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**Movement re-established but not restored: inferring the effectiveness of crossing mitigation by monitoring use**

Kylie Soanes\(^{a,b,*}\), Melissa Carmody Lobo\(^{a,b}\), Peter A. Vesk\(^b\), Michael A. McCarthy\(^b\), Joslin L. Moore\(^a\) and Rodney van der Ree\(^a\)

\(^a\) Australian Research Centre for Urban Ecology, Royal Botanic Gardens, Melbourne, VIC 3010, Australia

\(^b\) School of Botany, University of Melbourne, VIC 3010, Australia

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

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Abstract

Wildlife crossing structures are commonly used to mitigate the barrier and mortality impacts of roads on wildlife. For arboreal mammals, canopy bridges, glider poles and vegetated medians are used to provide safe passage across roads. However, the effectiveness of these measures is unknown. We investigate the effect of canopy bridges, glider poles and vegetated medians on squirrel glider movement across a freeway in south-east Australia. We monitored structures directly using motion-triggered cameras and passive integrated transponder (PIT) scanners. Further, post-mitigation radio-tracking was compared to a pre-mitigation study. Squirrel gliders used all structure types to cross the freeway, while the unmitigated freeway remained a barrier to movement. However, movement was not restored to the levels observed at non-freeway sites. Nevertheless, based on the number and frequency of individuals crossing, mitigation is likely to provide some level of functional connectivity. The rate of crossing increased over several years as animals habituated to the structure, with less than five crossings detected during the first 12 months of monitoring. We also found that crossing rate can be a misleading indicator of effectiveness if the number of individuals crossing is not identified. Therefore, studies should employ long-term monitoring and identify individuals crossing if inferences about population connectivity are to be made from movement data alone.
1. Introduction

Roads and traffic threaten the persistence of wildlife populations by fragmenting habitat, reducing gene flow and increasing mortality rates through roadkill (Bennett 1991; Fahrig and Rytwinski 2009; Forman and Alexander 1998; Holderegger and Di Giulio 2010). Wildlife crossing structures aim to mitigate these impacts by providing safe passage for wildlife across roads, yet in most cases their effectiveness has not been evaluated (Clevenger 2005; Forman et al. 2003; van der Ree et al. 2007; van der Ree et al. 2009). The first step in evaluating the effectiveness of crossing structures is to determine the frequency of crossing by target species and the number of individuals that use the structure (van der Ree et al. 2007).

The monitoring method employed and survey duration are critical as they are likely to affect the number of crossings detected and thus the perceived success of crossing structures (Hardy et al. 2003; Mateus et al. 2011). Thorough evaluation of the effectiveness of wildlife crossing structures is essential to ensure that successful measure are widely adopted, and unsuccessful ones are not repeated. Short-term studies which monitor the use of structures by wildlife without quantifying impacts of the road prior to mitigation, provide only a limited assessment of the extent to which wildlife crossing structures can restore, or maintain, connectivity (Hardy et al. 2003; van der Ree et al. 2007).

Arboreal mammals are highly susceptible to the mortality and barrier impacts created by large roads as they are often unable, or unwilling, to cross large gaps in tree cover (e.g. Asari et al. 2010; Goldingay and Taylor 2009; Laurance 1990; van der Ree et al. 2003). Road agencies increasingly rely on mitigation such as canopy bridges, glider poles or vegetated medians (retaining tall trees in the road median) to reduce the impacts on arboreal mammals, particularly on threatened species. However research on the use of these measures by wildlife
is limited to a few studies, including canopy bridges over rainforest roads (Weston et al. 2011), canopy bridges across a major freeway (Goldingay et al. 2012) and glider poles on landbridges which cross major roads (Goldingay et al. 2011).

