Author/s:
Soanes, K;Vesk, PA;Van Der Ree, R

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Monitoring the use of road-crossing structures by arboreal marsupials: insights gained from motion-triggered cameras and passive integrated transponder (PIT) tags

Kylie Soanes[ab*], Peter A. Vesk[b] and Rodney van der Ree[a]

[a] Australian Research Centre for Urban Ecology, Royal Botanic Gardens, Melbourne, VIC 3010, Australia

[b] School of BioSciences, University of Melbourne, VIC 3010, Australia

* corresponding author: Phone +61 (03) 8344 0146, Fax +61 (03) 9347 9123, e-mail: ksoanes@unimelb.edu.au.soanes@pgrad.unimelb.edu.au (K. Soanes)

Running head*:* Monitoring arboreal mammal crossing structures

Key words*:* Canopy bridge, glider pole, habitat fragmentation, barrier effect, road mitigation, connectivity, monitoring, individual identification

Abstract

Context: Wildlife crossing structures are installed to mitigate the impacts of roads on animal populations, yet little is known about some aspects of their success. Many studies monitor the use of structures by wildlife, but studies that also incorporate individual identification methods can offer additional insights into their effectiveness.

Aims: We monitored the use of wildlife crossing structures by arboreal marsupials along the Hume Freeway in south-east Australia to: 1) determine the species using these structures and their frequency of crossing; 2) determine the number and demographic characteristics of individuals crossing; and 3) use the rate of crossing by individuals to infer the types of movement that occurred.

Methods: We used motion-triggered cameras to monitor five canopy bridges and fifteen glider pole arrays installed at thirteen sites along the Hume Freeway. The five canopy bridges were also monitored with PIT tag readers to identify the rate of use by individuals.

Key results: Five species of arboreal marsupial were detected using canopy bridges and glider poles at eleven sites. Our analysis suggested that increasing the number and the distance between poles in a glider pole array reduced the rate of use by squirrel gliders. The PIT tag and camera footage revealed that the structures were used by adult males, adult females and juveniles, suggesting that all demographic groups are capable of using canopy bridges and glider poles. At two canopy bridges multiple squirrel gliders and common brushtail possums crossed more than once per night.

Conclusion: Given that previous studies showed that the freeway was a barrier to movement, and that many of the species detected crossing are subject to road mortality, we conclude that canopy bridges and glider poles benefit arboreal marsupials by providing safe access to resources that would otherwise have been inaccessible.

Implications: While the factors influencing crossing rate require further study, our analysis suggests that glider pole arrays with fewer poles placed closer together are likely to be more successful for squirrel gliders. The individual identification methods used in this study offer insights that are not possible from measuring the rate of use alone and should be adopted in future monitoring studies.

Introduction

Roads and traffic form barriers to animal movement when animals are unable to cross due to physical or behavioural limitations, or when those attempting to cross are killed by vehicles (Bennett 1991; Burnett 1992; Goosem 2001; Oxley et al. 1974). This process sub-divides habitat creating smaller, isolated populations with a higher risk of local extinction (Bennett 1991; Fahrig and Rytwinski 2009; Forman et al. 2003; van der Ree et al. 2015b). Road agencies often install wildlife crossing structures (e.g. culverts, tunnels, land bridges) to mitigate these effects and conserve roadside populations (Forman et al. 2003; Taylor and Goldingay 2010; van der Grift et al. 2013; van der Ree et al. 2007). Given the financial investment in crossing structures worldwide, and their use to meet environmental regulatory requirements, it is critical that we understand how well they work.

Many studies use cameras to monitor the use of crossing structures by wildlife and infer the effectiveness of the structures, or particular design features, based on how frequently animals use them (e.g. Clevenger and Waltho 2000; Grilo et al. 2008; Ng et al. 2004; van der Ree et al. 2007). However, few studies identify and record the number of individuals that use a structure (but see Baxter-Gilbert et al. 2013; Boarman et al. 1998; Chambers and Bencini 2015; Clevenger and Sawaya 2010; Dodd et al. 2007; Harris et al. 2010). Knowing the number and type of individuals crossing adds value to a monitoring program for two reasons. First, it can show whether a structure is used by all types of individuals or just a small subset of the population and whether demographic connectivity (i.e. affecting recruitment and reproductive rates) is facilitated. For example, if a crossing structure only benefits a small proportion of the local population, or use is dominated by one gender or age-class, then the structure is unlikely to facilitate demographic connectivity (Clevenger 2005; Herrod 2005; Olsson et al. 2008). Second, crossing structures, like corridors, may facilitate multiple types of movement, which can be categorised by different patterns of use. Movements to access resources within an animal's home-range are generally short and frequent, while dispersal movements over long distances tend to be infrequent or seasonal (Bennett 1999; Bissonette and Adair 2008; Van Dyck and Baguette 2005). These different types of movement can potentially be determined by identifying the individuals that use the crossing structure. For example, a camera may detect 20 crossings in a night, but only methods that identify individuals can reveal if the structure was used by 20 individuals crossing once each or a single individual crossing 20 times. This information can give valuable insights into how a crossing structure might benefit a population, e.g. through occasional dispersal or regular habitat access (Clevenger 2005; Foster and Humphrey 1995; van der Ree et al. 2007; van der Ree et al. 2009). Monitoring use is an important first step in evaluating the effectiveness of wildlife crossing structures and by making these initial studies more informative, they can better guide and complement future population-level studies (Soanes 2014; van der Grift et al. 2013; van der Grift and van der Ree 2015).

Canopy bridges and glider poles (hereafter referred to as 'bridges' and 'poles') are crossing structures installed around the world to mitigate the impacts of roads on arboreal mammals (Goldingay et al. 2013; Kelly et al. 2013; Mass et al. 2011; Soanes et al. 2013; Soanes and van der Ree 2015; Taylor and Goldingay 2012b; Teixeira 2013; Weston et al. 2011). In eastern Australia, road agencies increasingly rely on bridges and poles to mitigate the impacts of major roads on threatened arboreal marsupials, including the squirrel glider (*Petaurus norfolcensis*). In the absence of crossing structures, major roads create a barrier to glider movement and reduce the survival rate and viability of squirrel glider populations (McCall et al. 2010; Taylor and Goldingay 2012a; van der Ree et al. 2010). Canopy bridges and glider poles were installed along a freeway in south-east Australia to provide safe passage for squirrel gliders and other arboreal marsupial species. The effectiveness of these structures has yet to be determined. We monitored the use of canopy bridges and glider poles by arboreal marsupials with the following aims:

1) to determine the species using these structures and their frequency of crossing;

2) to determine the number and demographic characteristics of individuals crossing canopy bridges; and
3) to use the rate of crossing by individuals to infer the types of movement that occurred across canopy bridges.

