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A remarkably quick habituation and high use of a rope bridge by an endangered marsupial, the western ringtail possum

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Abstract

Rope bridges are being increasingly installed worldwide to mitigate the negative impacts of roads on arboreal animals. However, monitoring of these structures is still limited and an assessment of factors influencing the crossing behaviours is lacking. We monitored the use of a rope bridge near Busselton, Western Australia by the endangered western ringtail possums (*Pseudocheirus occidentalis*) in order to identify the patterns of use and factors influencing the crossings. We installed motion sensor cameras and microchip readers on the bridge to record the crossings made by individual animals, and analysed these crossing data using generalised linear models that included factors such as days since the installation of the bridge, breeding season, wind speed, minimum temperature and moonlight. Possums started investigating the bridge even before the installation was completed, and the first complete crossing was recorded only 36 days after the installation, which is remarkably sooner than arboreal species studied in other parts of Australia. The possums crossed the bridge increasingly over 270 days of monitoring at a much higher rate than we expected (8.87 ± 0.59 complete crossings per night). Possums crossed the bridge less on windy nights and warm nights probably due to the risk of being blown away and heat stress on warmer days. Crossings also decreased slightly on brighter nights probably due to the higher risk of predation. Breeding season did not influence the crossings. *Pseudocheirus occidentalis* habituated to the bridge very quickly, and our results demonstrate that rope bridges have a potential as an effective mitigation measure against the negative impacts of roads on this species. More studies and longer monitoring, as well as investigating whether crossing results in the restoration of gene flow are then needed in order to further assess the true conservation value of these crossing structures.

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Keywords
Road ecology, rope bridge, habitat fragmentation, *Pseudocheirus occidentalis*, wildlife crossing structures

Introduction

Roads can act as a barrier to movement and gene flow in wildlife populations and cause genetic isolation and fragmentation. This can results in lowered fitness and adaptability, which increases the risk of population extinction (Forman and Alexander 1998). To mitigate against these impacts, an increasing number of wildlife crossing structures are being installed worldwide because they have the potential to prevent road mortality and habitat fragmentation by providing animals with safe passages across roads (Clevenger and Wierzchowski 2006).

Arboreal species can be especially affected by roads because of their fidelity to canopies and naivety on the ground (Lancaster et al. 2011). Many rope bridges, or canopy bridges, have been installed worldwide to mitigate negative impacts on arboreal species, including several opossum, monkey, dormouse and squirrel species (Norwood 1999, Teixeira et al. 2013, Sonoda 2014). In the eastern parts of Australia, rope bridges have been built for gliders, possums, and koalas (*Phascolarctos cinereus*); however, monitoring of the use of these structures by the target species is still limited to a handful of cases (Weston et al. 2011, Goldingay et al. 2013, Soanes et al. 2013), and assessment of factors influencing the use of these structures is lacking.

In Western Australia a rope bridge was installed on Caves Road near Busselton in 2013. The bridge was targeted to provide safe crossing for the western ringtail possum (*Pseudocheirus occidentalis*), a nocturnal, folivorous, arboreal marsupial endemic to southwest Western Australia (Figure 1a). In a national action plan for Australian mammals in 2012, this species was classified as critically endangered due to a continuing dramatic decline in its numbers and range (Woinarski et al. 2014). Habitat destruction, habitat fragmentation and introduced predators such as red foxes (*Vulpes vulpes*) and feral cats (*Felis catus*) are thought to be the main causes of their decline (Department of Environment, Water, Heritage and Arts 2008, Morris et al. 2008). The Busselton region is considered to be one of few strongholds left for this species possibly because it still has a relatively high abundance of the species' main food source, the peppermint tree (*Agonis flexuosa* in the Myrtaceae Family). However, this area is also subject to rapid and large-scale developments, which threaten the persistence of the species (Jones et al. 1994a, Australian Bureau of Statistics 2014). These possums are highly sedentary and have home ranges as small as 0.31 ha in high density areas (Yokochi et al. 2015). They show a high fidelity to canopies, and Yokochi et al. (2015) found that Caves Road, a 15 m wide road was restricting their movements and home ranges. Trimming et al. (2009) also found this road to be a roadkill hotspot for this species (Figure 1b).

