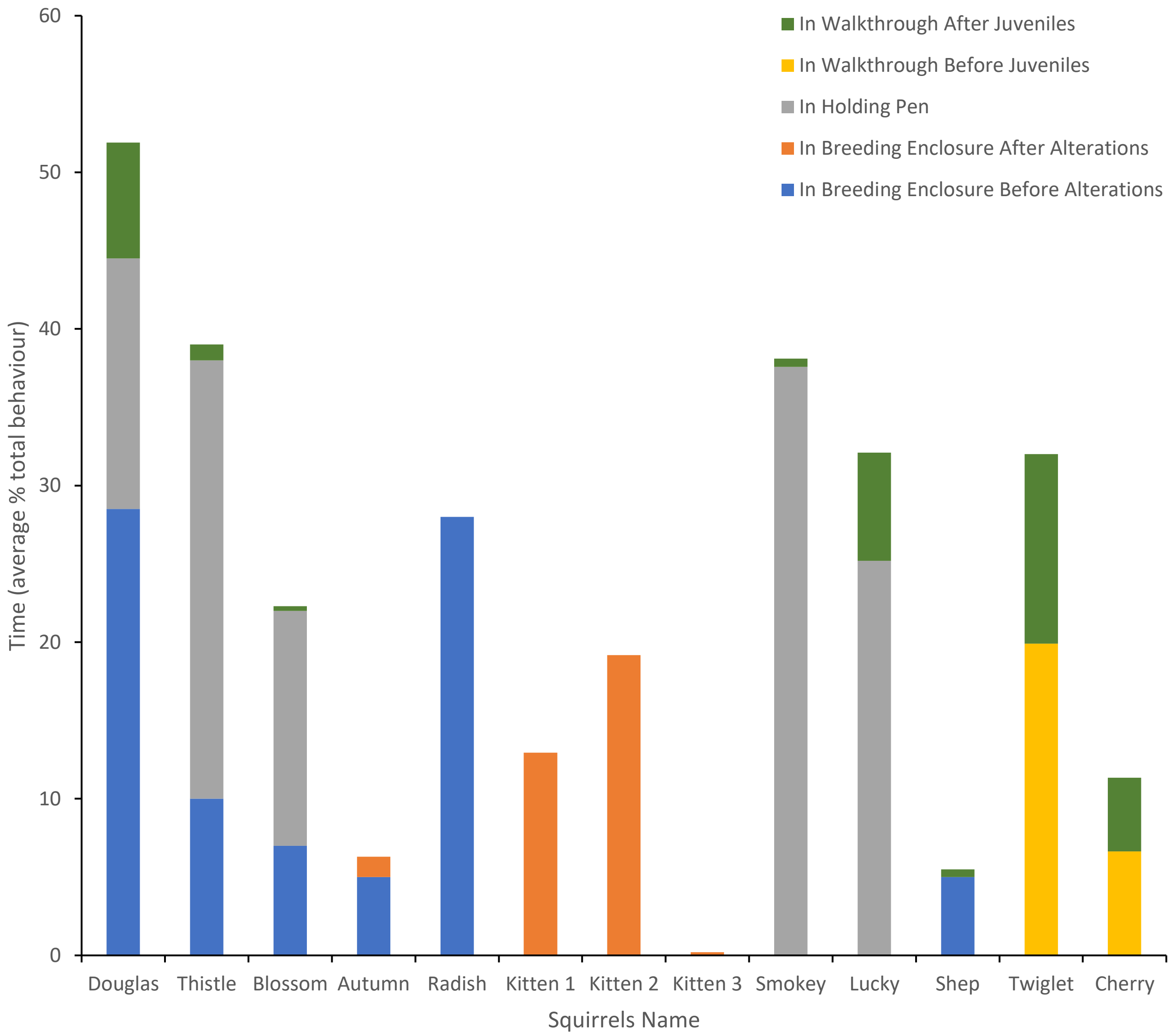


Figure 5b Individual pacing behaviour. Comparison of the average percentage of time spent (% total behaviour) in stereotypical pacing behaviour by each observed captive red squirrel, who was known to/observed pacing, between June 2019 and September 2020 (n=13/16). Error bars = standard deviation and the location they passed, in the breeding enclosure before or after alterations, in the holding pen or in the walkthrough before or after the juveniles (Douglas, Blossom, Thistle) were introduced

Twiglets pacing in particular took up a fifth of her observed activity budget before the juveniles were introduced into the walkthrough and reduced slightly to 12% after and Cherry's pacing reduced from 7% to 4.5% after the juveniles were introduced. Their pacing behaviour was affected by the juveniles being moved into their enclosure and a detailed account of the possible impact of these introductions is debated in the Discussion The onset of pacing was, for many individuals, directly recorded, and again, varied widely between individuals as did the pattern/occurrence of pacing as individual differences in pacing is described in detail in Table 5. In Figure 6 (a-d) the average time spent pacing each month is shown per individual.

Table 5. Reported/observed onset and occurrence of stereotypical pacing per individual. N=13. Either pacing onset was reported by keepers prior to data collection or was directly observed for the first-time during data collection.

Name	Stereotypical Behaviour
Autumn	Was never reported to pace significantly by keepers at either British Wildlife Centre or Wildwood Escot (Devon). Was observed by the resesarcher stereotypically pacing (at fouryears of age) at an average of just under 3% of total time, with maximal pacing during October 2019 (20% of total observed time, which reduced to an average of 9% of observed time in November 2019), and early February 2020 (5%), post planned enclosure alterations. Autumn's pacing always took the form of running back and forth by the door of the old side of the breeding enclosure.
Radish	Keepers reported pacing started at 10 weeks old in the breeding enclosure. Radish is a prolific pacer, already established behaviour when data collection started, spending on average of 28% of time pacing (before and after enclosure alterations), with maxinal pacing in June and September 2019 (up to 35% overall activity budget). His pacing remained high for many months but ceased after planned enclosure alterations in January 2020. Over the entire observation period, pacing accounted for 10% of his activity budget. His pacing took the form of running backwards and forwards opposite the enclosure doorway on the new side with food in his mouth, which he would then cache and re-pace over the caching location.
Twiglet	Reported by the keepers to have started to pace at 10 weeks old in the breeding enclosure. She was moved to the walkthrough enclosure at 23 weeks of age and still paced an average of 15% of all observed time, with peaks of 28% in August, 33% in September, 23% in October 2019 and 20% in March 2020. Whilst Twiglet tended to pace more than Cherry their pattern of pacing mirrored each other accross the months of observation, peaking and reducing in the same months .

	Her pacing in the walkthrough involved running the whole length of the enclosure (45-75 metres) on the two sides near the doorway (see enclosure drawing figure 3). She also sometimes went into the holding pen to pace.
Cherry	Reported by the keepers to have started to pace from 10 weeks old in the breeding enclosure. She was moved to the walkthrough at 23 weeks of age and rarely seen pacing at only an average of 4.6% of observed time, this pacing was seasonal, increasing to an average of 13% in August and 21% in October, then reducing to 4% in November 2019. Her pacing also increased again in March 2020 to an average of 13% of observed time. Her pacing involved either pacing along the outside or inside of the holding pen facing the boardwalk. (see figure 3).
Thistle	Started to pace from 14 weeks of age in the breeding enclosure at an average of just 0.6% of observed time, by 18 weeks her pacing had increased to an average of 29% of observed time and remained high at an average of 28%. This reduced to an average of less than 1% after release into the walkthrough September 2019 and ceased end of October 2019, making her average pacing 8.6% across all observations. Her pacing mirrored Blossom's in style.
Douglas	Started to pace from 10 weeks old in the breeding enclosure with a peak of 43% in June 2019. His pacing did reduce to just 4% at 14 weeks old and this is discussed later in the section. In the holding pen he paced an average of 16%, which reduced to less than 1% when released into the walkthrough in October 2019. His pacing did increase on release day to 35% of observed time. His overall average was 15.4% across all observations and his pacing mirrored that of his sisters in all settings.
Smokey	It is unknown when Smokey started to pace but keepers reported that he did pace at the Welsh Mountain Zoo when he was 1 year old. He paced on average 12.8% of all observed time but paced maximally (38%) in the holding pen. Once released into the walkthrough his pacing reduced to <1% of observed time. His pacing took the form of pacing backward and forwards around the internal or external perimeter of the holding pen inside the walkthrough enclosure.
Lucky	It is unknown when Lucky started to pace but keepers reported that he did pace at the Welsh Mountain Zoo when he was 1 year old. Lucky paced an average of 11% reaching double this (25%) in the holding pen and on release day (23%). His pacing reduced to less than an average of 1% in the walkthrough. Smokey and Lucky both paced the most at the start of the observations to the greatest degree in the holding pen, which then reduced once they were in the walkthrough, their pacing was very similar in style

Shep	Shep was reported by keepers to have started to pace at 10 weeks of age (5%) in the breeding enclosure. He was only observed for one day in the breeding enclosure as he killed his offspring so was moved the next day to the walkthrough. He was not given a soft release and was never in the holding pen as it was felt this may increase his stress and aggressive levels, so was released straight into the walkthrough and his pacing reduced to less than an average of 1% of observed time. In the breeding enclosure he paced around a box holding hazelnuts, and his pacing was similar to Radish, pacing with food in his mouth, caching where he paced and re- pacing over the cache location by the doorway to the breeding enclosure. In the walkthrough his pacing became similar to Lucky and Smokey.
Kitten 1	Started to pace in the breeding enclosure in July 2020 (18 weeks of age) (13% of total time observed) and paced maximally (27%) August 2020. This pacing initially took the form of pacing by the doorways to the breeding enclosure, but changed in format as he got older, around 24 weeks of age as due to COVID-19 pandemic the juveniles had not been moved from their parents. His pacing took the form of linear and a gambolling style in a figure of eight on the old and new side on branches that were on top of the boards
Kitten 2	Started to pace August 2020 (22 weeks of age) an average of 19% of total time observed, with peaks of 47% by late August 2020. His pacing mirrored that of kitten 1, changing in style the older he got.
Kitten 3	Started pacing end July 2020 in the breeding enclosure (20 weeks of age but only 0.5% of total time and took the form of pacing back and forth near the doorway on the new side. He died in August 2020.

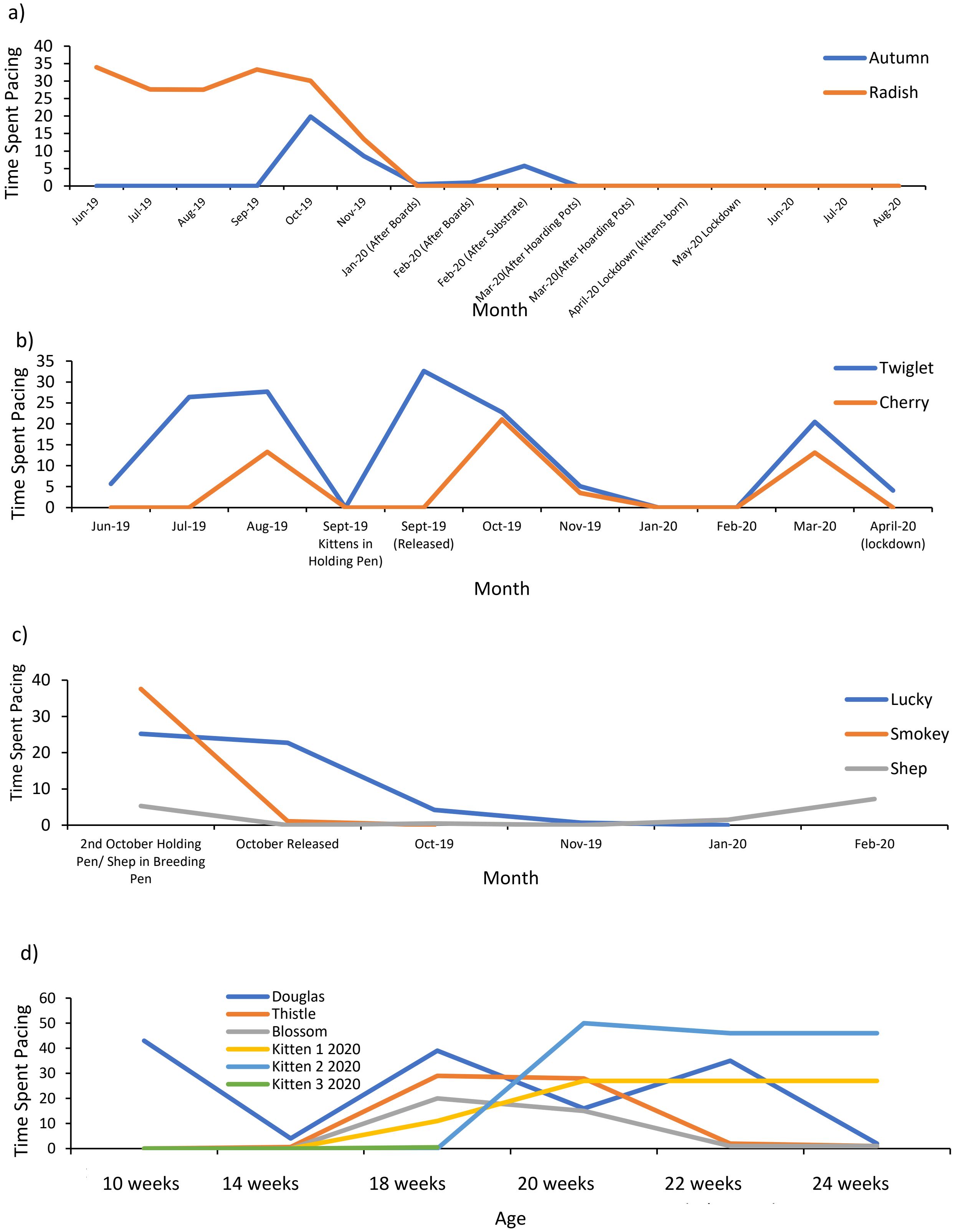


Figure 6: Time spent (% total time) stereotypically pacing. a) Radish and Autumn June 2019-August 2020, in the breeding enclosure at Wildwood Escot. b) Cherry and Twiglet June 2019-April 2020, in the Walkthrough at Wildwood Escot. c) Lucky, Smokey and Shep October 2019-February 2020, in the Walkthrough at Wildwood Kent. d) Douglas, Blossom and Thistle 2019 litter compared to Kitten 1, 2 and 3 2020 litter in breeding enclosure at Wildwood Escot. 2019 litter moved to walkthrough enclosure at 24 weeks.

Individual pacing behaviour ranged from being relatively infrequent (Shep, 1.45%) to very frequent (Kitten 2, 19.2%) though was consistently performed less often than feeding or locomotion by all individuals. Most individuals started pacing in July or August and most started between 10-18 weeks old (mean age of onset 14 weeks, +/-4.9weeks). Months when pacing peaked were September and October. Cage-mates sometimes shared pacing cycles (Autumn, Twiglet and Cherry), (Radish and Shep), (Douglas and Thistle), and (Kittens 1 and 2) where all acted similarly each month, while some cage-mates (2019 litter vs 2020 litter) acted very differently, but this could be because alterations to the enclosure occurred once the 2019 litter had moved to a larger enclosure and before the 2020 litter were born. The 2020 litter were also born in lockdown, so their behaviour could not be recorded by the observer during their first six weeks of emergence and they were also not exposed to any visitors until they were four or five months old and both these extraneous variable could have impacted on their behaviour and are considered further in the Discussion Large decreases in pacing were observed for Radish after alterations to the breeding enclosure, and Douglas, Thistle and Blossom reduced their pacing following an enclosure change. Twiglet and Cherry both increased their pacing on the week that Thistle and Blossom were released into the walkthrough enclosure where they were housed. Lucky, Smokey and Shep also reduced their pacing once they exited the holding pen into the walkthrough in Kent. Kittens 1, 2 and 3 from the 2020 litter began pacing later than the 2019 litter, (18 weeks vs 10 weeks) and increased their pacing the longer they were housed with their parents.

3.1.1.2 Sex differences in pacing

To test the effect of gender differences on the presentation of stereotypical behaviour a Mann Whitney U test was conducted to compare whether there is a difference between these two independent groups. Female red squirrel pacing was not significantly different from male red squirrel pacing (female median=4.63 range=11.79, male median=11.82 range=18.97, U=14, n=13, p=0.435). On average, female red squirrel pacing was observed a third less than male red squirrel pacing (7% vs 10.5%), Douglas (15.42%), and Kitten 2 (19.17%), both males, paced higher than the highest pacing seen by any female (Twiglet). However, there were exemptions to this, e.g., Kitten 3 paced 0.2% of observed time and Shep paced on average 1.45% of observed time (both males) compared to Twiglet at 14.49% (female). Douglas was the first male to start pacing at 10 weeks old in June 2019 and Thistle and Blossom did not start pacing until 18 weeks in July 2019 (although Thistle was seen pacing just 0.5% of time at 14 weeks in June 2019).

The similarity between the adult females is all their pacing increased in October and November 2019, Autumn's pacing increased to an average of 20% in October and reduced to 9% in November, Cherry's increased to 21% in October and reduced to 13% in November and Twiglet's increased to 23% in October and reduced to 5% in November 2019, however, Blossom and Thistle moved into the holding pen in the walkthrough in September 2019 and were released into the walkthrough in October 2019 so this could have impacted on Twiglet and Cherry's behaviour and is described in detail in the Discussion. The major similarity between males was that their pacing reduced in October, November, December, and January to almost zero. Lucky (23%) and Smokey (38%) increased their pacing in the holding pen, and although all four males (Lucky, Smokey, Shep and Douglas) reduced their pacing to just 1% once released into the walkthrough enclosure in Kent, they were still witnessed either pacing around the perimeter of the holding pen or entering the holding pen to pace. Radish's pacing was different in presentation to all the others, and although his pacing only averaged 10.37% of observed time, his pacing peaked to 35% in June and September 2019 before planned alterations to the enclosure and ceased once alterations were made (Figures 7/8).

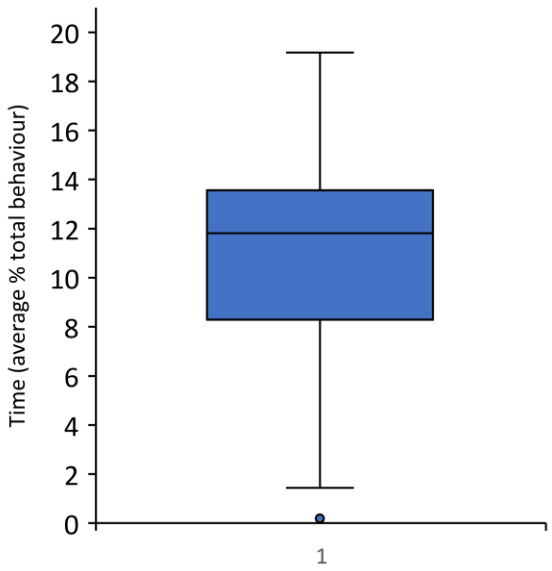


Figure 7 – Percentage of time (%total time) male red squirrels (Radish, Kitten 1, 2 and 3, Douglas, Lucky, Shep, Smokey) spent in stereotypical pacing between June 2019 and September 2020, including median, mean and range, and outlier Kitten 3 is being an outlier at 0.2%

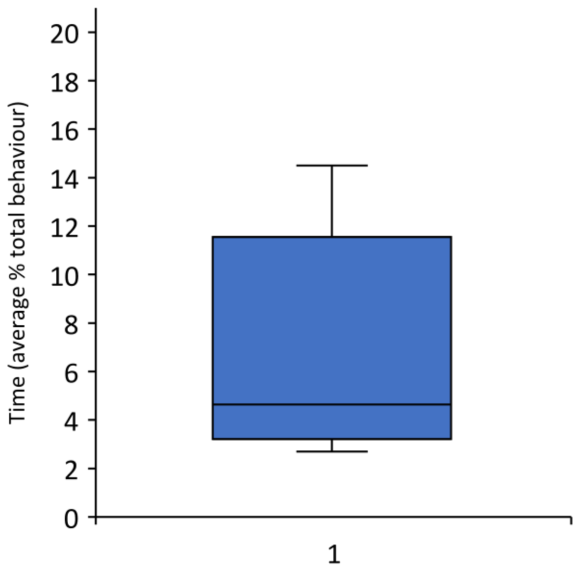


Figure 8 – Percentage of time (%total time) female red squirrels (Twiglet, Cherry, Autumn, Thistle and Blossom) spent in stereotypical behaviour between June 2019 and September 2020, including median, mean and range.

3.1.1.3 Life stage differences in pacing

Activity budgets were compared between adults and non-adults (juveniles and infants) using Chi^2 goodness of fit (Figure 9). Adult and juvenile activity budgets differed significantly (Chi^2=130.578, N=13, df=6, p=<0.001). Social behaviour was similar between adults and juveniles (9.23% vs 7.4%), as was stereotypical behaviour at 8.2% for the adults and 10% for the juveniles. Adults were observed drey building twice as much as the juveniles (1% vs 0.44%), however juveniles groomed twice as much as adults (2% vs 1%). There was a large difference in feeding and locomotive behaviour, with adults feeding at an average 39% and juveniles at an average of 30% of observed time and adults locomoting at an average of 30% and juveniles at 25% of observed time. The biggest difference was seen between adult and juvenile inactivity (12% vs 25%), however the juveniles of the 2020 litter were observed being very inactive (Kitten 1 and 2 at an average of 32% of observed time and Kitten 3 at an average of 59% of observed time). These juveniles were observed during the pandemic and subsequent lock-down and were not habituated to volunteers, visitors and the observer compared to the 2019 litter which were recorded at just 10% inactivity as they were habituated to these external stimuli.

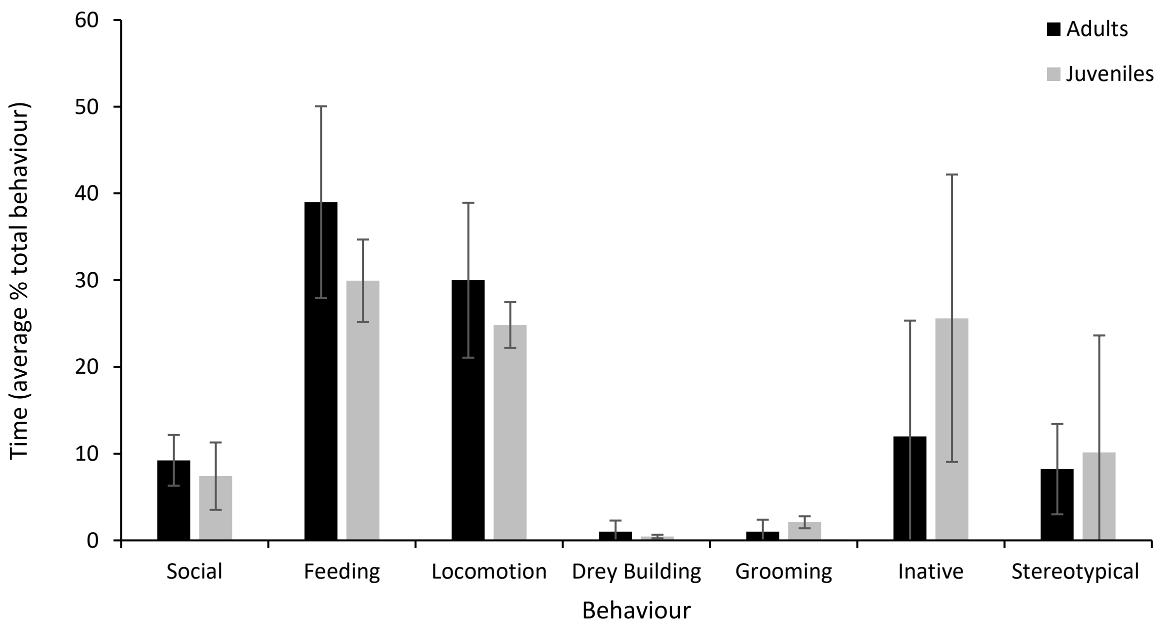


Figure 9 – Percentage of time (%total time) spent in behaviour by all adults (Basil, Lucky, Smokey, Shep and Radish (m) and Fern, Bracken, Twiglet, Cherry and Autumn (f) vs red squirrels juveniles (Douglas, Thistle, Blossom, (2019 litter) Kitten 1, 2 and 3 (2020 litter) between June 2019 and September 2020

To test whether age had an effect on the performance of stereotypical pacing a Mann Whitney U test was run and Figures 10 and 11 illustrate that stereotypical pacing was not significantly different between adults and juveniles (adult median 10.57, range = 13.04, juveniles median 10.78, range = 18.97, U=17, n=13, p= 0.628).

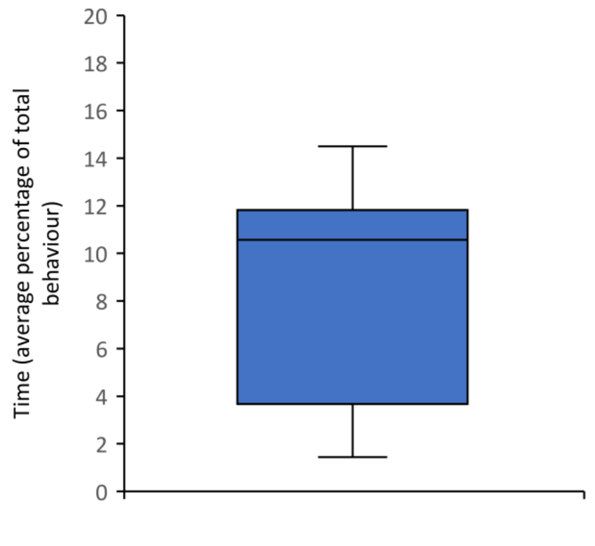


Figure 10 – Percentage of time (%total time) for all pacing adult red squirrels (Twiglet, Cherry, Autumn, Thistle and Blossom (f) and Lucky, Smokey, Shep and Radish (m)) spent in stereotypical behaviour between June 2019 and September 2020, including median, mean and range

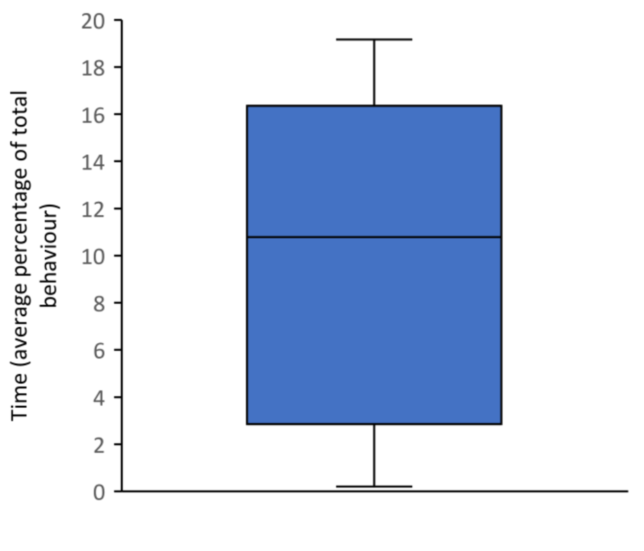


Figure 11 – Percentage of time (%total time) juvenile red squirrels (Douglas, Thistle and Blossom (2019 litter and Kitten 1, 2 and 3 (2020 Litter)) spend in stereotypical behaviour between June 2019 and September 2020, including median, mean and range.

3.1.1.4 Social group changes and pacing

To test if changing social groups altered pacing behaviour of the existing group of individuals results were also analysed using a Wilcoxson matched pairs test was applied to the two paired groups to determine any statistically significant difference between the data Figure 12 compares the behaviour of the resident four females in the Wildwood Escot walkthrough enclosure (Fern, Bracken, Twiglet and Cherry) before and after the introduction of two female juveniles (Thistle and Blossom). Stereotypical behaviour did not differ significantly (z=0, p=1) and consistently accounted for 4.5% of total time on average.

Social behaviour (z=-.730, p=0.465), feeding (z=-1.826, p=0.068), locomotion (-1.826, p=0.068), drey building (-1.461, p=0.144) and inactivity (z=-.730, 0.465) did not differ significantly suggesting a change in social group did not change behaviour in the resident females at Wildwood Escot long term, but there were changes during the week of release which is discussed in detail in the Discussion. This could not be conducted for Wildwood in Kent as the observer was unable to observe Basil, Lucky and Smokey's behaviour prior to the addition of Douglas. The juvenile's behaviour post social group change is examined later when hypothesis 5 is discussed.

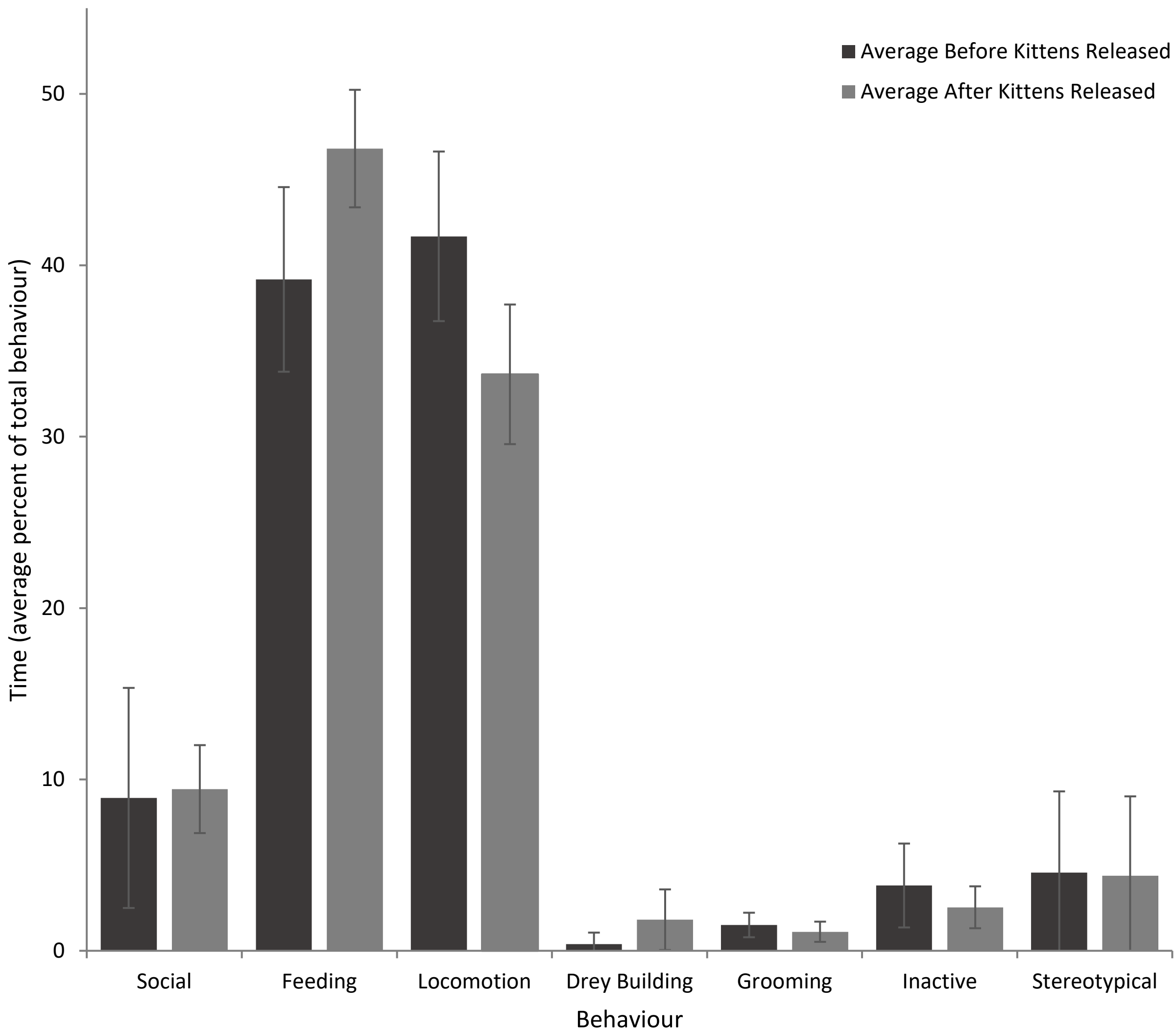


Figure 12 – Percentage of time (%total time) spent in behaviour by resident red squirrels (Fern, Bracken, Twiglet and Cherry) at Wildwood Escot walkthrough, before and after the introduction of the two female juveniles in September 2019 (Thistle and Blossom)

3.1.1.5 Differences in pacing between sites

The activity budgets, averaged across individuals, for squirrels at Wildwood Escot, and squirrels at Wildwood Kent are shown in Figure 13. Pacing was observed at both sites in multiple individuals, 83% and 75% of the population at Wildwood Escot and Wildwood Kent respectively. Both males and females, adults and non-adults at both sites paced. To test if zoo location had an impact on pacing a Mann Whitney U test was conducted and time spent pacing was not, on average, significantly different between the sites (Wildwood Escot median = 10.76, range =16.47, Wildwood Kent median = 6.01, range =11.88, U=19, n=16, p=0.599). The large error bars indicate large individual differences in individual behaviour particularly for feeding, inactive and stereotypical behaviour as previously discussed this is probably because in Devon the squirrels were recorded in both the breeding enclosure, holding pens and walkthrough and in Kent they were only observed in the holding pen and walkthrough and as already noted there are large differences in both group and individual behaviour when housed in difference enclosures as seen in Figure 14.

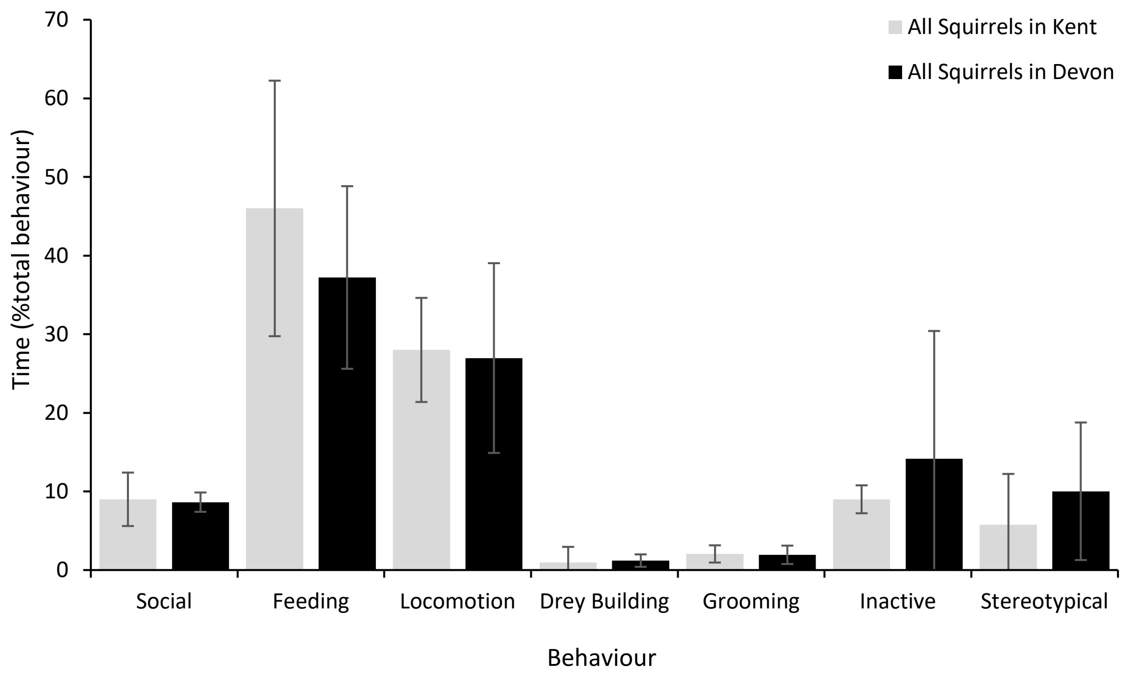


Figure 13: A comparison of time spent (%total time) in different behaviours between all red squirrels (N= 13) (Fern, Bracken, Twiglet, Cherry, Autumn, Radish, Douglas, Thistle, Blossom, Kitten 1, 2 and 3) housed at Wildwood Escot (Devon) and all red squirrel n=4 (Basil, Lucky, Smokey and Shep) housed at Wildwood Kent walkthrough between October 2019 and September 2020. Error Bars = Standard Deviation.