Radio-tracking of individuals has demonstrated that the Hume Freeway in south-east Australia is a barrier to squirrel glider (*Petaurus norfolcensis*) movement, while vegetated medians retained during construction facilitated road crossing (van der Ree et al. 2010). Canopy bridges and glider poles have been built to mitigate the impacts on squirrel glider movement, although the effectiveness of these measures is unknown. Here, we investigate the effect of canopy bridges, glider poles and vegetated medians on squirrel glider movement using remotely-triggered cameras, passive integrated transponder (PIT) scanners and post-mitigation radio-tracking of individuals. We also explore the effects of survey duration and monitoring method on detected crossing rates and how these factors influence the perceived effectiveness of crossing structures.

2. Methods

2.1 Site and study species

We studied a 70 km section of the Hume Freeway between the rural towns of Avenel (33° 42’ S, 148° 176’ E) and Benalla (36° 55’ S, 145° 98’ E) in south-east Australia (Figure 1). This section was upgraded to a four-lane divided freeway during the 1970-80s with an average width of 53 m (44 – 76 m) including a centre median (21 – 38 m wide). The average traffic volume is 10 000 vehicles per day (speed limit 110 km/hr) 25 % of which occurs between 10 pm and 5 am (VicRoads, unpub. data), when native mammal species are most active. The surrounding landscape is predominantly cleared agricultural land with less than 5 % of the original (pre-European) tree cover remaining (Figure 1). The majority (83%) of remnant box-gum wood land (*Eucalyptus* spp.) exists as a network of linear strips along
roadsides and waterways (van der Ree 2002). Where linear strips are bisected by the freeway mature trees occur 5 – 20 m from the road edge. During the freeway upgrade, vegetated medians containing trees 20 – 30 m tall were retained at some sites reducing the gap in tree cover across the road to <15 m. Sites without vegetated medians where the treeless gap exceeds 50 m are referred to as 'unmitigated'. Linear remnants in this region contain a high density of large, hollow-bearing trees providing critical habitat for the squirrel glider, a small (~250 g), nocturnal gliding marsupial (family Petauridae) which is threatened in south-east Australia (van der Ree 2002). A gliding membrane that extends from each wrist to each hind leg allows individuals to glide from tree to tree. The average glide length is 20 – 35 m with a maximum of approximately 70 m, depending on launch height (Goldingay and Taylor 2009; van der Ree et al. 2003). Squirrel gliders very rarely move along the ground (Fleay 1947).

2.2 Crossing structures

In July 2007, approximately 20 – 30 years after the highway was upgraded, crossing structures were installed at five sites where the treeless gap across the road exceeded 50 m: Longwood (canopy bridge), Violet Town (canopy bridge), Balmattum (glider poles), Baddaginnie (glider pole) and Warrenbayne (glider pole). Prior to mitigation, radio-tracking at these sites detected no road crossings by squirrel gliders (van der Ree et al. 2010). Structures were placed where a linear strip of remnant woodland (usually along a single-lane rural road) intersected the freeway (Figure 1).

Each canopy bridge is approximately 70 m long and 0.5 m wide, constructed of UV stabilised marine-grade rope in a flat lattice-work configuration (i.e. analogous to a rope
ladder laid horizontally). The canopy bridges are suspended between two timber poles placed near roadside habitat trees at a minimum height of 6 m above the road surface (Figure 2a). Single strands of rope extend from the terminal ends of the structure into the adjacent tree canopy to encourage access by arboreal mammals. Wooden glider poles act as surrogate trees to reduce the gap in tree cover allowing the road to be crossed in several short glides (Figure 2b). A 2 m long cross-beam fixed 50 cm below the top of the pole provides a suitable launch site. A single glider pole (12 – 14 m high, 40 – 50 cm diameter) was installed in the centre median at each site (Balmattum, Baddaginnie and Warrenbayne) reducing the maximum glide distance across the road to less than 35 m. A second pole was required in the road verge at Balmattum due to the absence of tall, roadside trees.