We monitored the use of this bridge by *P. occidentalis* and other fauna to identify the patterns of use and factors influencing the crossings. In previous studies, animals

Figure 1. Photographs of the western ringtail possum (*Pesudocheirus occidentalis*). **a** A possum at Locke Nature Reserve **b** A possum roadkill in Busselton, Western Australia.

have been observed to show reluctance towards wildlife crossing structures for a certain period of time before they habituated to them and started using them regularly (Gagnon et al. 2011). For example, possums and gliders in eastern Australia started using rope bridges after 7 to 17 months of bridge constructions, and the number of crossings increased over time until it reached asymptotes (Weston et al. 2011, Goldingay et al. 2013, Soanes et al. 2013). Therefore, we expected that *P. occidentalis* would avoid using the rope bridge for a certain period of time before it starts crossing, and that the number of crossings would increase over time and eventually reach an asymptote.

Several arboreal marsupials increase their activity ranges or change their movement patterns during the breeding season in search of mates and additional resources (Gentile et al. 1997, Broome 2001, Loretto and Vieira 2005). Other arboreal folivorous species have been observed to be less active on well lit nights with low temperatures, presumably to avoid the risk of predation and heat loss (Laurance 1990, Starr et al. 2012, Rode-Margono and Nekaris 2014). Greater wind speed also decreased the number of common brushtail possums (*Trichosurus vulpecula*) observed in open pasture (Paterson 1993). Wind speed did not influence the detection rate of *P. occidentalis* in a forest habitat (Wayne et al. 2005); however, the rope bridge in this study was completely exposed to the wind over the road, and strong wind could deter *P. occidentalis* from crossing the open bridge. Given this information, we also predicted that *P. occidentalis* would cross the bridge more during their breeding seasons and less on well lit, cold and/or windy nights.

Methods

Study area and rope bridge

In July 2013, a rope bridge was constructed across Caves Road about 9 km west of Busselton, Western Australia (33°39'32"S; 115°14'26"E) to connect peppermint trees in Locke Nature Reserve to those in a campsite across the road. Caves Road is a 15 m wide major road connecting popular tourist destinations in the region. The recorded daily traffic volume on this road was 6,000 cars in 2008, but it could vary up to 15,000 cars in the peak tourist season (Main Roads WA 2009, G. Zoetelief, Pers. Comm.). Locke Nature Reserve and its surrounding campsites are known to support the highest density of *P. occidentalis* in the Swan Coastal Plain, a region dominated by *A. flexuosa* vegetation, which is an ideal habitat for the possums (Jones et al. 1994a, Jones et al. 2007). Another possum species, the common brushtail possum, has also been observed in the nature reserve at a low density (Clarke 2011, Yokochi unpublished data).

The rope bridge was supported by an approximately 8.5 m tall wooden pole with a concrete foundation and two metal stay wires on each side of the road. The bridge was 300 mm in width and approximately 26.5 m in length. Two steel wires running between poles with nettings of marine grade ropes in between provided a flat surface for possums to cross (Figure 2). We employed the flat design over a box design because the