3.1.1.6 Enclosure type and pacing

At each site, squirrels could be kept in various enclosure types. At Wildwood Escot individuals were observed in either breeding enclosures, holding pens or walkthrough enclosures, at Wildwood Kent individuals were observed in breeding enclosure (and only Shep for just one day) holding pens or walkthrough enclosures. The walkthrough enclosures at both sites were comparable in terms of size and design/complexity, but the three enclosure types (walkthrough, holding pen, breeding enclosure) differed in space and complexity (Figure 14).

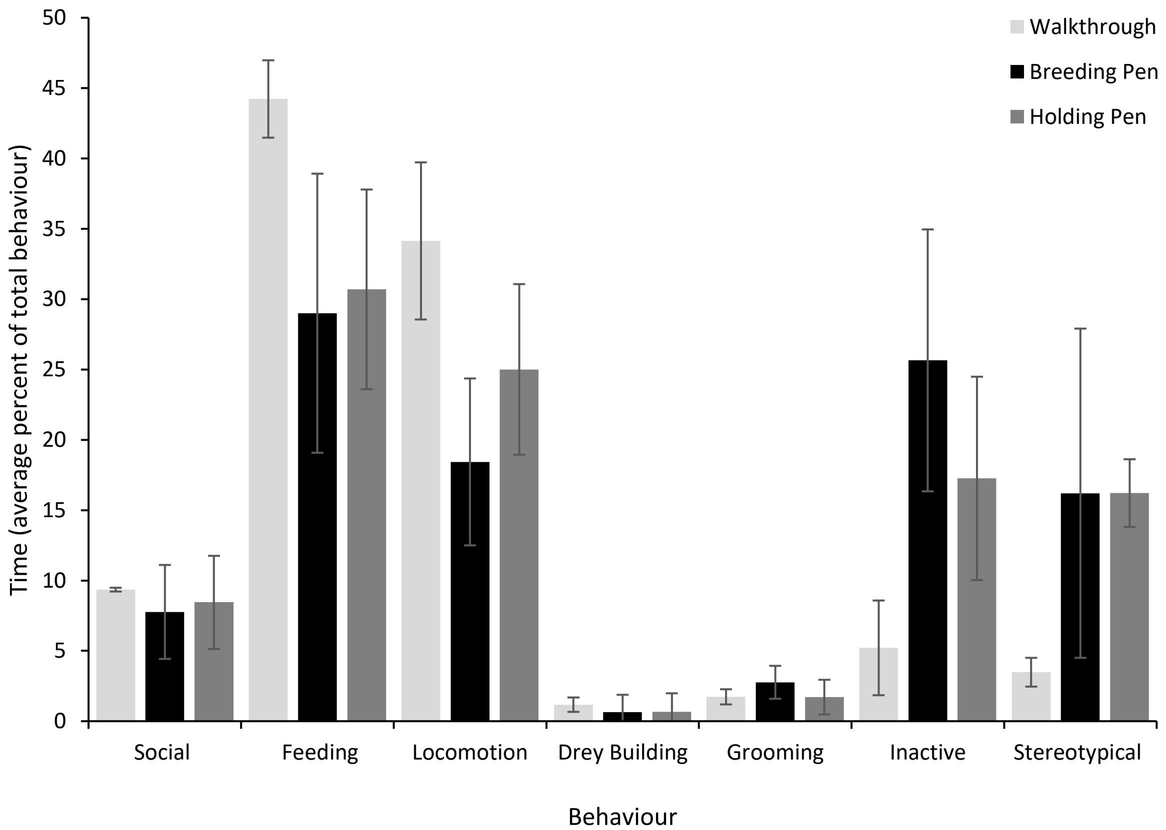


Figure 14: Time spent (%total time) in key behaviours by all squirrels ever housed in Escot walkthrough, Fern, Bracken, Twiglet, Cherry, Blossom, Thistle (Female) and Kent Walkthrough, Basil, Lucky, Smokey, Shep and Douglas (Male). Squirrels housed in breeding enc (before alterations) – Autumn, Blossom and Thistle (female) and Radish and Douglas (male). And all squirrels ever housed in the Holding pen in Escot (Devon), walkthrough (Blossom and Thistle) and Kent Walkthrough, (Basil, Lucky, Smokey, Shep and Douglas) during observations between June 2019 and September 2020). Error bars =standard deviation.

Across the three enclosure types, activity budgets (averaged across individuals) showed similarity in the pattern of drey building, grooming and social behaviour which were consistently the least frequently performed behaviours in all enclosure types. Noticeable differences in the average amount of time spent in locomotion, feeding, inactivity and stereotypical behaviour were observed between enclosure types (Figure 14). These differences impact on all the data analysis and are reflected in the large error bars around the average, but as the study took over 18 months, it would be considered that the mean is a fair estimate of the average time each squirrel spends in each behaviour in the study and full explanations of all extraneous variables and rationales are given in the Discussion.

Stereotypical behaviour was much higher in the breeding and holding pens than the walkthrough enclosures (16.2% vs 3.4%). In walkthrough enclosures, feeding (44%) and locomotion (34%) were the most performed behaviours accounting for nearly 80% of all behaviour observed, while inactivity (5%) and stereotypical pacing (3.5%) contributed relatively little to the overall activity budget. In both holding pens and breeding enclosures a four-fold increase in stereotypical pacing was observed as this behaviour accounted for almost a fifth of all behaviour observed (16%). A four- or five-fold increase in inactivity was also observed in holding pens and breeding enclosure respectively. In both breeding and holding pens, feeding and locomotion reduced compared to walkthrough enclosures. This makes an average of 47% and 55% of time spent in feeding and locomotive behaviour in the breeding enclosure and holding pen respectively, a reduction of 33% in the breeding enclosure compared to that seen in the walkthrough enclosures and 25% in the holding pen

To test whether the type of enclosure had an impact of the presentation of stereotypical pacing a Friedmans test was used to highlight statistical difference in activity budgets and there was a significant difference in stereotypy (Friedmans Chi^2=9.33, df=2, n=6, p=0.009), inactivity (Friedmans Chi^2=11.14, df=2, n=7, p=0.001), locomotion (Friedmans Chi^2=8.86, df=2, n=7, p=0.008) and feeding (Chi^2=8.86, df=2, n=7, p=0.008) behaviour between the activity budgets of the captive squirrels when housed in the breeding enclosure, the holding pen and the walkthrough.

To test where the differences in activity budgets were a Wilcoxon matched pairs test was run on each comparable behaviour in each location. Stereotypical behaviour was observed more in the holding pen and breeding enclosure than in the walkthrough (walkthrough median =0.81, holding pen median = 16.02, breeding enclosure median= 10.35) and differed significantly between the holding pen and the walkthrough (z=-2.201, p=0.031) and breeding enclosure and the walkthrough (z=-1.820, p=0.039), but not between the holding pen and the breeding enclosure (z=-.676, p=0.578).

Inactivity was observed most in the breeding enclosure, (walkthrough median =3.49, holding pen median = 8.31, breeding enclosure median= 32.1) and differed significantly between the walkthrough and the breeding enclosure (z=-2.521, p=0.008) and the holding pen (z=-2.2366, p=0.016) but not between the breeding enclosure and the holding pen (z=-1.690, p=0.109). Locomotion was higher in the walkthroughs (walkthrough median =36.62, holding pen median = 20.39, breeding enclosure median= 19.02) and differed significantly between the walkthrough and the breeding enclosure (z=-2.521, p=0.008) but not between the holding pen and the walkthrough (z=-1.690, p=0.109) or the holding pen and the breeding enclosure (z= -1.183, p=0.297). Feeding behaviour was higher in the walkthroughs (walkthrough median = 36.48, holding pen median = 21.84, breeding enclosure median= 28.46) and differed significantly between the walkthrough and the holding pen (z=-2.366, p=0.016) and the breeding enclosure (z=-2.240, p=0.023) but not between the holding pen and the breeding enclosure (z=-1.183, p=0.297). (See Figure 15). At both sites, most individuals were housed in or moved to walkthrough enclosures and most observations took place while individuals were in walkthrough enclosures at both sites, Stereotypical behaviour between Kent and Escot walkthrough did not differ significantly (Wildwood Escot median =2.75, range = 14.13, Wildwood Kent median = 3.72, range = 6.93, U=8, n=8, p=0.577).

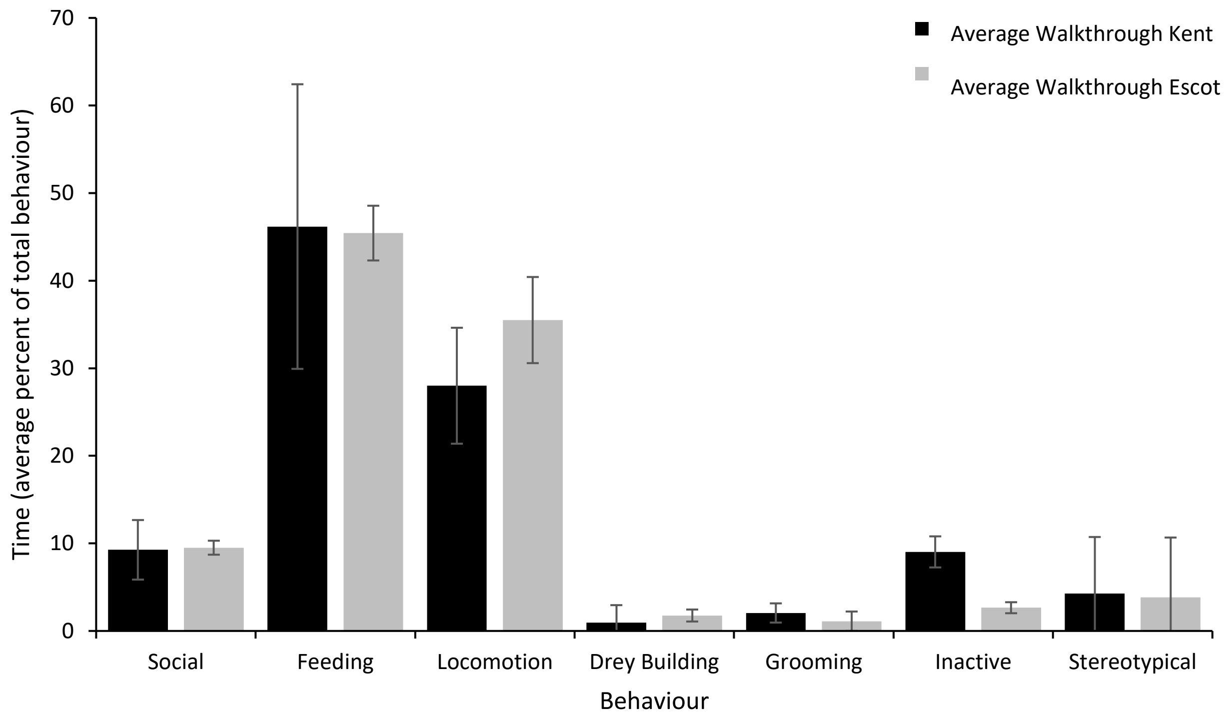


Figure 15: A comparison of time spent (%total time) in different behaviours between four female red squirrels (Fern, Bracken, Twiglet and Cherry) in Wildwood Escot (Devon) walkthrough compared to four male red squirrels (Basil, Lucky, Smokey and Shep) in Wildwood Kent walkthrough between October 2019 and March 2020. Error Bars = Standard Deviation.

3.1.2 Hypothesis 2 – Red Squirrel Feeding and Stereotypical Pacing

To test if feeding behaviour (%total time averaged across all individuals that paced) and stereotypical pacing behaviour (%total time averaged across all individuals that paced) were negatively correlated, a Spearman's rank correlation was used to measure the degree of association between these two variables. Feeding and pacing behaviour were found to be significantly, negatively correlated (r=-0.6, n=13, p=0.015), suggesting that when red squirrels pace their feeding behaviour reduces and vice versa (Figure 16 and 17). Every five-minute increase in feeding behaviour was associated with a seven-minute decrease in stereotypical pacing no matter where the squirrels were housed. Feeding behaviour was always performed more than pacing (between two and six times more over the entire study) but almost converged when stereotypical pacing was highest in August 2019. Conversely there was no statistical correlation for locomotion and stereotypical behaviour (r=0.002, n=13, p=0.126), social and stereotypical behaviour (r=0.132, n=13, p=0.213) or inactivity and stereotypical behaviour (r=0.063, n=13, p=0.650).

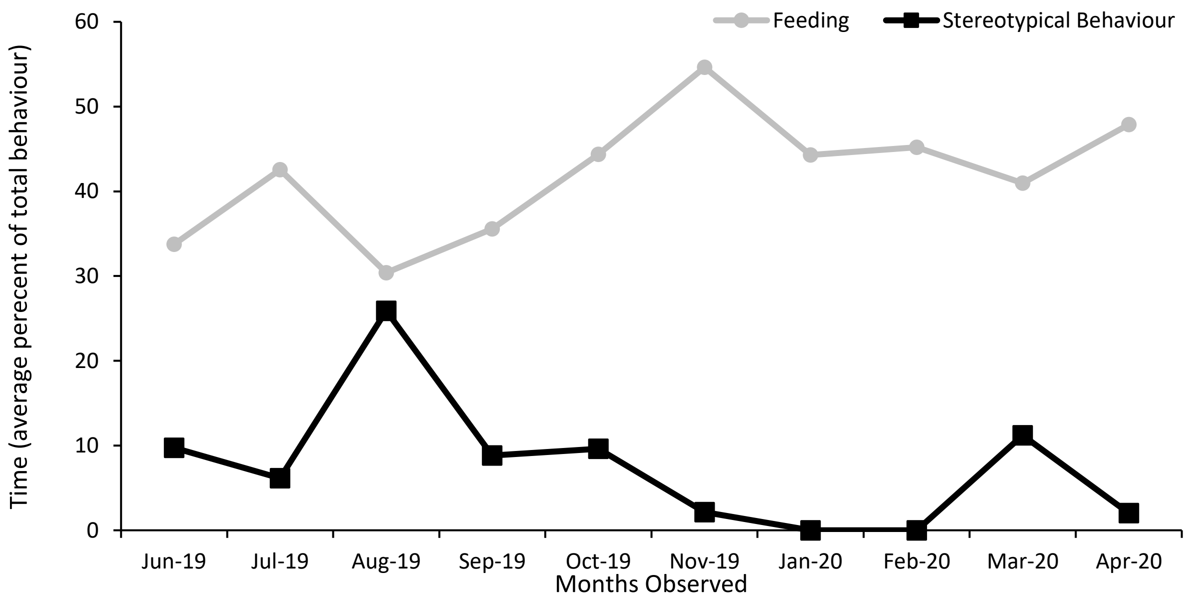


Figure 16 - The mean percentage of time spent (%total time) of feeding and stereotypical behaviour for all red squirrels that stereotypically pace (N=13, Twiglet, Cherry, Radish, Autumn, Douglas, Thistle, Blossom, Lucky, Smokey, Shep, Kitten 1, 2 and 3).

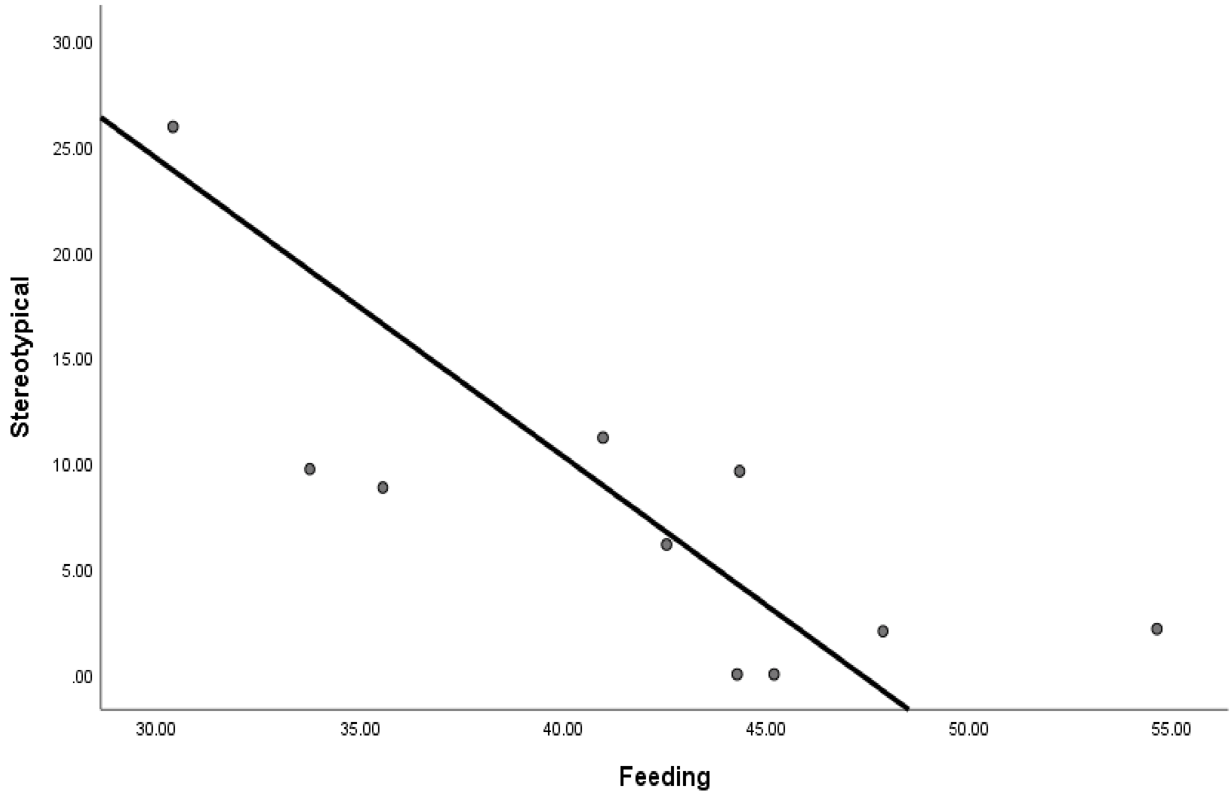


Figure 17 - Spearman's rank-order correlation illustrating a significant strong negative correlation between feeding and stereotypical behaviour, with coefficient was r=-0.6

3.1.3 Hypothesis 3 – Red squirrel anticipatory behaviour

To test if pacing was linked with anticipatory feeding behaviour a Wilcoxon matched pairs was used to analyse pacing behaviour before and after feeding times in the breeding enclosure and was found to not be statistically significantly different ($z= -1.014$, $p= 0.310$) Figure 18 illustrates the mean percentage of time spent in stereotypical behaviour from all red squirrels ever housed in the breeding enclosure at Wildwood Escot, before and after feeding, during 16 months of observations from June 2019 to September 2020. The percentage of stereotypical pacing observed was on average 10% (of observed time for all pacing squirrels) lower post feeding, (58% before feeding compared to 42% after feeding)., therefore suggesting that stereotypical pacing is not linked to anticipatory behaviour when red squirrels anticipate being fed. The error bars show there were individual differences in the amount of stereotypical pacing before and after pacing, so some form of anticipatory mechanism to stereotypy cannot be totally ruled out. It needs to be noted that this test was not run on the walkthroughs as feeding resources were always available through natural feeding opportunities so a direct comparison could not be made.

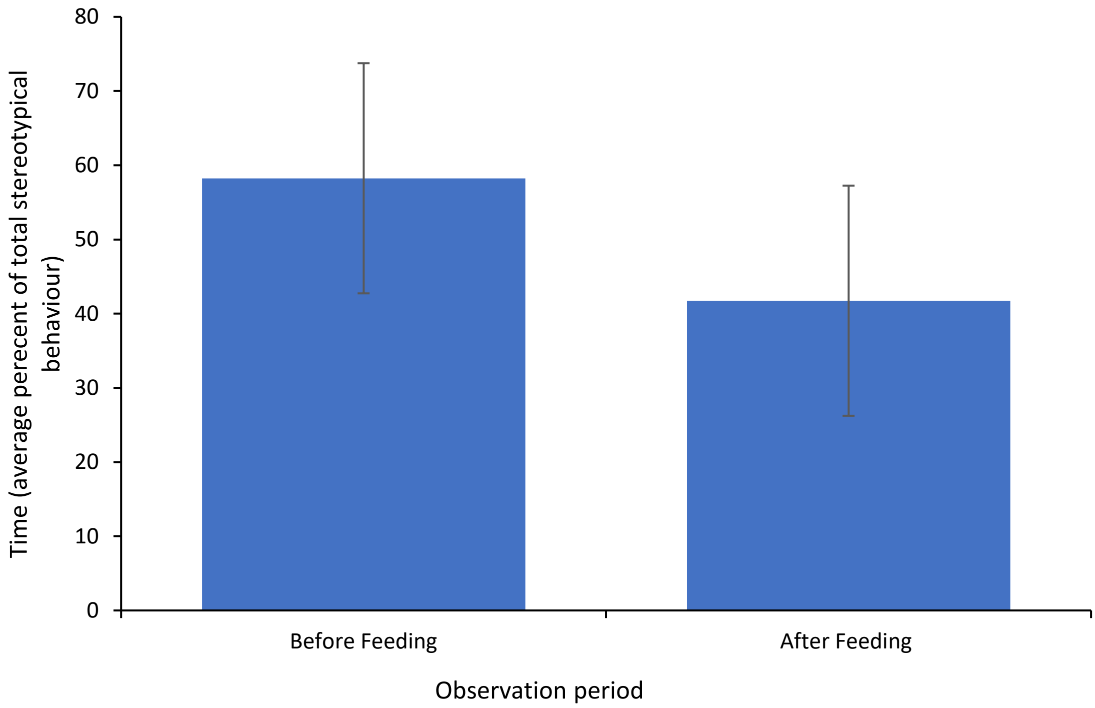


Figure 18 - The mean percentage of the total amount of time (10%total time) in stereotypical behaviour before and after feeding by all red squirrels in the breeding enclosure at Wildwood Escot, Autumn, Thistle and Blossom (females), Radish, Douglas, Kitten 1, 2 and 3 (males), between June 2019 and September 2020.

3.1.4 Hypothesis 4 – Red squirrel juveniles move from the breeding enclosure to a walkthrough enclosure

A comparison was made regarding the activity budgets of juveniles born in the 2019 litter when housed in the breeding enclosure and when subsequently housed in the walkthrough enclosure and when merged in with another social group (Figure 19). To test if location influenced the presentation of stereotypical pacing a Wilcoxon matched pairs test was used to analyse behaviour before and after moving enclosures. Pacing by red squirrel juveniles before and after the move was significantly different ($z=2.023$, $p=0.031$). Pacing was significantly higher in the breeding enclosure (16.21%) and reduced to 2.3% after the move. Importantly, once pacing was established in the breeding enclosure, stereotypical pacing tended to be retained in an individual's activity budget. In addition, feeding behaviour increased from an average of 30% of observed time to 41% and locomotion increased from an average, of 25% to 39% of observed time when juveniles were moved to the walkthrough enclosure.

Thus, making a total of 80% of time spent in feeding and locomotion, which is the same as seen in the wild. Inactivity was reduced from an average of 17% to just 4% after the move and stereotypical behaviour reduced from an average of 16.2% to 2.3%. It is important to remember that this analysis could only be conducted on the 2019 litter as the 2020 litter was not moved due to the pandemic.

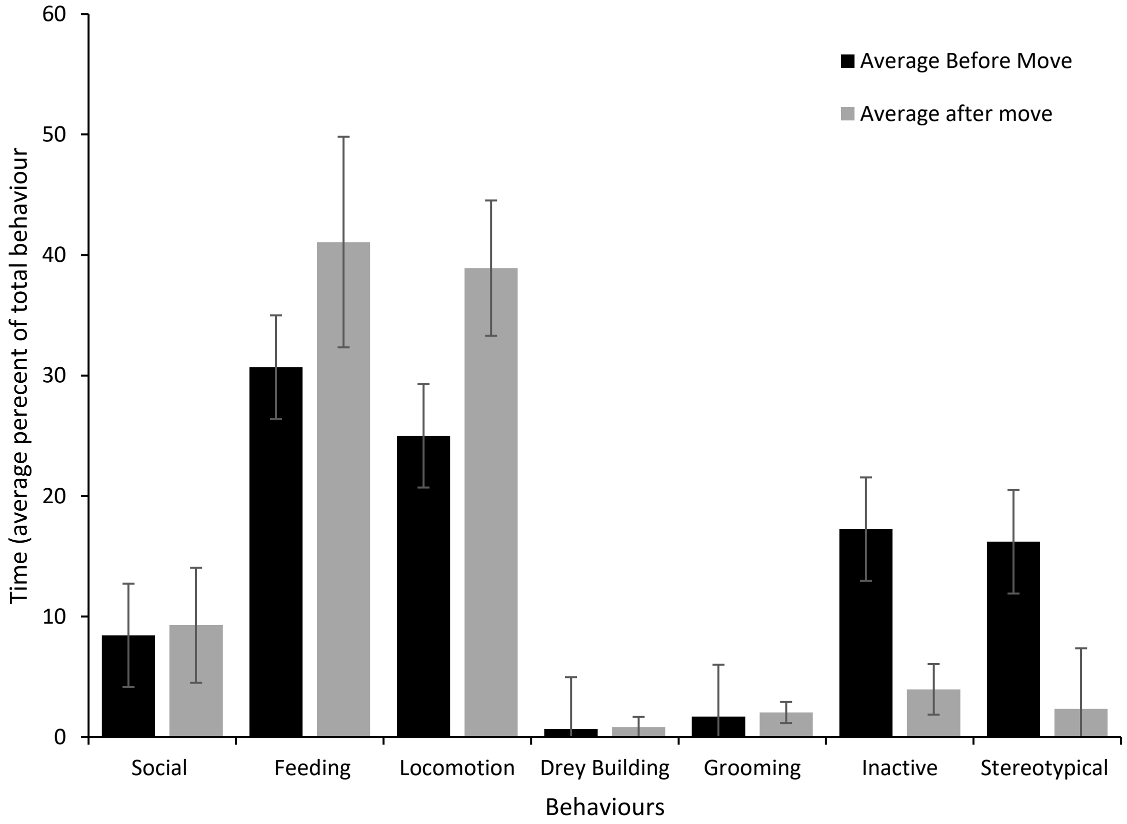


Figure 19 - The mean percentage of time spent (%total time) spent in all key behaviour by the 2019 Litter, Douglas, Thistle and Blossom, in the breeding enclosure at Wildwood Escot, and after their move, (Douglas moved to Wildwood Kent walkthrough and Blossom and Thistle moved to Wildwood Escot walkthrough). Error Bars = Standard Deviation.

Feeding was significantly different and increased after the move (before 31% vs after 41%) as did locomotion (before 25% vs after 39%). Inactivity significantly reduced after the move (before 17% vs after 4%). Drey building before and after the move (0.5% vs 0.8%) did not differ significantly, neither did social behaviour (8.4% vs 9.2%) or grooming (1.7% vs 2%) (Table 6).

Table 6 - the z score and p value for the Wilcoxon matched pairs statistical test for social, feeding, locomotion, drey building, grooming, inactivity and stereotypical behaviour for Douglas, Thistle and Blossom whilst housed in the breeding enclosure before and after the move to the walkthrough.

Comparison	z score	P value	Significant?
Social	-0.135	0.094	No
Feeding	-2.023	0.043	Yes
Locomotion	2.023	0.031	Yes
Drey Building	0..405	0.813	No
Grooming	-0.405	0.094	No
Inactivity	-2.023	0.043	Yes
Stereotypical Behaviour	2.023	0.031	Yes

3.1.5 Hypothesis 5 - Alterations to the breeding enclosure and impact on stereotypical behaviour in the breeding pair of red squirrels

Autumn and Radish's behaviour in the breeding enclosure was observed before and after the zoo's planned enclosure alterations (addition of screening boards and substrate, and then addition of hoarding opportunity) to investigate if the alterations changed behaviour. Figure 20 shows Radish was, at the start of observations, a prolific pacer and directly before Wildwood Escot decided to alter the enclosure, Autumn also started to pace. Figure 20 shows the monthly changes in pacing for Radish and Autumn. Before alterations to the breeding enclosure Radish stereotypically paced (on average) 28% of observed time, with a maximum occurrence of 35% of observed time, whereas after the alterations his pacing stopped. There was consequently an absolute change in pacing behaviour for this male. The keepers at Wildwood Escot stated that Autumn had never been witnessed pacing to any large degree, however in this study, Autumn was observed pacing in October 2019 (20%), November 2019 (9%), January 2020 (1%) and February 2020 (5%).

To test the effect the enclosure redesign had an impact on Autumn's stereotypical pacing a Wilcoxon matched pairs test was used, and results showed no statistically significant difference (z=-0.405, p=0.686) before compared to after the addition of boards and substrate but once the hoarding potential was increased by 10% there was an absolute difference in her behaviour as her stereotypical pacing ceased. However, as she died in August 2020, she could not be observed in the hoarding season in 2020, (Figure 20 and 21).

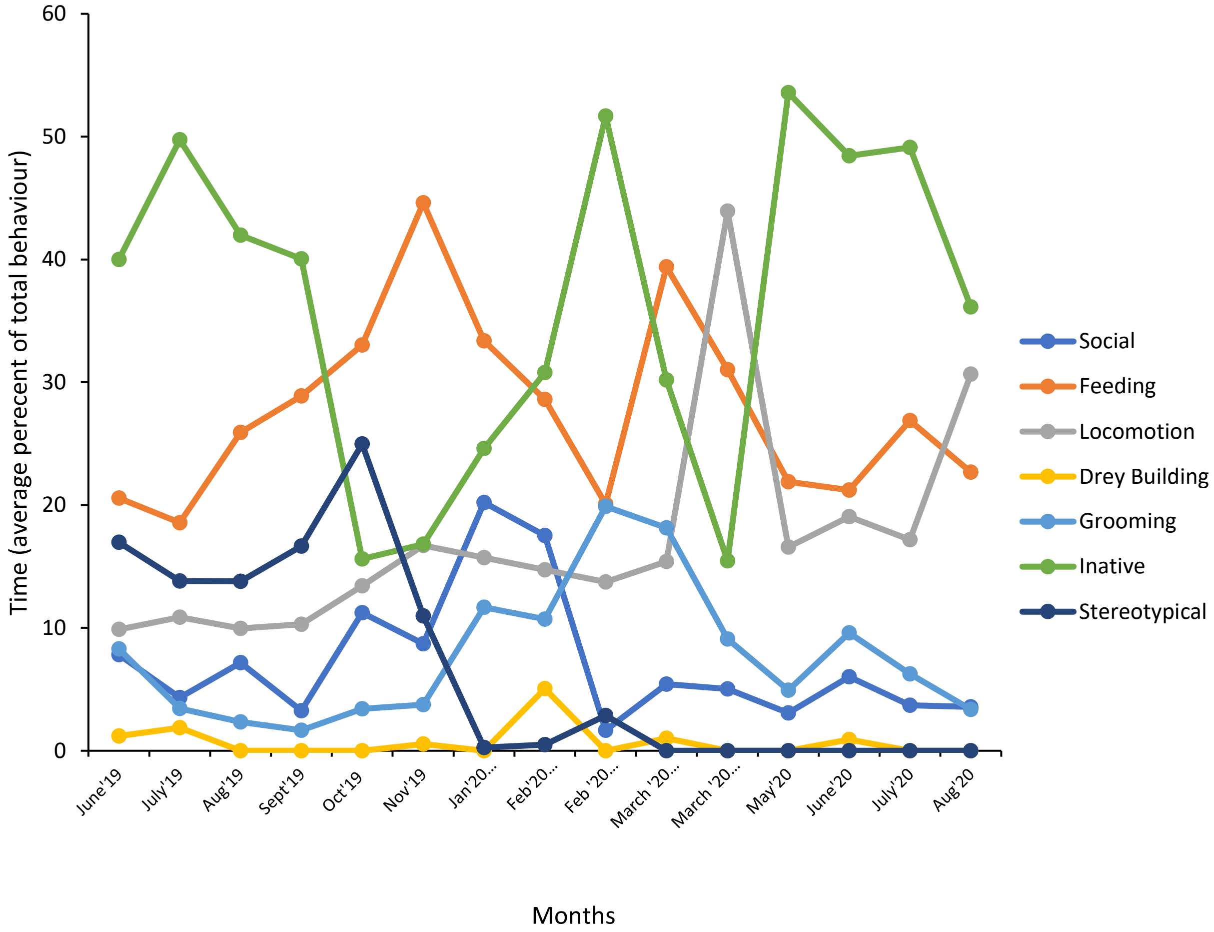


Figure 20: Time spent (%total time) in different behaviour of Autumn (female) and Radish (male) red squirrels before alteration in January 2020 and after alterations in March 2020 to the breeding enclosure

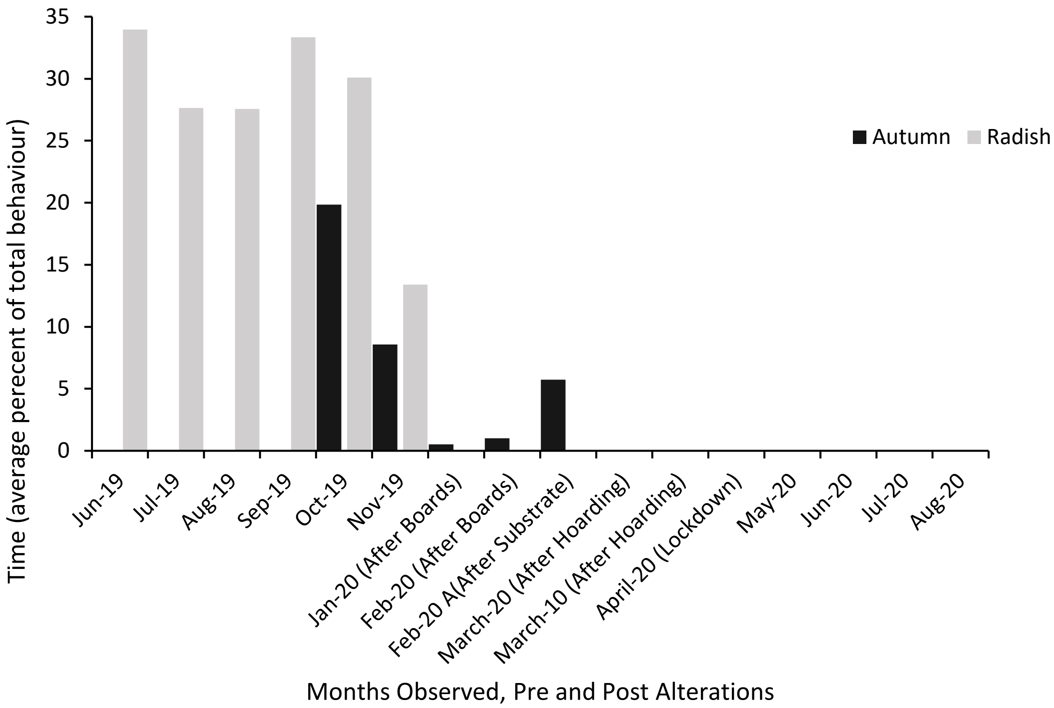


Figure 21: Time spent (%total time) in stereotypic pacing of Autumn (female) and Radish (male) red squirrels before and after enclosure alterations to the Wildwood Escot (Devon) breeding enclosure

3.1.6 Hypothesis 6 – Differences in stereotypical pacing between the 2019 litter (before alterations to the breeding enclosure and 2020 litter (after alterations)

The average time spent in key behaviours was compared between the 2019 litter (Douglas, Blossom and Thistle), before the alterations to the breeding enclosure, and the 2020 litter (Kitten 1, 2 and 3) after the alterations to the breeding enclosure (figure 22). The 2019 litter were moved at 22 weeks, Douglas to the holding pen in the Kent walkthrough, and Blossom and Thistle to the holding pen in the Escot walkthrough and released at 24 weeks where their pacing declined to an average of 1% by 28 weeks.

