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Site-specific incidence of ring-related injuries in Rufous Fantails *Rhipidura rufifrons*

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ABSTRACT
Although colour-ringing is generally considered safe, a small proportion of avian species experience significant ring-related injuries. We report the first description of ring-related injuries in a rhipidurid flycatcher, the Rufous Fantail, and show that, in a population on Rota in the Northern Mariana Islands, switching from plastic to metal rings reduced but did not eliminate ring-related injuries. Over 30% of Rufous Fantails experienced severe injuries (tarsal swelling and leg loss) while wearing plastic rings and 11.7% experienced injuries while wearing metal rings. Injuries were not caused by improper fit of the rings and the use of metal rings in a separate population in Iluka, Australia, did not appear to cause any injuries. We suggest that injuries are caused by the accumulation of spider silk around the tarsus. Rufous Fantails may be more susceptible to injuries on Rota than in Iluka due to the frequency with which they encounter spider webs. We recommend avoiding the use of plastic rings on rhipidurids and related species and, more specifically, developing alternative marking schemes for the potential reintroduction of Rufous Fantails to Guam, where spider webs are abnormally abundant.

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Bird ringing has transformed our understanding of avian ecology by elucidating patterns of movement, life history and social behaviour. Thus, the benefits of ringing to science are believed to outweigh the risks of injury to ringed birds. However, for those species that experience ring-related injuries, ringing presents both ethical and scientific concerns, since an injury can affect behaviour, reproduction, and survival (Sedgwick & Klus 1997). Ring-related injuries include foot entrapment (Berggren & Low 2004, Griesser *et al* 2012), tarsal swelling (Rothstein 1979, Splittgerber & Clarke 2006, Pierce *et al* 2007), inflammation (Griesser *et al* 2012), abrasion (Lingle *et al* 1999, Berggren & Low 2004, Freifeld *et al* 2016), and necrosis and amputation of the foot (Sedgwick & Klus 1997, Amat 1999, Lingle *et al* 1999, Pierce *et al* 2007, Hache *et al* 2016).

Flycatchers ringed with plastic colour rings appear to be especially susceptible to injuries. In six species studied intensively, injury rates of colour-ringed flycatchers ranged from 9.6% to 35.3% of ringed birds, with injuries occurring most often when birds were ringed with two plastic rings on the same leg (Sedgwick & Klus 1997, Pierce *et al* 2007). Ring-related injuries have also been reported for Ochre-bellied Flycatchers *Mionectes oleagineus* (Koronkiewicz *et al* 2005), Leaden Flycatchers *Myiagra rubecula* (S. Tremont pers comm), and some *Empidonax* and *Contopus* species (Haas & Hargrove 2003), indicating that ecology or tarsal morphology may predispose flycatchers to ring-related injuries. Although leg injuries in other passerines have been attributed to ill-fitting rings (Splittgerber & Clarke 2006, Griesser *et al* 2012), in several flycatcher species the plastic ring material itself appears to cause injuries (Koronkiewicz *et al* 2005, Pierce *et al* 2007).

Splittgerber & Clarke (2006) found that, unlike metal rings, celluloid and Darvic plastic colour rings both generate static electricity, which can attract shed tarsal scales and other debris. Leg injuries occur when debris becomes lodged beneath the rings, causing swelling and eventual constriction and amputation of the foot (Splittgerber & Clarke 2006, Pierce *et al* 2007). Griesser *et al* (2012) found that leg injuries in Purple-crowned Fairy-wrens *Malurus coronatus* seemed to be caused by spider silk accumulation under the rings. Using coloured metal rings instead of plastic rings is recommended for reducing the prevalence of ring-related injuries in Purple-crowned Fairy-wrens, and has also been successful in several other species (Koronkiewicz *et al* 2005, Pierce *et al* 2007, Griesser *et al* 2012). Here, we report observations of ring-related leg injuries caused by spider silk accumulation in a rhipidurid flycatcher, the Rufous Fantail *Rhipidura rufifrons*, and make recommendations for future colour-ringing studies in this and related species.