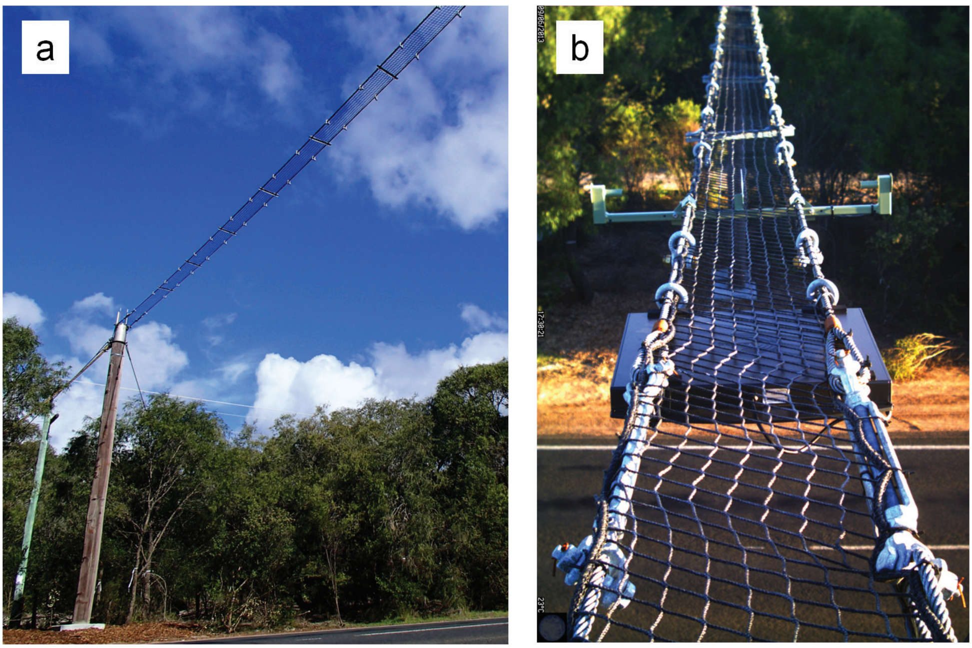

Figure 2. A rope bridge installed on Caves Road near Busselton, Western Australia. **a** Two stay wires and a rope extending from the pole of a rope bridge to nearby trees on South side of Caves Road **b** Close up of the bridge showing one of the sensors and microchip reader on the North side (taken by an infrared camera on the bridge).

box design was found to be unnecessary (Weston et al. 2011). One large rope extending from the top of each pole provided a passage between the bridge and surrounding trees, together with the metal stay wires that were in contact with nearby trees.

Monitoring

We captured 44 female and 53 male western ringtail possums within two 200 m x 200 m blocks on the North and South sides of the rope bridge site on Caves Road from March 2010 to April 2014 (Figure 3). To capture the possums, we used a specially built tranquiliser gun with darts containing a nominal dose of 12 mg/kg of Zoletil 100® (Virbac Australia, NSW Australia) following a method developed by P. de Tores and reported by Clarke (2011). A Trovan Unique ID100 Implantable Transponder (Trovan, Ltd., U.K.) was inserted subcutaneously between the shoulder blades of each captured possum.

Thirty days after the installation of the rope bridge, an infrared camera (BuckEye Cam Orion camera, BuckEye Cam Australia, Victoria), a microchip reader (LID650N / ANT612 system, Dorset Identification B.V., Aalten, Netherlands), and a pair of optical sensors were set up on each end of the bridge. When an animal moved past and blocked one of the sensors, this triggered the camera to take three consecutive photos and activated the microchip reader for a period of 30 seconds. Date and time were recorded on every photograph taken, and the microchip readers recorded the date, time and microchip code of individuals that used the bridge. Unfortunately, the microchip readers malfunctioned regularly, so we used photographic data from 270 nights of monitoring from August 2013 to May 2014 for further analyses.

A crossing was regarded "partial" if an animal was recorded on one side of the bridge only and returned back to its original side. A crossing was regarded "complete" if an animal was recorded leaving one side and then arriving on the other side within 10 minutes. We recorded the simultaneous crossing by two and three adults as two and three crossings respectively; however, a crossing by a pair of mother and young was counted as a single crossing. Species, type, and direction of the crossings were obtained from photographic data to calculate the number of complete crossings of the bridge by *P. occidentalis* on each night.

Data analyses

We used generalised linear models with a negative binomial distribution and log link function to identify the factors influencing the number of crossings per night because the crossing data were discrete and overdispersed (Byers et al. 2003). Based on our hypotheses, we constructed candidate models with variables such as days since the bridge installation, breeding season, daily minimum temperature, fraction of the moon lit, and daily maximum wind speed (Table 1). We set the breeding season as