2.3 Remote monitoring equipment

To detect arboreal mammals using the crossing structures we installed motion-triggered digital cameras on all canopy bridges and glider poles (Olympus, Faunatech Austbat, Pty Ltd). Cameras mounted to the supporting pole at each end of the canopy bridge were triggered by the movement of animals past two active infra-red sensor beams on the bridge (approximately one and four metres from the camera). At glider poles, cameras were mounted to a bracket providing a view of the cross-beam, where glides would most likely take place. Animals triggered the camera as they climbed past a set of sensor beams circumnavigating the pole below the cross-beam. Once triggered, all cameras recorded a series of images taken at three second intervals (five images at canopy bridges, nine images at glider poles) providing a sequence of crossing behaviour. The time and date of each image was also recorded. All camera systems were powered by a 12 V battery kept continuously charged by a solar panel. We downloaded images approximately once a fortnight, at which time we inspected the road and roadside within 100 m of each structure for dead squirrel gliders that may indicate predation or roadkill.
We monitored the canopy bridges from August 2007 – May 2011 and the glider poles from December 2009 – March 2011. Monitoring at the canopy bridges began one month after the structures were built. Glider poles were not monitored during the first 2.5 years as cameras suitable for long-term installation had to be designed and custom built. Due to false triggers (e.g. heavy rain, insects or debris) and equipment failure, data collection was not continuous throughout this period and the total monitoring effort varied at each structure. Of 1388 possible monitoring nights, cameras at the Longwood and Violet Town canopy bridges were operational on 56.7 % (787) and 62.9 % (873) of nights respectively. Monitoring effort at the glider poles was much lower. Of a possible 438 monitoring nights, cameras were operational on 19.8% (87) of nights at Balmattum, 8.4 % (37) of nights at Baddaginnie, and 5.0 % (22) of nights at Warrenbayne.

To investigate rate of use by individuals we trialled PIT scanning equipment at the Longwood canopy bridge. A single flatbed antenna connected to a decoder unit was installed at one end of the bridge (Trovan ANT-612 antenna and LID650 decoder, Microchips Australia, Pty Ltd). Tagged animals are detected as they pass over the antenna, and the time and date of crossings are recorded. The system was powered by a 12 V battery connected to a solar panel. The scanner was operational for 46 nights between November 2010 and April 2011.

2.4 Radio-tracking

Pre-mitigation radio-tracking was conducted along the freeway at unmitigated (n = 3) and vegetated median (n = 3) sites from December 2005 – May 2006 (see van der Ree et al. 2010). The pre-mitigation study also included control sites (n = 2) located over 6 km away from the freeway, where squirrel gliders had to cross single lane, low traffic-volume roads (less than 10 m wide, ~100 vehicles per day). Habitat quality and configuration were similar
at all site types. We replicated the pre-mitigation radio-tracking to determine the impact of
mitigation on squirrel glider movement. Post-mitigation radio-tracking was conducted at
vegetated median, canopy bridge, glider pole, unmitigated freeway and control sites (Figure
1, Table 1). All sites that were unmitigated during the 2005/06 survey had crossing structures
installed in 2007, so we included two additional unmitigated sites in this study. None of the
individuals collared in the pre-mitigation study were also collared in the post-mitigation
study. There was no difference in traffic volume pre- and post-mitigation.

Post-mitigation, squirrel gliders were trapped at 11 sites during 2575 trap nights
between November 2010 and March 2011 (using identical methods to van der Ree et al.
2010). The trapping effort varied between each site depending on the time taken to capture
and collar a sufficient number of animals. Wire-mesh cage traps (17 cm x 20 cm x 50cm)
were baited with a mixture of rolled oats, peanut butter and honey, and nailed to tree trunks at
a height of 2 – 4 m. Trapping transects extended along linear woodland strips intersecting the
freeway or local, low traffic volume roads (control sites). Traps were set on both sides of the
road intersection at approximately 50 m intervals, beginning at the road edge and extending
up to 250 m away.