Whereas the 2020 litter remained in the breeding enclosure and their pacing rapidly increased from 22 weeks onwards. It needs to be noted that Douglas' pacing was very high at (43%) from 10 weeks of age in June 2019 then reduced to just 4% in July 2019 (14 weeks). This is because Douglas was only seen for 77 out of a possible 630 counts (just 12% of total observations) in June, he was the first to emerge and was very nervous and spent half of this small amount of time pacing. Neither of his sisters were seen out of the nest during this time. In July his sisters had emerged, and he was seen socially interacting with them and all juveniles were observed being active for a larger amount of time and therefore other behaviours could be recorded.

These results show that the 2019 litter started pacing earlier at 10 weeks of age and increased until they were moved, but the 2020 litter started pacing 8 weeks later at 18 weeks of age and increased by 24 weeks as they were not moved to a larger enclosure. The alterations not only impacted on reducing and eliminating the parents stereotypical pacing in the breeding enclosure but appeared to increase the age at which the juveniles started to pace. However, as this litter emerged during lockdown, this delayed onset in pacing behaviour could be linked to either zookeepers missing the emergence of pacing behaviour as the observer could not record their behaviour directly or a lack of any visitors and only a limited number of staff conducting husbandry procedures and not necessarily linked to the alterations.   Conversely at 18 weeks the observer was once again able to visit and noted the pacing starting and also as the lockdown was removed and visitor numbers increased, the pacing behaviour also increased. It is difficult to conclude if the alterations had a direct impact of the presentation of stereotypical pacing in the 2020 litter.

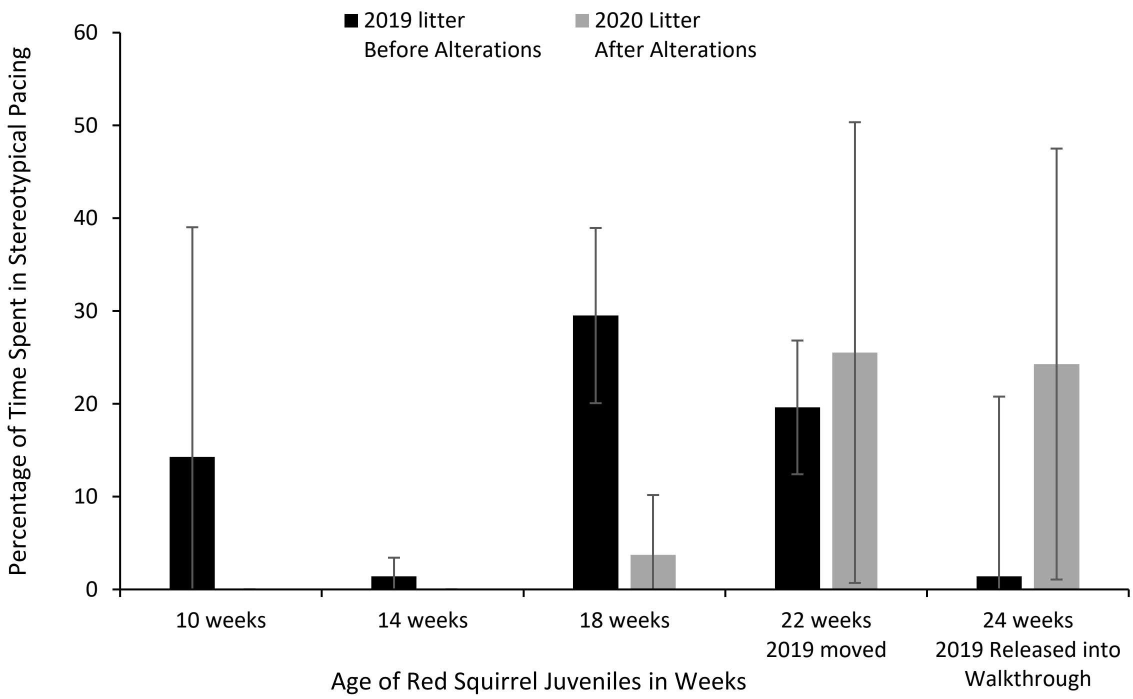


Figure 22 – Percentage of time (%total time) spent in stereotypical behaviour based on age in weeks for 2019 (Douglas, Thistle and Blossom) and 2020 (Kitten 1, 2 and 3) litter Error Bars = Standard Deviation.

3.2 Questionnaire Data

Eight out of ten zoos responded to the questionnaire, six of them were currently attempting to breed their captive squirrels (zoos 1-6) while zoo 8 stopped breeding four years ago and zoo 7 stopped breeding in 2019, but both zoo 7 and 8 still offered information on how their red squirrels were housed and husbandry and dietary details.

3.2.1 Red Squirrel Captive Population Characteristics

Each zoo provided information on the red squirrel population they housed during the observation period, between June 2019 to December 2020 (Table 7). Data were collected on 56 additional captive red squirrels (21 adults (10m, 11f), and 35 juveniles (19m and 14 f and 2 unsexed). (Table 7).

Table 7 - Red Squirrel Holding Number. Number (total count) and type (life stage and origin) of captive red squirrels currently held in the sample zoos between June 2019 and December 2020.

ZOO	**Females**		**Males**		**Juveniles**		**Survival Rate**	**Birth Origin**				**Pacing**	**Total**
	Number (died)	Mean age in Months (std+/-)	Number (died)	Mean age in Months (std+/-)	Number (died) M:F	Mean age in Months (std+/-)	Survival rate (Number that died)	Born free range	Born in breeding enclosure	Born in Wild	From another zoo	% that Pace	
1	6(2)	63(26)	1	1 (53)	6(2) 4:2	11.5 (3.8)	69% (4)	2	10	0	1 Breeding Female British Wildlife Centre (died)	10/13 (77%)	13
2	2(1)	60(33.9)	5(3)	36(30.4)	2(2) 1:1	15(0)	33.3% (6)		4	0	1-Pensthorpe (m died) 2-Private Breeder (m, 1 died) 1-Shepreth (m) 1-Cumbira (m) died	6/9 (67%)	9
3	2	60(0)	0	0	3 2:1	12(0)	100%		2	3		2/5 (40%)	5
4	1	24	2(1)	38(12)	5 0:3:2	12(5.5)	62.5% (3)		4		4-East Anglian Group (1f, 2m, 1 died) 1F-Wildwood moved to EARSG	3/8 (38%)	8
5	0		1	36(0)	7 6:1	15.2(4.8)	100%		2		6- private breeder	7/8 (87%)	8
6	0		1	40(0)	12(3) 6:6	11.7(5.6)	77%		13			2/13 (15%)	13

3.2.2 Pacing behaviour

The questionnaire results illustrated that, according to keeper reports, 54% (30) captive red squirrels stereotypical paced, 16 males (53%) and 14 females (47%). A third of these were adults (10, four males, six females), and two thirds were juveniles (20) with the majority (12) being males juveniles (60%) and 8 being female juveniles (40%). Out of the 14 females that paced, eight (57%) were juveniles and six (43%) were adults. Out of the 16 males that paced, 12 (75%) were juveniles and four (25%) were adults. Therefore, the group that paced most were male juveniles (Table 8).

Three adult males paced daily in breeding enclosures for about half the day for a few hours at a time, with two lateral running with food, and one without food. One adult male paced seasonally in an aviary, for a few minutes a day over half the day, displaying lateral running without food.

Two adult females paced in a walkthrough enclosure on a monthly/seasonal basis, for less than half the day and less than an hour at a time and this took the form of lateral running without food. The other four adult females paced in a breeding enclosure on a seasonal basis and only for a few minutes a day at isolated times and both involved figure of eight pacing. Six male juveniles paced in breeding enclosures and two thirds (four out of six) paced daily for about half the day between a few hours to an hour each day. Three juveniles displayed lateral running without food and three displayed figure of eight pacing. The other six male juveniles paced in an aviary on a monthly rather than daily basis, at isolated times for a few minutes a day, and all took the form of lateral running without food.

Seven female juveniles paced in a breeding enclosure and the majority (four) paced every day, with the other three pacing only seasonally. They paced between just a few isolated times to half a day with the median being less than half the day. They paced for varying lengths of time from an hour a day to just a few minutes, with the median being for less than an hour a day. All pacing took the form of lateral running without food with three figure of eight pacing.

Table 8 – Pacing numbers, adult and juvenile, males and females. The median, range of the frequency of pacing – daily (1), weekly (2), monthly (3), seasonally (4). The time of day – isolated (1), less than half a day (2), about half a day (3) Most of the day (4), All day (5) and the bout in length of time - a few minutes (1) less than an hour (2), an hour (3) a few hours (4), many hours (5). The mode of when the pacing started – was already established when red squirrel arrived (1) or emerged on site (2). The mode for the type of pacing - stepping side to side (1), lateral running without food (2), figure of 8 (3), spinning (4) lateral running with food (5)

Life Stage	Sex	Pacing Details		Enclosure Type	
	M/F	Pacing Characteristic	Walkthrough	Breeding Enclosure	Aviary
Adult	Males	Frequency		1 (3 squirrels)	4 (1 squirrel)
		Time		3	3
		Bout length		4(3-4)	1
		When		1	1
		Type		5	2
	Females	Frequency	3 (2-4) (2 squirrels)	4 (4 squirrels)	
		Time	1.5(1-2)	1	
		Bout	2.5 (1-4)	1(1-2)	
		When	2	1 (1-2)	
		Type	2	3	
Juveniles	Males	Frequency		1 (1-4) (6 squirrels)	4 (6 squirrels)
		Time		2 (1-3)	1
		Bout		2.5 (1-4)	1
		When		2	1
		Type		3	2
	Females	Frequency		1 (1-4) (7 squirrels)	4 (1 squirrel)
		Time		2 (1-3)	1
		Bout		2(1-3)	1
		When		2	2
		Type		2	2

3.2.3 Enclosure type and feeding information

The zoos which recorded two of the highest amounts of pacing (zoo 5 at 87% and zoo 2 at 67%) housed the red squirrels in the smallest enclosures, both under $100m^3$, with only woodchip as substrate, and offered neither artificial nor natural cover. However, the zoo with the second largest amount of pacing (zoo 1 at 77%) was $180m^3$, with both wood chip and leaf substrate and both artificial and natural cover. The major similarity amongst these three zoos being that the red squirrels were not fed seasonally and were the only zoos offering a basic diet of seeds, nuts, fruit and veg. The zoos with least recorded pacing (zoo 3 at 40%, zoo 4 at 38%, and zoo 6 at 15%, with zoo 7 stated that when they had squirrels, pacing was very minimal) all had enclosures between $200m^3$ ->$300m^3$. Both zoo 4 and 7 had sand baths in their enclosure and all offered both leaf and bark substrate. Zoo 3, 6 and 7 all have artificial cover of either walls or board and also natural cover of trees and branches. Although zoo 4 did not offer artificial cover they have the largest enclosure with three separate enclosures joined by tunnels and offering cover using branches and trees. Zoos which reported the lowest amount of pacing (zoo 3, 4 and 6) offered seasonal feeding, and the biggest difference between the zoos reporting the highest pacing and those reporting the least amount of pacing was in the complexity of the red squirrels' diet. Zoos 3, 4,6 and 7 offered a complex diet including natural browse, beechmast, hawthorn, cob and pine nuts, antlers and even live food, compared to the zoos recording the most pacing which fed a less complex diet.

A logistic regression was performed to ascertain if, age (adult or juvenile), enclosure type, enclosure size, enclosure complexity and feeding complexity had an impact on the likelihood that individuals would pace. The predictors were chosen given the observational data results and the literature research on stereotypy aetiology in rodents which together suggest pacing is most likely to occur in individual squirrels who are: kept in smaller enclosures, housed with multiple conspecifics, kept in enclosures without privacy boards and/or enough foraging and hoarding opportunities and inappropriate substrate, incorrect or inadequate husbandry procedures or feeding not meeting motivational requirements and or seasonal needs. Each predictor was given a numerical value to allow for summary statistics and comparisons between respondents. These numbers were then applied to each individual squirrel (n=56) (Appendix VIII) and used to run a multiple logistic regression model to predict the dependent data variable (pacing) by analysing the relationship between several different independent variables (enclosure, feeding, age, sex) (as discussed in the Methodology).

The overall logistic regression model was statistically significant for enclosure size (p=0.039) when compared to the null model, ($X^2(7)=28.843$, $p<0.05$), suggesting that increasing enclosure size is associated with reduction of occurrence of stereotypical pacing in captive red squirrels. Age, adult or juvenile (p=0.246), sex (p =0.675), enclosure type (p=0.494), feeding complexity (p=0.28) and enclosure complexity (p=0.7) did not significantly predict the presence or absence of pacing. All types of individuals in all enclosure types paced and therefore it must be questioned further if the significant predictor is a genuine predictor or if, though the data suggests it, some other factor may be at work, and this is critically analysed further in the Discussion.

3.2.4 Mortality rate and pacing

Seven zoos detailed information about birth and survival rates for three years of breeding, 2017-2020. As zoo 7 stopped breeding in 2019 their rates are for 2017 and 2018 only.

The survival rate for all squirrels ranged from 33%-100% and for juveniles under 18months from 50%-100% (Table 9). Zoo 1 had the highest recorded pacing at 77% of the population being reported at partaking in pacing behaviour but a survival rate at 75%, whilst Zoo 2 reported 67% of their population paced and only 50% survived, zoo 4 had 68% survival rate and just 38% pacing, zoo 5 had 67% survival rate and 87% pacing and zoo 6 had only 15% pacing and 77% survival rate. Zoo 3 was atypical with no deaths recorded. This zoo reported 40% of their red squirrel population paced (Table 9).

Table 9 - Mortality Rates for Red Squirrels Litters for three years of breeding. Total births, deaths and survival rates for seven zoos who completed the questionnaire and had bred within the last four years from 2017-2020

Zoo	2020 born	2020 survived	2019 born	2019 survived	2018 born	2018 survived	2017 born	2017 survived	Total	Deaths	Survived
1	3	1	4	3	5	5			12	3 (25%)	75% (3)
2	0	0	8	3	5	2	7	5	20	10 (50%)	50% (6)
3	0	0	11	11	8	8			18	0 (0%)	100% (1)
4	3	1	7	2	7	7			17	7 (41%)	69% (4)
5	4	3	5	3					9	3 (33.3%)	67% (5)
6	7	6	5	3					13	3 (23%)	77% (2)
7					4	4	9	7	13	2 (15%)	75% (3)

3.2.5 Breeding and Dispersal Ages

Collectively, zoos reported nine males and ten females bred. The mean age at which breeding was reported to occur was 18 months (+/-0.5) and the mean age at which keepers reported they stopped allowing individuals to breed was 72 months (+/- 0.9). Two zoos stated that the decision to stop breeding was dependent on the individual's health and breeding success and two zoos reported they stopped breeding only because of the death of the individual. Only half of the zoos answered the question as to when their juveniles were moved from the natal enclosure, with three zoos confirming they move juveniles between 12-14 weeks of age, whilst one zoo stated they did not move the juveniles until 23 weeks of age, and one zoo reported juveniles were 'sent for release' once they reached 250g body mass.

3.2.6 Seasonal Impact on the Presentation of Stereotypical Pacing

Six zoos reported that they believed the presentation of stereotypical behaviour was due in part to seasonality, however the months with the largest decrease or increase in this behaviour varies between each zoo. One respondent noted an increase in pacing in spring, one noted an increase in spring and summer, one reported an increase in summer and autumn, one stated they only saw an increase in September, and one noted an increase in pacing in autumn. Two respondents did not link increased pacing with a particular month directly but considered the start of pacing coincided with the time juveniles need to disperse.

Although one respondent reported a decrease in pacing in summer, the majority reported that winter saw the main decrease in pacing. Two respondents reported no seasonal change in pacing but noted a decrease in pacing when the juveniles are removed from their parents (see Figure 23).

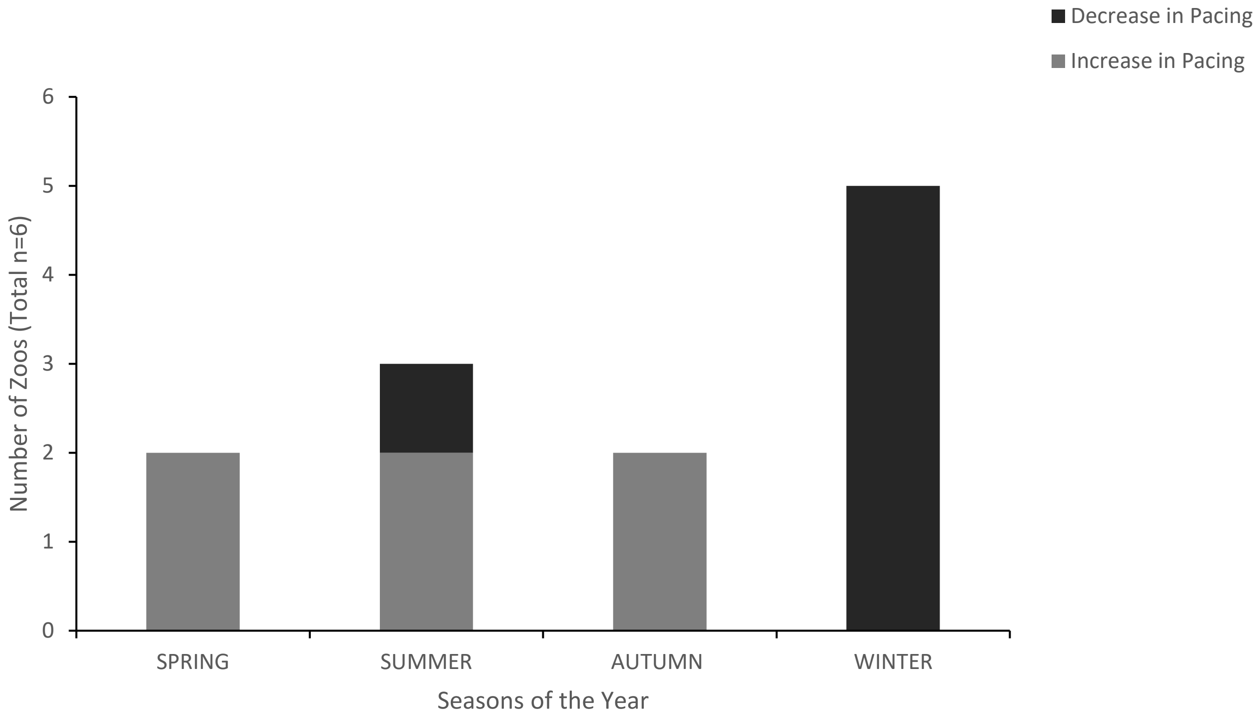


Figure 23 - Months when captive red squirrel pacing was reported by keepers to either increased or decreased throughout the calendar year.

3.2.7 Red Squirrel Juvenile Location and impact on Behaviour of in situ Adults

Five zoos gave details regarding behavioural changes when juveniles were moved away from the breeding enclosure to a new location. Three zoos reported no change in behaviour of the parents when the juveniles were removed from the breeding enclosure or any change in behaviour of the resident adults to the enclosure the juveniles were moved to.

One respondent reported less chasing, aggression and reduced pacing in the male of the breeding pair when then juveniles were removed. Only two respondents reported a change in the resident adult's behaviour when the juveniles were added to the new enclosure with increased territorial behaviour including chasing, increased social and aggressive behaviour.

Whilst both these respondents reported a reduction in pacing by the juveniles' they also witnessed an increase in pacing by stereotyping red squirrels already housed in the enclosure (Figure 24).

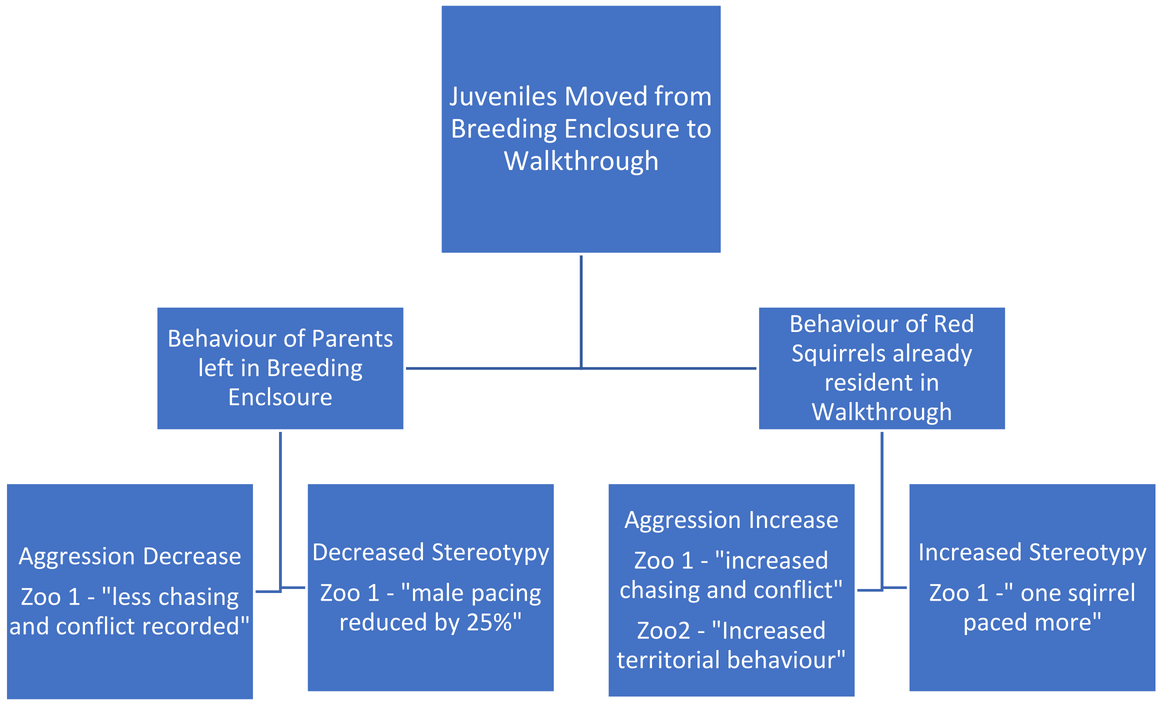


Figure 24 - Impact on behaviour of resident squirrels when the juveniles are moved from the breeding enclosure and the impact on behaviour of the resident red squirrels already housed in the new enclosure. Two zoos recorded changes in behaviour.

3.2.8 Methods to Reduce Stereotypical Behaviour in Captive Red Squirrels

All respondents had implemented several different methods to attempt to reduce stereotypical behaviour, these mainly involved changes to the existing enclosure or changes to the diet of the red squirrels.

Two major themes were identified – enclosure changes and dietary changes. Within the theme of ‘enclosure’ two minor themes were identified ‘cover’ and ‘substrate’, five zoos discussed ‘adding tree cover’ or ‘screens’ to essentially provide hiding opportunity and act as a visual barrier. Four zoos added wood or leaf piles, including hoarding containers to provide additional digging and caching areas and two zoos offered seasonal/wider diet and increased browsing opportunities, such as adding maple and hornbeam to the squirrel’s diet and adding feeding and behavioural enrichment.

Three respondents reported the most successful way they found to reduce or eliminate stereotypical pacing was by increasing floor space, either by adding hoarding containers or by moving the red squirrels to a larger, free roaming enclosure with less conspecifics.

Figure 25 and 26 shows content analysis of the specific type of action taken to reduce stereotypical pacing, all zoos are ‘adding’ opportunities for the action taken (no zoo has taken away opportunity by housing alone in a side pen), five are adding cover, four adding substrate and four adding to the diet, either using enrichment or seasonal changes.

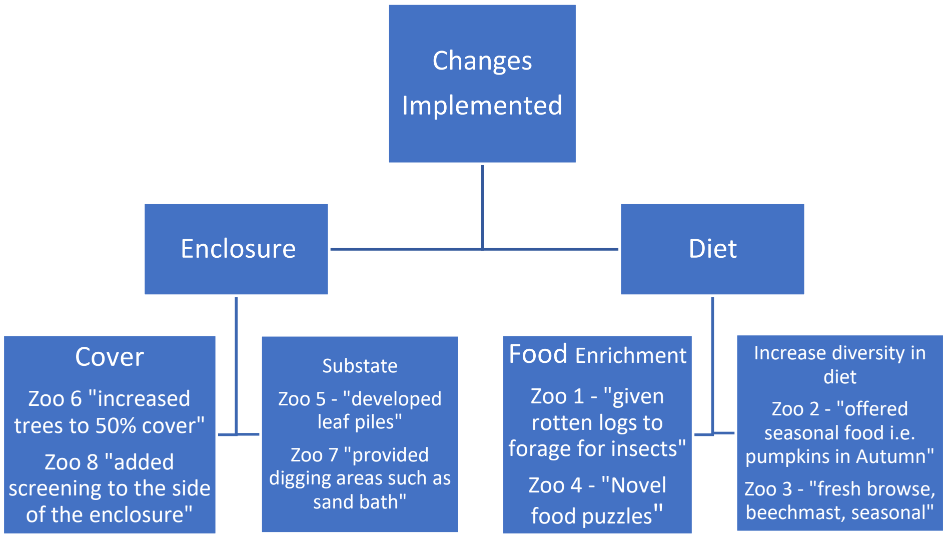


Figure 25 - Changes implemented by all 8 zoos in order to try and reduce or eliminate stereotypical pacing, involved changes to both the Enclosure and Diet.

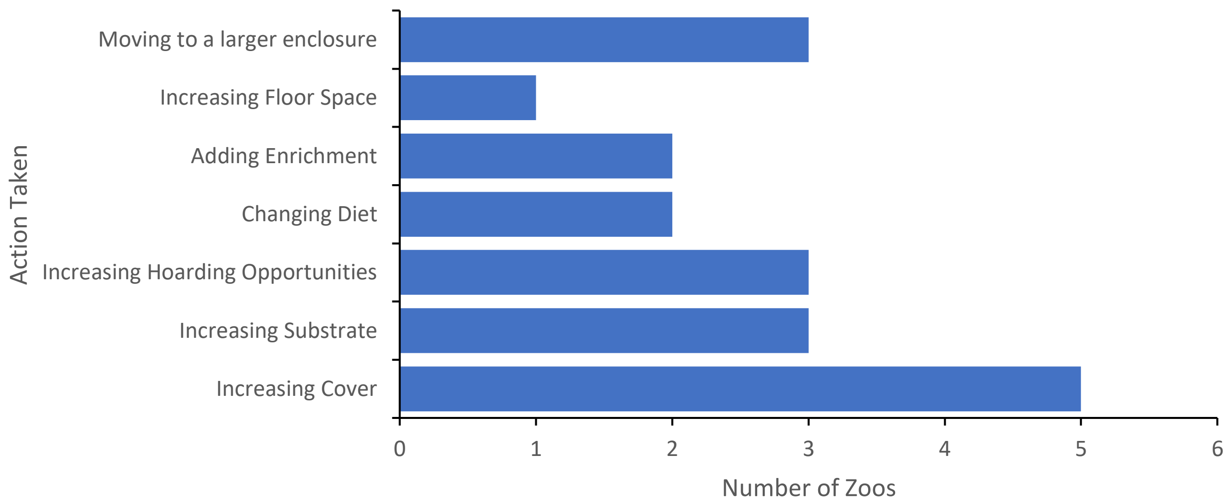


Figure 26 - Number of zoos that implemented different changes to either the enclosure or the diet of their captive red squirrels in attempt to reduce stereotypical pacing behaviour.

3.2.9 Potential Impact of Visitors/Pandemic on Red Squirrel Behaviour

When asked if the zoo believed the amount or type of visitor, or lack of visitors during the COVID-19 pandemic lockdowns had impacted upon the presentation of stereotypical behaviour, half reported increased pacing, more vigilant behaviour and increased hiding or avoidance behaviour in red squirrel behaviour when the zoo experiences increased visitor numbers and the other half noting no change in behaviour. Only two respondents noted a change in the behaviour of captive red squirrels due to the COVID-19 pandemic lockdowns, with a new litter of juveniles being more timid and hiding more, and pacing reduced in some red squirrels (although this could be related to reduced numbers of staff available to witness their pacing) (Figure 27).

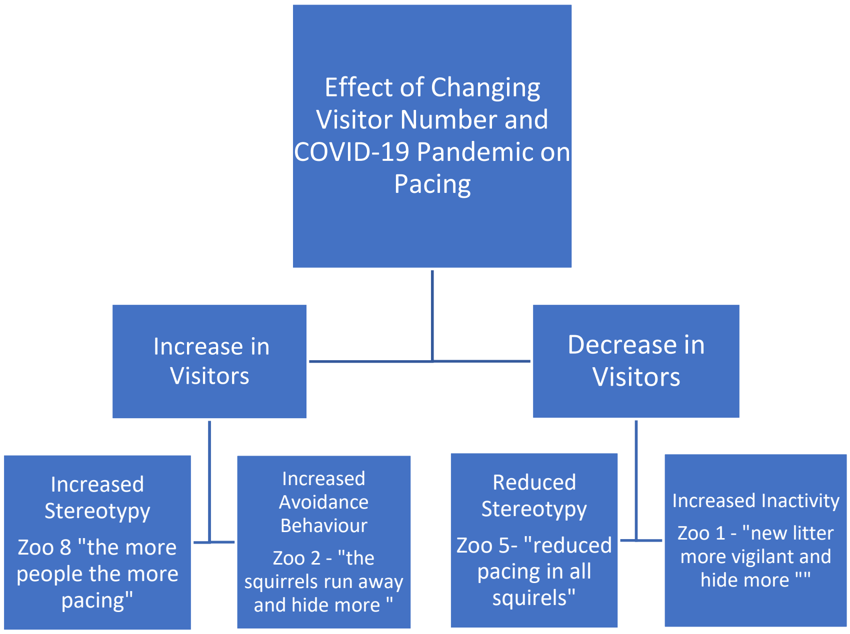


Figure 27 – Effects of changing visitor numbers and impact of COVID-19 pandemic and subsequent implementation and lifting of lockdowns on stereotypical behaviour of captive red squirrels

3.2.10 Why Stereotypical Behaviour Emerges, Manifests in Captive Red Squirrels

Respondents detailed what they believed caused red squirrel pacing in captivity with several potential causes for the emergence of stereotypical behaviour in red squirrels being given (figure 28). The main point findings were that collectively zoos identified nine reasons why stereotypical behaviour emerges. Most zoos suggest that dispersal needs in juveniles is the major contributory factor, along with hoarding/foraging stress. The main reason given for pacing in adult captive red squirrels was adverse internal and external stressors. Five respondents contributed stereotypical behaviour to the captive environment not meeting group or individual hoarding/foraging needs, key factors included increased competition for food and pilfering particularly when housed in large numbers and not enough food or caching sites. Inappropriate or inadequate foraging opportunities, including incorrect diet or inappropriate food presentation was also listed and two respondents linked this foraging stress to a lack of opportunity to display natural behaviour particularly those behaviour red squirrels feel highly motivated to perform.

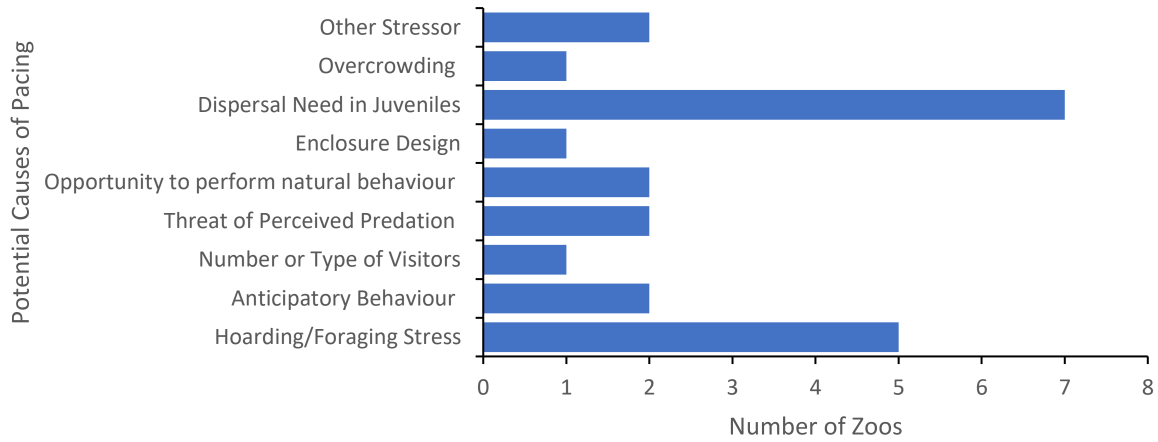


Figure 28 - Respondents answers as to potential causes of stereotypical behaviour in captive red squirrels

Two respondents believed the perceived threat from potential predators, including increased visitors near the animals and the inability to hide or escape contributed to the emergence of stereotypical behaviour   Two respondents only recorded stereotypical behaviour just before feeding times, indicating anticipatory feeding behaviour may be linked with increased abnormal behaviour. One respondent stated their belief was inappropriate, small enclosures, lacking complexity and too many conspecifics were a key reason for pacing in captive red squirrels.

3.2 11 Captive breeding for release programmes and limitations for release.

Zoo 3 and 5 breed for both wild reintroduction and captive conservation and educational purposes. Zoo 5 states they would like to release more animals in the wild but faced several limitations, however zoo 3 felt there were no limitations for captive release, (this zoo is not on mainland England). Zoo 2, 7 and 8 have bred for captive release in the past but now mainly breed for captive conservation and education due to limitations being faced with wild release programmes. None of the five zoos who responded believed that stereotypical pacing was a limiting factor for the release of captive red squirrels into the wild (Figure 29).