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Methods

We studied two Rufous Fantail (hereafter 'fantail') subspecies: *R. r. mariae* on the island of Rota, Commonwealth of the Northern Mariana Islands, USA (14°09'04"N 145°12'54"E) and nominate *rufifrons* in Iluka Nature Reserve, New South Wales, Australia (29° 24'19"S 153°21'34"E). Rota is a raised limestone island of volcanic origin, which experiences a tropical climate year round (average high temperature 28–30°C). This island receives most of its 244 cm of annual rainfall between July and January (NOAA 2017). Iluka is the largest remnant of littoral rainforest in New South Wales. Iluka receives an average of 146 cm of rain per year, most of which falls between December and March, and experiences a subtropical climate with average summer temperatures of 20–26°C (Grantley 2010). On Rota, fantails are resident breeders that occupy stable, year-round territories. They breed throughout the year, with a distinct peak in nesting between February and June (pers obs). In Iluka, fantails occupy breeding territories between September and February before migrating to overwinter in the tropics (Higgins *et al* 2006).

At both study sites, we captured fantails using mist nets (Avinet, Portland, USA). The first time a bird was captured, we measured tarsus length using digital callipers (to 0.01 mm), mass using a digital balance (to 0.01 g), and wing chord (flattened) and tail length, using a ruler, to the nearest 0.5 mm. We determined sex based on the presence of a brood patch (females) or the orientation of the cloacal protuberance (caudal orientation = female). We confirmed this method of sexing by observing copulations between colour-ringed fantails.

Rota

In 2013–14, we ringed fantails on Rota with a unique combination of size XF Darvic plastic colour rings (Avinet, Portland, USA; height x internal diameter 4.0 × 2.3 mm) and a size 0 United States Geological Survey (USGS) numbered aluminium ring (5.5 × 2.1 mm). We use the phrase 'plastic colour rings' to refer to the ringing scheme in which we placed a USGS metal ring on one tarsus with a colour ring above it and placed two colour rings on the other tarsus.

After resighting plastic colour-ringed birds with leg injuries (Fig 1) in April 2014, we ceased colour marking and aimed to recapture colour-ringed birds. For all birds recaptured, we evaluated the severity of injuries using a six-point rating scale (Table 1), from 0 (clean, healthy tarsus) to 5 (leg amputation). We evaluated stage 3–5 injuries by visual inspection of recaptured birds or during observations of free-flying individuals (see 'resighting' below). Stage 0–2 injuries could be evaluated only by capturing birds and inspecting their tarsi.

In 2014, we elected to remove all rings (USGS and plastic) from recaptured individuals that had sustained a significant injury (stages 3–5, Table 1) that was considered to be due to the presence of the rings. For the