Resident adults at each site were fitted with single-stage tuned-loop radio-collars (150
MHz; Sirtrack, New Zealand) weighing less than 5% of body weight (Table 1). To reduce the
chance that a collared animal had to cross through opposing home ranges to access the
crossing structure, only animals captured within 250 m of the road were collared. Radio-
tracking was undertaken on foot using a Regal 2000 receiver and a Yagi 3-element antenna
(Titley Electronics, Australia) using the same methods as the pre-mitigation study (van der
Ree et al. 2010). In brief, we collected homing fixes (the actual location of the animal to an
accuracy of ±10 m) as well as directional fixes from the road edge to determine which side of
the road the glider was on.
2.5 Data analysis

2.5.1 Camera monitoring of crossing rates

The crossing rate at canopy bridges and glider poles was calculated by dividing the number of crossings that occurred by the number of nights that the camera was operational. Placing a camera at each end of the canopy bridge allowed us to distinguish crossings from non-crossings by confirming that the animals passed both cameras. When a second camera was not functioning we inferred crossings based on the animal behaviour and direction of travel. We could not confirm crossings at glider poles as it was not possible to observe which side of the road an animal originated from or travelled to. Therefore, we may have overestimated the number of crossings recorded at glider poles if a large number of animals glided to the centre pole but returned to their original side without crossing.

We expected the crossing rate to increase over time and approach some asymptote as animals habituated to the structure. Therefore, we assumed the crossing rate $t$ years after the crossing structure was installed at site $i$ followed a logistic function of the form:

$$x_{i,t} = K_i/(1 + \exp(-a-bt)),$$

where $a$ and $b$ are coefficients that define how the crossing rate changes over time ($b$ is constrained to be positive) and $K_i$ is the asymptotic crossing rate, which is site specific. We assumed $a$ and $b$ were common for all sites because we had insufficient data to estimate these parameters separately for each site. We felt that $K$ was more likely to differ among sites, providing a better indication of structure effectiveness and there was no prior information to suggest how $a$ or $b$ would vary among sites.

The data used to estimate the model parameters were the number of crossings observed within each of 59 time periods during which the cameras were operating. These
periods varied in length. Let $t_1$ be the time at the start of a period, and $t_2$ be the time at the end of that period. Then the expected number of crossings within that period is given by the integral

$$\lambda_{i,t_1,t_2} = \int_{t_1}^{t_2} x_{i,t} \, dt$$

We modelled the actual number of crossings at site $i$ in the time interval $[t_1, t_2]$ as a sample from a Poisson distribution with parameter $\lambda_{i,t_1,t_2}$.

We used Bayesian inference to fit the model in Open BUGS 3.2.1 (Spiegelhalter et al. 2011). To reflect the lack of prior information we selected vague prior distributions for all parameters, using a normal distribution with mean 0 and standard deviation 1000 for each of $K_i$ and $a$, and a uniform distribution in the interval 0 to 100 for $b$. We included a prediction contrast comparing the post-habituation crossing rate of canopy bridge sites to glider pole sites to investigate the influence of structure type on crossing rate. Code is supplied as Appendix A. The model was run for 100 000 iterations after discarding a burn in of 50 000 at which time we were satisfied that the model had reached convergence.

2.5.1 Radio-tracking movements BACI

We conducted post-mitigation radio-tracking between November 2010 and May 2011, collecting 1335 fixes from 42 squirrel gliders (18 females and 24 males) that were used in subsequent analysis (Table 1). The pre-mitigation dataset included 1993 fixes from 47 squirrel gliders (23 females, 24 males). A crossing was recorded when two consecutive fixes (homing or directional) for an individual were obtained on opposite sides of the road (i.e. all four lanes and median were crossed). In contrast to van der Ree et al. (2010), we did not
include partial crossings (where an individual moved to the centre median and then returned to side of origin), as we were primarily interested in complete crossings of the road barrier. The proportion of individuals crossing at each treatment was calculated and compared with the results from the pre-mitigation study.