Methods

Study area

The Hume Freeway is a major interstate freeway in south-east Australia that has been progressively upgraded to a four-lane, divided freeway over the past 50 years. The width of the freeway from road edge to road edge varies from 40 to 80 m, depending on the width of the centre median, which ranges from 21 to 38 m. Each carriageway is 12 m wide (including emergency and travel lanes). The treeless gap across the Hume Freeway can exceed 100 m where mature trees have been cleared from the centre median and roadsides, presenting a barrier to the movement of arboreal marsupials. We studied two sections of freeway located approximately 200 km apart; a length of 63 km in the state of Victoria between the towns of Avenel and Benalla that was upgraded in the 1970s-80s, and a length of 70 km in the state of New South Wales between the towns of Albury and Tarcutta that was upgraded in 2009 (Figure 1). The traffic volume averages 10,000 vehicles per day at a maximum legal speed of 110 km/h (DOTARS 2007). The landscape surrounding the Hume Freeway is predominantly agricultural land and rural townships, with remnant and regrowth woodland occurring only in small patches, travelling stock reserves (TSRs) and linear reserves as described in van der Ree (2002) and Gibbons and Boak (2002).

[Figure 1]

Study species

The patches of *Eucalyptus* spp. woodland along the Hume Freeway are a critical resource for arboreal marsupials including: the squirrel glider; the sugar glider (*Petaurus breviceps*); the common brushtail possum (*Trichosurus vulpecula*); the common ringtail possum (*Pseudocheirus peregrinus*); the brush-tailed phascogale (*Phascogale tapoatafa*); the yellow-footed antechinus (*Antechnius flavipes*), and; the koala (*Phascolarctos cinereus*). While all of these species can be negatively affected by roads (e.g. Dique et al. 2003; Gulle 2006; Herrod 2005; Russell et al.

2009) the squirrel glider was the primary target of mitigation and monitoring. Listed as Threatened in Victoria and Vulnerable in New South Wales (Claridge and van der Ree 2004; DSE 2007), squirrel gliders move by gliding from tree to tree with an average glide length of 30–40 m, although glides of up to 70 m have been recorded (Jackson 2000; van der Ree et al. 2003; van der Ree et al. 2010.

Wildlife crossing structures

Crossing structures were installed where the Hume Freeway intersected mature woodland habitat and was likely to limit squirrel glider movement. That is, at sites where squirrel gliders were not expected to be able to safely cross the freeway using existing roadside trees (12–25 m tall) based on a glide ratio of 2.5:1 (Jackson 2000; van der Ree et al. 2010). The width of the treeless gap created by the freeway ranged from 60–380 m (Table 1). Previous studies show major roads with a treeless gap of > 50 m wide restrict squirrel glider movement (Soanes et al. 2013; van der Ree 2006; van der Ree et al. 2010). In 2007, crossing structures were retrofitted to five sites in Victoria. A further eight sites in New South Wales were mitigated during the freeway upgrade in 2009. The number of structures required at each site varied (between one and three) depending on the extent and configuration of roadside habitat (Table 1).

[Figure 2]

Canopy bridges were made of UV-stabilised marine-grade rope (~15 mm diameter) woven into a flat net 50 cm wide, resembling a long, narrow strip of cargo net. Two steel cables suspended the rope between hardwood timber poles on either side of the freeway. Four bridges, ranging from approximately 60 to 85 m long, were erected at a minimum of 6 m above the road (Figure 2). A fifth bridge (Yarra Yarra Creek), 170 m long (zig-zagged across a gap of ~150 m), was installed under an open-span road bridge at a minimum height of 4 m from the ground and 2 m from the underside of the bridge and was supported by six poles along its length. Canopy bridges were installed as close as possible to the existing roadside woodland, usually within 5 m of overhanging tree branches (up to 15 m from tree trunk). Additional ropes connected the ends of each canopy bridge to the branches of roadside trees to facilitate access by arboreal marsupials (1 to 3 ropes per end).

Glider poles (round, hardwood timber poles ~13–18 m tall, 40–50 cm in diameter) provide gliders with an alternative glide site to reduce the width of the treeless gap across the road (Soanes and van der Ree 2015). A timber cross-beam (10 cm x 10 cm x 2.4 m) was fixed horizontally 50 cm from the top of each pole (oriented parallel to the road edge) providing a 'branch-like' launch site. The number and height of poles required depended on the width of the gap across the freeway and the height of roadside trees relative to the height of the road (i.e. if road was in a cutting or raised). As such, each pole crossing could include a single pole in the centre median, or an array of multiple poles placed in the median and roadsides (Figure 2, Table 1). Glide paths were calculated in detailed schematics to ensure that glides in each direction were achievable within a recommended glide ratio of 2.5:1, allowing animals to pass safely above the maximum expected height of traffic. These designs took into account the terrain and the height and location of roadside trees to determine the appropriate height and spacing of glider poles in each array (e.g. Soanes and van der Ree 2015). Squirrel gliders and sugar gliders were the only species within the study area capable of using glider poles to cross the freeway.

Monitoring crossing structures

We used two methods to monitor the use of crossing structures by arboreal marsupials; motion triggered, infrared cameras (Orion, Buckeye Pty Ltd) and PIT tag reading systems (Trovan, Microchips Australia Pty Ltd). Cameras were installed on all crossing structures. PIT tag readers were only placed on canopy bridges, as a suitable design for glider poles was not available. Equipment was installed between April and June 2012 and the structures were monitored until February 2013, providing 9–11 months of monitoring per structure. This period occurred approximately five years after the crossing structures were installed in Victoria and three years after crossing structures were installed in New South Wales. The number of possible monitoring nights per structure ranged from 258 to 315, however, due to equipment malfunctions, the actual monitoring effort ranged from 75 to 279 nights per structure (Table 2). Detail on the monitoring set-up for each method is described below.

[Table 2]

Motion triggered cameras

We placed one camera at each end of each bridge (i.e. two cameras per bridge), and one on the centre median pole of each glider-pole array. At bridges, cameras were triggered by animal movement past a pair of active infrared sensors placed approximately 1 and 4 m from each camera. At poles, cameras were mounted on one end of the cross-beam, so that animals moving along the length of the beam or to the top of the pole would trigger the passive infrared sensor. Each time a sensor was triggered the cameras recorded 9–20 seconds of video. Cameras were powered by an appropriately sized lead-acid battery and solar panel.

Videos from the Victorian structures were transmitted wirelessly to the memory card of an on-ground unit from where they were downloaded during fortnightly field visits. Cameras on the New South Wales structures used an internal modem to transmit videos to the office computer each morning via the 3G mobile phone network. We inspected all videos for the presence of animals on bridges and poles. Each time an animal was detected, the date, time and species were recorded, as well as the number of videos the animal appeared in and direction of travel. Where observed, distinctive markings such as male scent glands, ear notches, or differences in body size were noted to determine the animal's approximate age and sex.