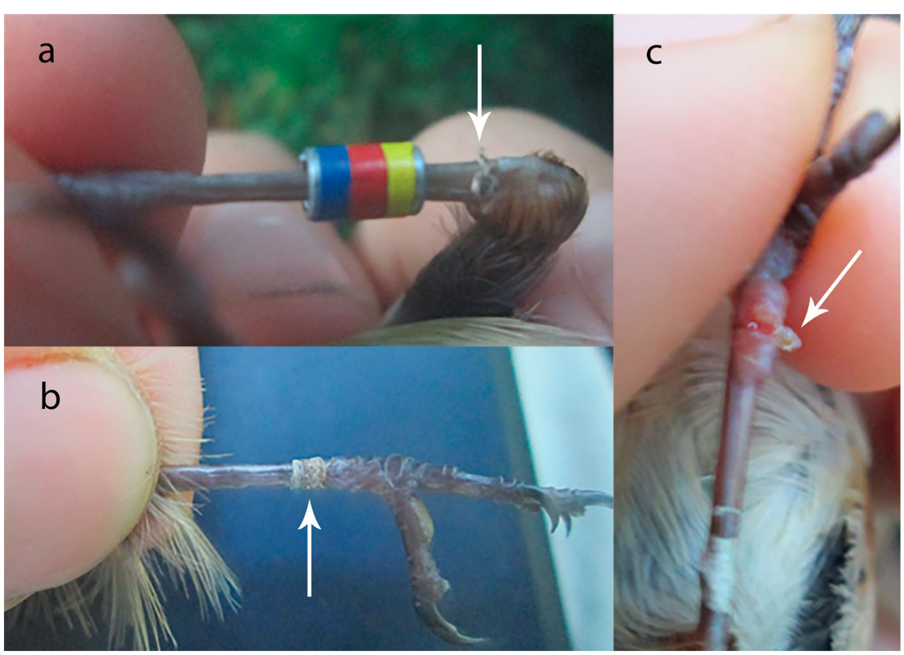

Figure 1. Progression of ring-related injuries in Rufous Fantails: (a) a loose bracelet of spider silk forms around tarsus (above a 'pin-striped' aluminium ring); (b) the bracelet adheres to the tarsus as more silk accumulates; and (c) swelling occurs under the ring and a silk bracelet cuts into the tarsus causing eventual amputation of the foot.

Table 1. Rating scale used to assess the severity of ring-related injuries. See Griesser *et al* (2012) for a description of the ringing-hazard rating scale.

Stage	Hazard points	Description
0	0	No injury or accumulation of material
1	0	Loose bracelet(s) of spider silk around tarsus
2	0	Spider silk wound tightly and adhered to tarsus
3	1	Swelling under and around rings
4	20	Loss of use of foot (difficulty perching)
5	20	Amputation of foot

remaining individuals, we removed any accumulated debris using forceps and removed only the plastic rings. These individuals were then ringed with a single 'pin-striped' aluminium ring (Fig 1a, Koronkiewicz *et al* 2005), on the leg opposite the USGS ring. We made pin-striped rings by wrapping a plain aluminium ring (National Band and Tag, Newport, USA; 5.5 × 2.1 mm) with strips of automotive pin-striping and coating the resulting coloured ring in clear epoxy (Koronkiewicz *et al* 2005). Our method for making the pin-striped rings departed from Koronkiewicz *et al* (2005) in that we used five-minute epoxy rather than flexible rod-wrapping epoxy due to the unavailability of the latter on Rota. We use the phrase 'pin-striped colour rings' to refer to the ringing scheme in which fantails were ringed with a pin-striped colour ring on one tarsus and a numbered metal ring on the other tarsus.

In 2015, we observed injuries caused by pin-striped colour rings, and we therefore elected to terminate ringing efforts. In 2015 and 2017 we aimed to recapture all remaining ringed fantails in order to remove their rings and did not ring any additional birds.

Iluka

From 2014 to 2016 we ringed adult fantails with a size 01 Australian Bird and Bat Banding Scheme (ABBBS) aluminium–magnesium alloy ring (5.5 × 2.0 mm) on one tarsus and the other tarsus with the same pin-striped rings that we used on Rota. To increase the number of unique colour combinations, ABBBS rings were anodised in gold, red, or blue by Australian Anodizing (Goonellabah, NSW) or were not anodised. In 2015 and 2016 we recaptured a total of 14 individuals to assess the prevalence of ring-related injuries. We did not ring any fantails with plastic colour rings in Iluka. We did not remove rings from any fantails in Iluka, because we never observed any ring-related injuries at this site.

Resighting

We resighted fantails six days per week for a total of 38 weeks on Rota (one week in 2013, 12 weeks in 2014, 14 weeks in 2015 and 11 weeks in 2017) and 33 weeks in Iluka (five weeks in 2014, 12 weeks in 2015 and 16 weeks in 2016). During each sighting of a ringed bird, we used binoculars to note whether the rings were moving freely on each tarsus and observed behaviour to confirm that the bird could perch on both legs.

Data analysis

We used t-tests to compare body-size measurements of males and females within each site and overall body-size measurements between sites. We compared Kaplan–Meier survival curves between sites using a Cox proportional-hazard model (Therneau & Grambsch 2000). Data were right-censored at the last time we resighted each individual, or uncensored for cases of ring removal. We included resighting effort (measured in weeks of field work after initial ringing) as a covariate to account for any differences in effort between sites or years. For birds that had their rings removed, we counted resighting effort only for the period for which they had carried a ring.