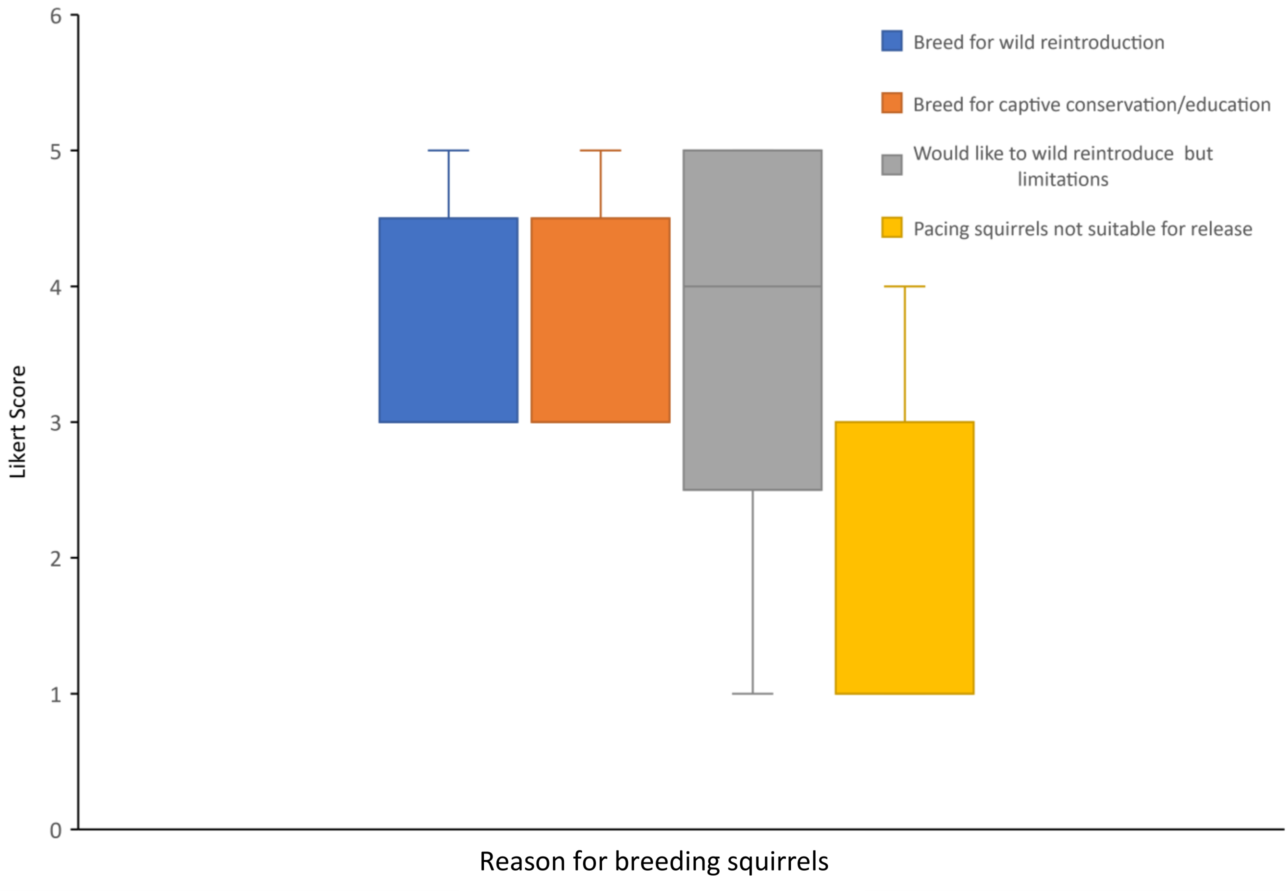


Figure 29 - How zoos rated four different answers as to how they view their role of breeding captive red squirrels using a Likert scale between 1-5, 1 being strongly disagree and 5 being strongly agree.

Zoos 2, 7 and 8 felt there were no suitable release sites, and too high a prevalence of disease in the wild population to enable them to now be involved in release programmes. Zoo 7 felt there were too many release sites with already established large populations of red squirrels, with all six zoos except zoo 3 highly rated the main limitation to wild release being not enough captive red squirrels to release. Zoos 2 and 7 also felt there was not enough support for the reintroduction of red squirrels into the wild, whereas zoo 3 strongly disagreed with this, and zoos 8 and 6 did not agree at all. Although zoos rated different limitations to red squirrel release at different levels of importance, the answers to these questions did not differ significantly (Chi^2=7.108, n=27, df=5, p=0.210), suggesting zoos collectively identify multiple reasons and all reasons are as likely.

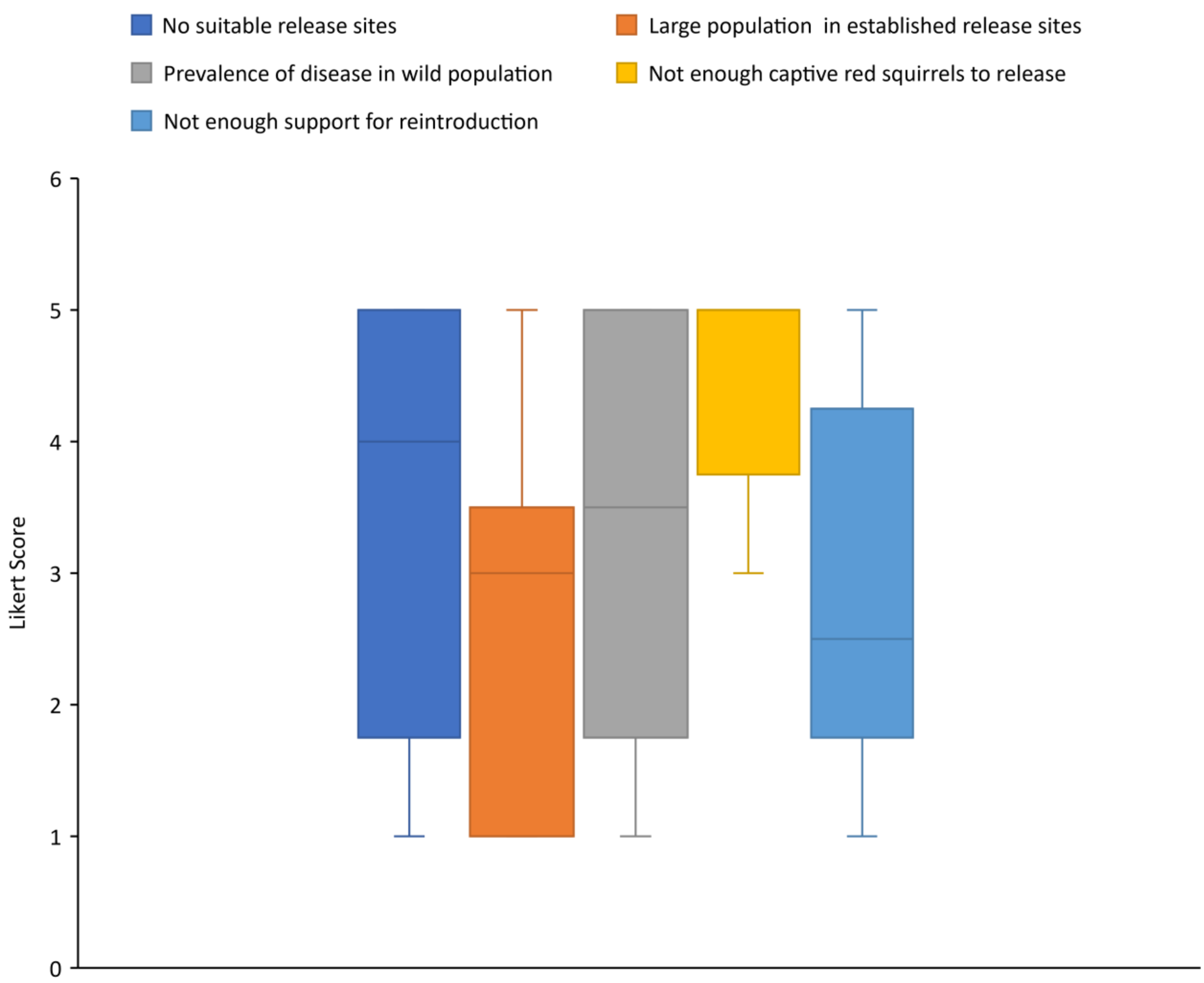


Figure 30 - How zoos rated five different answers for potential limiting factors that could affect the release of captive red squirrels into the wild. Likert scale scored between 1- 5 with 1 being strongly disagree and 5 being strongly agree

4. Discussion

Red squirrels are an endangered species in the UK. Translocation of the species outside its natural distribution, and captive breeding programmes need to be successful in order to prevent extinction of the UK wild population before 2040. It is therefore essential that individual animals enlisted in captive breeding programmes have good welfare and remain phenotypically viable for wild release. Uchida et al. (2016) states the only way to meet individual animals' species-specific needs in captivity and therefore understand an animal's intrinsic and extrinsic motivations to perform certain behavioural repertoires is to understand their in-situ behaviour and activity budgets in the wild. However, the biology and ecology of red squirrels is still not fully understood, yet comparisons between the activity budgets of wild and captive red squirrels is the best way to indicate which behaviour is most important to an animal to meet survival and fitness needs (Bertolino et al., 2004).

This study involved the observation of 16 captive red squirrels at two UK sites, Wildwood Escot (Devon) and Wildwood Kent over an 18-month period to investigate the occurrence of stereotypical pacing behaviour, possible causal factors and disturbance in activity budget compared to wild activity budgets. Wild red squirrel behaviour is categorised in the literature as Social, Feeding, Locomotion, Drey Building, Grooming and Inactivity (Holm, 2010) and these categories were therefore used to define the captive activity budget, with the notable addition of stereotypical pacing behaviour, an artefact of captivity. To further substantiate the observational data collected in this study, a survey of British red squirrel keepers was conducted to ascertain the scope and scale of stereotypical pacing in the wider captive red squirrel population, and keeper-reported possible causes of pacing and patterns of onset, as well as information on how the squirrels are kept, enclosure design, diet, and mortality.

4.1 Captive versus wild red squirrel behaviour

This research showed that, while some captive individuals in the observed population have a wild-type activity budget, these individuals are the smallest of minorities and are those squirrels that have not been observed partaking in stereotypical behaviour. Most captive individuals in the observed population (over 80%) displayed stereotypical pacing behaviour and significant differences in their activity budgets compared to wild individuals. Stereotypical behaviour can be a sign of reduced welfare and whilst an animal is partaking in stereotypical pacing behaviour, other species typical behaviour is being performed to a lesser degree, which may impact on the animals' fitness and survival rates (Mason and Latham, 2004).

Thus, even if stereotypy is considered a coping mechanism, the long-term maladaptation gives cause for concern and indicates that the presence of stereotypy in the behavioural repertoire means the individual(s) is experiencing negative welfare or has experienced negative welfare in the past. Evidence has suggested that established stereotypies are hard to eliminate (Coleme and Maier, 2010) particularly in older animals ((Wurbel, 2001) as animals get stuck in a positive feedback loop of stereotypical expression (Dantzer, et al., 2010). For example, Cherry and Twiglet started pacing in the breeding enclosure, probably due to limited resources and/or the need to disperse to a new territory or away from overcrowding, but this behaviour was maintained even when they were moved to a larger walkthrough enclosure with less squirrels, more territory and increased resources. The presence of stereotypical behaviour not only compromises the welfare of captive red squirrels, but can affect brain development, reduce immune response, and also effect breeding success and be detrimental to captive breeding and release programmes (Mason and Latham, 2004). Therefore, the observed 10% occurrence of stereotypical pacing in the activity budget averaged across all observed individuals in this study is concerning, especially when the large variation in individual performance of pacing is considered; at certain times of the year and stages of development pacing accounted for more than a quarter of the entire activity budget of some individuals, even up to 47% for one male.

This study, whilst gathering a great deal of data regarding 16 specific squirrels over 18 months could be considered unrepresentative of the greater captive population, however all captive zoos follow the Welsh Mountain Zoo's Husbandry Guidelines for housing red squirrels so this group would be a "true" sample of how British red squirrels are kept in captivity. The observations also used robust methods and by using mix-method research of also surveying eight UK zoos, the observational findings could be verified as a representative sample of the captive population as a whole. Survey responses also suggest that pacing is a concern in the wider captive red squirrel population, as 56% of the wider captive population were reported to pace. This percentage is lower than may be expected given pacing prevalence in the observed population was over 20% higher (80%). The seasonal variation in maximal pacing observed in all individuals may explain this; the months during which pacing is high, August, September, and October, are the hoarding and dispersal months for red squirrels, and these coincide with extremely busy times at the zoo calendar – school holidays and summer when visitor numbers are increased. It is at these times that zookeepers are busiest and spend the least time on their sections as there are visitor duties to typically perform. It may thus be a case of missed observation. Zookeepers are typically the best placed to consult on the behaviour of animals in their care (WMZ, 2019) but they are extremely busy and often split their time between sections or groups within the zoo, rarely being consistently present to observe one group of animals throughout the day.

This is one reason why designated observational studies are so helpful in understanding captive zoo animal behaviour and one of the biggest strengths of this study was that red squirrels were observed for several hours throughout every month of the year, so seasonal changes in behaviour could be recorded. Given that observations occurred at two sites, this, and the survey results, suggest the observed individuals are not outliers but rather indicative of a prevalent occurrence of stereotypy in captive squirrels.

As captive individuals typically spent significant amounts of time pacing, they were unable to spend time performing wild-type behaviours. Wild squirrels spend 80% of their active time in feeding or locomotive behaviour, rarely being inactive or engaging in social interaction apart from during the mating season (Wauters et al., 1992). Noticeable differences were recorded in the captive population that stereotypically paced compared to the wild red squirrel activity budget derived from the literature, as captive red squirrels feed and locomote significantly less, and are significantly more inactive than their wild counterparts. However, it must be noted that the large error bars in Figure 4 illustrates that wild, pacing and non-pacing individuals' behaviour varies considerably around the average and these changes are noted in feeding, locomotive and inactive behaviour. For example, the average feeding behaviour for wild red squirrels across the four studies averages almost 57% for feeding and 25% in locomotion, however across the studies some squirrels fed nearly 80% of their time compared to just 40% for other groups, and locomotion ranged from 10-50%. However, it needs to be considered that in the wild the species is much harder to locate, and all four studies took place over differing lengths of time ranging from 2 years to just 3 months, which could explain these large error bars. Future work could include conducting observational studies of a wild population over the same length of time as this study was conducted and this would give a truer representation of wild red squirrel activity budgets.

Wild red squirrel behaviour is driven by the changing seasonal abundance of food and activity levels are determined by both food choice and availability (Bertolino et al., 2004). Red squirrels need a balance between resting/sleeping in the nest to conserve energy and foraging and feeding enough for survival requirements (Wauters et al., 1992). Their daily activity is linked to sunrise and the number of hours spent active per day is based on daily sunlight, which increases as spring progresses into summer between May – September, when they spend between 8-11 hours per day out the nest. Feeding behaviour takes up half of this active daily budget and foraging behaviour averages between 54-85% of total observed feeding behaviour (Wauters et al., 1992).

Bertolino et al. (2004) states feeding behaviour and locomotion is directly linked and when food is in abundance red squirrels spend 95% of their time in less than half a hectare foraging for food, however if food availability is scarce, they will begin to randomly search for food and increase their travel to ¾ hectare. In Spring they locate themselves near their caches as this provides their main source of food but in summer, they travel further looking for scattered seeds as their caches are empty. Traveling in spring, accounts for an average of 10% of active time in wild red squirrels but increases in autumn (Wauters and Dhondt, 1987). During late summer and early autumn, the time spent foraging, and the distances travelled searching successfully for food are similar to those measured in early summer (Bertolino et al., 2004).

In captivity food is readily available throughout the year, often in nutritional abundance compared to the nutritional requirements of a squirrel. The food presented is typically of high nutrient value and therefore squirrels can spend less time eating. As food is presented directly to captive squirrels there is no need to travel or forage and this may account for the low locomotion time and increased inactivity in the captive population. In this study captive red squirrels spent an average of 10% of their activity budget in abnormal behaviour, in the form of pacing, and less than 60% of their time feeding and in locomotion, with only 31% of time spent in feeding behaviour, almost half that seen in wild red squirrels. This is discussed further in section 5.2.

In the wild, interactions comprised around 4% of total activity. Captive red squirrels, both pacing and non-pacing individuals displayed twice the amount of social behaviour, including aggressive behaviour as their wild counterparts, at an average of 8.5% vs 4.2%. This is probably due to being kept in increased numbers in smaller environments, increasing aggressive interactions potentially explaining both hoarding stress behaviours and why infanticide occurs in captivity, as witnessed in this study when Shep killed his offspring. In captivity there is increased social tension in a small group when individuals cannot disperse and this increase in social tension either leads to aggressions, or a great deal of time spent trying to ease social tension, whereas wild squirrels are able to feed in close proximity on temporarily abundant food resources with hardly any signs of aggression as they are able to move away from stressful social interactions (Wauters et al., 1992). This is discussed further in section 5.3.

Averaging four studies into wild red squirrel activity budgets an average of 57% of their time is spent in feeding behaviour, similar to that of non-pacing squirrels that averaged 51% of observed time, but 20% less in captive red squirrels that pace (Wauters et al., 1992). Whilst it is recognised that there is variation in the literature concerning wild activity budgets of red squirrels, it is extremely unlikely that any wild squirrel will ever stereotypically pace as abnormal behaviour is an artefact of captivity.

While seasonal changes in the other normal behaviours occur, they tend not to coincide with the activity budgets of the pacing captive squirrels, suggesting that captive red squirrels simply locate and eat too little and are too inactive.

Captive males and females' stereotypically pace and both adults and juvenile's pace. This indicates this kind of abnormal behaviour is systemic to every fragment of the captive population, a behaviour that is considered to be absent in the wild population and suggests compromised welfare in the captive population. However, determining the cause of the onset of stereotypy is not easy as individuals that paced showed a lack of commonality in the presentation of stereotypical behaviour. Some individuals paced 1-20% total time and others paced nearly half of their activity budget. This is represented by the large error bars and variation around the mean for individual pacing in Figure 5a and clearly illustrated in Figure 5b stacked bar charts which shows differing amounts of pacing in different enclosures, including Douglas, Thistle and Blossom move from the breeding enclosures to walkthroughs, the effect the move had on the resident squirrels in the walkthroughs and the impact on pacing the alterations on the breeding enclosure had on both Autumn and Radish behaviour and that of the 2020 litter.

However, whilst it was important to look at individuals this study also looked at different groups, such as males and females, adults and juveniles as certain groups of individuals are known to react to captivity in a similar way based on their age, birth origin, life experiences, husbandry conditions or how they are housed. Indeed, keepers highlighted nine different reasons why they believe red squirrels stereotypically pace and as finances and time are real constraints in zoos, they are more likely to tackle group abnormal behaviour, for example juveniles who start to pace because of dispersal needs, than a potential outlying individual that appears to have no obvious reason for pacing, therefore looking at the wider data set by using the mean to find average behaviour is justified and valuable in this kind of research.

Stereotypical behaviour also took on different patterns, from lateral running with or without food, to figure of eight pacing and repeatedly stepping from one foot onto the other in between pacing bouts. A real strength of this study was that these findings were also reflected in responses to the questionnaire, with the frequency, type of pacing, time and bout length spent on stereotypical behaviour often being dissimilar amongst different individuals. Herborn et al. (2010) state that different individuals, even within a homogeneous captive environment, explore and forage in different ways when confronted with the same environmental or behavioural stimuli, suggesting that personality also plays a part in behavioural variation between individuals and how individuals cope with different stressful stimuli.

Comparisons between different enclosures (walkthrough enclosure, breeding enclosure and holding pen) illustrate that the walkthrough enclosures, which represent a more similar wild type of habitat, with less conspecifics and natural environments with no roof, large coniferous and broadleaf trees, facilitated the most amount of feeding and locomotive behaviour, at an average of 80% of observed time, similar to that found in the wild.

Conversely squirrels housed in breeding and holding enclosures (Figure 14) recorded the least feeding and locomotion (47% and 55% respectively). This deficit in time being taken up in an increase in both pacing and inactivity. Wauters and Dhondt (1987) state wild red squirrels are maximally inactive for 8% of their total activity budget (this coincides with winter as moving can course heat loss). Similar captive non-pacing red squirrels were typically inactive for 5% of observed time, whereas captive pacing squirrels were inactive over double this at an average of 18.5% of observed time. Again, large error bars illustrate large differences in inactivity and stereotypical behaviour in the breeding and holding pens. Inactivity ranged from 15-35% of time in the breeding enclosure and 10-25% in holding pen and stereotypical behaviour ranged from 5%-25% of observed behaviour in the breeding enclosure. It is important to note that the highest amount of inactivity seen by any individual in the walkthrough was just 9% which was still lower than ever recorded in the breeding enclosure or holding pen and the highest amount of pacing in the walkthrough was recorded at an average of 3.7%, which is still lower than that recorded in the breeding and holding pens, suggesting that although the error bars are large for inactivity and stereotypical pacing, these two behaviour are still recorded at a much lower level in the walkthroughs than the breeding and holding pens.

Stereotypical behaviour was recorded at 16.2% in both the breeding enclosure and holding pen, but at just 4% in the walkthrough enclosures. Feeding, inactive and pacing behaviour were significantly different between the two walkthrough enclosures, and holding and breeding enclosure, but not between the holding and breeding enclosures. Although locomotion was significantly different between the walkthrough and the breeding enclosure, it was not significantly different between the walkthrough and holding pen. However, both holding pens are in the middle of the walkthroughs, and whilst the juveniles were shut inside them for two weeks awaiting a soft release, the other resident squirrels in the walkthrough spent a great deal of time running along the outside of the holding pens chasing those housed inside, so this could explain this increase in locomotion by the red squirrels housed in the holding pens.

An increase in locomotion can often be a sign of stress and can lead to or increase stereotypy as Morgan and Tromborg (2007) found stereotypical pacing accidently arose from increased autonomic activity and increases in metabolism encouraging the fight or flight response and Cooper et al. (1996) found that active rodents react to a stimulus by an active response and locomotion is linked to pacing, conversely, they are also the ones that become most inactive after enrichment is added to their cage.

When the juveniles were housed with their parents in the breeding enclosure the main differences in behaviour were in inactive and active behaviour (Figure 9). The large error bars again show large differences around the mean, which could be because the juveniles from the 2019 litter were recorded as being more active that the 2020 litter. Indeed they were recorded as being active 35%of observed time compared to just 17% by the adults, this is probably because juveniles move around more, playing and exploring their environment in order to learn about the resources available in their territory , and why they locate 14% more than the adults (Wauters et al., 1992). However, conversely the 2020 litter juveniles were recorded to be twice as inactive as the adults. This is probably because the 2020 litter spent up to a third of observed time in inactivity as they were born in the lockdown and were not habituated to the observer as the 2019 were (this is discussed in more detail later).

Once the juveniles were released into the walkthrough feeding and locomotive behaviour increased to an average of 80% of observed time, similar to that seen in wild and non-pacing and other red squirrels housed in the walkthroughs, suggesting that when offered a more complex environment and diet, similar to that found in the wild, species specific behaviour such as locomotion and feeding behaviour increases to be more in line with their wild counterparts and stereotypical behaviour decreases. Whilst these findings suggest that enclosure size and complexity does have an influence on the presentation of stereotypical behaviour because overcrowding can be a stressor in captivity (Dickens at al., 2010) it must be noted that individuals in all enclosure types paced, therefore enclosure size cannot be the only contributory factor in the performance of stereotypy; age, social groupings, hoarding and dispersal needs may all play a part.

It is also important to note the behaviour of the squirrels already resident in the walkthrough enclosures where the juveniles were released into at 23 weeks (Figure 12). At Wildwood Escot, Fern is an older squirrel, is dominant and territorial, Bracken is the second oldest, both of these squirrels were born in the walkthrough enclosure and neither of them stereotypically pace. Twiglet and Cherry are from Autumn and Radish's 2018 litter and were born in the breeding enclosure at Wildwood Escot.

Both Cherry and Twiglet paced in the breeding enclosure and have continued to do so even after being moved into the walkthrough enclosure, with Cherry pacing on average 5% of observed time and Twiglet an average of 14% of observed time (again both squirrels paced more during different times which is illustrated in the large error bars). Whilst Cherry's and Bracken's behaviour remained stable during the juveniles' soft release from the holding pen into the walkthrough enclosure, Fern's territorial behaviour did increase from a daily average of 11% - 16%, mostly due to aggressively chasing and engaging in conflict with the other squirrels. In contrast, Twiglet's pacing behaviour greatly increased from an average of 20% of observed time before the release of the juveniles to 33% on the day the juveniles were released. Whilst hierarchies and social statuses remain stable in a group, so does the behaviour of individuals, but when social groups and therefore the composition of hierarchy's changes, different status individuals react quickly, with an overall negative correlation between rank and exploratory tendency (Herborn et al., 2010; Beerya et al., 2020). Enforced social environments has been found to be one of the primary sources of captive induced stress, with the frequency and type of interactions between conspecifics effecting HPA axis activity.

In the wild there are often limited resources (e.g., food or mate selection) and those animals that are more dominant and able to effectively defend their territory are the ones that are more likely to survive (Dantzer et al., 2010). Territorial animals such as Fern may well cope better with enforced social environments as they can defend their territory whereas less dominant animals may struggle with these environments and incur distress, evidenced by stereotypical pacing may be linked with the need for the animal to escape such dominant conspecifics. Older females like Fern can become very territorial about their home range and monopolize resources defending them from intruders and sub-dominants so they can take over any potential alpha males, whereas less dominant squirrels, such as Twiglet will actively avoid these females or they risk being permanently attacked (Beerya et al., 2020). In the wild, less dominant females would move away to a new territory when faced with aggressive female counterparts, but in captivity they cannot do this so this may explain the increase in Twiglets pacing as stereotypical pacing is known to increase if escape attempts are thwarted (Herborn et al., 2010).

Interestingly Twiglet is one of the squirrels that has escaped the walkthrough enclosure several times and there is a link between lower hierarchy animals and increased pacing and escape attempts (Balcombe, 2010). Lewis and Hurst (2004) found escape behaviour is linked with social conflict in the environment causing stress to individuals and Balcombe (2010) found that stressed female rodents have a higher cortisone level than other females and spent more time stretching up against walls in a maze to escape.

Two respondents to the questionnaire found a change in the adults' behaviour when the juveniles moved into their enclosure, and these also included increased aggression, and chasing and increased pacing. However, there was no long-term change in the overall behaviour between the resident red squirrels in the Escot Walkthrough, before and after the juveniles were released.

There were limitations in comparing the behaviour between the resident squirrels in the Kent and Devon walkthroughs. Whilst there was no obvious change in the behaviour in Wildwood Kent resident squirrels when Douglas was released, it needs to be noted that all four adults, Basil, Lucky, Smokey and Douglas were being held in the holding pen as one squirrel had previously escaped so the walkthrough was being secured. They were therefore all released on the same day. The main difference was that locomotion and feeding behaviour almost doubled once they were in the walkthrough and stereotypical behaviour reduced from an average of 14% to just an average of 3% across all squirrels, in line with Mason et al. (2007) findings that motor stereotypies reduce when locomotion and feeding increases. Unlike the Wildwood Escot squirrels, no one particular behaviour for any one particular squirrel in the Kent walkthrough either increased or decreased on the release day, except Douglas whose stereotypical behaviour increased on the day of release but only because he could not find the exit point and appeared very stressed by this. Once he exited the holding pen, he was never seen pacing again in the walkthrough. Sadly, he did escape and was killed in February 2020.

Natural environments which encourage natural behaviour including breeding success, foraging behaviour, mate-seeking and predator avoidance, promote the expression of species-specific behaviour and decreases abnormal behaviour moving toward a more wild-type activity budget, particularly for those animals that are bred for reintroduction into the wild (Harrison et al., 2017). However logistic regression on analysis of responses from the questionnaire did not suggest that increasing enclosure complexity would reduce stereotypical pacing ($p=0.7$).

In summary, all types of individual pace, and squirrels housed in all enclosure types stereotypically pace. However, pacing is much less frequently performed in walkthrough enclosures. These are the biggest and most complex enclosure type and therefore pacing occurrence and performance may be linked in some way to being in a relatively small, relatively less complex enclosure. For rodents, literature on the size of enclosure and stereotypy is contradictory, and other factors may also be related to the causation of stereotypy therefore and the relationship between stereotypy and other behaviours must be further explored before those possible aetiologies can be determined.

4.2 Feeding Behaviour and Stereotypical Behaviour is Negatively Correlated

A strong significant negative correlation between feeding and stereotypical behaviour was found in the observed squirrels. Conversely no significant correlation was found between locomotion and stereotypical behaviour, social and stereotypical behaviour, or inactivity and stereotypical behaviour. These findings confirm that when feeding reduced stereotypical behaviour increased.

Whilst in the wild, a red squirrel may forage for up to 80% of observed time, in captivity this can be reduced to just 20% (Wauters et al., 1992). Indeed, the observed red squirrels in this research that paced the most (over an average of 10% of observed time) displayed the least amount of foraging time at just 30% on average. Previous research has suggested that the inability to display certain highly motivated behaviour correlates with emergence of stereotypical behaviour (for example, Siber, 2018). Feeding behaviour, including foraging, handling food and caching is a complex behaviour in red squirrels which they are highly motivated to perform. Other research suggests the inability to perform highly motivated behaviour negatively impacts on an animal's welfare and potentially causes the emergence or increased performance of stereotypical behaviour (Morgan and Tromborg, 2007; Mason and Latham, 2010). These results, while not definitive, suggest there may be a causal link between feeding behaviour and pacing as for every five-minute increase in feeding there was a seven-minute decrease in stereotypical pacing. To determine this in future, experimental work where feeding opportunity is systematically increased or decreased is needed.

In the wild, squirrel diets are highly diverse and may include seeds and berries, insects, and chicks (Bosch and Lurz, 2012), fallen fruit, tree buds, flowers and leaves, and in winter dried fungi found on tree branches (Wauter and Dondt, 1987; Wauters et al., 1992). Seasonal variation in food items is highly important, for example, from July – April pine seeds become their primary food source at up to 90% of their diet and secondary sources include berries in July to September. In April and May buds of coniferous and then oak tree shoots become the most important food source making up 23% of their diet (Wauters et al., 1992). Captive diets are rather more static and there is an abundance of highly nutritious food that is readily available. One possible mechanism by which feeding and stereotypical behaviour interact is the decreased opportunity to forage in captivity, leading to increased 'free time', boredom and frustration. Foraging opportunity is an important behaviour to maintain mental and physical health, and animals are intrinsically motivated to explore both familiar and unfamiliar resources and territories, spending a large portion of their daily activity budget in the search for, and consumption of food (Morgan and Tromborg. 2007).

Although there are obvious barriers to providing the same opportunities to captive red squirrels as their wild counterparts, for example cage size or access to wild food, it is recognised that welfare and behavioural needs are extrinsically linked and therefore zoos have a responsibility to try and meet these needs. It could be considered that thwarting highly motivated natural foraging and feeding behaviour could be linked to the emergence, increase and preservation of stereotypical behaviour (Manteca Vilanova, 2020) as the animal cannot fulfil appetitive behaviours because of a lack of consummatory opportunities in their environment (Appleby, 2005).

However, in captivity the majority of the red squirrel diet is commercial parrot mix, fruit and vegetables, being more condensed, higher in protein and different in texture to that found in the wild (Tarou and Bashaw, 2007). Shepherdson et al. (1998) found food generally contains all the nutrients for the day on one plate, offered on feeding tables and logs, twice a day at predictable times preventing animals from engaging in critical natural behaviour and reducing searching and handling time (Welsh Mountain Zoo, 2019). In this study although the squirrels in the breeding enclosure spent on average 28% of time in feeding behaviour, only 4% of that time was spent handling food, compared to 7.5% - 25% of total foraging behaviour seen in wild populations (Wauters et al., 1992). In the two walkthrough enclosures, which had availability of natural food to forage on, the average time spent on feeding behaviour was increased to an average of 44% of observed time.

Although zoos do attempt to add feeding enrichments and scatter feed to increase foraging opportunities, findings from Glenarm Castle Estate during the soft release of captive red squirrels into their estate, highlighted issues with not only the type of food being given to captive animals for release but also how the food is presented.   The red squirrels in the release programme had not eaten pinecones in captivity and did not recognise them as food items and it took them several days before one squirrel attempted to remove the scales to access the seeds inside.

Wild squirrels access berries and nuts not only from the floor but also by foraging in the trees, and it took these captive squirrels some time to understand how to reach food such as beechnuts, from the end of branches tied to the bars of the cage as they had not been exposed previously to this feeding presentation type in captivity. Captive red squirrels are also not exposed to different drey building materials, so Gamekeepers had to reproduce wild-type opportunities to harvest drey building materials. For example, to mimic natural moss growing on branches they had to place moss on beams in the soft release cages, so that the squirrels would understand how to access moss in the wild (Shuttleworth, 2019).

Preventing red squirrels from foraging in a natural way such as contrafreeloading not only causes problems for those squirrels bred to be released but was found to increase stereotypical behaviour and fearfulness in rodents, decreasing the performance of natural exploratory behaviour (Tarou and Bashaw, 2007). Shepherdson et al. (1998) states that using contrafreeloading is a type of operant conditioning that may improve welfare as the animal is provided with a means to control the outcome of its own behaviour, as a lack of control is linked to stereotypical pacing (Dawkins, 2003). Indeed, rodents, offered a buzzer to press to get food or a bowl of freshly prepared food, chose to use the buzzer and work harder and wait longer for a more indirect route for their meals (Brandao, 2011).

An example of contrafreeloading was found in the Kent breeding enclosure. Squirrels had constant access to a bird feeder full of hazelnuts, their favourite food, however this feeder remained full as the squirrels chose to forage for the nuts which were scatter fed around their environment during feeding times rather than eat the nuts from this feeder. This feeder full of hazelnuts caused several territorial fights. Male red squirrels are more aggressive and hierarchical than females, and male red squirrels will aggressively try and prevent others entering their caching space (Alphern et al., 2019), and higher incidents of infanticide are reported in smaller enclosures, due to the males protecting isolated resources (Wauters et al., 2001; Shonfield et al., 2012). The father (Shep) guarded this resource aggressively and was seen chasing and attacking his young when they approached either his cache or the feeder and was observed killing two of his offspring, with the other three being found dead the next day. Therefore, this research would suggest that providing different feeding opportunities, with the ability to contrafreeload may increase handling time and reduce stereotypical pacing and territorial behaviour over limited resources and the best way to improve welfare and reduce pacing in red squirrels would be to tackle this underlying frustration (Mason and Latham, 2010).

Although logistic regression showed no significant prediction between complex feeding and stereotypical pacing ($p=0.28$), four zoos that reported the least amount of pacing (3, 4, 6 and 7) used feeding enrichment, seasonal/wider diets and increased browsing opportunities, such as adding maple and hornbeam to the squirrels' diet and saw a reduction in stereotypical pacing, conversely the zoos recording the highest amount of pacing (1,2 and 5) were fed a less complex diet of nuts, seeds, fruit and vegetable and were not seasonally fed. Not being able to forage and feed in the way an animal desires and/or food not being presented in species-appropriate manner can result in undesired behaviours emerging (Brando and Buchanan, 2018).

If these abnormal, displacement, activities serve to decrease arousal, and lead to a reduction of pituitary adrenal activity or enhance arousal, associated with enhanced plasma levels of cortisol or adrenocorticotropic hormone, the animal can get stuck in a positive feedback loop which is hard to eliminate (Dantzer et al., 2010).

These findings support the theory that a wider range of foods, changed seasonally and displayed in a more natural way are factors that may not only reduce stereotypical pacing in the captive species but also enable squirrels to survive in the wild. Indeed, half of the respondents to the questionnaires stated they believe that lack of feeding and foraging opportunity was a major cause of pacing.

