

The University of Adelaide

Artificial nest boxes to aid eastern barn owl control of introduced house mice in the Australian wheat belt

A preliminary assessment of nest box design, external monitoring and prey intake measures



Honours in Ecology and Environment
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Acknowledgments

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Abstract

The introduced house mouse (*Mus domesticus*) causes significant economic damage to Australia's agricultural enterprises. Focussing on a key native predator of mice, the eastern barn owl (*Tyto alba delicatula*), we piloted a manipulative rodent biocontrol study as part of the Great Southern Ark project on the southern Yorke Peninsula (SYP). Here, we aimed to evaluate existing eastern barn owl populations, formulate an appropriate pole-mounted nest box design to enhance barn owl hunting capacity, and trial a novel method of monitoring prey intake. Pre-manipulation owl densities averaged 2.14 owls per 1000 hectares. Of the 11 nest boxes installed, 55 percent were colonised within one month, and 82 percent were colonised within seven months. Occupied nest boxes were actively used by paired owls for reproduction, resulting in up to 35 observed fledglings. A total of 3717 harvested prey items, of which 78 percent were the target prey, were recorded. Relative mouse abundance correlated with expected seasonal markers, rising from February to a peak of 116 active burrows per hectare in April, and steeply dropping into winter. Barn owl prey intake and energy requirements followed a similar trend of changes to mouse abundance. The highest number of prey items captured within 14 days at one site was 229. The trail camera monitoring system was successful at capturing important barn owl reproductive and behavioural milestones throughout the study, however accuracy of prey intake and prey identification was closely related to unstandardized camera settings and placement, with significant room for refinement in future studies. Barn owl numbers were not intentionally manipulated to effective densities in this study, however we noted significantly fewer active mouse burrows in grazed than non-grazed paddocks, which could have further beneficial implications for ecologically-integrated management of rodent pests.

1. Introduction

Rodent pests comprise an estimated 7% of the 2277 total rodent species recorded globally (Capizzi *et al.* 2014). Their role as a vector of disease (Perry & Fetherston 1997; Meerburg *et al.* 2009) and their ability to displace or threaten native species (Witmer *et al.* 2014; Smith *et al.* 2016) has been well documented. Internationally, rodent crop damage has been estimated to cost tens to hundreds-of-millions \$USD annually, and is often a primary limiting factor impacting crop yield (Stenseth *et al.* 2003; Baldwin *et al.* 2014; Capizzi *et al.* 2014). In Australia, plagues of feral house mice (*Mus domesticus*) reaching 800 - 1000 individuals per hectare, have been attributed to their flexible reproductive strategies, low competition, suitability to cropping regions and locally low disease burden. These eruptions have been occurring at intervals of 3.5-7 years across the country's wheat belt (Singleton *et al.* 2001; Singleton *et al.* 2005) and can result in losses of up to \$AUD60 million annually (Brown & Singleton 2000). These losses are the result of pre-and-post seed consumption, contamination of stored grain and crop damage following sowing (Singleton *et al.* 2005; Capizzi *et al.* 2014). Mitigating the effects of rodent pests globally has proven to be difficult, unsustainable and costly. Long-term use of rodenticides – the leading method of rodent control – has resulted in significant economic losses, especially for developing regions (Skonhøft *et al.* 2006), non-target species mortality (Cox & Smith 1990), and physiological (Thijssen 1995) or behavioural (Brunton *et al.* 1993) poison resistance, which has been observed in mice following ingestion of sub-lethal doses of zinc phosphide (Brown *et al.* 2002). Habitat modification can slow but not prevent rodent outbreaks (Brown *et al.* 2010), and research into immunocontraceptive or disease methods may not be feasible unless all non-target impacts can be eliminated (Redwood *et al.* 2008).

A more promising area of research is the implementation of ecologically-based integrated management systems (IMS), which encompasses elements of these methods with an increasing

understanding of complex rodent ecology, behaviour and movement (Makundi *et al.* 1999; Singleton *et al.* 1999).