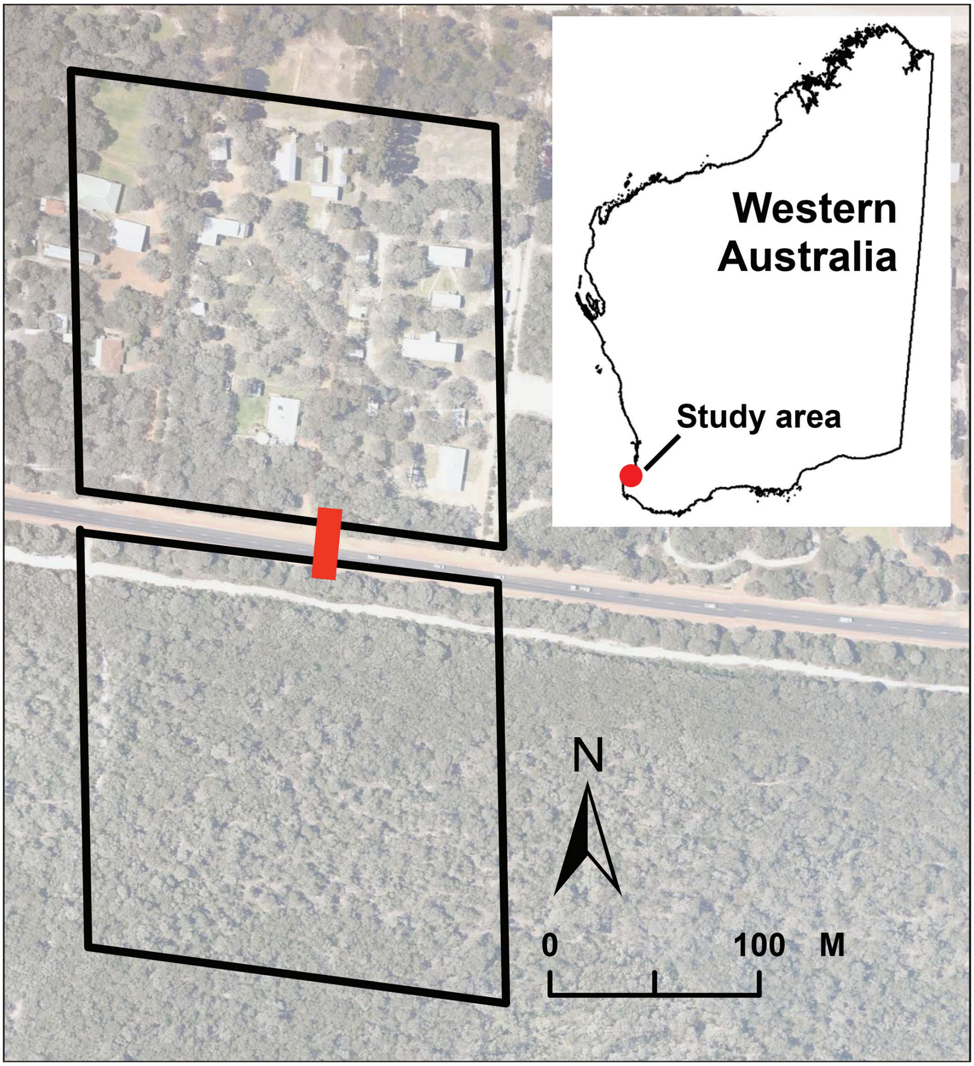

Figure 3. A map of the study area near Busselton, Western Australia. Black rectangles represent the areas where *Pseudocheirus occidentalis* were captured for tagging, and the thick red line represents the rope bridge across Caves Road.

April to July and September to November, which are the known breeding peaks for *P. occidentalis* in the Busselton region (Jones et al. 1994b). We obtained data from the Australian Bureau of Meteorology (2014) on daily minimum temperature and maximum wind speed at Busselton Regional Airport, which is approximately 15 km from the study site. Data on the fraction of the moon illuminated at 10 pm in Western Australia were obtained from The United States Navy Observatory (2014).

We ran generalized linear models using the package MASS v.7.3-35 (Ripley et al. 2014) on R v3.0.1 (R Foundation for Statistical Computing 2013, available at

Table 1. Candidate models used to analyse variables affecting the number of crossings of a rope bridge by *Pseudocheirus occidentalis*.

Variables	Hypothesis tested
Time	Crossings will increase over time.
Breeding	Crossings will increase in breeding seasons.
Min temp	Crossings will decrease on cold nights.
Moon	Crossings will decrease on well lit nights.
Wind	Crossings will decrease on windy nights.
Time + Breeding	Crossings will increase over time and in breeding seasons.
Time + Min temp	Crossings will increase over time but decrease on cold nights.
Time + Moon	Crossings will increase over time but decrease on well lit nights.
Time + Wind	Crossings will increase over time but decrease on windy nights.
Min temp * Moon	Crossings will decrease on cold nights if the moon is bright.
Null	The number of crossings varies randomly.