At bridges, the placement of a camera at each end of the bridge allowed us to confirm crossings. A crossing was confirmed when an animal was viewed moving out onto the bridge by one camera and away from the camera until it was out of sight, then detected by the camera on the opposite side of the bridge and viewed exiting the structure. When only one camera was operational, crossings were inferred based on the animal's behaviour and direction of travel.

For example, if an animal moved out onto the bridge (i.e. approaching the other side) until it was no longer visible and did not turn around and return within ten minutes we classified it as a crossing. This is based on the fact that more than 90% of crossings confirmed by both cameras were completed in less than ten minutes. In this way, we distinguished crossings from 'visits', in which an animal was detected moving on to the bridge before turning around and exiting the same side without completing the crossing.

Crossings could not be confirmed at glider poles. Animals can glide to the pole in the centre median from any number of roadside trees (or roadside poles) and land on the pole below the cross-arm, out of view of the camera. This means that it is impossible to determine the direction of travel. While we acknowledge that some animals may glide to the centre median pole and then return to the same side, we expect this to represent only a small proportion of detections, as there is no habitat in the centre median and therefore no reason for animals to repeatedly glide to the centre pole without completing the crossing. This is supported by previous radiotracking data, which showed that individuals detected on the centre median pole went on to cross the freeway (Soanes et al. 2013). Therefore, we refer to all detections on the centre median pole as crossings for the purposes of this study. However this may overestimate the true number of complete crossings where squirrel gliders glide to the centre median pole and return without crossing.

<u>Preliminary analysis of factors affecting crossing rate</u>

Site covariates and design attributes of the road and crossing structures can influence the use of crossing structures by wildlife. For each site, the road width (width of the treeless gap across the road as measured from trunk to trunk), the distance of the structure to roadside trees, bridge length, number of poles in a glider pole array and maximum glide distance (between two successive poles or pole and tree in an array of poles) were obtained from schematic diagrams, satellite imagery and site visits (Table 1). We also calculated an activity index for squirrel gliders (the target species) using data from a concurrent mark-recapture study to estimate the number of individuals that were active in the habitat adjacent to the mark-recapture study. The activity index was determined at the site level by dividing the average minimum number of squirrel gliders known to be alive (MNKTBA) by the area of habitat trapped at each site (details provided in Appendix 1).

Upon inspection of the data it became clear that it would not be possible to investigate all potential factors influencing crossing rates within the scope of this study. There were only five canopy bridge sites available, and of these only three were used by arboreal marsupials. This sample size was not suited to further statistical analysis, and so we limited our analysis to glider poles. As non-gliding species were restricted to using canopy bridges, and sugar gliders were detected at only three sites, we further restricted our analysis to squirrel gliders. At the time of this study, two glider pole arrays at Kyeamba TSR were >100 m from the nearest roadside squirrel glider habitat, as the habitat restoration works activities surrounding the structures have not yet successfully linked the crossing structure with the existing roadside trees. This distance is well beyond the maximum glide range of a squirrel glider, and therefore we excluded these two structures from further analysis. This left us with a sample size of 13 glider poles to include in statistical analysis.

Due to the limited dataset we restricted our analysis to two factors likely to influence the use of glider poles by squirrel gliders: the number of poles in an array (hereafter 'number of poles'), and the maximum glide distance required to cross the glider pole array (hereafter 'glide distance'). We chose to focus on these factors because they are both biologically meaningful and also relevant to managers as these design features are relatively easily to manipulate when installing crossing structures. We did not include the activity index as a parameter, as preliminary analysis revealed that its effect on crossing rate was highly uncertain. Inspection of the data showed that a high activity index did not correspond with a high crossing rate at that site. Further, multiple structures from the same site and hence the same activity index commonly varied widely in crossing rates.

We used a Poisson regression to investigate the effect of glide distance and the number of poles on the crossing rate. The data used to estimate the model parameters were the number of crossings observed within each of 26 time periods of varied length during which the cameras were operating. The expected number of crossings per night was modelled as the exponent of a linear model with a grand mean, and fixed effects for the number of poles and glide distance. Error terms were included to account for residual error and a random effect of each individual crossing structure on the crossing rate. The model was estimated using Bayesian inference in the program OpenBugs 3.2.1 (Spiegelhalter et al. 2011). We used vague priors for all parameters, using a normal distribution with a mean of 0 and a standard deviation of 1000 for the fixed effects and half-Cauchy distributions with scale 25 for the random effects (Gelman 2006). The model code and data are provided in Appendix 2. The model was run for 50,000 iterations after discarding a burn-in of 20,000. Convergence was assessed through the visual inspection of three independent chains.

<u>PIT tag scanners on canopy bridges</u>

We installed one flat panel antenna (ANT-612, Trovan, Microchips Australia Pty Ltd, Figure 3.2) at each end of each canopy bridge (i.e. two antennae per bridge). Antennae were approximately the same width as the bridge (40 cm) with a read distance of 35 cm. Data were stored in an attached decoder unit (LID650, Trovan, Microchips Australia Pty. Ltd.) fixed to the support pole. The antennae were not continuously operational due to constraints on the size of the battery and solar panel that could be installed on canopy bridges. Antennae on bridges in New South Wales were integrated with the camera's active infrared sensors and only operated when a sensor was triggered. In Victoria, we could not integrate antennae with the existing camera sensors and therefore we scheduled them to operate for half of the night. At these bridges one antenna was operational from 17:00 h until 23:59 h and the opposite from 00:01 h until 07:00 h. A full night of monitoring was achieved if both antennae were operational on the same night. Unfortunately, the units frequently malfunctioned and a full night of monitoring only occurred on 32 nights at the Longwood bridge and four nights at Violet Town. On all other nights the Victorian bridges were only monitored with one antenna (6–7 hours per night), leaving approximately 3–6 hours of the night unmonitored depending on the season, therefore our study is likely to underestimate the true number of individuals that used the canopy bridges.

Data from the Victorian PIT tag readers were downloaded from the decoder via a USB cable during fortnightly site visits. Decoders on bridges in New South Wales contained modems programmed to transmit data to the office computer each morning via the 3G mobile phone network. We recorded the time and date of each PIT tag reading and matched it with the corresponding video where possible. PIT tag readings were also cross-checked against mark-recapture records to identify each individual. Unless both PIT tag antennae at a bridge were simultaneously operational, it was not possible to confirm crossings using PIT tags alone. Therefore we cross-checked the time and date of all tag reads with the videos recorded by cameras to collect information on the crossing behaviour of tagged individuals.