We used a logistic regression to model the probability that a squirrel glider crossed the road as a function of the treatment and survey period (pre- or post-mitigation). There were insufficient data to fit an effect of sex, or investigate differences between the two crossing structure types (i.e. canopy bridges and glider poles were pooled). A Bernoulli distribution with parameter $p$ was drawn to model whether a squirrel glider, $i$, crossed or not:

$$\text{logit}(p_i) = \text{logit}(p) + b_t(t_i) + b_p(p_i) + \text{int}(t_i, p_i)$$

where $\text{logit}(p)$ is the intercept, $b_t(t_i)$ is the effect of treatment, $b_p(p_i)$ is the effect of period, and $\text{int}(t_i, p_i)$ is the interaction between periods for the each treatment. The categorical variables $b_t(t_i)$ and $b_p(p_i)$ were modelled using a reference class, set arbitrarily to zero for control sites and the pre-mitigation period. We were unable to fit a random effect for site due to insufficient data. We used Bayesian inference to fit the model (Open BUGS 3.2.1) using uninformative priors. The prior for parameter $p$ was a uniform distribution $[0,1]$. The priors for parameters $b_t(t_i)$, $b_p(p_i)$ and $\text{int}(t_i, p_i)$ (excluding interactions with parameters set to their reference state, which were set to zero) were normal distributions (mean 0, standard deviation 100). Code is supplied as Appendix B. The model was run for 100 000 iterations after discarding a burn in of 50 000, at which time we were satisfied the model had reached convergence.

3. Results

3.1 Camera monitoring of crossing structures
Cameras detected squirrel gliders at all five crossing structures with 1187 crossings from 1660 functioning camera nights at canopy bridges and 13 crossings from 146 functioning camera nights at glider poles (Figure 2). No signs of predation or mortality were observed during regular site surveys and no owls or other potential predators were recorded on or near the structures. Squirrel gliders were first detected crossing the Violet Town canopy bridge after eight months of monitoring (i.e. nine months after the structure was installed), while no crossings were detected at the Longwood canopy bridge during the first 13 months of monitoring. It was not possible to determine the date of first use at the glider poles as monitoring of these sites did not begin immediately after mitigation.

The statistical model predicted an increase in squirrel glider crossings over time at all sites before reaching a maximum, or post-habituation, rate (Figure 3). The median post-habituation crossing rates were highest at the Longwood canopy bridge (2.47, 95% credible interval 2.27 – 2.72) and Warrenbayne glider pole (0.35, 95% credible interval 0.16 – 0.65), with less than 0.10 crossings per night at all other sites (Figure 3). Crossing rates were slightly higher at canopy bridges than the glider poles, with the prediction contrast indicating a median of 1.07 as many crossings per night at canopy bridges, (95% credible interval, 0.93 – 1.23). However, this was primarily driven by the high crossing rate at the Longwood canopy bridge.

3.2 Monitoring using PIT scanners

The PIT scanner recorded crossings by three out of the six tagged squirrel gliders known to be present within 600 m of the Longwood canopy bridge. One adult female carrying two pouch young, one adult male, and one young male (recently independent) were detected crossing 63 times over 46 nights. The female and older male were also radio-collared, and tracking records show that they share a nest site within 200 m of the canopy
bridge. The average crossing rate for each individual ranged from 0.39 – 0.76 crossings per night during the six month period.