A generalised linear model with negative binomial regression was used to compare these candidate models. "+" denotes additive effects of variables and "*" denotes additive and interactive effects of variables.

http://R-project.org), and ranked the models based on Akaike Information Criterion (AIC) values. The model with the lowest AIC value was chosen as the best fit for the data. We considered models with ΔAIC values (the difference between the AIC value of the model and that of the highest ranking model) of less than 2 to have strong support, those with ΔAIC values between 2 and 7 to have weak support and those with ΔAIC values of greater than 7 to have no support from our data (Burnham and Anderson 2002). We also generated 95 % confidence intervals for each of the parameters to check for directionality and significant divergence from zero.

Results

Within a week of installation of the poles, an author (KY) observed two western ringtail possums on stay wires investigating the poles. This was even before the metal wires and rope nettings were installed between the poles (i.e. before the installation of the bridge was completed). Three separate partial crossings by *P. occidentalis* were recorded on 16 photos on the first night of monitoring on the North end of the bridge. The first complete crossing from North to South was recorded on the 6th night of monitoring, only 36 days after the installation of the bridge had been completed. During 270 nights of monitoring, cameras recorded 664 complete crossings from North to South and 636 complete crossings from South to North, totalling 1300 crossings. The number of complete crossings increased gradually over time (Figure 4), and *P. occidentalis* completely crossed the bridge at least three times a night for the last 100 nights of monitoring. The rate of crossings was 8.87 ± 0.59 (s.e.) complete crossings per night for the last 30 nights of monitoring. No other species, including common brushtail possums, was captured on cameras other than several

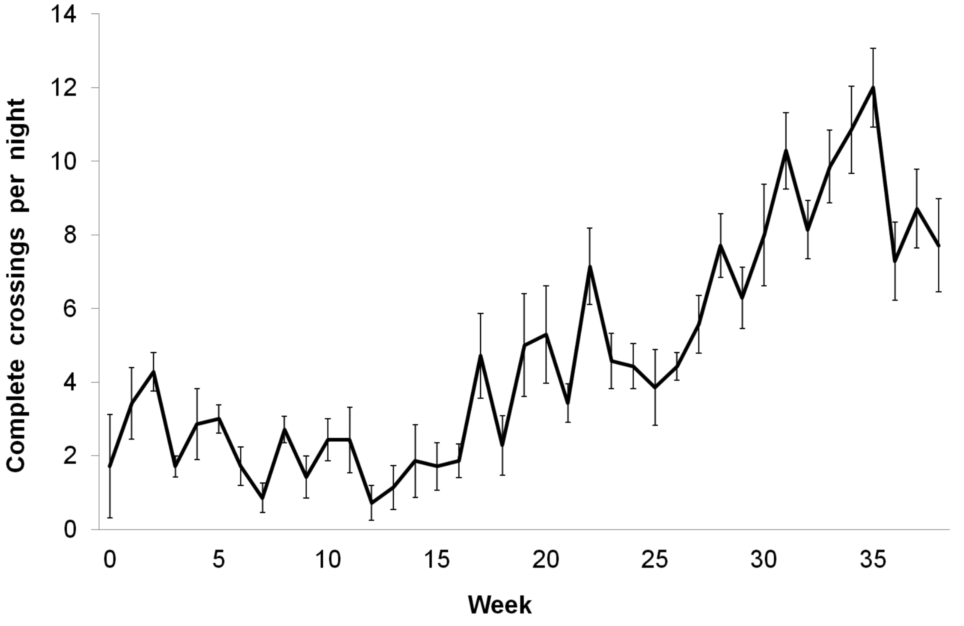

Figure 4. Weekly averages of the number of complete crossings by *Pseudocheirus occidentalis* on a rope bridge installed over Caves Road near Busselton, Western Australia. The thick line shows the weekly averages and thin vertical lines represent standard errors of the means.

birds, including Australian magpies (*Cracticus tibicen*), tawny frogmouths (*Podargus strigoides*), common bronzewings (*Phaps chalcoptera*), silvereyes (*Zosterops lateralis*), and red wattlebirds (*Anthochaera carunculata*) resting on the bridge.