Mark-recapture surveys were also conducted at all five canopy bridge sites (as described in Appendix 1). All squirrel gliders and common brushtail possums captured were implanted with a PIT tag under the skin between the shoulder blades (ID 100, Trovan, Microchips Australia Pty Ltd). Data from the existing mark-recapture data set suggests that the rate of tag loss is <0.05% (unpublished data), which corresponds to other studies on small mammals (Schooley et al. 1993). Other species were not marked with PIT tags, as they are rarely captured in wire cage traps. The average MNKTBA for common brushtail possums and squirrel gliders in habitat adjacent to each canopy bridge site was calculated as described for glider poles (Appendix 1, Table 1, Table 6).

Results

Rate of use by arboreal marsupials

Using motion-triggered cameras, we detected five species of arboreal marsupial using both types of crossing structure over 3,929 nights of camera monitoring: squirrel gliders (n=1,317 detections), common ringtail possums (n=394), common brushtail possums (n=241), sugar gliders (n=258) and brush-tailed phascogales (n=4) (Tables 3 and 4). Only 42 detections were classed as visits and excluded from further analysis. No koalas or yellow-footed antechinus were detected, despite occurring in the general area. Arboreal marsupials used crossing structures to cross the freeway at 11 of the 13 monitoring sites. Two canopy bridges and four glider pole arrays were not used by any species. Squirrel gliders were detected crossing the road using canopy bridges and glider poles. Both species of possum and phascogales were only detected crossing bridges. Sugar gliders were only detected using poles to cross the road.

[Table 3, Table 4]

The number of crossings per night varied widely among sites, with some structures used frequently and others not at all (Tables 3 and 4). For example, squirrel gliders were detected crossing the Longwood bridge, Warrenbayne pole, Sages pole 1 and Kyeamba Creek pole 1 more than once each night, while the Violet Town bridge and Sages pole 2 were used less than once every two months. Seven structures were not used by squirrel gliders, even though the species was present at all sites. Therefore while the mean crossing rate for squirrel gliders was 0.84 crossings per night at canopy bridges (± SE 0.84) and 0.38 at glider poles (± SE 0.17), the crossing rate at any one site ranged from 0.00 to 4.19 crossings per night. Crossing rates by other species ranged from 0.00 to 1.14 crossings per night for common brushtail possums (mean 0.23, ± SE 0.23); 0.00 to 1.36 crossings per night for common ringtail possums (mean 0.46, ± SE 0.28); and 0.00 to 0.93 crossings per night for sugar gliders (mean 0.07, ± SE 0.06). Brush-tailed phascogales were detected infrequently on one bridge, with 1 crossing per 50 nights of monitoring (0.02 crossings per night).

The regression analysis revealed that the number of poles in a glider pole array and the maximum glide distance required to cross had a negative effect on the use of glider poles by squirrel gliders. The point estimates for the effect of number of poles and glide distance were both negative (-1.26 and -0.12, respectively), although the credible intervals for both parameters overlapped zero reflecting uncertainty in the estimates, and rather more so for the distance than number of poles (Table 5). The wide credible intervals are not surprising given the low sample size available for analysis (*n*=13). Based on these data, the predicted mean rate of use for a glider pole array consisting of two poles with a maximum distance of 30 m apart was 0.11 crossings per night (95% CI 0.00–0.53). Note that this does not predict beyond the observed data and does not incorporate possible sources of uncertainty.

[Table 5]

The video footage showed that multiple individuals of both sexes used the crossing structures, including adults and juveniles. This finding was based on observed differences in body size, the presence of active scent glands and ear notches visible in 445 of the 2,291 of the occasions during which animals were detected. Both species of possum were observed carrying dependent young across the Longwood and Violet Town canopy bridges and adult squirrel gliders were seen carrying pouch young or accompanied by smaller individuals, presumably juveniles, on poles (Figure 3). Independent juveniles (i.e. not carried by parents) were also observed, with juvenile ringtail possums at the Violet Town bridge, and juvenile squirrel gliders at Kyeamba Creek pole 2, Sages pole 2, and the Warrenbayne and Blue Metal poles.

Rate of use by individuals

Three squirrel gliders and five common brushtail possums with PIT tags were detected crossing two canopy bridges over the 11 months during which PIT tag readers were in use. Three squirrel gliders and a common brushtail possum were detected crossing the Longwood bridge and four common brushtail possums were detected crossing the Violet Town bridge. A fourth squirrel glider was detected on the canopy bridge at Sages TSR but did not complete the crossing. All individuals were reproductively active adults. Due to equipment malfunctions, only

5–27% of detections by PIT tag reader could be confirmed as crossings. However, given that only 42 of the 1,112 camera detections at bridges were identified as visits, we are confident that a large portion of the detections by the PIT tag reader also represent complete crossings. The number of individuals observed using the canopy bridges was generally lower than the number present in the surrounding habitat (as indicated by the average MNKTBA), with the exception of the bridge at Longwood, where the number of squirrel gliders observed crossing was approximately equal to the average MNTKBA (Table 4). Canopy bridges at Yarra Yarra Creek, Blue Metal TSR and Sages TSR were not crossed by any tagged squirrel gliders or common brushtail possums despite the presence of tagged individuals in the adjacent habitat (Table 4).

Discussion

Use of structures by arboreal marsupials

Five species of arboreal marsupial were detected using crossing structures to cross the Hume Freeway, including structures that were retrofitted to existing sections of the freeway and those that were installed during freeway construction. Squirrel gliders, common brushtail possums, common ringtail possums, sugar gliders and brush-tailed phascogales are all affected by road mortality (pers. obs.; McCall et al. 2010; Russell et al. 2009; Taylor and Goldingay 2004) and structures that increase their safe passage across the road may help reduce rates of road-kill. There is now mounting evidence that canopy bridges and glider poles of various designs are used by a wide range of arboreal mammals, including monkeys, lemurs, and squirrels (Donaldson and Cunneyworth 2015; Goldingay et al. 2013; Kelly et al. 2013; Mass et al. 2011; Soanes et al. 2013; Taylor and Goldingay 2012a; Teixeira 2013; Weston et al. 2011) and these structures are likely to be useful for other species with similar behaviours and ecological requirements, including arboreal reptiles and amphibians. For example, mahogany gliders (*Petaurus gracilis*), mountain brushtail possums (*Trichosurus cunninghami*) and western ringtail possums (*Pseudocheirus peregrinus occidentalis*) have similar ecology and behaviour to species detected in this study and are negatively affected by roads and fragmentation (Asari et al. 2010; Taylor and Goldingay 2004; Trimming et al. 2009).