In addition to our injury scale (Table 1), we also used the ringing-hazard scale proposed by Griesser *et al* (2012) to facilitate comparison of injury prevalence and severity with other species for which ring-related injuries have been reported. We used chi-square tests to compare, first, the proportion of all captured individuals that had stage 1 injuries at the time of initial capture versus after wearing rings for at least 35 days at each site and, second, the proportion of individuals that had stage 1 injuries at the time of initial capture between sites. Since we removed all debris from the tarsi of every fantail we captured, these comparisons allowed us to determine whether rings augmented the accumulation of spider silk around the tarsus and whether the natural level of spider silk accumulation differed between Rota and Iluka. All statistical analyses were performed in R 3.4.1 (R Core Team 2017).

Results

We captured 81 fantails in Iluka (25 females, 48 males and eight of unknown sex) and 69 fantails on Rota (24 females, 35 males and 10 of unknown sex). Males had larger body-size measurements than females in both Iluka and Rota (Table 2). Fantails in Iluka were heavier and had longer wings and tails, but shorter tarsi than fantails on Rota (Table 2). Fantails survived for a median of 346 days (95% CI 323–438) in Iluka and 300 days (255–392) on Rota.

There was no difference in survival between sites (z = -1.01, P = 0.31).

Rota

Plastic colour rings

Of the 24 fantails ringed with plastic colour rings on Rota in August 2013, we resighted 16 individuals (66.7%) in April 2014. We ringed an additional 15 fantails with plastic colour rings in 2014 before our first resighting of an injured bird, making a total of 31 plastic-ringed individuals on our study plot in 2014, 24 of which we were able to recapture. Based on field observations of all resighted individuals, and in-hand evaluation of recaptured birds, 10 of 31 individuals (32.3%) had significant ring-related injuries (stages 3–5, Fig 2a), giving a hazard rating of 3.39 (Table 1). Restricting our analysis to include only birds that had carried rings for at least six months revealed a higher prevalence of stage 3–5 injuries (37.5%, Fig 2a) and a hazard rating of 5.19. Stage 3–5 injuries were more common on the leg ringed with two plastic colour rings (seven of 10 individuals). Sixteen recaptured individuals (66.7%) had stage 1 or 2 injuries, which we believe would eventually progress to a severe injury if the rings were not removed. Four recaptured birds (16.7%) had neither an injury nor accumulated debris. It is likely that these figures are an underestimate of the prevalence of ring-related injuries because stage 1–3 injuries could be detected with certainty only among recaptured birds.

Of the five individuals whose injuries affected their ability to perch (stages 4 and 5), two males attended active nests but disappeared shortly after nest failure, two males were unpaired and one female was paired