The correlation data suggests general locomotion and pacing are not a factor which is contrary to previous research on large carnivores; however, the red squirrel is a small rodent and maybe maximal foraging length per day is an important indicator of pacing in small rodents. In captivity and especially in larger enclosures, there is opportunity to locomote but regardless of the enclosure type, there is consistently a lack of foraging opportunity specifically and this needs further investigation to see if, like general feeding time, there is a specific link between foraging opportunity and pacing. This would require an experiment where the amount of food and foraging distance/time needed to be systematically increased and decreased and squirrel behaviour observed.

Understanding red squirrel foraging behaviour provides us with important knowledge for conservation and management of the species both in captivity and for release (Palmer and Koprowski, 2014). The results of this study suggest a possible link between feeding and stereotypy and increasing foraging opportunity and handling and eating time may be a beneficial management strategy when trying to reduce time spent performing stereotypy in the captive red squirrel population.

4.3 **Anticipatory Behaviour**

Animals are able to predict food delivery times, but they are not able to control this, and this absence of control can be a critical stress-eliciting factor in captive animals causing stereotypical behaviour to emerge (Wolfensohn et al., 2018). In red squirrels pacing is not part of natural feeding behaviour but if performed more prior to feed times, pacing could be considered an anticipatory behaviour and therefore have a function and not be stereotypical, as this is by definition functionless (Dawkins, 2003) (though pacing would still be considered abnormal and repetitive and a possible indicator of negative welfare).

Vinke et al. (2006) advocate the notion that 'stereotypical' pacing could be anticipatory behaviour, and, in this study, pacing emerged gradually and around doorways where food is delivered from. However, there was no significant difference in the amount of pacing behaviour observed before and after feeding when all 16 observed squirrels are considered collectively, suggesting stereotypy was not anticipatory. However, as Figure 18 illustrates the large error bars suggest that some individual squirrels did appear to perform possible anticipatory stereotypical behaviour. One female adult performed the vast majority of stereotypy (83% of total pacing time) pre-feeding and a further two juveniles performed more than two thirds of their total pacing time pre-feeding. Furthermore, two zoos reported that they only saw stereotypical pacing behaviour directly before feeding times, indicating anticipatory feeding behaviour may be linked with increased abnormal behaviour in certain individuals (Brando and Buchanan, 2018). However, there are limitations to these findings as the times when zookeepers observe animals the most is at feeding times, so other displays of pacing could have been missed.

The squirrels observed in this study patrolled (while displaying stereotypy) near the doors that were opened several times a day for cleaning, feeding and other husbandry purposes, which served to reinforce the behaviour. This behaviour can then develop into environment-directed stereotypy and gets replaced by self-directed stereotypies which leads to a reduction in both the diversity and number of the normal behavioural repertoires performed by an animal (Dantzer, et al., 2011). Vinke et al. (2006) suggests that anticipatory behaviour balances the underlying reward-related neuro-circuitry between reward and stress-inducing stimuli in the animals' environment. These findings would suggest that anticipatory stereotypical pacing would increase at feeding times as the squirrels anticipate the arrival of food (Morgan and Tromborg, 2007). This has been shown in other rodents previously; Nevison et al. (1999) found a gradual emergence of stereotypies in bar chewing rodents, when mice initially started chewing the bars that opened more strongly than the other bars. However, the vast majority of individual red squirrels observed in this study showed no increase in pacing or other stereotypy before, compared to after, feeding. This is similar to Vinke et al.'s (2006) findings that there was no significant correlation between anticipatory and stereotypical behaviour. Overall, it appears that for the minority of individuals stereotypy could be an anticipatory behaviour, while for the majority it is unlikely to play a causal role.

It is important to acknowledge certain limitations in the data collected for this study due to seasonal differences within activity budgets. The red squirrels were fed at the same time each day (9am-10am), but as they wake up at dawn, the length of time the squirrels are active before being fed differs.

For example, the squirrels wake up around 5am in summer and would have waited 4 hours before being fed. Observations were conducted from 8am every day so the red squirrels could have been pacing for several hours prior to the start of data recording. There is a positive relationship between food deprivation and the frequency of stereotypies (Dawkins, 2003; Zhao and Cao, 2009), though there is no data on how long the wait has to be before it would effectively be deprivation. In winter months, when squirrels were much later to wake up, the observation period now captured pre-feeding time. These seasonal differences could have affected the results. However, as the squirrels still paced even when food was left over in the enclosure, the key findings suggest that it is not anticipatory behaviour but the lack of foraging opportunities that cause pacing, and this reinforces the contrafreeloading hypothesis mentioned above, that these captive squirrels want to work (and specifically look for) food.

4.4 Red squirrel juvenile behaviour after moving from the breeding enclosure to a walkthrough enclosure

Enclosure type was not a significant predictor of pacing behaviour from the questionnaire data. This is not surprising given squirrels in all enclosure types were reported by keepers to pace. In the observed squirrels, pacing occurred in all three enclosure types, however significantly more pacing occurred in the breeding and holding pens. Some individuals paced 20-40% of the overall activity budget in holding/breeding pens compared to less than 4% in walkthrough enclosures. In the wild 1.3 red squirrels live in each hectare with home range of 0.356ha (Holm, 2010) whereas captive red squirrels are kept in cages of approximately 6mx9m and a minimum height of 3 metres (Welsh Mountain Zoo, 2019).

It might therefore seem obvious that moving from a small cage to a bigger enclosure with large trees and established canopy cover, would reduce any stereotypical behaviour because resources are more widely available and evenly distributed, whereas in small cages clustered resources increase stress and aggression (Akre et al., 2011). Indeed, free range large enclosures do give animals more choice and control over their environment and encourages natural behaviour (Shepherdson et al., 1998; Hosey, 2005; Davey, 2006). And naturalistic environments such as free roaming enclosures do introduce meaningful biological complexity, fulfil animals' ethological needs, and help to foster normal behavioural and brain development (Würbel, 2006).

A Swiss team of investigators has studied mice in large open-roofed outdoor pens measuring 400 metres^2, with hay-filled shelters and several wooden boxes and reported the rodents to have better body condition, appeared cleaner, were more inquisitive and friendly, reduced stereotypical behaviour and appeared less fearful than rats housed in small cages (Dell'Omo et al., 2000). Height is considered the most important factor when housing red squirrels as they have to partake in natural mating chases up trees in order to be able to successfully breed (Holm, 2010). Two zoos reporting the lowest pacing have enclosures of a height between 4 – 6 metres compared to other zoos with more pacing with just 3 metre height.

However, the main reason given in the questionnaire for why red squirrel juveniles begin to pace post weaning and continue to increase this behaviour until they are moved is their need to disperse and not that they are moved to a larger enclosure per se. In the wild in late summer, when they are fully weaned, juveniles start to increase their exploratory behaviour (Callard and Price, 2000) and make short forays off their natal territory in search of a vacant territory (Larsen & Boutin, 1994). They continue to do this until they have found a suitable territory, or their mother bequeaths some of her territory to them. This is very important behaviour for survival as it allows juveniles to gain knowledge about their environment and be able to recognize and select the optimum habitat for survival. Squirrels frequently do not stray far from the maternal drey for much of the summer, but the majority of juveniles will have dispersed by autumn. It is estimated that only 15-25% will survive their first winter so securing a good territory is vital for survival (Larsen and Boutin, 1994). Red squirrels reach sexual maturity by 9-10 months of age, so with sufficient food supplies and body condition, survivors can produce their own offspring in their first year (Wauters et al., 1997).

Verbeylena et al. (2009) found juveniles who settle in mature forests had a significantly higher chance of surviving their first year and raising juveniles of their own, than those than settled in thin edge forests with fewer resources. Similarly, a study of squirrels released into an Antwerp Park found those animals that dispersed quickly on release found the best areas and survived longer than those that stayed near the release site in poorer areas (Wauters et al., 1997). Finding a good quality territory is a key behaviour for red squirrels as resources are linked to breeding success; every three-six years white spruce (a major dietary component for wild red squirrels) have large crops called masting and red squirrel breeding success increases when masting occurs, whereas the following years see low cone production and therefore poor food availability, decreasing red squirrel abundance (McFarlane et al., 2014). High cone abundance creates intense competition for good territories and the bigger and stronger juveniles secure the best territories (Boutin et al., 2013).

Juveniles have an innate need to disperse as the best territories offer a higher chance of survival, with larger food resources and higher-than-average body mass than those that do not secure a good home range (Larvive et al., 2010).

Dispersing is also driven by sex as males and females disperse differently, with females trying to acquire territories with the most resources and males acquiring territories that overlap with as many female territories as possible so they can quickly access female home ranges during mating season (Fran et al., 2012). This could explain why Douglas, Shep and Radish started pacing first at 10 weeks in the breeding enclosure and then rapidly increased their pacing. These dispersal patterns are therefore very important, but in captivity although juveniles are still driven by intrinsic factors to engage in this behaviour, an innate drive to disperse, they are unable to do so and this causes dispersal stress, and this stress can easily turn into pacing near a perceived escape route (Nevison et al., 1999). Thwarting dispersal behaviour has been found to increase attempts of escape and stereotypical pacing to emerge in juveniles (Balcombe, 2006).

This need to disperse could explain why the pacing is reported by zookeepers to start at 10 weeks, (red squirrels are fully weaned by 10 weeks), and increases week on week, and then decreases if the juveniles are moved. In this study pacing was observed to begin from 10 weeks, when wild juveniles start to make small forays in order to disperse and increased until the squirrels were moved at 22 weeks. This motivation to disperse is also increased due to social pressure, the more conspecifics in the area, particularly the more juveniles there are, the more pressure there is for squirrels to secure a quality territory of their own (Price and Boutin, 1999). Juveniles were found to try and escape when housed with aggressive cage mates in an attempt to expand their home ranges to increase their chance of survival (Koprowski et al., 2008).

Other reasons why pacing may be reduced when the squirrels were moved to a walkthrough enclosure may be because in the wild red squirrels spend up to 80% of their wild activity budget in the canopy, both foraging and locomoting, moving from tree to tree using the trees as corridors, as they do not travel distances on the floor, due to the high risk of terrestrial predation (Holm, 2010). During observations the first thing all three juveniles did on release from the holding pen into the walkthrough enclosure was to run to the very top of the nearest tree. Walkthrough enclosures have more natural resources and offer more opportunities to partake in species specific behaviour, natural foraging opportunities and more drey building opportunities.

In the wild red squirrels build several dreys and move between them throughout the day (Wauters et al., 1992). In walkthrough enclosures drey building was seen twice as often than in the breeding and holding enclosure (2%-1%) as squirrels like to build dreys high up in the fork of trees. Walkthrough enclosures also offered a wider variety of suitable drey-building material like mosses, twigs, and grass which are used by wild squirrels to build a drey (Edelman et al., 2009). Such materials were not available in breeding enclosures and were limited in number in holding pens.

There is more opportunity to avoid social tension in larger enclosures, in captivity the probability of chance encounters with other squirrels is directly impacted by enclosure size, with smaller enclosures restricting natural ranging and increasing socials interactions. In larger enclosures, similar to the wild, social motivation is controlled by individuals who can choose how close a proximity they are to one another, whereas this is not the case in smaller enclosures, particularly where there are limited resources, for example juveniles housed with parents are faced with not only the need to disperse, but also hoarding needs from August to October and food is not increased to meet these needs so resources are limited and more territorial fights are observed (Chadwick et al., 2015).

In essence, it could be any of these specific factors or any combination of them that may have caused the decrease in stereotypical pacing when the juveniles moved to the walkthrough enclosures. Dispersal and the need to avoid social tension and establish a home range is most likely the reason but it is difficult to tell the exact course and further research is needed to experimentally test this. However, the social tension and dispersal hypothesis is reinforced by results in section 4.5 as privacy screens that also increased hoarding and caching opportunity also reduced stereotypy.

4.5 Alterations to the breeding enclosure and impact on stereotypical behaviour in the breeding pair of red squirrels

Alterations to the breeding enclosure, which included adding boards to the front and side of the enclosure (Appendix IV), and adding extra substrate of woodchip and dried leaves, and increasing hoarding potential by 10%, by adding hoarding containers stopped pacing in Radish from 35% of observed time at its' peak before the alterations to zero immediately after the alterations (Figure 20 and 21). Conversely Autumn's pacing was not significantly reduced, but she completely stopped pacing after the hoarding pots were added in March 2020. Before the changes to the enclosure Autumn only paced an average of 5% of her activity budget each day, this did escalate to 20% for October and 9% in November, but this was more than likely due to hoarding stress.

Dantzer et al. (2010) found that female stress hormones increase in autumn as they become more territorial during the hoarding season in an attempt to eat enough food to improve their body condition after parturition and lactation, but also hoard enough food to survive winter and be fit enough to mate in late December and January. Twiglet and Cherry also increased their pacing in the Wildwood Escot walkthrough enclosure during this time, with Cherry increasing her pacing from an average of 5% to 21% and Twiglet from an average of 16% to 23% of their activity budget. Zoo 3 stated their females also increase their pacing by 10% during autumn. Non pacing squirrels did not start pacing, and none of the males, either in the breeding enclosure or either walkthrough increased their pacing at this time.

Radish used to pace an average of 28% of observed times with a maximum of 35% of observed time recorded in June and September 2019 before the alterations. His pacing took on a strict pattern, he would pace along the same edge, opposite the doorway and always with food in his mouth. He would collect food, pace, cache the food and re-pace over the same area and then repeat this for several minutes and even hours a day. He never paced by the door or anywhere else in the enclosure or without food. The author had witnessed this previously, in male red squirrels in a breeding enclosure during previous research at another organisation and also witnessed this behaviour in Shep, the male in the breeding enclosure at Wildwood Kent. Three zoos partaking in the questionnaire also reported that their male squirrels only paced with food in their mouth and this pacing took place where they cached food.

Jenkins (2011) states that although males hoard more, they are less flexible in the way they hoard compared to females. Hirsch et al. (2013) found that wild red squirrels' cache along trails in their home range, which individuals patrol over several times a day, known as the 'memory enhancement hypothesis' which states red squirrels are twice as likely to walk past or near these caches than other locations and this surveillance is thought to reinforce their spatial memory without having to dig up or recache their food and risk conspecifics seeing and stealing their cache. This patrolling is thought to increase memory of their cache between 12-20 days and possibly up to 62 days. Therefore, this pacing style by male red squirrels may not only be linked with hoarding stress but also maladaptive patrolling behaviour.

After the boards were added to the enclosure in January 2020, Radish pacing immediately stopped. The reason for this could be two-fold, firstly red squirrels in captivity are known to suffer from hoarding stress as they do not like to cache in the presence of others, being conspecifics or other potential pilferers such as birds, other mammals or even humans (Lucas and Zielinski, 1998).

Female red squirrels have been found to cache less than males and therefore to pilfer more from male-made caches (Jansen et al., 2012), and juveniles pilfer 87% more than adults, probably because they are inexperienced at caching (Donald and Boutin, 2011). As Radish is housed with a female and his offspring this could have increased his hoarding stress and therefore explain this constant pacing with food in his mouth trying to ascertain the best time and place to cache his food, with the minimum risk in having his behaviour being witnessed and his cache stolen.

Secondly, red squirrels like to cache is shady areas and turn their backs to cover up their cache, but this leaves them open to predation (Dally et al., 2006; Alpern et al., 2019). In the wild red squirrels' can spend up to 80% of their time in the canopy so are only open to predation when they are on the floor, foraging or caching food (Petty et al., 2003). Red squirrels are highly arboreal in nature, and they spend limited time on the ground, not only due to food supply but also because of high predator pressure due to the presence of several terrestrial mammalian predators (Wauters et al., 1992). The addition of the boards offered two completely enclosed, shady areas on each side of the enclosure which was almost hidden from public view, where they could forage and cache in perceived privacy. Because there was a roof over the top of each of these areas the red squirrels were also protected from perceived aerial and/or ground attacks. The result being that Radish stopped caching in just one area but moved over to the other side of the enclosure to forage and cache, something he had not done before the alterations.

Alternatively using boards to block external stressful cues may have reduced stereotypical pacing as Brando and Buchanan-Smith (2018) found that adding a wall or other cover has an impact on breeding success in rodents. Five zoos (1, 3, 5, 6, 7) in the questionnaire stated they offered cover such as boards, walls or natural foliage which helped reduce pacing As rodents pick up olfactory clues from outside their cages being constantly aware of predators in their near vicinity, this could be increasing their stress levels, as neighbouring smells of predators may increase escape behaviour (Annie et al., 2013), solid boards may have eliminated or reduced these fearful smells, similar to Balcombe (2006) studies that Perspex cages have been found to reduce stereotypical behaviour by reducing outside odours. Two respondents to the questionnaire believed the perceived threat from potential predators, including increased visitors in close proximity to the animals and the inability to hide or escape contributed to the emergence of stereotypical behaviour. Woolway and Goodenough (2017) found visitor numbers increased feeding behaviour and reduced resting behaviour, and that the presence of pacing was linked with the type of visitor and not the amount, with noisy children having the greatest impact on behaviour.

Finnegan (2020) did not find a conclusive link between visitor number and stereotypical pacing. However, the two zoos in the questionnaire that did not offer any enclosure cover reported daily pacing from minutes to hours at a time.

Adding extra substrate also could have had a positive effect on reducing pacing in the breeding pair as Zong *et al.* (2014) investigated the importance of hoarding material and found Eurasian red squirrels preferred fallen leaves and moss as hoarding substrate as leaves fall under the dense canopy of trees so receive the thinnest layer of snow making retrieval easier. The addition of extra substrate such as woodchip or leaf piles on the floor and in the hoarding containers provided additional digging and potential caching areas and five respondents stated they saw a reduction in caching stress when extra substrate was added with zoo 4 and 7 seeing a reduction in pacing when red squirrels were offered large tubs filled with sand to dig, hoard and play in. The addition, the hoarding containers offered more opportunity to forage and hoard in the hope of reducing hoarding stress. After the alterations to the enclosure caching behaviour did not increase in either breeding squirrel even though they were given hoarding pots, increasing their physical floor space by 10%. Radish caching behaviour decreased, from an average of 4% of total activity budget per day to 1%, and Autumn remained the same at an average of just 1% of observed time each day. However, the alterations took place after the hoarding season in late January, so this could explain these statistics.

Autumn stopped pacing after the hoarding containers were added in March 2020 and her feeding increased from an average of 26% of observed time before the alterations to an average of 34% after the alterations, with ingestion increasing by 50%. The keepers started hiding food in the containers which encouraged both squirrels, but particularly Autumn to forage more in these containers for her food. Increasing the potential for her to fulfil her innate motivation to preform natural foraging behaviour may explain why she stopped pacing completely (Holm, 2010; Manteca Red Vilanova, 2020). Species that spend a relatively large amount of time foraging over a large area might be most sensitive to spatial restriction. Draper and Bernstein (1963) found that increasing available vertical space was effective at reducing pacing in rodents. However, care needs to be taken in this interpretation as this increase in feeding also coincided with the birth of the 2020 litter when she may have been increasing her nutrition in order to feed her offspring (Dantzer et al., 2010).

The other two major changes in behaviour pre and post alterations to the breeding enclosure were inactivity and locomotion. Radish locomotion increased from an average of 13% to 22% of observed time after the alterations, more in line with a wild-type budget, however caution needs to be taken in interpreting these findings. On 18th March Radish locomotion suddenly escalated to 56% of observed time for one day.

He seemed very stressed during this time, running back and forth between the two sides of the enclosure, and checking the nest boxes on the old side. The consensus is that this is the day Autumn went into parturition, as she was not seen until the evening when she looked to be lactating. During parturition in the wild, the male is not normally in the location of the birth and all nearby females become highly vigilant; it has been recorded those neighbouring relatives will take over the care of juveniles should the mother die during birth (Lane and Boutin, 2007). This timing fits in with when mating had been witnessed during the week of 4th February, being a gestation of five-six-weeks, and with emergence of the juveniles at around six weeks of age on 29th April 2020 and could explain Radish's hyper-vigilant state and the dramatic increase in this behaviour (Holm, 2010).

The biggest change in behaviour for Autumn was a decrease in inactivity after the alterations to the enclosure from an average of 51% of observed time to 31%. However, this may be because before the birth of the juveniles she spent a lot of her time sleeping on one of the swinging beams, so her inactivity was easily observed, but after the birth of the juveniles she was rarely seen sleeping outside the nest box. Presumably because she spent more time feeding and sleeping with her young, as attentiveness towards offspring is positively correlated with offspring growth and survival (Dantzer, et al., 2010).

It also needs to be noted that from May 2020 Radish inactive behaviour dramatically increased from an average of 17% to 46%. A reduction in Radish's pacing after the alterations whilst greatly welcomed, needs to be viewed with caution due to this sudden increase in inactivity. Mason, (2010) states that when stereotypical behaviour results from frustration, individuals displaying abnormal behaviour fare better in captivity than animals responding with inactivity as this can lead to apathy. Jones et al. (2012) supports this theory as mice that do not perform stereotypy in severely impoverished cages became atypically inactive and these animals were considered to have poorer welfare than their stereotypic cage mates as inactivity in animals can indicate a feeling of hopelessness which can greatly affect both the animal's physical and mental well-being (Mason et al., 2007). A lack of stereotypical behaviour could mean animals are too bored to do anything and Balcombe (2006) states that inactivity can restrict species-specific behaviour, impairing animals' anticipatory responses and giving rise to frustration and depression when all attempts to cope have been unsuccessful. Mason et al. (2007) state inactivity can mask poor welfare and Lierop et al. (2010) state voles with pronounced stereotypical behaviour often show better welfare than non-stereotypical individuals, including reduction in heart rate, and better survival and reproductive success. Cooper et al. (1996) found that stereotypic voles ceased stereotyping after being transferred from a barren to an enriched cage but also noted increased immobility.

Jones et al. (2012) states that in environments where animals feel no control and cannot fulfil highly motivated behaviour, non-stereotypic individuals are generally atypically inactive (Meyer-Holzapfel 1968; Altman 1999), and often seem to have poorer welfare than their stereotypic cage-mates (Mason & Latham 2010). This suggests that in adverse captive conditions, inactivity is an alternative response to pacing, perhaps because resting/sleeping is secondary to 'apathy' (defined here as a lack of interest or motivation) (Marin, 1990).

It is difficult to know whether this inactivity was a way to cope with the potential stress the enclosure changes had on Radish as he was only observed in March 2020 immediately after the alterations and then not again until May 2020 due to COVID-19 pandemic restrictions, also red squirrel activity moves to bimodal in April and throughout the summer to unimodal in winter. The alterations were finished by the end of March when the red squirrels start to become most active early mornings (after sunrise) and late afternoon, times when the observer was not always able to record the behaviour.

Additionally, before March 2020 Radish was observed an average of 209 counts per week and after May 2020 this increased to 236 counts per week, an increase of 13% of time observed. Before the enclosure alterations Radish slept in one particular nesting box, or in a drey he built in the corner, where he was unseen, and his inactivity could not be recorded. After the alterations and the birth of the 2020 litter his drey was removed and as there were only four nesting boxes and five squirrels he had to choose to sleep in sight of the observer. Indeed, his vigilant behaviour did not change at just an average of 8% before and 9% after the alterations, but his resting behaviour increased from an average of 4% before alterations to 18% after and his sleeping behaviour increased from an average of 5% of observed time before to 19% after alterations. The observer also witnessed an increase in Autumns aggressive behaviour towards Radish, preventing him from accessing the nest boxes where she or her offspring were sleeping.

It could therefore be safely suggested that this was not necessary an increase in inactive behaviour but an increase in the amount of time Radish was observable while being inactive and the reduction in pacing meant the alterations had a positive effect on his welfare. Analysis of the questionnaire also supports the success of these alterations as all respondents had attempted to reduce stereotypical behaviour in their captive populations and found the best was to reduce pacing was to change the existing enclosure, including adding natural or artificial screens, substrate such as leaf piles or sand baths and increasing food enrichment.

4.6 Differences in stereotypical pacing between the 2019 litter (before the alterations to the breeding enclosure) and the 2020 litter (after the alterations)

In the wild, red squirrel juveniles start to explore around their natal territory once they are fully weaned at 10 weeks of age. This has guided husbandry practice and three zoos confirmed that they move their juveniles away from their parents between 12-14 weeks of age, when pacing starts. All respondents also confirm that pacing increases the longer the juveniles are housed with their parents. Following the observations and replies to the questionnaire there is evidence to suggest that pacing in red squirrel juveniles is linked with thwarted dispersal needs and this behaviour gets gradually worse the longer an individual is kept with their parents. In the litter of 2019 (before the enclosure alterations) one red squirrel started pacing at 10 weeks of age, two were pacing at 14 weeks of age and all three squirrels were pacing by 18 weeks of age. Whereas in the litter of 2020, no pacing was observed until 18 weeks, and then only at 10 % of total observed time. (Figure 22).

The difference in the presentation of stereotypical pacing between the 2019 and 202 litter may be because the 2019 litter were moved at 22 weeks to the holding pen in the walkthrough enclosure and released at 24 weeks where their pacing dramatically declined. Whereas the 2020 litter remained in the breeding enclosure and their pacing rapidly increased from 22 weeks to an average of 37% of observed time, with one juvenile pacing almost 50% of observed time by the end of August 2020. These findings are in line with recent research by Finnegan (2020) that found a positive relation between juvenile development and stereotypical behaviour, from just 3% at 14 weeks to 45% of observed time from week 16. The changes to the enclosure may have delayed the onset of stereotypical pacing, from 10 weeks to 18 weeks of age, suggesting that if the juveniles were moved before this time, as they are in the wild, this pacing may never have started. Finnegan (2020) found that enclosure size significantly affected the amount of stereotypical behaviour observed with the smaller enclosure eliciting the largest amount of stereotypical pacing in juveniles.

This is in line with findings from the questionnaire that less pacing was observed in walkthrough enclosures in this study compared with breeding and holding enclosures. Increasing hoarding/ foraging and floor space by 10% by introducing hoarding containers could explain this delayed onset of stereotypical pacing. Indeed, three zoos in the questionnaire reported that increasing floor space either using vertical containers or moving juveniles to large free roaming enclosure was the most successful way to reduce or eliminate stereotypical pacing, as not only were they offered a more wild-type home range but also had less conspecifics as overcrowding is known to be a stressor in captivity (Shepherdson et al., 1998).

However, the effects on visitor numbers from the COVID-19 pandemic needs to be considered. When the 2020 litter juveniles first emerged at 6 weeks old on 29th April 2020, the whole country was in lockdown and therefore there were no visitors to the zoo and all voluntary work was suspended, with only the keepers accessing the squirrels during their twice daily husbandry procedures. This lockdown continued until 4th July 2020, when the juveniles were approximately 16 weeks of age. During April the keepers were responsible for recording behaviour and could have missed discrete behaviour, or the early emergence of pacing. The researcher was able to conduct day visits in May and June, and although no pacing was witnessed it must be noted that the 2020 litter appeared much more fearful of her presence, having not been habituated to observations as the 2019 litter were. Indeed, most of their time was spent inactive and hiding up to 52% of observed time compared to the 2019 litter where activity only accounted for an average of 11% of observed time over the first three months of emergence.

Haughland and Larsen (2004) found juveniles in the wild are recorded to be most active days after emergence from the nest. As previously discussed, this inactivity is more likely to have been a stress response, despite stereotypical behaviour usually indicating poor group welfare, the fact that wild-caught striped mice were less stereotypic than captive-bred animals did not necessarily mean that they had better welfare as wild-caught mice were more anxious and fearful and higher circulating levels of corticosterone. Similarly, as the red squirrels had emerged during the pandemic, arousal or fear evoked by extraneous stimulations they were not habituated to, such as interactions with zookeepers and numerous volunteers, the observer watching them for several hours at a time, sudden loud noises, visitors to the zoo in July and stimuli from neighbouring animals, all may have evoked this inactive stress response in the juveniles (Catlard et al., 2000), however, the error bars for both litters show a large individual difference in pacing behaviour with some individuals pacing upto half their activity budget and some only pacing 1% of observed time. This is because all squirrels started pacing at different times and although all their individual pacing increased, it did so to different degrees over time, which reinforces the findings from this study that stereotypical pacing is linked to individuals' ability to cope with captivity and that it needs to be dealt with at both an individual and group level.

4.7 Implications for Red Squirrel Welfare

This study took place over 18 months, and the observational data illustrated that the behaviour of the captive population, similar to the wild population, is seasonally driven. It is clear from the findings of this thesis that stereotypical behaviour in the form of pacing is a behaviour that is prolific in captive red squirrels and yet a behaviour not seen in the wild population. A behaviour such as stereotypical pacing that takes up potentially a third to half of a captive animal's activity budget impacts on the time they spend on other species-specific behaviours which are essential for survival, such as feeding, locomotion, dispersal and reproducing and therefore expression of stereotypy in the captive population is considered in this thesis to indicate a welfare problem either because it shows animals are currently or have previously been distressed or because the animal's overall activity budget is maladaptive given the dearth of wild-type behaviours expressed.

Whilst it is often difficult for zoos to provide the same opportunities afforded to wild counterparts, due to constraints on space, resources, food availability and most importantly cost, animal welfare is still a legal and ethical responsibility for zoos. Animal welfare needs to be assessed at not only a species and group level but also an individual level, as there may be many different reasons why one individual may pace when others seem to be unaffected by stressors in the same captive environment. Personality differences influence how animals adapt to their environment, for example where an individual's personality sits on the shy/bold axis. Bolder animals have been found to be more aggressive so less affected by different social groups and being more flexible and adaptable within their environment and less likely to be affected by environmental stressors (Dantzer et al., 2010; Patton et al. 2014). Whereas shyer, more docile animals have less behavioural flexibility and adapt less well to captivity and are more likely to display stress behaviours such as stereotypical pacing (Callard and Price, 2000). This has implications for animal welfare as stereotypical behaviour cannot be dealt with at a group level but to be effective needs to be considered and treated at an individual level (Dalliare et al., 2006).

Animals are kept in zoos for many reasons, including for breeding and release programmes. In order for animals to be successful in the wild they need to learn how and where to forage for food and which territories offer the most resources and therefore the best chance of surviving and breeding viable offspring. If captive animals are to be bred for release into the wild, they need to be given the opportunity in captivity to learn these skills, for example not only be offered food they will find in the wild, but also have food presented in a wild-type way.

Captive breeding of red squirrels is important for the conservation of the species in the wild, however the high prevalence of stereotypical behaviour in these animals is a welfare issue and the continued breeding of a species which displays a large number of behavioural problems in captivity becomes an ethical concern for many collections (Shuttleworth *et al.,* 2015). The Five Domains Model of Welfare acts as a foundation to define the fundamentals of animal welfare and these findings, which suggest animals may suffer from food deprivation, persistent thwarted motivation to forage and cache, or disperse to their own territory, together with inappropriate diet and food presentations (lack of contrafreeloading) all contribute to a fixed environment that offers the animal little choice or control. Many of these animals are bred for release but exposure to prolonged stress can also affect the brain, which effects learning tasks, increases cognition decline and anxiety and the animals' ability to learn and adapt, and the loss of species-specific behaviour necessary for survival in the wild (Courtney-Jones et., 2017).

There is not just one reason why animals develop stereotypies, and not a "one size fits all" approach to solving this behaviour as there is an individual reaction to stressors in captive environments, and individual traits that effect how animals cope with captivity (Brando and Buchanan Smith, 2018). This study reiterates the literature and suggests several potential separate reasons why stereotypies may emerge in red squirrels: dispersal needs, thwarting foraging needs, and attempted escape.

Red squirrels, like all animals, are sentient beings and although there is a great deal of research into animal welfare, these are often based on few specific animals and red squirrels are still a very understudied species. There is a gap in knowledge and conflicting information regarding the best way to house red squirrels, husbandry care including feeding and their social groupings. Research is often short term and does not consider seasonal changes in behaviour. Stereotypical behaviour is prolific throughout the captive collections, and yet the research on this behaviour is very minimal.

Zoos are uniquely placed to highlight the plight of the red squirrels in order to change public perception and behaviour, but stereotypical behaviour has been shown to have a negative impact not only on the welfare of the animals but also on the visitor's perception and overall satisfaction (Karanikola et al., 2020). With the evolution of the animal welfare movement (Powell and Watters, 2017) more needs to be done to tackle stereotypical behaviour and individuals given choice and control over some aspects of their captive environments to offer them the opportunity to reflect their own behaviour repertoire and be able to breed in situations that mimic that found in the wild.

4.8. Husbandry Recommendations

This study shows aetiology of red squirrel stereotypy is multifactorial and potentially linked to individuals and their individual ability to cope and adapt to captivity and therefore it is not easy to make blanket recommendations that would be effective in reducing stereotypical behaviour in all the captive species as a whole. However, for the best opportunity to breed red squirrels that do not develop or perform less stereotypy the following recommendations should be followed:

4.8.1 Diet and nutrition

The research showed that there was a strong negative correlation between feeding and stereotypical behaviour, and that a five-minute increase in foraging behaviour saw a seven-minute decline in stereotypical pacing. A recommendation would therefore be to encourage captive red squirrels to forage, feed and cache as they would in the wild as resource availability and breeding success is positively linked, therefore, food abundance and variety in captivity needs to be increased to maximise breeding success. Although two zoos did not link the emergence of stereotypical behaviour with a particular season, six out of the eight zoos confirmed that they witnessed changes in behaviour which appeared to be dependent on the changing seasons, therefore as foraging and caching behaviour is considered a highly motivated behaviour in red squirrels to meet this need feeding seasonally, to reflect the diet seen in the wild, by offering flowers and buds in spring, nuts and berries in summer and fungi and pinecones in autumn is recommended. In the wild red squirrels also eat live food and will take baby bird eggs and insects, so offering live food such as meal worms would ensure this dietary requirement is met and calcium and other mineral intake can be increased by giving antlers or cuttle fish.

Zoos should also provide temporal variation in the presentation of food and be presented in a way to mimic natural food distribution including multiple high yield areas dispersed with no yield and low yield areas. Food needs to be offered in a wild-type manner, particularly for those squirrels set for release, branches with leaves and buds on could be offered as could fallen pine trees to forage in. All study squirrels were observed foraging on the roof of the breeding and holding enclosures attempting to reach the buds and leaves from the trees above, and the squirrels in the walkthrough all foraged in the canopies, so to mimic this wild-type behaviour, branches with fruit and buds on could be placed on the top of the enclosures to encourage this species typical foraging behaviour.

Increasing the amount and type of food during hoarding season by at least 10% may help to reduce hoarding stress, currently only a few hazelnuts are placed in the breeding enclosures and because these are highly valued there were constant aggressive chases and even fights over these foods. Offering many different natural feeding opportunities, increasing handling time by offering more complex foods such as pinecones allows animals to contrafreeload and may reduce aggressive fights and reduce caching stress and high levels of pilfering. Offering hoarding opportunities using containers or pots full of substrate can also increase natural foraging and caching behaviour.