Microchip readers malfunctioned regularly, and not all possums using the bridge were microchipped, so only five microchipped individuals were recorded on eight nights. The North reader recorded one female partially crossing the bridge four times on one night, and the possum was also photographed on the bridge with her young on multiple occasions. After gaining independence, her young was recorded crossing the bridge on its own. Other mothers and their young as well as pairs of a male and a female were also regularly captured by the cameras while crossing the bridge together (Figure 5).

The number of crossings by *P. occidentalis* had a strong positive correlation with time since bridge installation (Table 2). At the same time, the number of crossings decreased on nights with greater maximum wind speed. The number of crossings was found to increase on colder nights although this effect was not as strong as that of wind (ΔAIC = 1.4). The fraction of the moon lit had a considerably weaker negative effect on the number of crossings compared to wind speed and minimum temperature (ΔAIC = 4.9). The correlation between the number of crossings and breeding season was even weaker (ΔAIC = 5.4) and 95 % confidence interval of its parameter estimate included zero, indicating that the breeding season did not affect the number of crossings. The interaction effect between the moonlight and minimum temperature also had no support (ΔAIC = 159.5).

Table 2. Generalised linear model analysis on number of crossings of a rope bridge by *Pseudocheirus occidentalis*.

Variables included in the model	AIC	ΔAIC	Parameter estimates
Time + Wind speed	1291.9		Time = 0.007*, Wind = -0.011*
Time + Min temp	1293.3	1.4	Time = 0.007*, Min temp = -0.027*
Time + Moon	1296.8	4.9	Time = 0.007*, Moon = -0.211*
Time + Breeding	1297.3	5.4	Time = 0.007*, Breeding = -0.141
Time	1299.1	7.2	
Wind	1430.0	138.1	
Breeding	1448.7	156.8	
Null	1448.7	156.8	
Min temp	1449.2	157.3	
Moon	1449.6	157.7	
Moon * Min temp	1451.4	159.5	

Akaike Information Criterion (AIC) values and ΔAIC values for all candidate models and parameter estimates for the models with ΔAIC < 7.0 (i.e. models with at least a weak support). An asterisk (*) next to a parameter estimate indicates that its 95 % confidence intervals excluded zero. The variable "Time" indicates the number of days since the installation of the rope bridge, "Wind" is a variable representing the daily maximum wind speed, "Min Temp" is the daily minimum temperature, and "Moon" is a variable representing the fraction of the moon lit. For the breeding season variable, the estimate is for the non-breeding season.

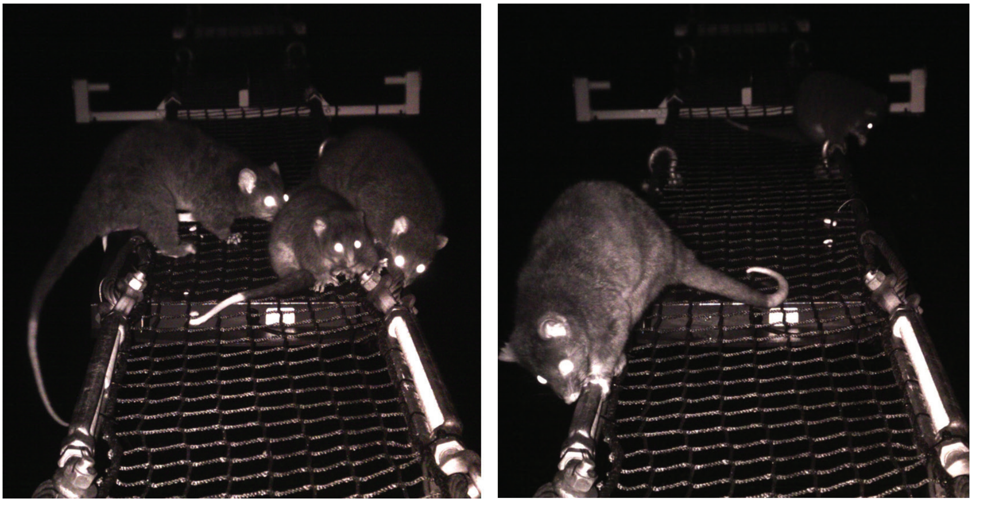

Figure 5. Photographs of mother and young *Pseudocheirus occidentalis* crossing the road using the rope bridge near Busselton, Western Australia. The left photograph is of a mother and her young with another adult possum.