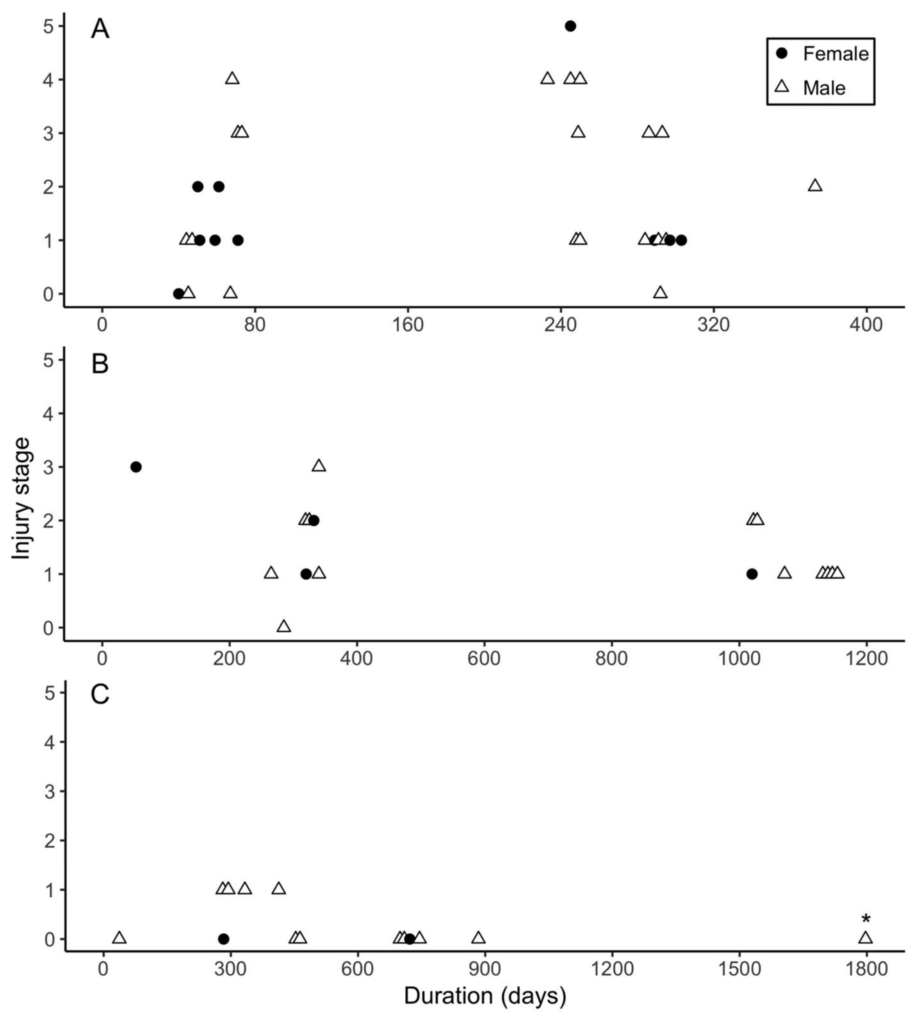

Figure 2. Ring-related injuries were (a) most severe among Rufous Fantails wearing plastic rings on Rota, (b) intermediate among fantails wearing metal rings on Rota and (c) rare among fantails wearing metal rings in Australia. Injury stage corresponds to the rating scale described in Table 1, where 0 indicates no injury and 5 indicates complete amputation of the foot. Overlapping points have been horizontally offset. Note the different x-axis scales. The individual marked with an asterisk had initially been captured in 2010 by a different ringer.

but did not initiate a nest despite constant harassment from her mate (males chase females aggressively prior to initiation of nest-building). One of the unpaired males sustained a stage 4 injury within 68 days of ringing (Fig 2a). All five severely injured individuals were shy and generally unresponsive to our efforts to recapture them and three of them disappeared before we were able to remove their rings.

Pin-striped colour rings

We ringed 40 fantails in 2014 with pin-striped colour rings: 20 were individuals whose plastic colour rings were replaced with a pin-striped ring and 20 were captured and ringed for the first time. Of these, 25 (62.5%) were resighted in 2015 and eight (20%) of the birds resighted in 2015 were also seen in 2017. In 2014, we recaptured a female with pin-striped rings that sustained a stage 3 injury within 53 days of her initial ringing (i.e. she was never ringed with plastic rings). Due to the rapid development of this injury, we elected to terminate ringing efforts on Rota. During 2014–17, we recaptured 17 of 25 resighted individuals to remove their pin-striped and USGS rings: two had stage 3 injuries (11.7%), 14 had stage 1 or 2 injuries (82.4%), and one had neither injury nor accumulated debris (Fig 2b), yielding a hazard rating of 0.12. Despite the trend for pin-striped rings to reduce the severity of injuries (Fig 2), birds with pin-striped rings (n = 16) were more likely to have spider silk accumulation on their tarsus than unringed birds (n = 17, $\chi^2 = 5.02$, df = 1, $P = 0.025$).

Iluka

Of the 60 birds ringed in 2014 with pin-striped colour rings, we resighted 43 (72%) in 2015 and 26 (43%) in 2016. Of the birds resighted in 2015, 26 (43%) were also seen in 2016. We ringed an additional 11 birds in 2015, eight (73%) of which were resighted in 2016. During resighting, we did not observe evidence of stage 3–5 injury in any individual. Among recaptured fantails, we did not observe any instances of stage 2–5 injury and four of 14 (28.6%) individuals had a stage 1 injury. Despite the apparent low risk of ring-related injury in this population, ringed individuals (n = 14) were more likely to have spider silk accumulation on their tarsi than unringed birds (n = 79, $\chi^2 = 5.64$, df = 1, $P = 0.018$), indicating that ringing may elevate the risk of leg injuries. Unringed fantails in Iluka (n = 79) were less likely to have tarsal spider silk accumulation than unringed fantails on Rota (n = 17, $\chi^2 = 23.45$, df = 1, $P < 0.001$).