4.8.2 Enclosure design

This Logistical Regression showed that the size of enclosure had a direct impact on the presentation of different behaviour, with large enclosures over 300 metre^2 and higher than 4 metres reporting the least amount of pacing. Indeed, animals observed in large, open range walkthroughs and those moved into walkthroughs had a much more similar wild-type behaviour repertoire, with increased feeding and locomotion and decreased inactivity and stereotypical pacing than those housed in smaller breeding and holding pens. All enclosures should therefore ideally be as large and high as possible, preferrable based around established trees, both deciduous and coniferous with large canopies so red squirrels are able to locomote and forage in a more natural way. If increasing floor/height space is not possible increasing vertical space and cage complexity using foraging/hoarding pots of various sizes and type was also shown to reduce pacing. Species that spend a relatively large amount of time foraging over a large area like red squirrels might be most sensitive to spatial restriction and. increasing available vertical space is effective way to reduce pacing in rodents

Adding cover, both natural (trees, shrubs, and branches) and artificial (such as wooden boards or metal sheets) allows animals to cache in privacy without the concern of perceived predation as they can block both stressful visual and olfactory cues from outside their cage. Natural foliage should be changed regularly and reflect the seasons, for example adding Christmas trees during winter, and should be removed before they die, as this foliage not only offers cover but also foraging opportunities and drey building materials. Squirrels use lots of different materials to build dreys so as much variety as possible should be offered, including lichen, moss, wool, sticks, leaves and grasses so that they are able to partake in this natural behaviour. Housing red squirrels in complex walkthroughs that mimic the complexity of their natural habitat and given choices to both feed, locomote and nest in the way they would in the wild is preferable to small breeding enclosures.

However, if squirrels need to be housed together in smaller cages, these should offer as much complexity as possible, including drey building opportunities, offering shelves and trees to nest in. Offering different substrate such as sand baths, wood chip, leave piles, rotten logs, grass and pine needles, would also encourage natural caching and foraging behaviour and also choice and control over where to forage. Resources need to be spread evenly throughout the enclosure to prevent aggressive interactions.

4.8.3 Animal movement

Several keepers highlighted that they believed the need to disperse is one of the major contributory factors as to why post-weaned kittens start to pace from 10 weeks of age, an age when in the wild they would normally start to look for a new territory. Keeping juveniles with their parents beyond what is seen in the wild and not allowing them to disperse and establish their own territory, not only leads to the emergence of stereotypical pacing, but the longer juveniles are kept with their parents and siblings the more frequent and intense the pacing becomes. This need to disperse may cause the juveniles to explore more intensively near doors that open for husbandry procedures and this investigation may easily turn into patrolling behaviour and manifest into pacing as the door occasionally opens reinforcing the expression of this behaviour. Other husbandry recommendations would include moving the juveniles either to a walkthrough enclosure or be sent for release at the age when they would naturally be fully weaned and start to disperse in the wild, from 10-14 weeks of age and before pacing becomes established.

Another addition would be to allow males to leave the main enclosure during parturition and the raising of any offspring, as is seen in the wild. Two enclosures in the study have tunnels between enclosures which can be shut off so that different squirrels can be isolated in different areas in order to reduce conflict over limited resources or territories, so adding these to enclosure gives animals choice and control over where they want to spend their time and if they want to move away from other conspecifics. The holding pens are currently used for soft release into the walkthrough, where new squirrels are housed for two weeks before being released, but the squirrels in these enclosures seem to increase their chasing and aggressive behaviour and also their pacing, additionally the squirrels in the walkthrough were seen pacing outside these holding pens when other squirrels were housed in them, and again aggressive chasing took place between those in the pen and those in the walkthroughs.

In Kent one male was released straight into the walkthrough and this aggressive behaviour and increased pacing did not occur so collections need to consider if this soft release is really necessary or whether it just adds to the red squirrels' stress

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4.9 Further Work

Findings from both the observations and responses from zookeepers did support many of the observational findings. However, it is clear that more research needs to be conducted to attempt to isolate the main environmental and dietary changes required in order to attempt to prevent stereotypical behaviour emerging and reduce pacing in those animals that currently pace. In this study changes were made one at a time, firstly the boards were added, then the substrate and then the hoarding pots. It was clear that the boards stopped Radish pacing, but Autumn did not stop until hoarding pots were added. It is not clear if the sole addition of the hoarding pots would have stopped Radish pacing so quickly.

Further research would involve monitoring red squirrels in different zoos before any changes to the enclosure, then changing one thing in the enclosure, such as adding boards and then observing any changes in their behaviour, before removing the boards and seeing if the squirrels immediately reverted to their original behaviour. It would also be valuable to try to do the additions in the project to multiple locations amongst different groups and different ages. Similarly, this could also be done with a seasonal diet, adding certain foods during the season and comparing behaviour before and after the addition of these foods.

Additionally, more research using a Spatial Participation Index (SPI) would give vital information on enclosure usage by captive red squirrels and the different types of behaviour they partake in over different areas of the enclosure. It would also be useful to follow different wild and captive litters using a mixed effects model that includes a random factor, such as random observation times and considering a repeated measure approach of the analyses to prevent a lack of independence that multiple observations of the same population can introduce and is not considered in regular statistical tests.

Another area requiring further research is when exactly to move the juveniles from their parents. This would involve locations that have several litters a year and house their squirrels in similar breeding enclosures so to reduce the number of variables. Juveniles could then be moved from 10-14 weeks and any pacing before and after the move recorded. This may give a more definitive answer as to when juveniles should be moved away from the breeding enclosures.

4.10 Limitations

Ideally more of the captive red squirrel population would have been observed but this was logistically impractical. The squirrels observed are considered representative of the target population and importantly, observing these individuals over multiple months has allowed development of behaviour and seasonality to be investigated.

The COVID-19 pandemic not only caused difficulties for the observer to carry out the observations, due to several lockdowns being put in place during the study, but also prevented the observer from visiting different zoos to conduct interviews. Although questions were written with little ambiguity, they were still open to misinterpretation or different interpretation by respondents. Zookeepers are also very busy and so their reliance in providing accurate information may be limited. However, with the findings of the pilot scheme and the researcher answering specific questions each respondent had regarding the questionnaire, any confusion or ambiguity was reduced. Respondents were also able to expand on their answers and were able to add other details to the end of the questionnaire. Zookeepers are also best placed to supply information about the animals in their care, as they see their animals every day and have a greater understanding of their behaviour than anyone else.

Another limitation for the research was the observer being unable to physically see the enclosure designs and having to rely upon descriptions of each enclosure. The researcher requested pictures of the enclosure to try and overcome these limitations and most of the respondents were able to facilitate this request. As stereotypical behaviour is considered a potential indicator of poor welfare, respondents may not have felt willing to be completely honest about the amount of stereotypical pacing they witness. The observer did try to make it clear that this was not a judgement and during the observational research had found this behaviour prolific in the species. The findings of the observational research were disseminated to all respondents, with the express consent from Wildwood Escot, to encourage an environment of honesty and knowledge sharing (Appendix IV).

5. Conclusion

The key findings of this research were that captive red squirrels that do not stereotypical pace have a similar time budget to wild red squirrels, spending 80% of their time on feeding and locomotive behaviour. Whereas captive red squirrels that stereotypically pace spend half of this time on feeding and only 57% of their time on feeding and locomotive behaviour with stereotypical behaviour taking up over 10% on average of their time budget. Enclosure size is linked to pacing, with larger walkthrough enclosures eliciting the least amount of stereotypical pacing behaviour. Red squirrels housed in these walkthroughs having a similar time budget to wild and non-pacing captive red squirrels. The logistical regression model also supports these findings that zoos also state larger, walkthrough enclosures see the least amount of stereotypical pacing in their captive red squirrels.

Changing the complexity of smaller enclosures by adding privacy boards, increasing substrate and any vertical enrichment such as hoarding pots decreases stereotypical behaviour even in animals that pace up to a third of their activity budgets. These changes also appeared to delay the onset of pacing in post weaned juveniles. Another key finding was that feeding and stereotypical behaviour are significantly negatively correlated and by increasing food complexity and handling time by five minutes elicited a seven-minute reduction in pacing behaviour.

Whilst no one particular reason was found for the aetiology of stereotypical pacing in captive red squirrels, both the research and respondents to the questionnaire found several different reasons why red squirrels may pace, including overcrowding, inappropriate environment and diet, escape attempts, visitor noise and enforced social groups, but the overall findings are the need for juveniles to disperse to a territory of their own.

These findings are important as Keepers have overwhelmingly stated that they are struggling to find appropriate release sites for captive bred animals to be released into, because of no suitable habitat, too many grey squirrels, no financial support and prevalence of disease and therefore the industry needs to consider if red squirrels are a species that will possibly become extinct in the wild and only be able to be seen in captivity. This raises a real ethical issue; especially as stereotypical pacing is so prevalent in the captive group. Zookeepers need to look at not only enclosure design, but the whole husbandry care of their red squirrels, how they are housed together, foraging and hoarding opportunity, food and feeding presentation, availability of natural foliage and access to appropriate habitat to ensure their welfare is not compromised.

This study is of real importance to the wider world as the researcher has been studying the captive species over the past 7 years, in which time the British red squirrel has moved from a species of concern to being listed as an endangered animal on the IUCN Red List 2020, with extinction in the wild expected by 2040. Zoos and conservationists need to consider whether it is possible to ethically release animals into the wild, where the pressure from the grey squirrel means red squirrel are outcompeted for food and resources and carry the squirrel pox which causes extinction of the localised population. Even in Northern Scotland, a previous stronghold for red squirrels, has seen the emergence of the grey squirrel, even as far as Inverness, as the COVID-19 pandemic prevented the culling of grey squirrels which had previously been holding the line between the South and North. With forest fragmentation, there are few corridors for red squirrels to travel and even measures such as contraception and introducing the pine marten may not be enough to hold back to wave of grey squirrels.

For zoos to continue to produce suitable animals for release or for exhibition, whilst keeping welfare standards high and giving visitors not only an enjoyable but also an educational experience, stereotypical behaviour needs to be given priority and time and resources given over to try to ascertain the source of the stress and the best means to try and reduce or preferably eliminate it at an individual level.

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7. Appendices

APPENDIX I – Ethics Forms and Research Ethics Approval

Wildwood Escot
Ottery St Mary
Devon
EX11 1LU
escotkeepers@wildwoodtrust.org

Visiting Researcher Application Form

Full Name: Pamela Adams-Wright

Full home address:

30 Quakers Mede Haddenham Aylesbury Bucks HP17 8EB

Contact address (if different from above):

Mobile Number: 07516 297045

Email Address: pamelaadamswright@btinternet.com

Next of kin full name: Mr Mark Adams-Wright

Next of kin relationship to self: Husband

Contact number for next of kin: 07718 186878

Name and address of Academic institute:

Faculty of Humanities and Social Sciences
University of Winchester
Winchester
Hampshire
SO22 4NR

Course of study: MPhil TBC

Supervisor name and email address: Lisa Riles PhD, MSc, MRes, MSc, FHEA (Lisa.Riley@winchester.ac.uk)

Anticipated dates of zoo visits: 7th-9th June, 1st-5th July, 11th-16th August, 8th-12th September. (Then probably the 2nd or 3rd week of every month until approximately June 2020. I will also attempt to be there for the moving of the kittens to the main enclosure - to be agreed with Kerry Church).

Anticipated duration of visit: 5/6 days at a time

Project title: An Investigation into whether red squirrel pacing at Wildwood Escot is a learnt behaviour and after the kittens are moved from a breeding pen to the main enclosure and to the British Wildlife Centre, does this pacing increase, decrease or remain constant? What effect does this pacing have on the behaviour of other red squirrels already in the enclosures and does this potentially impact on the ability to be able to ultimately release them into the wild?

Common name of species: British Red Squirrels

Scientific name of species: Sciurus Vulgaris

Introduction and project methods (1000 words max)

Red squirrel (Sciurus Vulgaris) numbers have declined from 3.5 million to just over 140,000 since the 18th Century due to both economic and ecological issues, together with the introduction of the grey squirrel (sciurus carolinensis) from North America in 1876 which competes for both resources and carries the highly transmittable squirrel poxvirus disease which is fatal to red squirrels. There are approximately 180 captive red squirrels worldwide, a third of which are involved in captive breeding programmes to attempt to boost red squirrel numbers supported by different institutions including The Forestry Commission and The British Wildlife Centre. However captive animals face different environmental challenges caused by confinement stress, restricted movement, limited space and close proximity to humans which can reduce complexity of behaviour and increase both vigilance and hiding behaviour which can lead to abnormal stereotypical behaviour. The definition of stereotypical behaviour is repetitive, unvarying and apparently functionless behaviour patterns which can be an indicator of reduced welfare.

This study is an investigation into the cause of the emergence of stereotypical behaviour in red squirrel kittens at Wildwood Escot, and to observe if and why this behaviour continues once the kittens are separated and moved from the breeding pen to the main enclosure at Wildwood and to the British Wildlife Centre. The investigation will also look at the impact this behaviour has on the red squirrels currently at both locations and the behaviour the parents in the breeding pens display when the kittens are in or away from the breeding pen.

Methodology

The experimental design will be to compare the results of two different litters born at Wildwood, one litter born in May 2019, and the other litter which should be born in February/March 2020. Preliminary observations will record the diversity of both state and event behaviours using ad libitum sampling from both the red squirrels in the breeding pen and the main enclosure to design appropriate robust ethograms and marking sheets.

The observations will then move to instantaneous sampling at intervals of 2 minutes for red squirrels in both enclosures for state behaviour and event behaviour will be recorded using ad libitum/all occurences sampling as it occurs.

Approximately 500 hours of observations will take place at Wildwood between June 2019 and June 2020. Observations will be approximately 8 hours a day between 8:30am and 5:30pm, dependant on seasonal changes.

Before the kittens are moved to the main enclosure:

3/4 days observing adults and kittens in the breeding pen once a month.
2 days observing red squirrels in the main enclosure once a month.

The kittens and parents behaviour will be observed during the move from the breeding pen to isolation. The other squirrels in the main enclosure and the kittens behaviour will be observed when moving from isolation to the main enclosure.

When the kittens move in the main enclosure:

4 days observing red squirrels in the main enclosure
2 days observing the parents in the breeding pen

The squirrels that are moved to the British Wildlife Centre will be observed for the same amount of time to ensure robust data collection.

Please note: this is correct at time of writing as it has been agreed to try and start observations as quickly as possible (7th-9th June) so still needs to be fully verified by my Supervisor and these timings may change very slightly.

The dependent variable will be the display of behaviour, the controllable variables will be kept to a minimum, including taking observations in the same place, during the same time each month. The uncontrollable variables such as noise and public interactions will be noted on the marking sheets.

The research will use quantitative methods based on qualitative data collection from marking sheets to examine potential relationships between quantified variables. The mean duration of each behaviour element will be measured to work out an activity budget and the data will then be normalised to prevent masking of any significant differences in the data.

Inferential statistics will be used to compare observed frequencies in each category to test hypothesis.

Ethical Considerations and Health and Safety

To reduce potential stress all observations will take place from a quiet location near the enclosure with no direct interaction with the squirrels. A radio or phone number for the main reception will be kept by the observer in case of any health or safety issues should occur.

Intended outcomes of research (300 words max)

The rational for this research is there is very little research into how red squirrel stereotypical behaviour evolves, if it is a learnt behaviour and once it has manifested if it can be eradicated. The outcome of the research is intended to illustrate whether once red kittens are moved from a breeding pen to a larger enclosure if this behaviour increases, decreases or remains constant and how this impacts on the behaviour of the red squirrels already in the enclosure, both at Wildwood and The British Wildlife Centre. The study will also show any changes in the parent's behaviour in the breeding pen when the kittens are in the pen and once they have been moved. As captive breeding is essential in order to boost red squirrel numbers in the wild it is hoped the outcome will also highlight the impact the display of stereotypical behaviour has on the potential release of red squirrels in the wild, which is the ultimate desired outcome for breeding red squirrels in captivity.

I hereby agree to adhere to all health and safety protocols and to follow any instructions given by staff members at all times during my research.

Full name and signature: Pamela Adams-Wright

Date: 3rd June 2019

Personal Information

Your name:	Pamela Adams-Wright

Are you over 18?	Y	y	N	

Home telephone:	01844 290781	Mobile:	07516 297045

Contact email:	pamelaadamswright@btinternet.com

Home address:	30 Quakers Mede Haddenham Bucks HP17 8EB

Emergency details (Please fill in the following sections if you plan to collect data onsite)

Emergency contact 1	
Name:	Mark Adams-Wright
Telephone:	01844 290781
Mobile:	07718 186878

Emergency contact 2	
Name:	Olivia Adams-Wright
Telephone:	01844 290781
Mobile:	07590 995494

Doctor's details	
Name:	Dr Halcot
Telephone:	01844 293300
Address:	Haddenham Medical Surgery Stanbridge Road Haddenham, HP17 8JX

Medical conditions (Please fill in the following sections if you plan to collect data onsite)

Anyone at Wildwood may come into contact with hay, straw, sawdust, nuts, fungi, pollen, animals of all kinds (we have adders, which are venomous snakes), other materials and tools during their activities. Do you have any medical / behavioral conditions, disabilities and / or medication that we should be aware of that might be affected by these (e.g. hay fever, allergies, anaphylaxis, asthma, diabetes, epilepsy, animal phobias, etc.)? If yes, please specify…

Are you up to date with your tetanus vaccination	Y		N	N	Date	

University /College Information

University /College Name:	University of Winchester
University /College Address:	Sparkford Road Winchester SO22 4NR
Level of programme: (eg BSc, MSc, HND)	MPhil
Course title:	Sociology and Biology
Name of supervisor:	Lisa Riley
Supervisor's email:	Lisa.Riley@winchester.ac.uk

Research Proposed

Research Project title:	An Investigation into whether red squirrel pacing at Wildwood Escot is a learnt behaviour and after the kittens are moved from a breeding pen to the main enclosure, does this pacing increase, decrease or remain constant? What effect does this pacing have on the behaviour of other red squirrels already in the enclosures and does this potentially impact on the ability to be able to ultimately release them into the wild?						
Preferred start date (data collection):	September 2019						
Preferred finish date (data collection):	Approx May 2020						
Project submission date:	2021						
When do you intend to collect your data:	Mon	Tue	We d	Thu	Fri	Sat	Sun
(Please circle/highlight)	See	Below					
	AM		PM		All day		

Method Statement/Abstract (Please use a separate sheet if required)

Red squirrel (Sciurus Vulgaris) numbers have declined from 3.5 million to just over 140,000 since the 18th Century due to both economic and ecological issues, together with the introduction of the grey squirrel (sciurus carolinensis) from North America in 1876 which competes for both resources and carries the highly transmittable squirrel poxvirus disease which is fatal to red squirrels. There are approximately 180 captive red squirrels worldwide, a third of which are involved in captive breeding programmes to attempt to boost red squirrel numbers supported by different institutions including The Forestry Commission and The British Wildlife Centre.

However captive animals face different environmental challenges caused by confinement stress, restricted movement, limited space and close proximity to humans which can reduce complexity of behaviour and increase both vigilance and hiding behaviour which can lead to abnormal stereotypical behaviour. The definition of stereotypical behaviour is repetitive, unvarying and apparently functionless behaviour patterns which can be an indicator of reduced welfare.

This study is an investigation into the cause of the emergence of stereotypical behaviour in red squirrel kittens at Wildwood Escot, and to observe if and why this behaviour continues once the kittens are separated and moved from the breeding pen to the main enclosure at Wildwood Escot and Wilwood Devon. The investigation will also look at the impact this behaviour has on the red squirrels currently at both locations and the behaviour the parents in the breeding pens display when the kittens are in or away from the breeding pen.

Methodology

The experimental design will be to compare the results of two different litters born at Wildwood, one litter born in May 2019, and the other litter which should be born in February/March 2020. Preliminary observations have recorded the diversity of both state and event behaviours using ad libitum sampling from both the red squirrels in the breeding pen and the main enclosure to design appropriate robust ethograms and marking sheets.

The observations have moved to instantaneous sampling at intervals of 2 minutes for red squirrels in both enclosures for state behaviour and event behaviour is recorded using ad libitum/all occurences sampling as it occurs.

Approximately 500 hours of observations will take place across both locations (at Devon and Canterbury) between June 2019 and June 2020. Observations are approximately 8 hours a day between 8:30am and 5:30pm, for 3 days each month to observe any effect of seasonal variations on behaviour.

Whilst the kittens were in the breeding pen at Escot the following observations have taken place and an activity budget for the parents, kittens and squirrels already in their enclosure has been designed. The observations were as follows:

3 days observing adults and kittens in the breeding pen once a month from June-September 2019.
2 days observing red squirrels in the main enclosure once a month from June-September 2019.
2 days observing red squirrels in the main enclosure whilst the two kittens were in the holding pen September 2019.

I am going to observe the two red kittens being released into the enclosure on Monday 23rd September until 25th September. I will then observe them 3 days a week each month until approximately May 2020.

When the kittens move into the main enclosure at Wildwood Kent.

2 days observing red squirrels in early October 2019 (whilst Douglas is in the holding pen) - Dates to be agreed with Judi
Once Douglas is released 3 days once a month observing all the red squirrels in the main enclosure until approximately May 2020.

The dependent variable will be the display of behaviour, the controllable variables will be kept to a minimum, including taking observations in the same place, during the same time each month. The uncontrollable variables such as noise and public interactions will be noted on the marking sheets.

The research will use quantitative methods based on qualitative data collection from marking sheets to examine potential relationships between quantified variables. An Activity budget for each individual squirrel will be deviced and Inferential statistics will be used to compare observed frequencies in each category to test the hypothesis. I will also be sending out a questionnaire to approximately 15 other breeding programmes to gain insight into the experiences they have had breeding red squirrels and the development of any stereotypical behaviour they have seen in their animals.

Ethical Considerations and Health and Safety

To reduce potential stress all observations will take place from a quiet location near the enclosure with no direct interaction with the squirrels. A radio or phone number for the main reception will be kept by the observer in case of any health or safety issues should occur.

The rational for this research is there is very little research into how red squirrel stereotypical behaviour evolves, if it is a learnt behaviour and once it has manifested if it can be eradicated. The outcome of the research is intended to illustrate whether once red kittens are moved from a breeding pen to a larger enclosure if this behaviour increases, decreases or remains constant and how this impacts on the behaviour of the red squirrels already in the enclosure, both at Wildwood Escot and Wildwood Kent. The study will also show any changes in the parent's behaviour in the breeding pen when the kittens are in the pen and once they have been moved. As captive breeding is essential in order to boost red squirrel numbers in the wild it is hoped the outcome will also highlight the impact the display of stereotypical behaviour has on the potential release of red squirrels in the wild, which is the ultimate desired outcome for breeding red squirrels in captivity.

Please return the completed form to: vicki@wildwoodtrust.org
Vicki Breakell, Conservation Officer, Wildwood Trust, Herne Common, Herne Bay, Kent CT6 7LQ

On Sat, 1/6/19, Kerry Church wrote:

Subject: Re: Red Squirrel enquiry
To: "Pam Adams-Wright"
Cc: "escotkeepers"
Date: Saturday, 1 June, 2019, 15:47

Hello
Pam,
It was great to see you again today, I'm really excited about your research ideas!
Please find attached the Visiting Researcher Application Form as promised. Could you fill it in and email it back to me please.

I will speak to my manager, George about times you are able to arrive and get back to you.

See you next
week!
Best
wishes
Kerry

Pam Adams-Wright
To: Kerry Church
03/06/19 12:17

Sent
30.2 KB
Save all as ZIP

Hi Kerry

I enclose the application as requested. I think it will be a dynamic and agile project as we may have to change bits and pieces as the project develops anyway?

Thank you again and I hope this is ok, I've had to knock something up very quickly today so if you think It needs more detail please let me know?

If you let me know about timings for this weekend that will be great, l will try and get there on Friday at the agreed time, but obviously it is slightly traffic dependant.

Kind regards
PAM

On 4 Jun 2019, at 17:38, Kerry Church wrote:

Hello Pam,

Thank you for the completed application form, it looks great. We fully understand that there may be some amendments. The only other request we have which I forgot to mention, could we have a copy of your study when its complete please.

As you'll be on site for a long period of time and working alone, I'll need to ask you to read a few documents regarding emergency procedures, radio protocol, first aid etc. I'll get these together and email them to you if that is ok? I'll have a form ready on your first day for you to sign to say you've read and understood them.

I have spoken to my manager, he is happy for you to arrive at 8.30am. When you arrive please could you give me a call on xxxxxxxx and I'll come and meet you. I'll show you where to sign in and I'll give you a radio to use. After your first day you are more than welcome to sign yourself in, grab a radio and just radio to let us know you have arrived before heading to the enclosure. The walk-through is kept locked until we go to feed them at about 9.30am but we can 'pretend' lock it if we know which days you wish to study those squirrels so you can head down earlier.

I hope that answers all of your questions. If there is anything else, feel free to email. I am cc'ing in the keepers so please 'reply all' when you respond so that they are kept in the loop.

Best wishes

Kerry

Kerry Church
Senior Keeper

Wildwood Escot
Escot Park
Ottery St Mary
EX11 1LU

From: Pamela Adams-wright <
Date: 9 September 2019 at 07:31:27 BST
To: judi.dunn
Subject: Further to last weeks ABWAK Workshop

Hey Judi

It was great to meet you last week and the presentations and workshops were really great and I learnt a lot and got a lot of contacts which should really help me in my research.

So very briefly my research is based on researching stereotypical behaviour in red squirrel kittens, particularly to research how it manifests, develops, and whether a change in environment changes its presentation and also the impact it has on both the parents and the other reds in the enclosure they move into.

So far I have observed the parents and kittens in their breeding pen and also the four females in the big enclosure in Devon for one week per month since May . I have an activity budget for each individual squirrel to use as a baseline. My intention is to now observe the kittens in the release pen in Devon this week and when they are released in two weeks time. I will then continue to observe them for several months and then hopefully observe the next set of kittens when and if they have them just to try and make the research as robust as possible.

I would therefore really like to watch the reds already in the pen you will be putting the male into before he goes into it in order to get a baseline of their behaviour and then come and watch him when he goes into the holding pen and released. Then probably a few days each month until next year. However I am extremely tied up with work and research at Devon so we may just have to do the best we can. If this is all ok with you could you possibly keep me as updated as you are able to about the dates etc you will be moving him in so we can try and work out the best times for me to come and do the observations?

Alongside this will run a questionnaire which I will send out to other establishments so we can get a big a picture as possible about of other collections experiences.

Well I hope this is all ok, obviously once I've done my findings I will share everything with you.

I look forward to hearing from you soon and seeing you all again in the bit too distant future.

Kind regards

Pam

From: judi dunn
Date: 18 September 2019 at 14:21:45 BST
To: Pamela Adams-wright
Cc: Vicki Breakell
Subject: Re: Observing the male kitten moving from Escot

Hi Pam
Yes that is all great, Escot's squirrel Douglas arrived today. He is in our inner pen of the walkthru on his own. The plan is to have him in there for maybe four to six weeks. I may have some more males to go with him but don't know yet as they were born late July and haven't been sexed yet.

So initially you can observe him late September thru to mid October on his own. If we have another young male he will be going in with Douglas mid October in the inner pen. Then I will release both into the walk thru but can't give you a date for that yet. Probably towards the end of October. Douglas is a strapping six month old male and ours are only only 3 months so it will be interesting to see how they would get on.

The young that may join Douglas I have not experienced pacing yet. The squirrels they will be going into the walk thru with, are four males aged 7 years, 5 years (7 year old born at Pensthorpe, 5 year old born here) and two eighteen month old (both born to private breeders in the North of England and passed on via Welsh Mountain Zoo. I don't have any other details on them) All have shown pacing behaviour, but none in the walk thru just in the inner pen. I hope this helps and is not confusing please feel free to ask further questions.

I attach a research application form. Could you fill this in for our records and send it to our conservation and research officer Vicki Breakell on her email? Many thanks

Kind Regards
Judi

Pam Adams-Wright
To: Vicki Breakell
21/09/19 10:11

DearVicki Judi has asked me to complete the attached form. It is rather confusing as I have already started my research at Escot. But in brief I am following the red squirrels at Escot for one year to see how stereotypical behaviour manifests in their kittens and once the kittens move to a larger enclosure if this continues, and if it has an impact on either the parents in the breeding pen or the other reds in the large enclosure. Two females are staying at Escot and I believe Douglas the male is now with you. Judi is happy for me to start observing him in the holding pen in the main enclosure in early October and then to come a few days a month to observe him until spring next year to see if his pacing reduces or has an impact on the others in the enclosure. This research is for an MPhil I hope to start in September 2020. I hope the application is satisfactory, but please do not hesitate to contact me

UNIVERSITY OF WINCHESTER

RESEARCH ETHICS FORM 1

WHAT LEVEL OF REVIEW DO I NEED?

GUIDELINES

This form is for staff and students. It will help you identify the level of review needed for your project. Before completing it, you need to:

1. Read *The University Research Ethics Policy*.
2. If you are a student, discuss the ethical aspects of your project with your supervisor.

It is your responsibility to follow the University's Policy on the ethical conduct of research and to follow any relevant academic guidelines or professional codes of practice pertaining to your study when answering these questions.

The questions and checklist in this proforma are intended to guide your reflection on the ethical implications of your research. Explanatory notes and further details can be found in the Policy document.

SECTION 1

DETERMINING WHETHER YOU REQUIRE ETHICS REVIEW

YOUR RESEARCH
Project title: As investigation into how and why stereotypical pacing starts and how it manifests in captive post-weaned red squirrels.
Your name: Pamela Adams-Wright

1.	**Is the proposed activity classified as Research or Audit /Service Evaluation or similar?**	
	☒ Research	☐ Audit or Service Evaluation
	Use the Policy to help you answer this question. If the proposed activity meets the definition of ***research*** *(see the policy), CONTINUE.* *If the activity is an* ***audit*** *or a* ***service evaluation****, STOP. You do not need to seek ethics approval, but you do need to formally register your project with UREC, along with a project outline. To do this complete Form 2.* *If you are unclear what type of activity you are undertaking, please refer to the Policy for additional types.*	
2.	**Does the research involve living human participants, human samples or data derived from individuals who may be identifiable through that data?**	
	☐ Yes	☒ No
	Use the Policy to help you answer this question. *If you answer* ***NO****, SKIP to QUESTION 6 and CONTINUE.* *If you answer* ***YES****, CONTINUE.*	
3.	**Is the research being conducted for a medicinal purpose?**	
	☐ Yes	☐ No
	Use the Policy to help you answer this question. See Appendix 2 - FAQs and definitions. *If you answer* ***YES****, and think your research comes under the definition of 'for a medicinal purpose,' it will need to be scrutinised by the Committee. Please email the Committee Chair (ethics1@winchester.ac.uk) for further guidance on what to do.* *If you answer* ***NO****, CONTINUE.*	

4.	**Does your research require external ethics approval or review?**	
	☐ Yes	☐ No
	For example, from the NHS or another overseeing body. Use the Policy to help you answer this question. *If you answer **NO**, CONTINUE.* *If you answer **YES**, you need to formally register your project with UREC, along with the relevant external ethics approval. To do this complete Form 2.*	
5.	**Is the project underway and, the researcher or PI, has moved institution to Winchester?**	
	☐ Yes	☐ No
	*If you answer **YES**, please read the following:* *If the research began when the PI was employed at another institution but has subsequently moved to Winchester, and the project has previously been subjected to ethics scrutiny at that institution, then it need not go through ethics review again. The outcome of ethics review by that institution should be communicated to UREC for formal recording. To do this complete Form 2 and include evidence of the previous ethics approval.* *HOWEVER, if there have been significant changes to the original research design which have ethical implications or recruitment of a cohort of participants will be undertaken through Winchester, then the project will require ethics review and you should apply for approval, CONTINUE.* *If you answer **NO**, CONTINUE.*	
6.	**Is the research collaborative?**	
	☐ Yes	☒ No
	*If you answer **YES**:* • *where the Principal Investigator (PI) of the research is located at another institution, it is their responsibility to seek ethics approval, including partner research sites. The outcome of ethics review by that institution should be communicated to UREC for formal recording. To do this complete Form 2 and include evidence of the previous ethics approval.* • *where the PI is located at Winchester, then the project will undergo scrutiny as per Winchester's Ethics Policy, CONTINUE.* *If you answer **NO**, CONTINUE.*	
7.	**Is the research being conducted in another country?**	
	☐ Yes	☒ No

	*If you answer **YES**, please read the following:* *Where a project is conducted in another country, the researcher should consider if it is possible to obtain ethics review by a local research ethics committee or other relevant body. The outcome of such a review by that institution should be communicated to UREC for formal recording, along with a project outline. To do this complete Form 2.* *If this is not possible, the project should be reviewed by the University of Winchester, either at Faculty level or Committee depending on the nature of the proposed work, so CONTINUE.*	
8.	**Does the research involve the use of documentary material, papers, literary works or archive documents <u>in the public domain</u>?**	
	☐ Yes	☒ No
	Use the Policy to help you answer this question. *If you answer **NO** because the works are in a private archive or closed collection, do the following: complete Form 2, including details of the nature of the private /closed collection and provide evidence of the permission to use this material for research purposes.* *If you answer **YES**, you need to formally register your project with UREC, along with a project description. To do this complete Form 2.*	
9.	**Does the research involve the animals?**	
	☐ Yes	☒ No
	*If you answer **NO**, CONTINUE.* *If you answer **YES**, you need to formally register your project with UREC, along with a copy of the relevant licence (if required). To do this complete Form 5.*	
10.	**Does the research involve environmental interventions?**	
	☐ Yes	☒ No
	*If you answer **NO**, CONTINUE.* *If you answer **YES**, you need to formally register your project with UREC, along with a copy of the relevant licence (if appropriate). To do this complete Form 2*	
11.	**Does the data you will collect contain *any* information that could be linked back to participants or that might identify them (e.g. name, address, photo, voice, email)?**	
	☒ Yes	☐ No

SECTION 2

DETERMINING THE LEVEL OF ETHICS REVIEW REQUIRED

Please mark with an ☒ as appropriate	**YES**	**NO**
Does the research involve individuals who are vulnerable? *For example: vulnerable children, over-researched groups, people with learning difficulties, people with mental health problems, young offenders, people in care facilities, including prisons. For a note on research with children, see Appendix 2 of the Policy.*	☐	☒
Does the research involve individuals in unequal relationships e.g. your own students? *Please note:* 1. *students recruited via SONA are not considered 'your own students.' If you intend to recruit widely across the University or your Faculty (e.g. through snowball sampling or a mail shot) you do not need to consider such students as your own, even if some participants may be students you are directly involved with. Only tick "yes" if you are targeting your own students specifically.* 2. *if you are an undergraduate or postgraduate student carrying out research with children in either a school or early years setting, these DO NOT come under the category of your 'own students.'*	☐	☒
Will it be necessary for participants to take part in the study without their knowledge and consent at the time? *For example: covert observation of people in non-public places, use of deception. See Appendix 2 of the Policy.*	☐	☒
Will the study involve discussion of sensitive or personal topics? *For example: (but not limited to) participants' relationships, emotions, sexual behaviour, experience of violence, mental health, gender, race / ethnicity status or experience, political or religious affiliations. Please refer to the Policy.*	☐	☒

Is there a risk that the highly sensitive nature of the research topic might lead to disclosures from the participant concerning their own involvement in illegal activities or other activities that represent a threat to themselves or others which may need onward reporting? *For example: sexual activity, drug use, illegal activities or professional misconduct.*	☐	☒
Might the research involve the sharing data or confidential information beyond the initial consent given?	☐	☒
Might participant anonymity be compromised at any time during or after the study? *For example: will the research involve respondents using the internet, social media, or other visual /vocal methods where respondents may be identified?*	☐	☒
Is the study likely to induce severe physical harm or psychological distress?	☐	☒
Does your research involve tissue samples covered by the Human Tissue Act (2004)?	☐	☒
Is there a possibility that the safety of the researcher may be in question? *For example: research in high risk locations or with high risk groups.*	☐	☒
Does the research involve creating, downloading, storing or transmitting material that may be considered to be unlawful, indecent, offensive, defamatory, threatening, discriminatory or extremist? *If you answer **YES** to this question, you must also contact the Director of IT Services, who must provide approval for the use of such data.*	☐	☒

Answering **NO** to ***all*** these questions means your project is eligible for Faculty level ethics review. You now need to complete Form 3.