An important and often overlooked aspect of rodent ecology is avian predators (Kross *et al.* 2016; Krijger *et al.* 2017). Raptors exist naturally where rodent populations occur and their positive relationship with agricultural systems has been noted since as early as the 1870's (Kronenberg 2013). Most raptor biocontrol studies have occurred in the past 35 years, and are typically divided into two main categories; manipulative studies, which attract the target predator to the study site with nesting or perching resources; and non-manipulative, observational studies, set up to identify predator-prey relationships (Labuschagne *et al.* 2016). Raptor impact is typically assessed by prey composition (pellet analysis), measures of crop damage, and changes in rodent abundance (rodent trapping success). Using these measures, the 29 studies reviewed by Labuschagne *et al.* (2016) demonstrated an average 29.6% decline in trapped rodents and a 7.6% decrease in crop damage. Three manipulative studies found a combined 5.4-fold decrease in rodent abundance following avian predator manipulation (Ducket 1991; Munoz-Pedreros *et al.* 2010; Paz *et al.* 2012).

These studies, however, demonstrated a lack of either replicable experimental treatments, controls, standardisation of variables or length of study. Changes in rodent abundance were attributed to raptor presence but failed to address more significant variables, such as rodent food abundance (Labuschagne *et al.* 2016). The nature of these field studies made evaluating predator effectiveness difficult to accurately measure, suggesting that alternative methods may be necessary to further this field of research - in particular, establishment of a measure of prey intake, matched to a suitable native avian predator, preferably one with anatomical and behavioural attributes best-suited to the target prey.

Prior to 2018, no manipulative study using a nocturnal avian species to target the nocturnal nature of house mice had been undertaken in Australia. Australian observational studies have

focussed primarily on the effects of avian predation on house mice population regulation (Sinclair *et al.* 1990); mouse size, age and sex dynamics; and predator preferences for subordinate and weaker rodent individuals (Dickman *et al.* 1991). The only published manipulative study in Australia used perches to attract two native diurnal raptors, nankeen kestrels and black-shouldered kites, to soy bean crops in NSW (Kay *et al.* 2004). Contrastingly, outside Australia, the barn owl (*Tyto alba*) has been the focus of 86% of all raptor studies (Labuschagne *et al.* 2016).

Australia's native equivalent, the eastern barn owl (*Tyto alba delicatula*, Gould, 1837) (Parker 1977) would make an ideal candidate for a comparative manipulative study in Australia, as it can flourish in agricultural landscapes where rodent prey is abundant (Baxter 1995) and nesting sites are available (McLaughlin 1994). Over 90% of the eastern barn owl's diet consists of introduced house mice (Mortin & Martin 1979; Baker-Gabb 1984; McLaughlin 1994) but it is capable of eating other species opportunistically if mouse numbers are low (Tores *et al.* 2005; Avery *et al.* 2005; Kitowski 2013) and fly up to 10 km from a roost to hunt (Hyem 1936). It is able to produce up to 3-4 clutches annually when food is abundant, and reaches sexual maturity 95 days after hatching (McLaughlin 1994). Owlets grow rapidly, with higher energy requirements than adults (McLaughlin 1994; Durant & Handrich 1998). In natural settings, barn owls nest an average 1.4 km apart (McLaughlin 1994; Wendt and Johnson 2017) but can live in much higher densities if food is abundant (McLaughlin 1994; M. Browning unpublished data, 2017). Thus, if a sound method of evaluating prey intake and rodent pest impacts could be determined, manipulation of barn owl numbers could be optimised for inclusion into ecologically-based integrated management systems.

In Australia's wheat belt, eastern barn owls are limited primarily by the availability of suitable nesting cavities. These regions are dominated by mallee scrub, characterised by sparse, mostly cleared, narrow-trunked *Eucalyptus*-dominated habitats, and inhabited by competing cavity

nesters such as galahs (*Eolophus roseicapilla*) and brush-tailed possums (*Trichosurus vulpecula*) (McLaughlin 1994) and feral honey bees (*Apis mellifera*), which can negatively impact nesting success by barn owls (Charter *et al.* 2010a). By providing nesting cavities and perching spaces to the native barn owl on mouse-affected properties, its hunting impact can be greatly increased. However, some previous nest box studies have had serious negative implications for barn owl welfare and reproduction, likely due to human factors (Martin 2009) and nest-box design faults (Klein *et al.* 2007). Additionally, only a select few studies have discussed the design and positioning of nest boxes, with regards to temperature control, safety to owlets, ventilation and ease of access for researchers and landholders alike (Lambrechts *et al.* 2012).