Discussion

Our observations suggest that fantails on Rota are highly sensitive to ringing with plastic colour rings. The rapid development of injuries (< 3 months, Fig 2a) is also indicative of fantails' susceptibility: ring-related injuries in less sensitive species appear 1–3 years after ringing (Sedgwick & Klus 1997, Splittgerber & Clarke 2006, Hache *et al* 2016, but see Armstrong *et al* 1999, Lingle *et al* 1999, Pierce *et al* 2007 and Griesser *et al* 2012 for descriptions of injuries occurring after 2–12 weeks). Although there are reports of successful breeding following leg loss in other species (i.e. stage 4–5 injury, Lingle *et al* 1999, Pierce *et al* 2007, Hache *et al* 2016), none of the five fantails in this injury category bred successfully and only two initiated a nest. Thus, in addition to being among the species most susceptible to ring-related injuries, the fitness consequences to injured fantails may be high.

While switching from plastic to pin-striped colour rings reduced the injury rate on Rota (Fig 2), fantails still experienced injuries while wearing pin-striped rings (hazard rating = 0.12, Fig 2b). For comparison, a hazard rating of 0.11 was sufficient for researchers to switch from plastic to metal rings for Purple-crowned Fairy-wrens due to ethical concerns (Griesser *et al* 2012). Therefore, our observations suggest that ringing *per se* may constitute a significant risk to fantails on Rota. Improperly fitting rings cause injuries in other species (e.g. Splittgerber & Clarke 2006, Griesser *et al* 2012), but this did not appear to be the case in fantails. On Rota, an average of 73.2% of the metal ring's internal diameter (66.8% of the plastic ring's internal diameter) was occupied at the midpoint of the tarsus, which is within the suggested range for small passerines (Griesser *et al* 2012).

Instead, our observations suggest that rings promote the accumulation of spider silk, which eventually causes tarsal irritation and swelling (Fig 1; see also Greisser *et al* 2012). Splittgerber & Clarke (2006) found that plastic colour rings generate static charge, which causes them to attract more foreign material than metal rings. This might explain why we observed a higher incidence of severe injuries among fantails wearing plastic colour rings than among those wearing pin-striped rings on Rota (Fig 2). However, we also found that the presence of pin-striped rings appeared to increase the probability that a fantail would have a stage 1 (i.e. debris-only) injury, relative to previously unringed birds. This indicates that the presence of rings, regardless of material, might interfere with a fantail's ability to remove debris from the tarsus. Additionally, interference with tarsal preening could

Table 2. Mean (SE) body measurements of male and female Rufous Fantails in Iluka and Rota. Statistically significant differences between males and females within each site and the overall difference between sites are indicated in the P_{sex} and P_{site} columns, respectively.

	Iluka				Rota				
	Female	Male	P_{sex}	Overall	Female	Male	P_{sex}	Overall	P_{site}
Tarsus (mm)	20.95 (0.10)	21.63 (0.08)	***	21.39 (0.07)	21.55 (0.08)	22.14 (0.07)	***	21.90 (0.06)	***
Wing (mm)	70.32 (0.34)	74.34 (0.29)	***	72.96 (0.29)	64.92 (0.25)	67.92 (0.19)	***	66.69 (0.23)	***
Tail (mm)	82.88 (0.45)	85.80 (0.34)	***	84.79 (0.29)	77.14 (0.45)	78.75 (0.29)	**	78.10 (0.26)	***
Mass (g)	9.08 (0.09)	9.90 (0.06)	***	9.64 (0.07)	8.50 (0.14)	8.86 (0.07)	*	8.74 (0.07)	***

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

reduce the application of uropygial gland secretions to the tarsus, which have been shown to have anti-microbial properties *in vitro* (Moreno-Rueda 2017). The fact that reducing the proportion of tarsus length covered by rings decreased injury rates in other species (Haas & Hargrove 2003, Amirault *et al* 2006) provides further support for the idea that rings restrict access to the tarsus.