Answering **YES** to ***any*** of these questions means your project will require Committee ethics review. You now need to complete Form 4.

UNIVERSITY OF WINCHESTER

RESEARCH ETHICS FORM 3

FACULTY REVIEW

GUIDELINES

This form is for staff and students. It will help you set out the ethical aspects of your project that need to be reviewed. Before completing it, you need to:

3. Read *The University Research Ethics Policy*.
4. If you are a student, discuss the ethical aspects of your project with your supervisor.

It is your responsibility to follow the University's Policy on the ethical conduct of research and to follow any relevant academic guidelines or professional codes of practice pertaining to your study when answering these questions. This includes providing appropriate information sheets and consent forms and ensuring confidentiality in the storage and use of data.

The questions in this proforma are intended to guide your reflection on the ethical implications of your research. Explanatory notes and further details can be found in the Policy document.

If any aspect of your project changes during the course of the research, you must notify the Chair of UREC.

SECTION 1

YOUR DETAILS			
1.1.	Your name**: Pamela Adams-Wright**		
1.2.	Your department: CAW – Department of Interprofessional Studies		
1.3.	Your Faculty: Health and Wellbeing		
1.4.	Your status**:**		
		☐ Undergraduate Student	☐ Staff (Professional Services)
		☐ Taught Master	☐ Staff (Academic)
		X Research Degree Student	☐ Other (please specify below)
1.5.	Your university email address**: p.adams-wright.20@unimail.winchester.ac.uk**		
1.6.	Your telephone number**:** 07516297045		
	<u>For students only</u>:		
1.7.	Your degree programme**: MPhil**		
1.8.	Your supervisor's name**: Dr Lisa Riley**		
1.9.	Your supervisor's department**: CAW – Department of Interprofessional Studies**		
1.10.	Your supervisor's email**: Lisa.riley@winchester.ac.uk**		

SECTION 2

YOUR RESEARCH				
2.1.	Project title: *An investigation into why post weaning stereotypical pacing develops in red squirrel kittens at Wildwood Escot and Wildwood Kent.*			
2.2.	Start date**: September 2020**			
2.3.	Expected completion date**: December 2021**			
2.4.	Expected location of data collection: **Zoos and Animal Parks** (e.g. school, workplace, public place, University premises etc.)			
2.5.	Has funding been sought for this research**?**			
		X Yes		☐ No
2.6.	If so, where have you applied for funding? **Student Finance England**			
2.7.	Has the funding been granted?			
		X Yes	☐ No	☐ Pending
2.8.	Is the research collaborative? (e.g. co-investigators from another institution, at or with another organisation or colleagues in another department)			
		☐ Yes		X No
		If yes, which?		
2.9.	Is Disclosure and Barring Service clearance required for your study? It is your responsibility to contact the Disclosure and Barring Service (DBS) to confirm whether or not clearance is needed prior to commencing recruitment or data collection. More information here			
		☐ Yes		X No
2.10.	Will your research be informed by guidelines from a professional association or specific, agreed standards of practice?			
		☐ Yes		X No

SECTION 3

PROJECT DESCRIPTION

Please provide a brief description of your project in non-technical language (between 500-1000 words). This should include details of the research rationale, aim(s), research question(s), context (linking to some relevant literature), and methods (including details of participants, data collection (including examples /descriptions of any audio or visual stimuli to be presented to participants), data analysis) to be used. You should state any ethical issues that you have identified and how these will be dealt with. This overview should contain sufficient information to acquaint the reader with the principal features of the proposal. A copy of the full proposal may be requested if further information is deemed necessary.

Please use this section to list documentation that may be relevant to your application and append it to the submission (e.g. consent forms, information sheets, questionnaires etc.).

Red squirrel (*Sciurus vulgaris*) numbers in the UK have declined by approximately 60% over the past three generations (Mathews & Kubasiewicz 2017) to ~140,000 individuals. The decline is attributed to habitat loss and fragmentation (IUCN, 2019) and the introduction of the grey squirrel (*Sciurus carolinensis*) in 1876 (Holm, 2010), which carries squirrel pox virus and causes high mortality in red squirrels leading to localised extinction (Battersby, 2005; IUCN, 2019). This decline is set to continue with over 50% further loss predicted over the next three generations (Mammal Society, 2019); conservation action is needed to prevent extinction of the wild population by 2040 (White *et. al.*, 2014; Wildlife Trust, 2018; IUCN, 2019).

Several red squirrel breeding and release programmes exist in the UK (Scottish National Heritage, 2014; Forestry Commission, 2018) however stereotypical behaviour (linear and figure of eight pacing) is recorded in captive individuals (Würbel, 2006) and can be an indicator of poor animal welfare (Broom and Fraser 2007; Shepherdson *et. al.*, 1988). The reason for this pacing is often unknown and frequently starts in post weaned red squirrel kittens and could potentially affect their ability for release.

This research is an investigation into why post weaning stereotypical pacing develops and how it manifests in red squirrel kittens at Wildwood Escot and Wildwood Kent and involves behavioural observations of red squirrels at both locations. This research is non-invasive but has involved an intervention (changing the enclosure to provide environmental enrichment with the aim of improving animal welfare as indicated by a reduction in stereotypical pacing).

The main aim of the research is to determine major predictors of the onset of post-weaning stereotypical behaviour in captive red squirrel kittens and to investigate if changes to the squirrels' enclosure can reduce or eliminate stereotypical pacing. Multiple red squirrel kittens have been observed throughout the first year of life to note the development of stereotypical behaviour and how or if it increases or decreases once the red squirrels are moved to different enclosures (as per captive breeding requirements – the population is managed by a Studbook keeper to avoid inbreeding and encourage outbreeding) and any impact on their potential release into the wild.

During observations and discussions with other zoos, several alterations to the breeding pen at Escot have been discussed to try and reduce the pacing, these included putting up boards to potentially decrease stress of predation from outside and increasing substrate and hoarding possibilities by 10 percent. This was completed in three stages, boards put up in January 2020, substrate added in February 2020 and hoarding pots added in March 2020.

I have been observing captive red squirrels for seven years. I have developed this study independently and collected data for the past 15 months (from June 2019-September 2020). I have now registered as an MPhil student and will continue to collect data and all of the data will contribute towards the MPhil.

The method used is approximately 500 hours of observations over several days each month for 18 months between Wildwood Escot and Wildwood Kent, from June 2019 – December 2020. These observations use robust marking sheets to record behaviour via instantaneous sampling at intervals of 2 minutes for state behaviours and ad libitum/all occurrences sampling method for event behaviour to produce monthly activity budgets for each squirrel..As I am using a mixed methods approach, a questionnaire has been designed to send out to the 10 current red squirrel breeding associations (see attached). I would prefer to email this to the participants for them to complete and return to me but as it requires animal records it might be preferable to the respondents to have either an online or physical meeting/ to discuss their experiences and complete the questionnaire with them regarding red squirrel pacing. In the meeting/interview, the interviewer will simply read out the questions on the questionnaire and type the answers for the zoo keeper. I would also like for them to either send me pictures or for myself to take pictures of their different enclosures to compare their findings with those at the two Wildwood sites. . It is important to gain keepers opinions of animal behaviour as it was not possible, during lockdown to collect data directly and the prevalence of pacing in the UK captive population needs to be ascertained. .

This observational research uses quantitative methods to analyse behavioural data collected from marking sheets for individuals to work out individual activity budgets for each squirrel (as the definition of welfare used is 'an individual's perception of how it is doing'). Where normal distribution is not found, non-parametric regression modelling will be used to predict the occurrence of pacing. The questionnaire will generate both quantitative and qualitative data from a series of scale-based and open questions to investigate differences behaviour between collections and determine prevalence and severity of pacing across the target population

There are a three major groups where potential ethical issues need to be addressed, being the red squirrels themselves, the general public and the staff at the different zoos.

The red squirrels could feel stressed by my presence and alter their behaviour. I have not and will not interact with them at all, touch them or feed them nor be involved in any husbandry procedures and all observations are quietly completed in a partly hidden position. All alterations made to enclosures were only temporary so could be immediately removed if they caused the animal's distress. Alterations were completed gradually, over two months, so not to impact on the breeding season and unsettle the animals. Boards were erected outside the enclosure (rather than attached to inner walls of the enclosure) to minimise any stress and observations took place after each alteration to highlight any potential negative reactions. I allowed time for the parent and kitten relationship to develop by not observing too close to the enclosure. Although I recommended changes to the enclosures, all alterations went through the zoo's ethical committee before being put into place

There are a great number of members of the public visiting both zoos and I ensure I keep my interactions with the public to a minimum, so as not to share private information or opinion with them or gain information that was incorrect or erroneous. I do not ask for their thoughts or any personnel information about them and have not or will not share any information given to me in private by the zookeepers or try to influence the public's opinion.

I gained permission from both zoos before completing my observations and submitted a project request which was agreed to by the zoos' ethical committees. I ensure any information is not passed to the public or shared between individual zoos. I have a radio with me at all times and follow all the guidance regarding the zoo's lone working policy. When I visit other zoos, to complete my questionnaire, I will not share any information between other zoos or members of the public, unless I have specifically gained permission from the zoo to do so. I will not take any personal information from zookeepers or member of the public and members of staff will not be individually identified. Zoos will be given a unique number so that I can understand where the data was collected, but the zoos identity will not be shared. I have completed an information sheet and consent form for members of staff involved in the gathering of information (attached) to be completed before my visits and zoos will be able to withdraw from the research at any point with all their information being destroyed.

All information in physical forms and pictures will be locked in a secure environment and kept electronically on a password protected computer and all activities will be conducted in accordance with GDPR.

SECTION 4

REFINING THE LEVEL OF ETHICS REVIEW REQUIRED

Please mark with an ☒ as appropriate		**YES**	**NO**
1	Does the research involve members of the public in a research capacity as co-researchers? (I.e. as in participant research where involvement extends beyond data collection)	☐	**X**
2	Is there a risk of over-disclosure that may put the participants at risk or cause them any anxiety?	☐	**X**
3	Will tissue samples (including blood) be obtained from participants?	☐	**X**
4	Will the study require the co-operation of a gatekeeper for initial access to participants? (E.g. to students at school, to members of self-help group.)	☐	**X**
5	Is the right to withdraw from the study withheld at any time, or not made explicit?	☐	**X**
6	Is there any reason participants may feel obliged to participate in the study against their will?	☐	**X**
8	Will the research involve administrative or secure data that requires permission from the appropriate authorities before use?	☐	**X**
10	Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?	☐	**X**
11	Are there payments to researchers /participants that may have an impact on the objectivity of the research?	☐	**X**
12	Is there any cause for uncertainty as to whether the research will fully comply with the requirements of the General Data Protection Regulation (GDPR) (2018)?	☐	**X**
13	Does any part of the project breach any codes of practice for ethics in place within the organisation in which the research is taking place?	☐	**X**
14	Are drugs, placebos or other substances (e.g. food substances, vitamins) to be administered to the study participants? Please note: for fast track review, it is	☐	**X**

	expected that the study will not involve invasive, intrusive or potentially harmful procedures of any kind.		
15	Is pain or more than mild discomfort likely to result from the study?	☐	**X**
16	Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life? (E.g. involve prolonged or repetitive testing.)	☐	**X**

If you answer YES to *any* of these questions, please use the next section to indicate which question you have said yes to, describe the ethical issue in the context of your study and how you will address it. If you have answered NO to all questions, complete section 6.

SECTION 5

ADDITIONAL INFORMATION AND AMENDMENTS
Use this space to address ethical issues highlighted by the checklist in section 4, or to amend an original submission.

SECTION 6

DECLARATION
I have read and understood the University of Winchester Research Ethics Policy and confirm that adequate safeguards in relation to the ethical issues raised by this research can and will be put in place. I am aware of and understand University procedures regarding Health and Safety. I understand that the ethical aspects of this project may be monitored by the University Research Ethics Committee. I understand my responsibilities as a researcher as described in the University of Winchester Research Ethics Policy. I declare that the answers above accurately describe the research as presently designed and that a new application will be submitted should the research design change in a way which would alter any responses given in Form 1 or here.
X I confirm that if a Risk Assessment is required I will complete it and have it co-signed by my Supervisor or Head of Department before data collection takes place.
X I confirm that, if DBS clearance is required for my project, then I will seek it before the start of my project.
X I confirm that my research does not include risks that might cause it to be excluded from coverage by the University's insurers.
X I confirm that I have appropriate insurance for this research.

Researcher's signature: Pamela Adams-Wright	Date: September 2020
In addition, for **students** (undergraduates, masters, postgraduate, research): The student has the skills to carry out the proposed research. I undertake to monitor the student's adherence to the relevant research guidelines and codes of practice.	
Supervisor's signature: Lisa Riley	Date: 24th Sept 2020

Please submit this form along with Form 1 to your Faculty Head of RKE or nominee (staff /PGR) or your supervisor (taught postgraduate students).

UNIVERSITY OF WINCHESTER

Tel: 01962 841515
Fax: 01962 842280
www.winchester.ac.uk

Winchester
Hampshire
SO22 4NR

Pam Adams-Wright
Faculty of Health & Wellbeing
University of Winchester

10/11/2020

Dear Pam,

Re: An investigation into how and why stereotypical pacing starts and how it manifests in captive post-weaned red squirrels (HWB_REC_20_17_Adams-Wright)

Thank you for returning your updated ethics application to me and rebuttal responses to the reviewer questions. Your answers comprehensively address the queries posed and clearly highlight the changes made to the documentation. I have now reviewed these and I am pleased to confirm that your project has received a favourable opinion.

If the nature, content, location, procedures or personnel of your approved application change, please contact me directly.

I wish you well in taking your project forward.

Best wishes,

Miss Amy Wright
Ethics Lead, Health and Wellbeing
University of Winchester
Email: Amy.Wright@winchester.ac.uk; Tel: +44 (0)1962 624910

APPENDIX II – Risk Assessment

Wildwood Risk Assessment

Activity	**RA Ref**	**Date**
Working with Red Squirrels		17/4/2019

RISK ASSESSMENT GUIDE

Likelihood (L)	**Factor**	**Severity (S)**
Almost Never	1	Minor
Unlikely	2	< 3 days absence
Likely	3	> 3 days absence
Very Likely	4	Major injury
Certain	5	Death

RISK FACTOR (RF) CALCULATION (Likelihood x Severity)

Risk Factor	**Category**	**Action**
1 – 2	Acceptable	Routine long term monitoring
3 – 9	Acceptable	Caution required. Monitor to improve
10 – 15	Marginal	Reduce the risk. Address all safety systems
16 – 20	Undesirable	Stop work immediately

	Risk Pre-Controls					***Risk Post-Controls***		
Hazard	***L***	***S***	***RF***	***Persons at Risk***	***Controls***	***L***	***S***	***RF***
Slips, trips and falls	4	3	12	Staff, volunteers and general public	Leaves must be cleared from board walk regularly Mesh fitted to majority of the boardwalk to provide slip resistant surfaces Mesh checked regularly for damage Suitable footwear to be worn at all times by staff and volunteers Always take care and pay attention when moving around the enclosure, especially in wet and icy conditions Mud and ice/snow cleared away as necessary General public restricted to the boardwalk Always leave service areas clean and clear of equipment Tidy equipment as you go, do not leave equipment lying around after use	2	2	4

					Be aware of surface conditions before proceeding			
Animal bites and scratches	3	3	9	Staff, volunteers and general public	All staff to be informed of the capabilities of the animals Be aware of the location of the animals when working in the enclosure Squirrels only fed by or under the supervision and guidance of a keeper or volunteers may feed the squirrels on their own once they have undergone sufficient training and under instruction of an experienced keeper Do not reach hands out to the squirrels unless you are feeding them and be careful of fingers when feeding the squirrels Make no attempt to touch (unless for medical or management purposes by an experienced keeper) or grab the squirrels Long trousers must be worn at all times Upon training a safety briefing must be given to all staff and volunteers before entering any part of the enclosure Squirrel behaviour to be observed and acted upon accordingly Tend to any injuries immediately Wash hands thoroughly after working in the enclosure	1	2	2
Contact with hazardous animal by-products	3	3	9	Staff, volunteers and general public	Wash hands thoroughly after working in the enclosure When cleaning the water bowls, ensure the water does not come into contact with your eyes and mouth Suitable gloves to be worn when cleaning Handrail, door handles and other ‘contact’ surfaces cleaned regularly to remove faeces/urine. Hand sanitiser and signs recommending use provided at exit points	1	2	2
Electricity	3	4	12	Staff, volunteers and general public	Ensure you have dry hands when using the sockets and ensure it is fully covered after and during use Ensure to keep all electrical equipment at a safe distance from water Cover any electrical equipment from the rain Electric fence fitted at a suitable height to be out of reach of children. ‘Warning electric fence’ signs fitted at regular intervals	2	3	6

Visitor experience sessions	3	2	6	Staff, volunteers and general public	Participants to be supervised at all times. One keeper to a maximum of two members of the public when off the boardwalk. No participants under the age of 8 years Only food provided by the keepers to be fed All feeding under guidance/supervision of a keeper Safety briefing given to all participants before starting the session. Squirrel behaviour observed and acted upon accordingly – e.g. leave if the animals become stressed or aggressive	2	2	4
Training	3	2	6	Staff and volunteers	Caution with hands must be taken when feeding the squirrels, especially with multiple squirrels around Observe the behaviour of the squirrels and act accordingly All training equipment should be removed after use, cleaned and disinfected	2	2	4

Name of Assessor: Updated for Wildwood Escot by Senior Keeper Kerry Church – April 2019

Date of Next Review: April 2020

APPENDIX III – Example Marking Sheet

Date		time		weather						
Activity	**scription TIME**	2	4	6	8	10	12	14	16	18	20
STATE	chasing										
social	allogrooming										
	conflict										
	play										
	scent marking										
	touching										
	vocalisation										
feeding	foraging										
	ingestion										
	manipulation										
	drinking										
	bark stripping										
	caching food										
locomotion	walking										
arboreal	running										
	jumping										
	climbing										
ground level	walking										
	running										
	jumping										
Drey Building	collecting foliage/ materials										
	carrying foliage										
	using foliage to build drey										
	manipulating foliage drey										
Other	Grooming										
inactivity	resting										
	vigilent										
	sleeping										
stereotypical	pacing										
	rocking										
	stepping from side to side										
unseen	out of sight										

APPENDIX IV

Letter of Consent from George Hyde – General Manager at Wildwood Escot to share information and results from observations as part of MPhil research.

WILDWOOD ESCOT

Escot Park
Ottery St Mary, EX11 1LU
01404 822188
escot@wildwoodescot.org
www.wildwoodescot.org

23rd September 2020

Dear Pam,

Re: MPhil research project findings

Further to your recent submission, I am happy to grant permission for you to share the results of your observations on our red squirrels conducted from June 2019 to December 2020, as necessary for the requirements of your course.

Kind regards,

George Hyde
General Manager
Wildwood Escot

Wildwood Escot is a trading name of Wildwood Escot Ltd, a subsidiary of The Wildwood Trust, a registered charity: No 1093702

Wildwood Escot Breeding Enclosure, Old and New Side Before and after Alterations

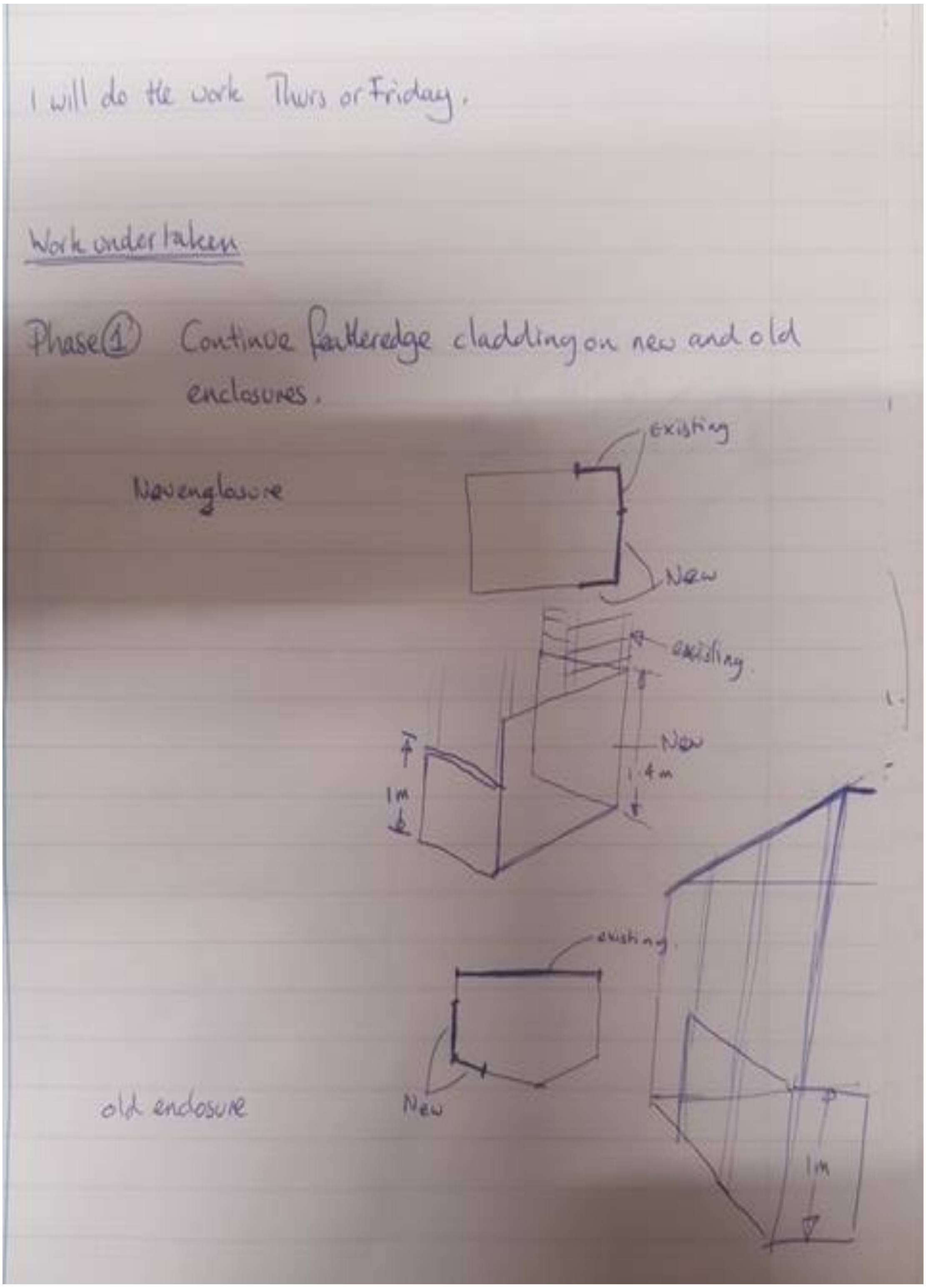

Plate 1 – Carpenter’s drawings of alterations to be made to the breeding enclosure

Plate 2 - New and old side with connecting run before alterations

Plate 3 - New and old side with connecting run before alterations

Plate 4 - Wildwood, Escot Breeding Enclosure – Outside the old side before alterations

Plate 5 - Wildwood, Escot Breeding Enclosure – Inside the old side before alterations

Plate 6 - Wildwood Escot Breeding Enclosure –Outside the new side before alterations

Plate 7 - Wildwood Escot Breeding Enclosure –Inside the new side before alterations

Plate 8 - Wildwood, Escot Breeding Enclosure – Outside the old side after alterations

Plate 9 - Wildwood, Escot Breeding Enclosure – Inside the old side after alterations

Plate10 - Wildwood, Escot Breeding Enclosure – Outside the new side after alterations

Plate 11 - Wildwood, Escot Breeding Enclosure – Inside the new side after alterations

Plate 12 - Hoarding container along the back of the old side of the Breeding Enclosure

Plate 13 - Hoarding pot at the front on the new side of the Breeding Enclosure

Plate 14 - Red squirrels using hoarding containers at the back of the new side

APPENDIX V - **Pilot Study for Data Collection**

Aim and Rational: to design robust, appropriate ethograms for data collection

Subjects: Autumn and Radish

Behavioural Sampling for Ethograms

Initially preliminary observations using ad libitum observations were used in May 2019 to ascertain the exact different types of behaviour the red squirrels engaged in. The squirrel in the breeding enclosure were watching for 7 hours and all behaviour displayed was noted down. Although ad libitum sampling is criticised because only conspicuous behaviour is often recorded and brief behaviour can be missed, it is considered a useful tool during preliminary observations to record different behavioural repertoires, including potentially rare or often unseen behaviour (Bernstein 1991, Martin and Bateson 2007). Understanding these limitations, extra rows were inserted into the marking sheets so other behaviour noted during primary data collection could be added to the ethograms/marking sheets if required (Martin and Bateson 2007).

Two different types of sampling were then considered for the data collection, continuous focal sampling and instantaneous scan sampling in order to understand the most effective means to observe and record behaviour of red squirrels for this study. Continuous focal sampling (Altman, 1974) was used in Palmer and Koprowski (2014) research on feeding behaviour and activity patterns of Amazon red squirrels (*Sciurus spadiceus*). This type of sampling focusses on one animal at a time, recording all the behaviours that an individual displays, with each behaviour being timed from start to finish. This method works well in dense forests where red squirrel sightings are rare, however there are limitations with this method as when the animal goes out of sight the sampling must stop. This can create a bias as animals often produce certain behaviour, such as grooming or caching when out of sight, so this type of sampling does not represent behaviour as a whole (Martin and Bateson, 2007). Instantaneous scan sampling was used by Wauters et al. (1992) in their study into activity budgets and foraging behaviour of red squirrels in coniferous forests. This method is more effective when observing multiple squirrels as behaviour can be recorded in timed intervals to devise an activity budget for each individual squirrel. Time can be allocated to each behaviour and then expressed as a percentage of total observed behaviour for individual squirrels each month, or to compare each squirrel behaviour against other conspecifics in the observed group.

The limitation of Instantaneous scan sampling is that it does restrict observations to recording one behaviour at a time, however scan sampling does allow the observer to obtain data that is evenly representative when recording individual behaviour at different times of the day and over different seasons so that behavioural comparisons can be drawn between different squirrels (Ruiter 1986, Martin and Bateson, 2007).

Pilot Questionnare

A pilot questionnaire was designed in August 2020 after 16 months of observations so that the observer could clearly understand the type of data required from different establishments in the field. A, highly structured questionnaire was used to collect data expressed as numbers (Easterby-Smith et al, 2008) as the results are measurable against other variables and directly comparable as number-based data can be directly compared whereas word-based date cannot (Saunders et al, 2007). This was sent to Wildwood Escot for their feedback and permission was granted by the Escot to share the research findings with other zoos.

The structure was changed to prevent ambiguity in certain questions and more multiple-choice questions added, particularly in questions 18 and 19 regarding why zoos were involved in captive breeding and rating limitations with release opportunities using a Likert scale. Open-ended questions were added to allow the respondent an opportunity to build on their answers and add additional, valuable information the observer may not be aware of. For example, why keepers felt pacing started and anything they had done to try and reduce it. An information sheet and consent form were agreed with by the ethical committee at Winchester University and by Wildwood Trusts (Appendix VI).

APPENDIX VI– Master Questionnaire, Information Sheet and Consent Form

CAPTIVE RED SQUIRREL QUESTIONNAIRE

1) Please complete this table stating the different captive red squirrels you have, or have had from 1st January 2020, what ages they are, if you still have them and from where they originate?

Squirrel Name	Sex	DoB	Age you got them	Do you still have them? If not can you confirm the reason, ie if they have died or moved elsewhere	Origin including the type of enclosure they were in (Free roaming/breeding pen)

2) Are you currently breeding your red squirrels or have you done so in the past?

Yes - Currently

Yes – In the Past

No – Please move to question …

3) What age do you breed your squirrels from and what age do you retire them?

Gender	Age at the start of breeding	Age when breeding ends
Males		
Females		

4) What age do your kittens move out from their parents?……

5) Could you complete the table below regarding mortality over the past 3 years,

Name of Parents		2020 (1st Litter)	2020 (2nd Litter)	2019 (1st Litter)	2019 (2nd Litter)	2018 (1st Litter)	2018 (2nd Litter)
	No of Kittens in Litter						
	No of Kittens that survived						
	No of Kittens in Litter						
	No of Kittens that survived						
	No of Kittens in Litter						
	No of Kittens that survived						
	No of Kittens in Litter						
	No of Kittens that survived						

6) Could you tell me a little more about your enclosure/s

ENCLOSURE 1

Type of Enclosure (Free Roaming/Breeding Pen/Other)	
Open to the Public Y/N?	
Is the Enclosure Used for Breeding?	
Squirrel Number Composition, Adult ID and number of kittens	
Approx Floor Area, Height and Width	
How is the enclosure constructed (Netting, Roof, Solid walls or coverage to the sides or roof?	
What type of substrate do you use?	
Types of nests (Nest Boxes, Drey Building Opps)	
Climbing Options (Trees, Branches, Artificial Structures)	
Foraging Options Numbers/ Types (Feeding Stations, Trees, Natural Foraging etc)	
Types of Enrichment added	
Indoor Provision (Describe design, size, resources and access	

ENCLOSURE 2

Type of Enclosure (Free Roaming/Breeding Pen/Other)	
Open to the Public Y/N?	
Is the Enclosure Used for Breeding?	
Squirrel Number Composition, Adult ID and number of kittens	
Approx Floor Area, Height and Width	
How is the enclosure constructed (Netting, Roof, Solid walls or coverage to the sides or roof?	
What type of substrate do you use?	
Types of nests (Nest Boxes, Drey Building Opps)	
Climbing Options (Trees, Branches, Artificial Structures)	
Foraging Options Numbers/ Types (Feeding Stations, Trees, Natural Foraging etc)	
Types of Enrichment added	
Indoor Provision (Describe design, size, resources and access	

7) Stereotypical pacing is described as apparently functionless behaviour that serves no obvious purpose and often takes the form of figure of eight or apex pacing, bouncing, somersaulting or weaving. Please could you give as much information as possible regarding any stereotypical pacing you have witnessed in your adult red squirrels

Name of Squirrel	Type of Enclosure, eg free roaming/ breeding pen	Does this squirrel pace?	Frequency of Pacing? (Every Day/Most Days/Seasonal?) At specific times in the year (4) Monthly (3) Weekly (2) Daily (1)	Do they pace all day or just part of the day? Do they pace All day (4) Most of the day (4) About half the day (3) Less than half the day (2) Only at an isolated time (when?) (1)	Do they pace for hours at a time or just minutes? Many hours (5) A few hours (4) An hour (3) Less than an hour (2) A few minutes (1)	When did this start? E.g. before they came to you, from emergence? Or did they use to pace and now stopped?	Describe the pacing. Figure of eight, spinning, only with food?

8) Please could you give as much information as possible regarding any stereotypical pacing you have witnessed in your kittens – answer this question with a typical kitten in mind . If there was more than one litter per year, please add an extra row.

Year of Litter	Name of Parents	How many of this litter pace? All (4) Most (3) A few (2) One (1) individual (who?)	Age pacing started and current age in years and months	Frequency of Pacing? (Every Day/Most Days/Seasonal)? At specific times in the year (4) Monthly (3) Weekly (2) Daily (1)	Do they pace all day or just part of the day? All day (5) Most of the day (4) About half the day (3) Less than half the day (2) Only at an isolated time (when?) (1)	Do they pace for hour at a time or just minutes? Many hours (5) A few hours (4) An hour (3) Less than an hour (2) A few minutes (1)	Describe the pacing. Figure of eight, spinning, only with food?	Do you know where they moved to and if they continue to pace?
2018								
2019								
2020								

9) Does this pacing increase or decrease with seasonal variation? **YES/NO**

If Yes - In which months does it increase?...................... If Yes - In which months does it decrease?...

10) Have you noticed any impact on the behaviour of the parents in the breeding pen when the kittens are removed? **YES/NO.**

If yes please describe…….

11) Have you noticed any impact on the behaviour of the other squirrels in the enclosure the kittens move to? **YES/NO**.

If yes please describe…….

12) Is your male housed with the female all the time or separated after kittens born? **YES/NO**

13) Have you taken action to try and reduce this pacing behaviour? **YES/NO**.

If Yes Please elaborate and state any success you have had……

14) Do you believe visitor numbers impact on red squirrel pacing/behaviour? **YES/NO**.

If Yes please elaborate………

15) Did the COVID-19 shutdown had an impact on red squirrel pacing/behaviour? **YES/NO**.

If Yes please elaborate………

16) What do you believe is the cause of red squirrel adult and/or kittens pacing in captivity? ……………………………………………………………………………………

17) Are you currently part of a reintroduction into the wild programme **YES/NO**

If Yes, what organisation are you linked with and where was the release site?...

If No have you done so in the past? **YES/NO**

If Yes, what organisation were you linked with and where was the release site?

………

How many squirrels have you successfully reintroduced?

Age of Squirrel	Sex of Squirrel	Date of release	Release Site	Was the squirrel tracked?	Is the squirrel still alive?	Is it still in the same location?

18) Please rate the following statements, 5 being strongly agree and 1 being do not agree at all.

Our breeding programme is primarily for reintroduction into the wild

Our breeding programme is primarily for captive conservation and education of the public

Our breeding programme would like to reintroduce squirrels into the wild but there are limitations in us doing this

Our breeding programme would not feel that animals displaying stereotypical behaviour would be suitable for release.

19) What do you feel are the factors causing limitations for release into the wild?

a) No suitable release sites.
b) Too large a population in established release sites to allow anymore reintroductions.
c) Prevalence of disease in known wild populations.
d) Not enough captive red squirrels to release.
e) Not enough support for reintroductions.
f) Other

20) Is there anything more you would like to add to that may be of help to me? ………..

Ethics Consent Form

UNIVERSITY OF WINCHESTER

Consent for Participation in Interview Research

Study Title: An Investigation into Why Post-weaning Stereotypical Pacing Develops and How it Manifests in Captive Red Squirrels.

I volunteer to participate in the above Research Project conducted by Mrs Pamela Adams-Wright. Please read the statements below and sign at the bottom of the form.

1) My participation in this research and voluntary and I can withdraw at any time up to 31st January 2021. After this time, I know I am unable to withdraw as the data I will have supplied will be included in analysis.
2) I have had a chance to discuss any issues I have with the researcher.
3) I have read the Participant Information Sheet and understand I will be one of approximately ten other captive red squirrel breeders/carers completing a questionnaire.
4) I can either choose to complete the questionnaire myself or the researcher can set up a physically meeting or virtually on Microsoft Teams so that the researcher can simply go through the questions with me and type in my answers.
5) I understand that the questionnaire takes around one hour to complete.
6) I have a right to decline to answer any question on the questionnaire.
7) If I choose to meet with the researcher, I agree to notes being taken during the meeting.
8) I understand the researcher will not personally identify either the animals, myself or the organisation, all data collected will be shared with anonymity so will not be identifiable to a specific person or organisation.
9) I agree to photographs being taken of the enclosure and animals and I understand that these may be included in reports and publications.
10) I understand how my data will be collected and stored.
11) I understand that I will not receive personal feedback or results from this study.

I agree to take part in this research and give me permission for the data to be used for research and teaching purposes. and associated publications and presentations.