While small sample sizes limited the statistical analysis possible within this study, we were able to identify some general principles for the design of crossings structures for arboreal marsupials. Arboreal marsupials used all structure types monitored in this study, including bridges up to 85 m long and roadside glider pole arrays consisting of up to four poles. While no animals were detected using the under-road canopy bridge in our study, previous work by Goldingay et al. (2013) detected common brushtail possums, common ringtail possums and feathertail gliders (*Acrobates pygmaeus*) using an under-road bridge, suggesting that this design is also useful. Our analysis suggests that the use of glider pole arrays by squirrel gliders decreases as the number of poles and the distance between the poles increases. This is supported by studies of glide capacity, which show that glide success decreases as glide distance increases (Ball and Goldingay 2008; Goldingay and Taylor 2009; Jackson 2000; van der Ree et al. 2003). Though we could not analyse it in our study, a similar principle is likely to apply to canopy bridges, with shorter spans likely to be more successful than longer ones, particularly for species that prefer closed canopy (e.g. Goosem et al. 2006; Weston et al. 2011). Crossing structures for arboreal mammals are therefore more likely to be successful if the width of the treeless gap across the road is minimised during construction, or structures are retrofitted to locations where the existing gap is narrowest. This will allow glider pole arrays to consist of fewer poles that can be placed closer together, or shorter-span canopy bridges.

Identifying the number and type of individuals

Collecting data on the age and sex of the animals using crossing structures provides valuable information on the ability of crossing structures to benefit the wider population (Clevenger 2005; Olsson et al. 2008; Sawaya et al. 2013). For example, Sawaya et al. (2013) identified eight female and nine male black bears (*Ursus americanus*) using crossing structures in Banff National Park, Canada and concluded that these structures provided demographic connectivity.

Conversely, Olsson et al. (2008) concluded a highway overpass was unlikely to affect the population demographic rates of moose (*Alces alces*) populations based on the low number of crossings made predominantly by males. In our study, PIT tags and body markings observed in video footage revealed that males and females, including females carrying dependent young, used canopy bridges and glider poles to cross the freeway. That is, no demographic group was excluded from using either structure type to cross the freeway. A comparison of the crossing rates obtained through video and PIT tag readers suggests that the crossing rate of common brushtail possums and squirrel gliders at the Violet Town and Longwood canopy bridges was almost entirely due to the repeated movements of a few individuals (1–4 individuals of each species per structure). Population-level analyses are required to assess whether or not the number of individuals crossing is enough to maintain demographic and genetic connectivity for the wider population. However based on our findings it is unlikely that there are any features of the current crossing structure designs that would prevent crossing structures from facilitating demographic and genetic connectivity.

Inferring types of movement

Identifying patterns of use by individuals allowed us to infer the types of movement that crossing structures facilitated. For example, daily movements are generally associated with foraging and accessing resources within an individual's home range (Bennett 1999; Bissonette and Adair 2008; Van Dyck and Baguette 2005). The high average crossing rates (more than one crossing per night) of squirrel gliders and common brushtail possums at canopy bridges as detected by cameras, were revealed by the PIT tag reader to be 3–4 individual animals crossing repeatedly (often multiple times each night). Prior to the installation of the Longwood and Violet Town canopy bridges, squirrel gliders and common brushtail possums did not cross the freeway regularly at these sites (Gulle 2006; Soanes et al. 2013; van der Ree et al. 2010). In our study, two squirrel gliders at the Longwood bridge and one common brushtail possum at the Violet Town bridge crossed almost every night, gaining nightly access to habitat on both sides of the freeway. Such regular use suggests that these individuals incorporate the crossing structure within their home range. Therefore it is possible that the high crossing rate at these sites is driven by a few individuals frequently crossing in order to access portions of their home range on either side of the road. It is likely that the high frequency of crossings (~ 1 crossing/night) by squirrel gliders at glider poles such as Warrenbayne, Little Billabong, Sages TSR pole 1 and Kyeamba Creek pole 1, common ringtail possums at the Violet Town bridge, and sugar gliders at the Little Billabong pole also reflect repeated crossings by a few individuals. Radiotracking studies that investigate home-range movements could be used to confirm this theory. Canopy connectivity is critical to maintain daily movements of arboreal species that are gap-limited or unwilling to cross open spaces (e.g. Anzures-Dadda and Manson 2007; Asari et al. 2010; Laurance and Laurance 1999; van der Ree et al. 2010; Wilson et al. 2007). This supports the idea that crossing structures can benefit wildlife by providing access to resources that would have been unavailable, or dangerous to reach, if crossing structures were not present (Bennett 1999; Eigenbrod et al. 2008).

Despite the advantages of using PIT tags to identify individuals, it did not help us infer other types of movement, such as dispersal, or allow us to detect effects at the population level. Dispersing individuals may be sourced from a large number of source populations, and tagging all potential dispersers that might use the structure would require an intensive and extensive survey effort. Furthermore, movement across a structure does not necessarily result in population-level effects (van der Grift et al. 2013; van der Ree et al. 2007; van der Ree et al. 2011). For example, territorial behaviour by resident individuals in roadside habitat can limit the reproductive success of dispersing individuals, creating a social barrier to gene flow despite movement (e.g. Riley et al. 2006, Corlatti et al. 2009, Simmons et al. 2010). Similarly, while the detection of animals crossing suggests that those individuals crossed safely and that roadkill is less likely to occur, we cannot say that population-level survival rates have improved. Ultimately, mark-recapture surveys collecting information on populations size, gene flow and survival rates before and after crossing structures are installed are required to determine whether the patterns of crossing behaviour yield population-level benefits (Corlatti et al. 2009; Sawaya et al. 2014; Simmons et al. 2010; van der Grift et al. 2013; van der Ree et al. 2007; van der Ree et al. 2011).

Limitations of studying arboreal crossing structures

Investigating the optimal dimensions of crossing structures and factors influencing use (e.g. structure placement, habitat quality and needs of the local population) guide best-practice mitigation and are a critical aspect of road ecology (van der Grift and van der Ree 2015). However, our study had limited statistical power due to the low replication and low crossing rates by some species. Our study almost certainly underestimated the true number of crossings and longer-term surveys may reveal that more of these structures are used by wildlife over time (e.g. Bond and Jones 2008; Clevenger and Waltho 2003; Gagnon et al. 2011; Soanes et al. 2013). While longer survey periods are likely to increase the number of crossings observed, increasing the number of canopy bridges and glider poles is more difficult. It is unlikely that a study along a single stretch of road, or mandated monitoring from a single construction project will have sufficient replication to investigate how structural and site attributes affect rates of use for different arboreal species (Rytwinski et al. 2015; van der Ree et al. 2015a). A promising avenue to improve replication in future studies is for road agencies and researchers to combine multiple projects into a coordinated monitoring program, thus enabling a thorough investigation of the factors that influence the effectiveness of crossing structures for arboreal mammals. Alternatively, the experimental manipulation of structural attributes in field trials could be used to explore the optimal structure designs (Rytwinski et al. 2015; van der Ree et al. 2015a).