Discussion

As expected, the use of the rope bridge by *P. occidentalis* increased over time; however, the possums started crossing the bridge much sooner and at much higher rates than we expected. They started investigating the bridge even before the installation was completed, and the first complete crossing was recorded only 36 days after the installation, which is remarkably shorter than seven months – the shortest time elapsed prior to other possum and glider species starting to use rope bridges in other parts of Australia (Weston et al. 2011, Goldingay et al. 2013, Soanes et al. 2013).

The rate of crossings was also considerably higher than those previously reported for other possums and gliders. Possums and gliders crossed the Pacific Highway in New South Wales using rope bridges at a rate of 0.02-0.08 crossings per night per species (Goldingay et al. 2013). On the Hume Highway in Victoria, squirrel gliders (*Petaurus norfolcensis*) used one of the rope bridges at a rate of 2.47 crossings per night after habituation (Soanes et al. 2013). In Queensland, the pooled crossing rate of three possum species was up to one crossing per 150 minutes (i.e. 4.8 crossings per 12 hours, Weston et al. 2011). The crossing rate of *P. occidentalis* recorded in this study (8.87 crossings per night) is considerably higher than these previously reported rates, and it did not reach a clear asymptote during the monitoring period. This could be due to the high density of the species in the study area and/or their particular lack of avoidance behaviour towards unfamiliar objects such as the new rope bridge (Wayne et al. 2005, Jones et al. 2007, Clarke 2011, Yokochi, K. personal observation). Moreover, possum species studied by Weston et al. (2011) lived in rainforests that generally have greater canopy cover than our study site, and their fidelity to a dense canopy may have made these possums more reluctant to cross exposed bridges. Uneven numbers of crossings by *P. occidentalis* in different directions suggest that some individuals crossed the bridge and remained on the other side. Use of the bridge by two generations of possums is also an encouraging sign that it will be used over generations and that it will be able to help increase gene flow across the road. This suggests that *P. occidentalis* can learn to use this type of wildlife crossing structure very quickly, and shows that rope bridges have the potential to be a very effective mitigation measure against the negative impacts of roads on this critically endangered species.

The number of bridge crossings decreased on windy nights, as expected. Being exposed to strong wind on the bridge may have discouraged possums from crossing due to the higher risk of being blown away. A higher risk of heat loss could be another reason for possums to cross the bridge less on windy nights (McCafferty et al. 2011); however, this is unlikely to be the case given that the number of crossings actually increased on colder nights, contrary to our expectation. It seems that heat loss is not as big problem for *P. occidentalis* as for other arboreal mammals studied by Laurance (1990), Starr et al. (2012), and Rode-Margono and Nekaris (2014). These researchers studied species in tropical regions such as Northern Queensland, Cambodia, and West Java, and their study species might have been more susceptible or less adapted to cold conditions compared to *P. occidentalis*. On the other hand, the lower number of crossing by *P. occidentalis* on warmer nights may be due to their susceptibility to overheating as they are prone to overheat and known to suffer physiologically at an ambient temperature of 35 °C or above (Yin 2006). In the study area, days with higher minimum temperatures generally experienced higher maximum temperature, which might have placed the possums under heat stress. Several mammalian species have been observed to decrease their food intake and activity under heat stress in order to reduce their heat production (Terrien et al. 2011). *P. occidentalis* may employ similar behavioural coping mechanisms and thus reduce their activity, including bridge crossings, on warmer nights.

The moonlight had a weak effect on the number of crossings, and fewer crossings were recorded on brighter nights. Whether this trend is caused by possums generally reducing their activities on bright nights or possums being discouraged to cross the exposed bridge on brighter nights cannot be known from our data. Wayne et al. (2005) reported that the moon or wind had no effect on the number of possums seen by spotlighting in a forest; however, possums are likely to act differently in a completely exposed environment such as on a rope bridge compared to an environment with greater cover from predators such as the canopy in a forest. Native owl species, such as the masked owl (*Tyto novaehollandiae*) are thought to be present in the region (Clarke 2011), and they prey on similar sized possum species in New South Wales (Kavanagh 1996). Therefore, it is possible that *P. occidentalis* reduced their activities on the exposed rope bridge on bright nights in order to reduce the risk of predation by birds of prey.