3.3 Radio-tracking before-after-control-impact (BACI)

The proportion of squirrel gliders crossing a road during the post-mitigation radio-tracking study was highest at control sites (70%), with less than 50% of individuals crossing at any mitigated highway site and no squirrel gliders crossing the unmitigated highway (Table 1). This is reflected in the logistic regression model, which shows that the probability of squirrel gliders crossing a road was higher at control sites than at any type of freeway site (Figure 4). Installing crossing structures (canopy bridges and glider poles) along the freeway increased the probability of crossing to a similar level as vegetated medians, while the probability of crossing the unmitigated freeway remained very low during both periods (Figure 4). The uncertainty around the parameter estimates was broad because less than 50 squirrel gliders could be captured and collared during both the pre- and post-mitigation surveys.

3.4 Crossing detectability of different methods

From November 2010 – May 2011 both canopy bridges and two glider poles were monitored using cameras and radio-tracking, and a PIT scanner was also operational at one canopy bridge (Table 2). Radio-tracking underestimated the crossing rate at all sites except for Violet Town, where the crossing rate was very low. Two individuals at the Longwood canopy bridge were both PIT-tagged and radio-collared and the PIT scanner detected a higher crossing rate than radio-tracking for both animals (Table 2).

4. Discussion

4.1 Movement re-established but not restored
Canopy bridges and glider poles can re-establish squirrel glider movement across a major road. We detected squirrel gliders using all five crossing structures installed at locations where the Hume Freeway was previously a barrier to movement (van der Ree et al. 2010). Uptake of the crossing structures was rapid considering the freeway has potentially been a barrier for approximately 30 years. Within four years of their installation, the probability of a squirrel glider crossing the freeway using crossing structures was similar to that at vegetated medians which have been present since the freeway was upgraded. All mitigation measures improved crossing by squirrel gliders relative to unmitigated sites, which remained a barrier to movement.

Despite the increase in freeway crossing, no mitigation strategy restored movement to the levels observed at control sites. This suggests that the gap in tree cover is not the only factor influencing road crossing by squirrel gliders. Squirrel gliders at control sites readily crossed low traffic volume roads with very little nocturnal traffic, while freeway sites have approximately 2500 vehicles per night, which may create enough noise and light disturbance to reduce crossing. Noise and traffic volume were found to reduce the use of crossing structures by other species (Clevenger et al. 2001; Olsson et al. 2008) and it may be that crossing structures cannot completely mitigate road impacts where the target species is vulnerable to traffic disturbance.

While movement across the freeway was not fully restored, if mitigation increases gene flow and reduces roadkill then it is likely to improve the viability of roadside populations. Previous mark-recapture research found that the survival rate of squirrel glider populations living adjacent to the Hume Freeway is 60% lower than at control sites, suggesting that any reduction in roadkill as a result of mitigation would be beneficial (McCall et al. 2010). Furthermore, a population viability analysis completed for the greater glider (*Petaurroides volans*) found that even low dispersal rates would prevent the extinction of sub-
populations separated by a road (Taylor and Goldingay 2009). We detected multiple individuals of both sexes and all ages using crossing structures, therefore it is likely that some level of functional connectivity is provided (Bissonette and Adair 2008; Clevenger 2005; Vucetich and Waite 2000). The next step is to determine what proportion of road crossing resulted in gene flow, and if roadside populations are now viable as a result of mitigation (Clevenger 2005; Corlatti et al. 2009; Riley et al. 2006; van der Ree et al. 2007).

**4.2 Monitoring method influences the detection of crossings**

Short sampling windows can lead to inaccurate conclusions about the effectiveness of mitigation (Clevenger 2005; Gagnon et al. 2011). We found that the crossing rate for squirrel gliders increased over time as animals habituated to the structures over several years of monitoring. For example, if we had stopped monitoring the canopy bridges after 12 months we would have detected only three crossings at one site, despite almost continuous camera monitoring during that period. Based on that evidence it would be hard to argue that canopy bridges were an effective form of mitigation. Most species show an adaption to crossing structures over time, with some taking up to a decade to habituate (Bond and Jones 2008; Clevenger and Waltho 2003; Gagnon et al. 2011; Olsson et al. 2008; Weston et al. 2011). Long-term monitoring ensures that animals have had time to habituate to the structure and increases the chance of detecting infrequent dispersal movements (Bissonette and Adair 2008; Corlatti et al. 2009; Hardy et al. 2003).