Monitoring arboreal crossing structures also presents a unique set of challenges when compared to culverts or land-bridges (Gregory et al. 2014; Taylor and Goldingay 2014). Cameras, sensors, batteries and solar panels must be securely installed 6–20 m high above an active roadway (storing power sources at height reduces risk of vandalism and theft). In our study, access to the monitoring equipment was only possible with an elevated work platform, which requiring a licensed operator, and traffic management to close highway lanes at approximately $5,000–$10,000AUD per day. To our knowledge, this is the first study to use wireless download technology and PIT tag readers on arboreal crossing structures. Unfortunately, though we tested the equipment extensively prior to its installation there were several unexpected technical issues once the monitoring program was in place. Due to the difficulties and costs associated with accessing the equipment, it was not feasible to conduct regular repairs, leading to a loss of data at some sites. These issues should be carefully considered when planning a monitoring study of arboreal structures, and additional maintenance costs should be factored into monitoring budgets.

Conclusions

Improving the quality of information gained from monitoring studies is crucial if we are to understand the conservation value of wildlife crossing structures. We found that individual identification provided insights that would not have been possible through monitoring the rate of use alone. While we used PIT tag readers, other methods such as non-invasive genetic sampling (e.g. Sawaya et al. 2013), radio- or GPS-tracking (e.g. Dodd et al. 2007; Olsson et al. 2008) or coat pattern recognition (e.g. Mendoza et al. 2011; Trolle and Kery 2003) could be used to obtain similar information. The most appropriate method will depend on the species (e.g. not all species can be recognised by coat pattern). Canopy bridges and glider poles were used by several species of arboreal marsupial, allowing safe access to resources that would have been otherwise unavailable. In our study crossing structures were primarily used by a small proportion of the population to regularly access habitat on both sides of the freeway. However, while this benefits the small number of individuals that use the structure, it is unknown whether this is enough to provide demographic or genetic connectivity for the wider population. Still, if even a few dispersing individuals successfully cross and reproduce, and the structures reduce rates of roadkill, then they are likely to benefit populations (Taylor and

Goldingay 2012a). Based on this and previous studies, these structures are likely to have a positive effect on a wide range of arboreal mammals, particularly gap-limited species or those that are frequent victims of road mortality. Canopy bridges can benefit a wider range of species than glider poles, and should be the preferred mitigation method where feasible. Methods such as PIT tag readers are underutilised and should be widely adopted in studies monitoring the use of structures by wildlife (Gibbons and Andrews 2004; van der Ree et al. 2007).

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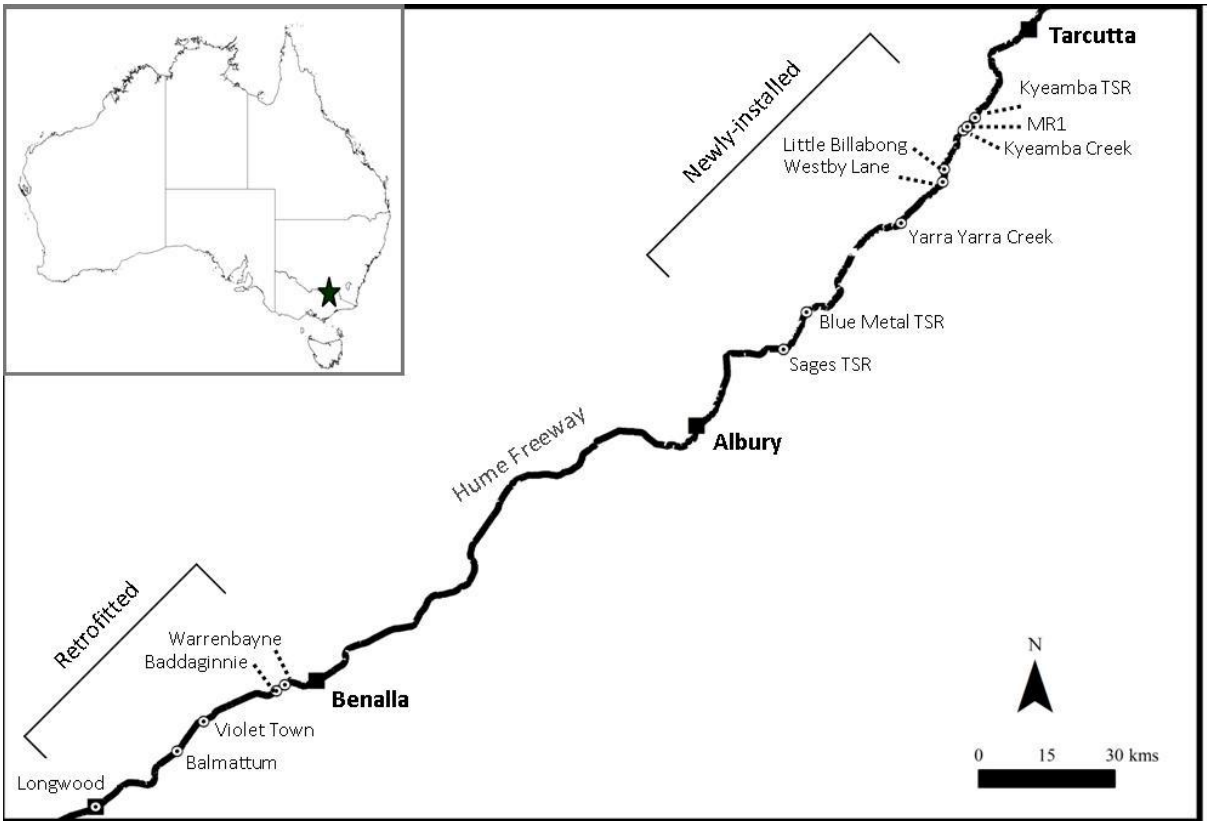

Figure 1. Location of the study area along the Hume Freeway in south-east Australia (inset). Major towns (black squares) and the position of retrofitted and newly-installed crossing structures (circles) are indicated. At some sites, multiple structures are present as identified in Table 1.

Figure 2. Canopy bridges (top left) and glider poles (top right) along the Hume Freeway in south-east Australia. Monitoring equipment (bottom left and right) included motion-triggered cameras (1), active-infrared sensors (2), PIT tag antennae (3) and power supply (4).

Figure 3. Video screen-shots of squirrel gliders on the cross-beam of the glider pole at Little Billabong with male head gland (left) and ear notch (right) visible.

Table 1. A description of the crossing structures present at each site. Road width measures the width of the treeless gap created by the road corridor (trunk to trunk). Squirrel glider activity index refers to average number of individuals per hectare based on concurrent mark-recapture surveys (described in Appendix 1). Max. glide distance refers to the maximum distance between glider poles and/or roadside trees.