Contrary to our expectation, the number of crossings did not increase during the breeding seasons. Home ranges of *P. occidentalis* in the same area also did not change during the breeding seasons (Yokochi et al. 2015), suggesting that *P. occidentalis* do not expand their areas of activities to search for mates or extra resources during the breeding season. A longer monitoring period would be required to assess the effect of breeding season on the crossing behaviour more thoroughly because only two breeding seasons could be monitored and the rate of crossings did not reach an asymptote in this study.

Malfunction of the microchip readers made it impossible for us to identify all individuals using the bridge; however, the data still revealed that at least five different individuals used the bridge and that these individuals were using the bridge regularly. We must be cautious when interpreting the number of crossings on this bridge because a few individuals contributed to many of the crossings. At the same time, however, this also means that those individuals incorporated the bridge into their regular movement, which yet again suggests their high adaptability to this type of structure. To identify exactly how many individuals are benefitting from the bridge, we need to improve the monitoring system or develop a more reliable way of identifying individuals.

Multiple years of monitoring of the rope bridge in this study will also be necessary to investigate the long-term seasonal and yearly changes in the use of this bridge by *P. occidentalis* as well as to identify the asymptotic rate of crossing. Gagnon et al. (2011) found that elks (*Cervus elaphus*) adapted and habituated to terrestrial crossing structures over years, and some factors, such as season, time of the day and length of monitoring, that influenced the crossing frequencies in the first year of monitoring, became insignificant after four years. Given the remarkably quick habituation shown by our study species, we may be able to identify the factors influencing their long term crossing behaviours even in less than four years.

We also need to study the use of rope bridges in other areas in order to further assess the effectiveness of these structures as a wildlife crossing structure for *P. occidentalis*. Only one bridge was installed for this study due to financial constraints, and crossing patterns and characteristics are likely to differ in other areas with different population densities, habitats, and road characteristics or even for different kinds of artificial linear structures. For instance, it took *P. occidentalis* 18 months before it was recorded on another bridge installed across a newly constructed highway in Bunbury, located only 60 km away from the study area (B. Chambers, Pers. Comm.). This is possibly due to the lower density of the species in the area, recent disturbance caused by the road construction, and the greater length of the bridge (Bencini and Chambers 2014). Although it is probably unnecessary for the rope bridge in our study because of its high crossing rate, alteration of the design would be possible for the bridge in Bunbury or future bridges if the possums do not appear to habituate to them. A design to reduce the exposure and the effects of wind and moonlight may encourage possums to start using the bridges. In another study conducted in the same study area in Busselton, we found that an artificial waterway nearby was causing greater genetic divergence among *P. occidentalis* than Caves Road (Yokochi 2015); therefore, installation and monitoring of a rope bridge across this waterway is strongly recommended given the willingness of the possums to utilise these crossing structures in this area. Crossing behaviours of *P. occidentalis* is also likely to differ in areas where more competitive arboreal species, such as common brushtail possums, exists in higher densities than at our study area because they are thought to limit the activities of *P. occidentalis* (Clarke 2011).

Using individual based analyses such as parentage testing and Bayesian cluster analysis, Sawaya et al. (2014) found that grizzly (*Ursus arctos*) and black bears (*Ursus americanus*) using terrestrial crossing structures were breeding on the other side of a highway and achieved enough gene flow to avoid genetic isolation. A similar investigation into whether the crossings of the bridge by possums result in reproduction on the other side and restore the gene flow is essential in order to assess the true conservation value of rope bridges (Corlatti et al. 2009). We also need to assess whether the rope bridge provides a safe passage for dispersing juveniles, therefore assisting the restoration of gene flow. A study focusing on this life stage needs to be conducted, as well as genetic investigations to assess the change in the level of gene flow before and after the bridge construction.

Conclusion

Roads pose negative impacts on wildlife and their impacts need to be mitigated by providing safe passages especially for threatened arboreal species. The critically endangered *P. occidentalis* habituated to a rope bridge remarkably quickly, and the bridge is now regularly used by multiple individuals at a high rate every night. This shows a high potential of rope bridges as an effective mitigation measure against the negative impacts of roads on this species. More studies and longer monitoring, as well as genetic investigations into whether crossing individuals are breeding across the road and resulting in the restoration of gene flow are needed in order to assess the true conservation value of these crossing structures.

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