Variation in how often fantails encounter spider webs could explain the different injury rates between our study sites (Fig 2). We rarely captured unringed birds with spider silk on their tarsi in Iluka (5.1% of captures), but this was a common occurrence on Rota (52.9% of captures). This result could be due to differences between our sites in the ambient density of spider webs (see Rogers *et al* 2012 for quantification of spider web density on Rota). While we did not measure spider web density, we did observe fantails using spider webs to construct their nests at both sites. Fantails appear to use their feet to shape the nest cup (pers obs), which could explain how they accumulate spider silk on their tarsi. Year-round breeding on Rota, compared to seasonal breeding in Iluka may influence the accumulation of spider silk and other debris. However, fantails in Iluka still build 2–5 nests per year, suggesting that this explanation may not fully explain the differences between sites in injury risk. The possibility that longer tarsi (Table 2) somehow increased the probability of spider silk accumulation is another potential explanation for the higher incidence of stage 1 injuries on Rota.

We do not think that differences in forest substrate (Lingle *et al* 1999, Freifeld *et al* 2016) or foraging behaviour (Rose 1997, Berggren & Low 2004, Griesser *et al* 2012) influenced the differences in ring-related injuries between sites. Our observations suggest that fantails use primarily aerial and gleaning foraging manoeuvres at both our study sites and we did not observe any other differences in behaviour that could explain the prevalence of ring-related injuries on Rota. While the different material of the metal rings issued is a potential confounding variable (USGS rings were aluminium, ABBBS rings were alloy), fantails wore a pin-striped (i.e. aluminium) ring on one tarsus at both sites. Therefore, if differences between sites were caused by the material of the metal rings, we would expect to see twice as many injuries on Rota as in Australia, since fantails on Rota wore two aluminium rings on Rota and one aluminium and one alloy ring in Australia. Instead, we found a more-than-threefold difference in the prevalence of debris accumulation or injury (stage 1–5 injuries) between fantails wearing pin-striped rings on Rota (94.1%) and in Australia (28.6%; Figs 2b & c). Although our sample size is small, this result suggests that differences in the material of government-issued rings did not cause the differences in injury rates between our study sites.

Fantails in Iluka are migratory and we resighted newly ringed fantails for a median of only four weeks prior to migration each year. Thus it is possible that we underestimated the injury rate, if injuries prevented fantails from returning to our study site. However, other small migratory passerines have successfully returned to breed after experiencing significant ring-related injuries during the non-breeding season (Sedgwick & Klus 1997, Hache *et al* 2016). Additionally, the proportions of ringed birds we observed in subsequent years (72% and 73% in 2015 and 2016, respectively) are similar to those reported for other migratory and resident Australian forest birds (59–87%: Robinson 1990, Rowley & Russell 1991, Bridges 1994, van Dongen & Yocom 2005). Huggett (2000) found that <40% of fantails ringed with plastic colour rings (Darvic size XF) returned in subsequent breeding seasons, but he did not observe any ring-related injuries in two years of resighting and recapturing (pers comm). It is unclear whether the difference in return rate between Huggett (2000) and the present study is due to resighting effort, habitat quality, or differences in injury rate mediated by ring material. Regardless, our observations of significant ring-related injuries on Rota, combined with our small sample of recaptured fantails and limited resighting effort prior to migration in Iluka, suggest that future studies should closely monitor ringed populations of fantails and related species.

Conclusion

Our research indicates that assessment of stage 1 injuries in unringed birds may allow ringers to forecast risks of injury in previously unstudied populations, at least for those species in which spider silk accumulation influences injury development (e.g. Griesser *et al* 2012). Additionally, we encourage future studies on the effects of life history on the prevalence of injuries in certain species (Pierce *et al* 2007). For example, comparative studies examining whether the type of nest material used, length of the breeding season or method of nest construction influences injury risk could allow *a priori* identification of at-risk species or populations. In the event that fantails are reintroduced to the island of Guam, where they were extirpated by an introduced snake (Savidge 1987), we recommend extreme caution with ringing efforts due to the high density of spider webs that has followed the extinction of nearly all forest birds (Rogers *et al* 2012). Finally, developing alternative auxiliary markers for small passerines should be prioritised and piloted prior to attempting future colour-marking of fantails on Rota.

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