Crossing rate is often used as an indicator of crossing structure effectiveness, yet we found that monitoring crossing rate alone can be misleading. Though the crossing rate for squirrel gliders at the Longwood canopy bridge was much higher than any other structure, the PIT scanner revealed crossings were made by only three out of six tagged individuals known to be present within 600 m of the structure. Squirrel gliders actively defend their territory
from members of neighbouring social groups and there is little overlap of home ranges of animals within linear strips (van der Ree and Bennett 2003). Radio-tracking and mark-recapture surveys suggest that the three animals using the canopy bridge belong to the same social group and incorporate the structure as part of their territory, crossing regularly to access resources on both sides of the road. While this is a positive outcome for those individuals, if a structure benefits only a select few it is unlikely to improve connectivity and survival for the whole population and therefore unlikely to be effective despite a high observed crossing rate (Corlatti et al. 2009; Riley et al. 2006; Simmons et al. 2010). The social organisation and territorial behaviour of target species should be considered when evaluating the effectiveness of crossing structures as it is likely to influence the number of individuals able to access a structure.

Similarly, low crossing rates do not necessarily mean a structure is ineffective. Many studies relate crossing rates to the presence of roadside habitat, local population abundance or crossing structure design (Cain et al. 2003; Clevenger and Waltho 2000; Ng et al. 2004). In our study there was no difference in these factors that could explain why some structures had comparatively lower crossing rates. The site with the lowest crossing rate, Violet Town, had the highest population density (unpub. data) and five individuals regularly located within 50 m of the structure were never detected crossing (despite high camera monitoring effort). Where roadside habitat already provides adequate resources, individuals are unlikely to depend on the crossing structure for daily movements. In these cases the structure may only be used infrequently for dispersal or re-colonisation and can still be effective despite a low observed crossing rate.

We found that radio-tracking detected fewer crossings and fewer individuals than directly monitoring the structure using cameras or PIT scanners. This is not surprising, as it is rarely possible to collar and continuously monitor all animals likely to encounter the
structure. Furthermore, BACI radio-tracking studies require an intensive field effort and large sample sizes that may not be feasible when working with rare species. Cameras and PIT scanners can provide continuous, long-term monitoring of structures, recording the timing and direction of crossings, the frequency at which they occur, and the identity and demographic characteristics of the individuals crossing (Ford et al. 2009; Mateus et al. 2011; Olsson et al. 2008). Combining these techniques with non-invasive genetic sampling could allow stronger inferences about the effectiveness of crossing structures to be made in the absence of intensive population monitoring (Clevenger and Sawaya 2010; Simmons et al. 2010).

4.3 Conclusion

This study shows that canopy bridges and glider poles can rapidly re-establish the movement of squirrel gliders across a road barrier. Based on the number of individuals and frequency of crossings, it is likely that canopy bridges, glider poles and vegetated medians provide some level of functional connectivity for squirrel gliders. However, the impact of the freeway on movement was only partially mitigated relative to non-freeway sites, suggesting other factors such as traffic disturbance may influence crossing behaviour. Long-term studies which identify the number of individuals using a structure and their demographic characteristics are essential when inferring the impacts of mitigation on connectivity in the absence of population data (e.g. Clevenger and Waltho 2003; Gagnon et al. 2011). Our work suggests that monitoring periods of at least two years are required to allow squirrel gliders adequate time to habituate to retrofitted crossing structures. Longer-term research is required to determine if the current crossing rates at canopy bridges, glider poles and vegetated medians are enough to restore gene flow and improve survival rates in roadside populations.
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**Figure captions**

**Figure 1.** Map of the study area surrounding the Hume Freeway in south-east Australia showing the location of crossing structures and pre- and post-mitigation radio-tracking sites (DSE 2011).