---	--
My signature	Date
---	--
My printed Name	Name of Zoo

RESEARCH INFORMATION SHEET

UNIVERSITY OF WINCHESTER

Participation Information Sheet

Study Title: An Investigation into Why Post-weaning Stereotypical Pacing Develops and How it Manifests in Captive Red Squirrels – Research Degree

Supervisory Team: Dr Lisa Riley (Lisa.riley@winchester.ac.uk)

Dr Dave Raper (David.raper@winchester.ac.uk)

Dr Colin Goble (Colin.goble@winchester.ac.uk)

This study has been approved by the Faculty of Health and Wellbeing at the University of Winchester. This sheet gives you detailed information regarding the study and why you have been asked to take part in this research. Please read this information before signing the consent form, if you have any questions do not hesitate to contact me.

Purpose of Study

Red squirrel numbers in the UK have declined by approximately 60% over the past three generations to 140,000 individuals. This decline is set to continue with over 50% further loss predicted over the next three generations conservation action is needed to prevent extinction of the wild population by 2040 and the red squirrel has now been listed as endangered on the Mammal Society Red List. Conservation of the species is critical, and several red squirrel breeding and release programmes exist in the UK, however stereotypical behaviour (linear and figure of eight pacing) is recorded in captive individuals and can be an indicator of reduced animal welfare. The reason for this pacing is often unknown but frequently starts in post weaned red squirrel kittens and could potentially affect their ability for release. This research is an investigation into why post weaning stereotypical pacing develops and how it manifests in red squirrel kittens and whether anything can be done to reduce it.

Why has my organisation been chosen and do we/I have to take part?

You have been chosen as one of approximately ten different red squirrel breeding establishments. Participation is totally voluntary, and this information sheet will give you detailed information about the study so you can decide whether to take part. You will need to sign an attached consent form, but you are able to withdraw from the study at any time up to 31st January 2021. After this date, the data will be analysed so you will not be able to withdraw.

What is required from me/my organisation in order to take part in the study?

I would like to send you a questionnaire to complete (taking approximately 1 hour). Alternatively as I will require information from animal records I am happy to either visit your zoo/institution or set up a Microsoft Teams meeting to run through the questions with you and type in your answers for you, taking additional notes as we go through. I will need to gather breeding information from three years 2018/19/20.. This information will be related to the age of your squirrels, how many litters they have had, mortality rates, where any kittens have moved to and any stereotypical behaviour you have noticed? Alternatively, your organisation can share ZIMS data or other animal records with me. I would also like to be able to have pictures of your enclosures. During the COVID-19 pandemic it may not be possible for me to conduct the meeting in person, in this case I can send the questionnaire to you and ask you to send me photographs and conduct an on-line meeting at a time convenient with yourself.

How will my/ my organisation's data be stored and my/ my organisation's privacy protected?

You will not be personally identified in the study. Your organisation will not be named but will be given an allocated number to make it clear for me to understand where the data relates to. Individual squirrels within your organisation will not be identified and given an alphabetised letter to ensure no-one recognises your establishment by the squirrel's names. All information in physical forms and pictures will be locked in a secure environment and kept electronically on a password protected computer and all activities will be conducted in accordance with GDPR. Any information given will not be passed onto any other collections or members of the public without your written consent. The information given may be used in the final MPhil dissertation, presentation or peer reviewed papers.

What are the possible risks of taking part?

There are no particular risks foreseen in your participation of the study.

What is the desired outcome for the collection of this data?

I have been studying captive red squirrel behaviour for over seven years and observed the prevalence of stereotypical pacing in many collections. I hope that by collecting this data and using my many years of experience, including five hundred hours of behavioural observations I have carried out over the past eighteen months of several different collections, I will be able to produce my MPhil dissertations, peer-reviewed papers and conference presentations in order to devise husbandry guidelines to potentially reduce stereotypical pacing in captive red squirrels.

How to voice any concerns?

Please feel free to contact me at anytime, Mrs Pamela Adams-Wright, p.adams-wright.20@unimail.winchester.ac.uk. If you remain unhappy and wish to complain formally please contact the Amy Wright Amy.Wright@winchester.ac.uk

Privacy Notice for Research Participants

The University of Winchester treats very seriously both the personal data and the sensitive personal data it processes on behalf of primarily its students and staff members, and also a wide range of other people who it works with and has contact with, including alumni.

The University has been and is continuing to work hard to comply fully with the new General Data Protection Regulation (GDPR) which is enforceable from 25 May 2018. The GDPR makes a number of key changes to data protection law in the United Kingdom and within the European Union (EU) and potentially beyond the EU. More information on these changes, which include strengthening of some individual rights and some new individual rights can be found on The Information Commissioners' Office (ICO) website at: https://ico.org.uk/

Research at the University of Winchester also conforms to GDPR. The University processes both personal data and sensitive personal data under a range of different 'lawful bases' depending on the nature of the respective 'processing purposes'. For the 'personal data' of Research Participants, the 'lawful basis' here is 'Consent'.

In addition, before we collect your personal data, we will always:-

i) provide you with information about the study in advance
ii) tell you how and why we will use your data
iii) tell you who will have access to your data
iv) tell you how long your data will be stored for
v) ask for signed written consent to collect your data (via a consent form).

We will only ask for personal data which is necessary for our stated research purposes and we will never sell your personal data to any 3rd party.

The 'purposes of the processing' for research include:

- Retaining proof of written consent to participate in our research.
- To manage your appointment(s) within the study; during initial sign up for the study and, subsequently, where there arose a need to cancel or re-arrange an already agreed time-slot.
- For the purposes of testing research predictions (Hypotheses) in order to answer the research question(s) as described on the study Participant Information sheet

Linked to its above mentioned 'processing purposes', the University processes a specific amount of participants' personal data, which includes, for example: A participants name; student number, email address; demographic information; visual and/or audio data; questionnaire responses.

Personal data will be kept for no longer than necessary, and these decisions will be made in line with legal requirements, the relevant University policies and in light of relevant best practices.

There are a number of individual rights available, and more information on these can be found at https://ico.org.uk/ In particular, as 'consent' is being used here as the lawful basis for the particular 'processing purpose' for Research Participants, there is a right to withdraw consent (if applicable). If you wish to withdraw your consent for the processing of your personal data for Research Participants, you should in the first instance, contact the researcher(s) named on the Participant information sheet.

Even after signing a consent form, you still have the right to withdraw your consent, at any time during your research participation and for a specified period of time after participating in a study. The time period for withdrawing varies between studies, but will always be stated on the information sheet and/or the consent form.

All research projects at the University of Winchester are reviewed and approved by either a Departmental Research Ethics committee or the University Research Ethics committee.

There is a right to lodge a complaint with a supervisory authority. This is the ICO, who can be contacted in various ways as listed at: https://ico.org.uk/global/contact-us/

We review and update (where necessary) this policy statement in line with current guidance and developments.

The data protection officer for the University is:

Joseph Dilger, Data Protection Officer,

The University of Winchester, Sparkford Road
Winchester, Hampshire.
SO22 4NR.

United Kingdom.

Tel: +44 (0) 1962 841515, Ext. 7306.

Email: Joseph.Dilger@winchester.ac.uk

The name and contact details of our organisation are:

The University of Winchester,

Sparkford Road
Winchester, Hampshire.
SO22 4NR.United Kingdom.

Tel: +44 (0) 1962 841515

Fax: +44 (0) 1962 842

APPENDIX VII – Details for Regression Statistics from Questionnaires

Survival Rate	Zoo	Open to Public	Compostition	Enclosure Size	Design	Feeding	Do Adults/parents pace	Kittens pace	Frequency	Times of Day	Hours or Minutes
100% success rate	3	2 not open	1(m) +2(f) main 1x1(m)+1(f) satelite	5m*5m*6m high	3 (2 main interconnected/ 1 satellite). Welded heavy durty mesh on all sides, 1cm by 5cm with boards and cover Substrate of soil, bark and leaf litter, numerous perching at all levels and xmas trees used for climbing and cover Multiple nest boxes, build drey but don't use for breeding	Two feeding stations, nuts, seeds, fruit, seasonally fed ie beechmast given. Fresh browse, seasonal feeding, antlers, animal hair,	No but female rarely paced when maintenace carried out or when disturbed	Not all kittens pace, only a few from each litter, linked with dispersal	Seasonally	Isolated times	Few minutes
91% success rate	7	Yes (only for 1 hour a day through guided tours)	1(m) + 1(f)	9m*7m*4m high and 130msq catchup	L shape, cover from wall along the bac, small wall approx 50 cm high from the floor all around the edges and front of enclosure, quarter inch mesh and wooden frame.Bark chip and two areas just soil and dirt, range of leaf and twigs. Have climbing branches, log piles, sand pit and nest boxes	Two feeds, varied seasonal diet, given rodent mix and ferret food via feeding staitions, and scatter fed. Given live food. Fresh browse, antlers,	No Only one female paced jumpinig back and forward along side of enclosure by the door, only happened before food, particularly live food	Kittens started pacing at 6 months if not moved/ dispersed	seasonally	Isolated times	Few minutes
83% success rate	1	Yes	1(f) + 1(m)	Old Side 26.2msq 7.4*m 3.8m* 4.1m high New side, 14.6msq 6.2*2.5m *4.3m high	Old Side 26.2m2, 7.4 m long 3.8m wide 2.3m high with sloped roof to 4.1m high New side, 14.6m2. 6.2m long, 2.5m wide, 4.3m high with tunnel interconnecting both sides Fully meshed sides, small roof on half of new side and roof over back of old enclosure, double entry doorways. Cover from 1m boards around the ends of each side. Bark, leaves, soil and natural plants ropes and branches. Four nest boxes, two on each side, given wool, hay , straw, thistle to build dreys	Sevveral feeding places on branches, logs Fed twice a day with parrot food, nuts, seeds, fruit and veg.	Yes Male paced upto 40% of observed time mostly with food before alterations to cage, then stopped. Female paces about 10% of observed time during hoarding season.	2018 and 2019 litters started pacing at 10 weeks, 2020 litter after alterations only started pacing at 4 months of age.	before daily, after seasonally	Before more than half a day, after isolated times	Before for many hours and after a few minutes
75% success rate	6	Yes	1(f) + 1(m) 3(f)	1x 40msq 8m*5m*5m high and 1x 30msq 6m*5m*5m high	Cover by wall at the back, and 50cm wall around the edge and tree and branches Mesh fitted around. Nest boxes given and deep bark substrate. Also have ropes.	Fed once a day via two feeding stations. Given antlers and hawthorn.	No - Squirrels pace very very rarely (Figure of 8)	12-14 weeks start to pace due to dispersal and stop when moved	Daily from 10 weeks then stops when moved	Isolated times	Few minutes
67% success rate	3	No	1(m) + 1(f) 6 (m) in one aviary	not given	Cover by 1m wooden panels at the front of the cage, Mesh fenching and tin roof. Branches and rope given for climbing bark substrate. Have nest boxes but giving build their own dreys	3 metal bird feeder	All squirrels pace	pacing in a line against the fence.	Weekly, wo	Isolated times	Few minutes
59% success rate	4	Yes	2 x 1(f) 1x 1(m)+1(f)	8m*6m*3.5m high	All three enclosures are joined and can be opened and closed as required and two have adjoining sheds. Wooden structure with 1 inch weldmesh and 2 build around a tree. Concrete foundation (No cover) Natural substrate, bark ship and sandNatural climbing in trees and holly and laurel planting, branches and ropes, Nest boxes and given materials to make dreys.	Natural foraging including cob nuts, branches and cones. Fed on stumps and feeding stations.	All squirrels pace	Long repetive runs, often with food in mouth, over door	Daily (worse	Isolated times	Few minutes
50% success rate	2	No	2X 2(m)	4m*3m*3m high	Quarter inch mesh and wooden frame, no roof or sold walls, brick foundation (no coverage) Woodchip on top soil, branches and ropes Two nest boxes in each enclosure shelves for drey building	Feeding station, logs and stumps, fed twice a day, dry feed mix, maize, sunflower seeds, fruit and veg. Seasonal, pumpkins, different trees	Pacing observed by the male with food in his mouth, next the the opening of the door taking up 30% of observed time. (note: he was then moved to the walkthrough and only paced when shut in holding pen, or very rarely outside the edge of the pen	Kittens from 2017 and 2018 litter start pacing 14-16 weeks	Daily	more than half the day	many hours

Squirrel	1-Adult 2 - Juvenile	1 - Male 2 – Female	1- Walthrough 2- Breeding Enclosure 3 - Other	1-<100m3 2- 100m3-200m3 3->200m3	Enclosure Complexity	Feeding Complexity	Substrate 1 - Concrete 2- Leaf 3 -Bark 4 Both 5-Sand 6 - All three	Cover 1-None 2-Natural 3-Man 4-Both	climbing 1 - none 2 - natural 3 - man 4 - both	Enrichment 1 - None 2 - Daily 3 - Weekly 4 - Monthly	Number of Nest boxes	Drey Bldng 1 - no 2- yes	Seasonal 1 - no 2 - yes	Variety 1 - basic 2 – complex	Timings how often fed	Presentation 1-feeding stations 2-scatter 3-both	Pace 1 =Yes 2=No
1	1	2	1	3	21	9	4	4	4	3	4	2	2	2	2	3	2
2	1	2	1	3	21	9	4	4	4	3	4	2	2	2	2	3	2
3	1	2	1	3	21	9	4	4	4	3	4	2	2	2	2	3	2
4	1	2	1	3	21	9	4	4	4	3	4	2	2	2	2	3	1
5	1	2	1	2	21	9	4	4	4	3	4	2	2	2	2	3	1
6	1	2	2	2	21	7	4	4	4	3	4	2	1	1	2	3	1
7	1	1	2	2	21	7	4	4	4	3	4	2	1	1	2	3	1
8	2	2	2	2	21	7	4	4	4	3	4	2	1	1	2	3	1
9	2	2	2	2	21	7	4	4	4	3	4	2	1	1	2	3	1
10	2	1	2	2	21	7	4	4	4	3	4	2	1	1	2	3	1
11	2	1	2	2	21	7	4	4	4	3	4	2	1	1	2	3	1
12	2	1	2	2	21	7	4	4	4	3	4	2	1	1	2	3	1
13	2	1	2	2	21	7	4	4	4	3	4	2	1	1	2	3	1
14	1	1	1	3	22	9	4	3	4	4	5	2	2	2	2	3	2
15	1	1	1	3	22	9	4	3	4	4	5	2	2	2	2	3	2
16	1	1	1	3	22	9	4	3	4	4	5	2	2	2	2	3	2
17	1	1	1	1	16	7	3	1	4	4	2	2	1	1	2	3	1
18	2	1	2	1	16	7	3	1	4	4	2	2	1	1	2	3	1
19	1	1	2	1	16	7	3	1	4	4	2	2	1	1	2	3	1
20	1	2	2	1	16	7	3	1	4	4	2	2	1	1	2	3	1
21	1	2	2	1	16	7	3	1	4	4	2	2	1	1	2	3	1
22	2	2	2	1	16	7	3	1	4	4	2	2	1	1	2	3	1
23	1	2	2	2	21	9	2	4	4	3	6	2	2	2	2	3	1
24	1	2	2	2	21	9	2	4	4	3	6	2	2	2	2	3	1
25	2	1	2	2	21	9	2	4	4	3	6	2	2	2	2	3	2
26	2	1	2	2	21	9	2	4	4	3	6	2	2	2	2	3	2
27	2	1	2	2	21	9	2	4	4	3	6	2	2	2	2	3	2
28	1	1	2	3	21	8	6	2	4	3	4	2	1	2	2	3	1
29	1	1	2	3	21	8	6	2	4	3	4	2	1	2	2	3	1
30	2	2	2	3	21	8	6	2	4	3	4	2	1	2	2	3	1
31	1	2	2	3	21	8	6	2	4	3	4	2	1	2	2	3	2
32	2	2	2	3	21	8	6	2	4	3	4	2	1	2	2	3	2
33	2	2	2	3	21	8	6	2	4	3	4	2	1	2	2	3	2
34	2	3	2	3	21	8	6	2	4	3	4	2	1	2	2	3	2
35	2	3	2	3	21	8	6	2	4	3	4	2	1	2	2	3	2
36	1	1	2	1	18	4	3	2	4	3	4	2	1	1	1	1	2
37	2	2	2	1	18	4	3	2	4	3	4	2	1	1	1	1	1
38	2	1	2	1	18	4	3	2	4	3	4	2	1	1	1	1	1
39	2	1	2	1	18	4	3	2	4	3	4	2	1	1	1	1	1
40	2	1	2	1	18	4	3	2	4	3	4	2	1	1	1	1	1
41	2	1	2	1	18	4	3	2	4	3	4	2	1	1	1	1	1
42	2	1	2	1	18	4	3	2	4	3	4	2	1	1	1	1	1
43	2	1	2	1	18	4	3	2	4	3	4	2	1	1	1	1	1
44	2	1	2	3	21	9	4	4	4	3	4	2	2	2	2	3	1
45	2	2	2	3	21	9	4	4	4	3	4	2	2	2	2	3	1
46	1	1	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
47	2	1	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
48	2	1	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
49	2	1	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
50	2	1	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
51	2	1	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2

52	2	2	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
53	2	2	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
54	2	2	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
55	2	2	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2
56	2	2	2	3	21	9	4	4	4	3	4	2	2	2	2	3	2

Number of Squirrels Pacing, Enclosure Type and Feeding Information. Zoo identification - 1-7, gender - m:f:j. Pacing squirrels – number of squirrels pacing/total number of squirrels and percentage. (Enclosure) Size - smaller than 100m^3 -1, larger than 100m^3 but smaller than 200m^3 - 2, larger than 200m^3 - 3. Substrate, concrete - 1, leaves/ leaf litter – 2, woodchip/barkchippings - 3, both leaves and woodchip - 4, sand only - 5, leaves, woodchip and sand - 6. Cover, no cover -1, natural cover/trees and branches - 2, artificial solid cover/walls and boards - 3, natural and artificial - 4. Climbing, no climbing opportunities -1, artificial/ropes only - 2, natural/branches and trees - 3, both natural and artificial – 4. Enrichment, none given - 1, daily - 2, weekly - 3, monthly - 4, Nest boxes, number given 1-6. Drey building opportunities given, no - 1, yes - 2. (Feeding), seasonal, no - 1, yes - 2. Variety, basic seeds, nuts, fruit and vet – 1, complex, cob nuts, hawthorne, browse and antler - 2. Timings, once a day - 1, twice a day - 2. Presentation, in feeding stations - 1, scatter fed/hidden in logs and enrichment -2, both - 3.

Zoo	No of pacing squirrels	Size	Design						Feeding			
			Substrate type	Cover	climbing	Enrich	Nest boxes	Drey Bldng	Seasonal	Variety	Timings	Presentation
1	10/13	2	4	4	4	3	4	2	1	1	2	3
2	6/9	1	3	1	4	4	2	2	1	1	2	3
3	2/5	2	2	3	4	3	6	2	2	2	2	3
4	3/8	2	6	2	4	3	4	2	1	2	2	3
5	7/9	-	3	2	4	3	4	2	1	1	1	1
6	2/13	3	4	4	4	3	4	2	1	2	2	3
7	n/a	2	6	4	4	3	4	2	2	2	2	3

APPENDIX VIII – Observational Data

Behaviour for 2020 Litter	June'19	July'19	Aug'19	Sept'19 HP	Released	Oct'19	Nov'19	Jan'20	Feb'20	March'20
Douglas Social	0.00	6.17	6.51	23.79	5.74	10.87	13.94	15.38	12.75	0.00
Thistle Social	8.05	8.09	13.83	7.97	11.25	19.81	0.00	13.73	0	0
Blossom Social	9.09	5.02	5.18	7.62	11.29	10.99	4.26	15.64	5.71	6.47
Average Social	5.71	6.43	8.51	13.13	9.43	13.89	6.07	14.92	9.23	2.16
Douglas Feeding	19.48	48.15	26.82	21.84	14.35	35.87	60.10	35.38	22.55	
Thistle Feeding	40.23	43.93	30.04	17.61	32.50	45.28	34.31			
Blossom Feeding	32.32	40.54	24.87	22.54	34.41	60.99	59.57	40.78	52.00	45.32
Average Feeding	30.68	44.21	27.24	20.66	27.09	47.38	51.33	38.08	37.27	45.32
Douglas Locomotion	18.18	14.20	21.84	20.39	35.89	43.48	24.04	41.54	50.00	
Thistle Locomotion	35.63	23.12	20.16	36.54	43.33	28.30	38.24			
Blossom Locomotion	19.19	24.71	22.28	43.81	43.55	25.82	34.04	36.31	41.71	43.17
Average Locomotion	24.34	20.68	21.43	33.58	40.92	32.53	32.11	38.93	45.86	43.17
Douglas Drey Building	0.00	0.00	0.00	6.31	0.00	0.00	0.00	0.00	0.00	
Thistle Drey Building	0.00	0.00	0.00	0.00	0.83	1.89	4.90			
Blossom Drey Building	0.00	0.00	0.52	1.27	5.38	0.00	0.00	1.12	0.00	0.00
Average Drey Building	0.00	0.00	0.17	2.53	2.07	0.63	1.63	0.56	0.00	0.00
Douglas Grooming	0.00	1.85	2.68	6.80	2.39	4.35	0.96	3.85	0.98	
Thistle Grooming	1.15	0.00	0.79	1.66	3.33	1.89	3.92			
Blossom Grooming	2.02	1.16	0.52	1.90	0.00	1.10	2.13	2.79	0.57	1.44
Average Grooming	1.06	1.00	1.33	3.45	1.91	2.44	2.34	3.32	0.78	1.44
Douglas Inactive	19.48	25.93	3.07	4.85	6.70	3.26	0.96	3.85	13.73	
Thistle Inactive	14.94	24.28	5.93	8.31	7.08	1.89	4.90			
Blossom Inactive	37.37	28.57	26.42	7.94	4.30	0.00	0.00	3.35	0.00	3.60
Average Inactive	23.93	26.26	11.81	7.03	6.03	1.72	1.95	3.60	6.86	3.60
Thistle Stereotypical	0.00	0.58	29.25	27.91	1.67	0.94	0.00			
Blossom Stereotypical	0.00	0.00	20.21	14.92	1.08	1.10	0.00	0.00	0.00	0.00
Average Stereotypical	14.29	1.43	29.51	19.62	12.56	1.41	0.00	0.00	0.00	0.00

Breed Pen Autumn &Radish	June'19	July'19	Aug'19	Sept'19	Oct'19	Nov'19	Jan'20 After Board	Feb'20 After Board	Feb '20 After subs	Mar '20 After Hoard	Mar -20 After Hoard	April'20 lockdown	May'20	June'20	July'20	Aug'20
A Social	4.33	3.38	3.51	4.88	13.24	9.05	18.21	17.29	2.09	1.78	5.68	4.00	1.21	4.39	3.54	0.00
R Social	11.32	5.26	10.85	1.67	9.22	8.38	22.22	17.72	1.25	9.09	4.40	0.00	4.92	7.65	3.85	7.14
Average	7.82	4.32	7.18	3.27	11.23	8.71	20.21	17.51	1.67	5.43	5.04	2.00	3.07	6.02	3.69	3.57
A Feeding	24.17	12.80	22.22	21.95	28.68	46.19	38.21	32.58	27.68	41.42	48.86	28.00	31.52	23.20	37.40	
R Feeding	16.98	24.34	29.62	35.83	37.38	43.02	28.53	24.60	12.50	37.37	13.19	23.81	12.30	19.27	16.35	22.69
Average	20.58	18.57	25.92	28.89	33.03	44.60	33.37	28.59	20.09	39.40	31.03	25.90	21.91	21.23	26.87	22.69
A Loco	5.60	6.28	7.02	9.76	17.65	16.67	14.62	12.53	17.49	15.68	31.82	12.00	17.58	20.69	14.17	
R Locom	14.15	15.46	12.90	10.83	9.22	16.76	16.82	16.93	10.00	15.15	56.04	19.05	15.57	17.43	20.19	30.67
Average	9.87	10.87	9.96	10.29	13.44	16.71	15.72	14.73	13.75	15.42	43.93	15.52	16.57	19.06	17.18	30.67
A Drey	0.25	0.24	0.00	0.00	0.00	0.00	0.00	4.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
R Drey	0.94	1.64	0.00	0.00	0.00	0.56	0.00	0.79	0.00	1.01	0.00	0.00	0.00	0.92	0.00	0.00
Average	1.20	1.89	0.00	0.00	0.00	0.56	0.00	5.05	0.00	1.01	0.00	0.00	0.00	0.92	0.00	0.00
A Groom	3.56	0.48	0.58	0.00	2.94	0.95	7.18	7.27	18.02	10.06	9.09	0.00	0.00	5.02	4.33	
R Groom	4.72	2.96	1.76	1.67	0.49	2.79	4.50	3.44	1.88	8.08	0.00	0.00	4.92	4.59	1.92	3.36
Average	8.28	3.44	2.34	1.67	3.43	3.75	11.68	10.71	19.89	18.14	9.09	0.00	4.92	9.60	6.25	3.36
A Inactive	62.09	76.81	66.67	63.41	17.65	18.57	21.28	25.06	28.98	31.07	4.55	56.00	44.85	46.71	40.55	
Radish Inactive	17.92	22.70	17.30	16.67	13.59	15.08	27.93	36.51	74.38	29.29	26.37	57.14	62.30	50.15	57.69	36.13
Average	40.01	49.75	41.98	40.04	15.62	16.83	24.60	30.79	51.68	30.18	15.46	56.57	53.57	48.43	49.12	36.13
A Pacing	0.00	0.00	0.00	0.00	19.85	8.57	0.51	1.00	5.74	0.00	0.00	0.00	4.85	0.00	0.00	
R Pacing	33.96226	27.63158	27.56598	33.33333	30.09709	13.40782	0	0	0	0	0	0	0	0	0	0
Average	16.98	13.82	13.78	16.67	24.98	10.99	0.26	0.50	2.87	0.00	0.00	0.00	2.42	0.00	0.00	0.00

2020 Litter Behaviour	8hrs May'20	6th & 24th June'20	22nd-23rd July '20	30th/31st Aug '20	13th & 14th Oct'20
Kitten 1 Social	4.35	0.00	5.58	9.52	14.15
Kitten 2 Socail	1.83	0.00	10.48	4.36	8.19
Kitten 3 Social	0.00	2.38	4.20	0.00	0.00
Average Social	2.06	0.79	6.75	6.94	11.17
Kitten 1 Feeding	26.09	12.99	34.52	20.63	33.66
Kitten 2 Feeding	8.26	21.18	37.10	17.17	27.71
Kitten 3 Feeding	3.09	33.33	35.66	0.00	0.00
Average Feeding	12.48	22.50	35.76	18.90	30.68
Kitten 1 Loco	20.29	16.88	30.46	20.63	17.07
Kitten 2 Loco	11.01	30.59	23.39	20.71	10.12
Kitten 3 Loco	2.47	16.67	20.28	0.00	0.00
Average Locomotion	11.26	21.38	24.71	20.67	13.60
Kitten 1 Drey	0.00	0.00	0.00	0.00	0.00
Kitten 2 Drey	0.00	0.00	0.00	0.00	0.00
Kitten 3 Drey	0.00	0.00	0.00	0.00	0.00
Average Drey Building	0.00	0.00	0.00	0.00	0.00
Kitten 1 Grooming	0.00	0.65	3.05	3.57	0.73
Kitten 2 Grooming	1.83	1.18	3.23	1.91	3.37
Kitten 3 Grooming	0.00	0.00	4.90	0.00	0.00
Average Grooming	0.61	0.61	3.72	2.74	2.05
Kitten 1 Inactive	49.28	69.48	15.23	18.65	7.80
Kitten 2 Inactive	77.06	47.06	25.81	6.27	4.34
Kitten 3 Inactive	94.44	47.62	34.97	0.00	0.00
Average Inactive	73.59	54.72	25.33	12.46	6.07
Kitten 1 Stereotypical	0.00	0.00	11.17	26.98	26.59
Kitten 2 Stereotypical	0.00	0.00	0.00	49.59	46.27
Kitten 3 Stereotypical	0.00	0.00	1.00	0.00	0.00
Average Stereotypical	0.00	0.00	4.06	38.29	36.43

Wildwood Escot Walkthrough Behaviour	29th/30th Jun'19	3rd July '19	13th/14th Aug '2019	10t/11th Sep '19 hp	23rd/24th/26Sept '19 (Release Date)	29th/30th Oct 2019	26th/28th Nov 2019	21st/22nd Jan 2020	4th/6th Feb 2020	3rd/5th Mar '20	April 2020
Fern Social	20.41	2.97	9.05	9.87	15.44	11.31	13.30	9.73	8.64	10.48	1.67
Bracken Social	22.64	1.82	2.55	10.26	10.42	8.18	13.39	4.82	11.97	10.17	2.74
Twiglet Social	26.94	1.89	2.17		9.93	11.76	11.30	9.09	10.00	6.02	4.08
Cherry Social	0.00	5.56	6.64	9.91	12.57	6.32	12.06	11.46	8.55	8.20	10.53
Average Social	17.50	3.06	5.10	10.01	12.09	9.39	12.51	8.78	9.79	8.72	4.75
Fern Feeding	29.25	59.41	44.44	32.89	39.38	56.55	48.28	48.67	37.04	52.38	60.00
Bracken Feeding	31.13	45.45	46.94	36.54	52.08	52.73	51.79	42.17	47.01	39.83	38.36
Twiglet Feeding	28.50	26.42	32.07		26.24	41.18	48.59	56.82	54.12	32.53	51.02
Cherry Feeding	48.28	53.70	38.17	37.84	49.10	38.42	50.25	54.17	52.14	45.08	44.74
Average Feeding	34.29	46.24	40.41	35.76	41.70	47.22	49.73	50.46	47.57	42.46	48.53
Fern Locomotion	34.01	34.65	38.68	51.32	42.08	14.88	31.53	30.09	49.38	35.24	33.33
Bracken Locomotion	43.40	49.09	48.47	47.44	36.81	39.09	32.14	48.19	36.75	48.31	38.36
Twiglet Locomotion	23.83	43.40	30.98		29.79	19.85	32.77	28.03	31.76	32.53	32.65
Cherry Locomotion	48.28	40.74	37.34	46.85	32.93	28.42	28.64	31.25	31.62	31.15	34.21
Average Locomotion	37.38	41.97	38.87	48.53	35.40	25.56	31.27	34.39	37.38	36.81	34.64
Fern Drey Building	0.00	0.00	0.00	3.29	0.39	13.69	0.49	0.88	0.00	0.00	0.00
Bracken Drey Building	0.00	0.00	0.51	0.00	0.69	0.00	0.00	2.41	3.42	0.00	15.07
Twiglet Drey Building	0.00	0.00	0.00		0.00	0.00	1.13	0.00	0.00	1.20	4.08
Cherry Drey Building	0.00	0.00	0.00	0.90	0.60	1.58	1.51	1.04	2.56	0.00	0.00
Average Drey Building	0.00	0.00	0.13	1.40	0.42	3.82	0.78	1.08	1.50	0.30	4.79
Fern Grooming	4.08	0.99	4.53	1.97	0.00	2.38	2.46	2.65	3.70	0.95	5.00
Bracken Grooming	0.00	0.00	1.53	0.64	0.00	0.00	1.79	0.00	0.00	0.00	0.00
Twiglet Grooming	0.00	0.00	1.53	0.64	0.00	0.00	1.79	0.00	0.00	0.00	0.00
Cherry Grooming	4.66	1.89	0.54		0.00	0.74	0.56	2.27	1.18	3.61	2.04
Average Grooming	2.19	0.72	2.03	1.09	0.00	0.78	1.65	1.23	1.22	1.14	1.76
Fern Inactive	12.24	1.98	3.29	0.66	2.70	1.19	3.94	7.96	1.23	0.95	0.00

Bracken Inactive	2.83	3.64	0.00	5.13	0.00	0.00	0.89	2.41	0.85	1.69	5.48
Twiglet Inactive	10.36	0.00	6.52		1.42	3.68	0.56	3.79	2.94	3.61	2.04
Cherry Inactive	3.45	0.00	2.90	4.50	2.99	2.11	3.52	2.08	0.85	1.64	10.53
Average Inactive	7.22	1.40	3.18	3.43	1.78	1.74	2.23	4.06	1.47	1.98	4.51
Fern Stereotypical	0	0	0	0	0	0	0	0	0	0	0
Bracken Stereotypical	0	0	0	0	0	0	0	0	0	0	0
Twiglet Stereotypical	5.70	26.42	27.72		32.62	22.79	5.08	0.00	0.00	20.48	4.08
Cherry Stereotypical	0.00	0.00	13.28	0.00	0.00	21.05	3.52	0.00	0.00	13.11	0.00
Average	1.42	6.60	10.25	0.00	8.16	10.96	2.15	0.00	0.00	8.40	1.02

Kent Walkthrough Behaviour	2nd-3rd Oct -19	10th Oct '19	19th-20th Oct '19	5th-6th Nov '19	28th-19th Jan 2020	14th-15th Feb 2020
Basil Social	7.89	9.09	6.98	2.58	8.33	2.46
Smokey Social	17.70	11.58	0.00			
Lucky Social	21.14	8.18	7.04	14.77	4.76	
Shep Social	14.63	15.15	11.85	26.09	4.41	
Average Social	15.34	11.00	6.47	14.48	5.84	2.46
Basil Feeding	36.84	72.73	53.49	75.48	69.70	64.75
Smokey Feeding	18.01	38.95	13.04			
Lucky Feeding	17.48	23.64	45.07	51.68	47.62	
Shep Feeding	34.55	18.18	57.35	38.04	32.35	
Average Feeding	26.72	38.37	42.24	55.07	49.89	64.75
Basil Locomotion	19.08	18.18	39.53	17.42	17.42	20.49
Smokey Locomotion	13.35	31.58	65.22			
Lucky Locomotion	27.24	40.00	28.17	25.50	26.19	
Shep Locomotion	13.41	60.61	24.17	32.61	44.12	
Average Locomotion	18.27	37.59	39.27	25.18	29.24	20.49
Basil Drey Building	0	0	0	0	0	0.82

Smokey Drey Building	0	0	13.04			
Lucky Drey Building	0.00	0.91	0.00	2.68	0.00	
Shep Drey Building	0	0	0	0	1.47	
Average Drey Building	0	0.227273	3.26	0.89	0.49	0.82
Basil Grooming	0.66	0.00	0.00	1.94	0.76	2.46
Smokey Grooming	0.31	2.11	0.00			
Lucky Grooming	2.44	1.82	1.41	0.67	7.14	
Shep Grooming	0.81	6.06	0.47	1.09	5.88	
Average Grooming	1.06	2.50	0.47	1.23	4.59	2.46
Basil Inactive	35.53	0.00	0.00	2.58	3.79	9.02
Smokey Inactive	13.04	14.74	8.70			
Lucky Inactive	6.50	2.73	14.08	4.03	14.29	
Shep Inactive	31.30	0.00	5.69	2.17	10.29	
Average Inactive	21.59	4.37	7.12	2.93	9.46	9.02
Basil Stereotypical	0.00	0.00	0.00	0.00	0.00	0.00
Smokey Stereotypical	37.58	1.05	0.00			
Lucky Stereotypical	25.20	22.73	4.23	0.67	0.00	
Shep Stereotyical	5.28	0.00	0.47	0.00	1.47	
Average	17.02	5.94	1.17	0.22	0.49	0