Site	Structures present	Road width (m)	Max. distance to roadside trees (m)	Bridge length (m)	No. of glider poles	Pole heights (m)	Max. glide distance (m)	Squirrel glider activity index	General description
Victoria – retrofitted to existing freeway									
Longwood	Bridge 1	80	9	73	-	-	-	0.33	Above road canopy bridge.
Violet Town	Bridge 2	94	9	86	-	-	-	0.23	Above road canopy bridge.
Balmattum	Pole 1	80	-	-	2	14–16	47	0.34	One pole in centre median, one on roadside.
Baddaginnie	Pole 2	62	-	-	1	16	31	NA	Pole in centre median
Warrenbayne	Pole 3	75	-	-	1	15	39	0.13	Pole in centre median
New South Wales – installed during freeway construction									
Sages	Bridge 3	70	12	63	-	-	-	0.18	Above-road canopy bridge.
	Pole 4	76	-	-	2	15–18	30	“	One pole in centre median, one on roadside.
	Pole 5	88	-	-	2	16–18	35	“	One pole in centre median, one on roadside.
Blue Metal TSR	Bridge 4	75	5	58	-	-	-	0.26	Above-road canopy bridge.
	Pole 6	103	-	-	4	15–17	28	“	Two poles in centre median, two on roadsides.
Yarra Yarra Creek	Bridge 5	151	4	140	-	-	-	1.05	Under-road canopy bridge.
Little Billabong	Pole 7	60	-	-	1	14–18	29	0.10	Pole in centre median.

Site	Structures present	Road width (m)	Max. distance to roadside trees (m)	Bridge length (m)	No. of glider poles	Pole heights (m)	Max. glide distance (m)	Squirrel glider activity index	General description
Westby	Pole 8	66	-	-	3	13–18	26	0.05	One pole in centre median, two on roadsides.
	Pole 9	66	-	-	3	13–18	30	“	One pole in centre median, two on roadsides.
Kyeamba Creek	Pole 10	56	-	-	2	13–18	28	0.59	One pole in centre median, one on roadside.
	Pole 11	68	-	-	2	16	29	“	One pole in centre median, one on roadside.
MR1	Pole 12	69	-	-	1	15–18	38	0.23	Pole in centre median.
Kyeamba TSR	Pole 13	93	-	-	4	15–18	28.5	0.57	Two poles in centre median, three on roadsides.
	Pole 14	382	-	-	5	14–17	184	“	Two poles in centre median, three on roadsides.
	Pole 15	244	-	-	5	15–17	110	“	One pole in centre median, three on roadsides.

Table 2. Monitoring effort at each site with motion-triggered cameras and PIT tag readers. At canopy bridges, camera nights shows the number of nights at least one camera was operational, and PIT reader nights shows the number of nights at least one PIT tag reader was operational. The number of nights that units at each end of the bridge were simultaneously operating is given in parentheses. Hyphen indicates no monitoring effort.

Site	Structures present	Camera nights	PIT-tag reader nights
Longwood	Bridge	108 (102)	*151 (0)
Violet Town	Bridge	199 (79)	*99 (0)
Balmattum	Pole	234	-
Baddaginnie	Pole	270	-
Warrenbayne	Pole	107	-
Sages TSR	Bridge	265 (102)	240 (30)
	Pole 1	231	-
	Pole 2	128	-
Blue Metal TSR	Bridge	235 (43)	271 (73)
	Pole	133	-
Yarra Yarra Creek	Bridge	161 (81)	272 (125)
Little Billabong	Pole	258	-
Westby	Pole 1	152	-
	Pole 2	152	-
Kyeamba Creek	Pole 1	75	-
	Pole 2	279	-
MR1	Pole	268	-
Kyeamba TSR	Pole 1	258	-
	Pole 2	268	-
	Pole 13	148	-

*PIT tag readers were operational for 6–14 hours per night

Table 3. The number of crossings detected by cameras at each glider pole, ranked in order of nightly crossing rate (number of crossings per night of camera operation) by squirrel gliders and sugar gliders. No animals were detected at Westby Lane (2), MR1, Kyeamba TSR (2) or Kyeamba TSR (3). Hyphen indicates 'not applicable'.

	Kyeamba Creek pole 1	**Warren -bayne**	**Sages TSR pole 1**	**Little Billabong**	**Blue Metal TSR**	**Baddaginnie**	**Kyeamba Creek pole 2**	**Balmattum**	**Sages TSR pole 2**	**Kyeamba TSR pole 1**	**Westby Lane pole 1**
Squirrel glider											
Number of crossings	144	160	269	224	10	18	10	4	2	1	0
Nightly rate	1.92	1.50	1.16	0.87	0.08	0.07	0.04	0.02	0.02	<0.01	-
Sugar glider											
Number of crossings	0	16	0	240	0	0	0	0	0	0	2
Nightly rate	-	-	0.15	0.93	-	-	-	-	-	-	0.01

Table 4. Monitoring results from cameras and PIT tag readers at each canopy bridge for each species, including: the nightly rate (number of crossings per night of operation); the percentage of crossings that were confirmed by units at each end of the bridge; the average number of tagged squirrel gliders and common brushtail possums present in the habitat surrounding each canopy bridge (detail in Appendix 1); and the identity (ID) of individuals detected by the PIT tag reader (♂=male, ♀=female). *indicates 'visit' only. Hyphen indicates 'not applicable'

Site	**Longwood**	**Violet Town**	**Sages TSR**	**Blue Metal TSR**	**Yarra Yarra Creek**
Squirrel glider					
Average MNKTBA	2.3	3.0	4.3	8.3	4.0
Crossings detected by camera					
Total number	453	2	0	0	0
% Confirmed	82%	0%			
Nightly rate	4.19	0.01	-	-	-
Crossings detected by PIT reader					
Total number	244	-	0*	-	-
% confirmed	9%				
Nightly rate	1.62	-	-	-	-
No. indiv.	3	0	1	0	0
ID: nightly rate	♂H1: 0.67 ♀P3: 0.79 ♀Z1: 0.15	-	♀J5: 0*	-	-
Common brushtail possum					
Average MNKTBA	11.7	14.3	1	7.8	4
Crossings detected by camera					
Total number	2	227	0	0	0
% Confirmed	100%	35%	-	-	-
Nightly rate	0.02	1.14	-	-	-
Crossings detected by PIT reader					
Total number	19	120	0	0	0
% Confirmed	5%	27%	-	-	-
Nightly rate	0.13	1.21	-	-	-
No. indiv.	1	4	0	0	0
ID: nightly rate	♀B1: 0.13	♂98: 0.22 ♀92: 0.95 ♂M1: 0.02 ♀Z6: 0.03	-	-	-
Common ringtail possum					
Crossings detected by camera					
Total number	94	270	0	22	0
% Confirmed	85%	26%	-	45%	-
Nightly rate	0.87	1.36	-	0.09	-
Brush-tailed phascogale					
Crossings detected by camera					
Total number	2	0	0	0	0
% Confirmed	0%	-	-	-	-
Nightly rate	0.02	-	-	-	-

Table 5. Parameter estimates from the Poisson regression of use of glider poles by squirrel gliders. SD is standard deviation.