**Figure 2.** Canopy bridge (a,c) and glider pole (b,d) installed along the Hume Freeway in south-east Australia.

**Figure 3.** The predicted change in the median crossing rate (number of crossings per night) of squirrel gliders over time since installation of the Longwood canopy bridge (diamonds) and Warrenbayne glider pole (squares) with 95% credible intervals indicated by the dotted line. The post-habituation median crossing rate at the Violet Town canopy bridge (cross) and the Balmattum and Baddaginnie glider poles (triangle and circle, respectively) is also shown (error bars indicate 95% credible intervals).

**Figure 4.** The mean predicted probability of radio-collared squirrel gliders crossing the road at each treatment type pre-mitigation (closed circles) and post-mitigation (open circles). Canopy bridges and glider poles were grouped as 'crossing structure'. It was assumed that the likelihood of individuals crossing at a crossing structure prior to mitigation was the same as at unmitigated sites. Error bars are 95% credible intervals.
Figure 2
Table 1. Summary of post-mitigation radio-tracking effort of 42 squirrel gliders along the Hume Freeway and control sites in south-east Australia.

<table>
<thead>
<tr>
<th>Treatment and sex</th>
<th>No. of individuals tracked</th>
<th>Mean no. of tracking nights&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean no. of fixes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of individuals crossing</th>
</tr>
</thead>
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<td><strong>Control (n = 3)</strong></td>
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<td>Male</td>
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<td>23.8 ± 2.6</td>
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<td>Female</td>
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<td>19.2 ± 2.8</td>
<td>27.8 ± 2.9</td>
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<td><strong>Freeway, vegetated median (n = 2)</strong></td>
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<td>Male</td>
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<td>30.7 ± 0.7</td>
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<tr>
<td>Female</td>
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<td><strong>Unmitigated (n = 2)</strong></td>
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<td>8</td>
<td>29.1 ± 2.2</td>
<td>34.9 ± 1.7</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>16.3 ± 0.5</td>
<td>30.3 ± 1.5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Canopy bridge (n = 2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>29.0 ± 2.1</td>
<td>34.0 ± 2.6</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>19.8 ± 3.4</td>
<td>31.8 ± 1.3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Glider pole (n = 2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>28.2 ± 2.8</td>
<td>33.8 ± 1.7</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>13.5 ± 2.5</td>
<td>30.0 ± 4.0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Where values are averages, ± 1 SE is shown.
Figure 3

Predicted median crossing rate

Number of days since crossing structure installed
Figure 4

![Graph showing predicted probability of road crossing for different conditions.](image)

- Control
- Vegetated median
- Crossing structures
- Unmitigated

**Y-axis:** Predicted probability of road crossing

**X-axis:** Freeway conditions

- Control
- Vegetated median
- Crossing structures
- Unmitigated
Table 2. Comparison of crossing rate of squirrel gliders detected by motion-triggered camera, PIT scanner and radio-tracking between November 2010 and May 2011.

<table>
<thead>
<tr>
<th>Structure type</th>
<th>Camera</th>
<th>Radio-tracking</th>
<th>PIT scanner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longwood</td>
<td>Canopy bridge</td>
<td>2.66</td>
<td>0.40</td>
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<tr>
<td>Female</td>
<td>-</td>
<td>0.31</td>
<td>0.76</td>
</tr>
<tr>
<td>Male</td>
<td>-</td>
<td>0.32</td>
<td>0.39</td>
</tr>
<tr>
<td>Violet Town</td>
<td>Canopy bridge</td>
<td>0.02</td>
<td>0.021</td>
</tr>
<tr>
<td>Warrenbayne</td>
<td>Glider pole</td>
<td>0.38</td>
<td>0.23</td>
</tr>
<tr>
<td>Balmattum</td>
<td>Glider pole</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>