Parameter	**Mean**	**Standard error**	**95% credible interval**
Grand mean	-3.11	1.45	[-6.23, -0.63]
Effect of number of poles	-1.26	1.33	[-3.92, 1.45]
Effect of gap width	-0.12	0.23	[-0.58, 0.38]
SD Residual error	1.10	0.49	[0.42, 2.35]
SD Structure error	3.89	1.41	[1.97, 7.39]

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Appendix 1 – Mark-recapture surveys at crossing structure sites

We obtained data on the local squirrel glider and common brushtail possum populations using data from a concurrent mark-recapture study. Data were available for 12 of the 13 sites. Each site was surveyed between one and four times between September 2011 and February 2013, with the exception of Baddaginnie, where no surveys were conducted. The trapping surveys covered the majority of the mature woodland within ~600 m of the crossing structure (or crossing zone, where multiple structures were present at a single site) on both sides of the freeway.

Wire-cage traps (17 cm x 20 cm x 50cm, Wiretainers Pty Ltd) were baited with a mixture of honey, oats and peanut butter and placed 3–5 m high on tree trunks. The mean number of traps set was 26 (range 8–38) per site per survey depending on the shape and extent of available habitat surrounding the crossing structure. Traps were spaced at 50–100 m intervals and arranged in a grid or transect extending up to 600 m away from the crossing structure. Traps were set for an average of six nights per survey (range 4–8). The trap effort at each site varied from 125–635 nights (mean 406).

Traps were checked each morning and all animals were weighed and their tooth-wear, gender and reproductive status recorded. Animals were marked with a unique tattoo in the ear flap and two 2 mm tissue biopsies were taken from the ear margin for use in genetic analysis for another study. Each squirrel glider and common brushtail possum captured was implanted with a PIT tag under the skin between the shoulder blades (ID 100, Trovan, Microchips Australia Pty Ltd). We did not implant PIT tags in other species during these surveys (e.g. common ringtail possums) because they are rarely detected in surveys using wire-cage traps.

Mark-recapture detected 267 animals during 4,883 trap nights. Species detected included squirrel gliders (n=88), common brushtail possums (n=147), common ringtail possums (n=1), sugar gliders (n=12), brush-tailed phascogales (n=3) and yellow-footed antechinus (n=16). We calculated the Minimum Number of Animals Known To Be Alive (MNKTBA) for squirrel gliders at each site and for common brushtail possums at canopy bridge sites. Where multiple surveys were conducted, we used the average MNKTBA across all surveys at that site (Table A1). This information was used to calculate the activity index for squirrel gliders presented in Table 1 of the main text. The activity index was determined by dividing the average MNKTBA by the area of habitat trapped at each site. The area of habitat trapped (in hectares) was calculated as the area in which traps were set plus a buffer zone of 50 m to include any habitat immediately adjacent to the trapping area (Table A1). The average MNKTBA for both squirrel gliders and common brushtail possums at canopy bridge sites was used to compare the number of individuals present at each site (i.e. the number of animals likely to have access to the canopy bridge at any one time) with the number that were detected crossing the canopy bridge by the PIT tag reader. This data is presented in Table 4 of the main text.

Table A1. Mark-recapture trapping effort at each site. The average MNKTBA for squirrel gliders and common brushtail possums is shown (+/- 1 SE in parentheses).

Site	Trapping area (ha.)	Number of surveys	Total number of trap nights	Squirrel glider activity index (indiv./ha.)	Average MNKTBA	
					Squirrel gliders	**Common brushtail possums**
Longwood	6.9	3	532	0.33	2.3 (0.3)	11.7 (2.0)
Violet Town	10. 3	4	460	0.23	2.3 (0.5)	10.8 (1.0)
Balmattum	10.4	2	125	0.34	3.5 (1.5)	-
Warrenbayne	15.7	1	140	0.13	2 (NA)	-
Sages TSR	23.4	3	575	0.18	4.3 (0.7)	1 (0.4)
Blue Metal TSR	32.3	3	633	0.26	8.3 (0.3)	7.7 (0.9)
Yarra Yarra Creek	3.8	3	166	1.05	4.0 (0.6)	0.3 (0.3)
Little Billabong	19.1	3	456	0.10	2 (0.6)	-
Westby Lane	19.2	3	517	0.05	1 (0.6)	-
Kyeamba Creek	9.6	3	439	0.59	5.7 (0.7)	-
MR1	8.8	3	205	0.23	2 (0.6)	-
Kyeamba TSR	18.3	3	635	0.57	5 (0.6)	-

Appendix 2 - Model code

```
model{
 for (i in 1:Nobs) {
  x[i] ~ dpois(lambda[i])
  lambda[i] <- theta[i] * t[i]
  theta[i] <- exp(mu[i])
  mu[i] ~ dnorm(phi[i], prec[1])

  phi[i] <- max(min(GM + npoles.eff * (npoles[i] -2) + gap.eff * (gap[i] - 30)  + str.eff[str[i]],99),-
99)
 }

 for (k in 1:Nstr) { str.eff[k] ~ dnorm (0, prec[2])   }

 GM ~dnorm(0,0.0001)
npoles.eff ~dnorm(0,0.0001)
 gap.eff ~dnorm(0,0.0001)

 for (m in 1:2) {
  prec[m] <- 1/sd[m]/sd[m]
  sd[m] ~ dt(0, .0016, 1)T(0,)
 }

 pred.mean.rate <- exp(GM)

}

}
END MODEL

DATA
list(Nobs=26, Nstr=13)
t[]   x[]   str[]   npoles[]   gap[]
228   3   1   2   47
6   1   1   2   47
30   36   2   1   39
77   124   2   1   39
206   7   3   1   31
59   10   3   1   31
5   1   3   1   31
217   246   4   2   30
14   23   4   2   30
114   2   5   2   35
114   0   5   2   35
119   4   6   4   28
14   6   6   4   28
258   224   7   2   29
138   0   8   3   26
14   0   8   3   26
138   0   9   3   30
14   0   9   3   30
61   118   10   2   28
14   26   10   2   28
```

```
279 10 11 2 29
60 0 12 1 38
208 0 12 1 38
70 0 13 4 28.5
20 1 13 4 28.5
168 0 13 4 28.5
END
```