At the time of writing, the Northern and Yorke Natural Resources Management Board is working with stakeholders to implement plans for the Great Southern Ark Rewilding project. The aims of this project are to repopulate areas of the southern Yorke Peninsula of South Australia with the region's lost native flora and highly-interactive native fauna, whilst eliminating destructive invasive species. As a part of this project, we performed the area's first pilot study on barn owls, one of the Rewilding project's key native predators. Our aims were to: (i) design a nest box for eastern barn owls, which would be readily colonised by the target species, support reproductive success and ease of monitoring and (ii) to trial a novel method of evaluating the effectiveness of nest boxes at reducing mice numbers, by means of a minimally-invasive trail camera, installed near the nest box to observe the owls, their behaviours and prey intake. The methods and findings of this study are documented and discussed in this thesis.

2. Methods and Materials

2.1. Study area and site selection

The study took place between November 2017 and August 2018 at a total of eleven sites (Figure 1) surrounding the towns of Warooka and Point Turton on southern Yorke Peninsula, South Australia (34.99° S, 137.40° E). These sites covered an area of approximately 12 km x 12 km. The area has a semi-arid, Mediterranean climate, with hot, dry summers and cool, wet winters. The region is flat and exposed, in some places gently undulating, where the small townships intersperse large, mostly cleared farming enterprises. Crops contain common grains or legumes, often rotated with livestock or fodder crops. Remnant scrub, characterised by native grasses, low-growing mallee (*Eucalyptus* spp.) and *Casuarina* sp., exists on road verges and windbreaks. The study area is bordered to the east by a large saltpan. Site locations details are described in Figure 1 and Table 1.

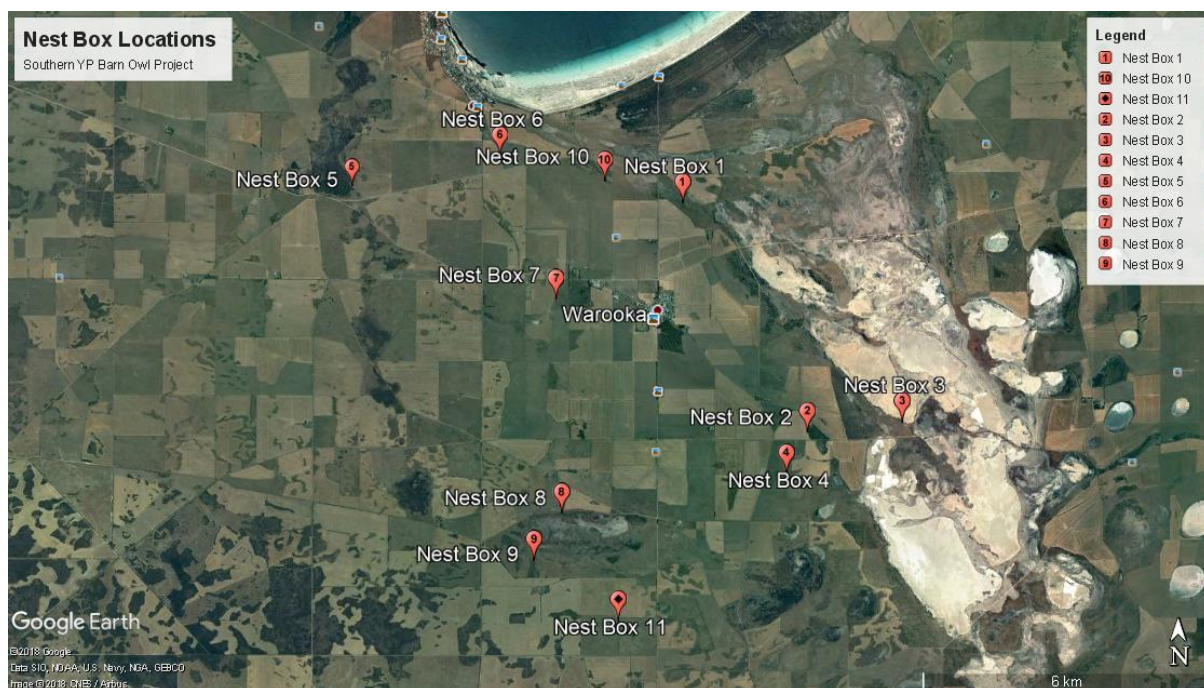


Figure 1. The locations of nest box sites (S1 to S11) within the study area of the Southern Yorke Peninsula, South Australia. Each site represents one nest box and a mouse survey site, located nearby. The sites were positioned on 9 properties offered for study by 7 volunteer landholders, and selected based on their juxtaposition to trees > 5 m tall, proximity to representative mouse survey sites (≤ 1000 m), presence of a stone pile for future studies, vehicle accessibility, crop type – focusing on wheat or barley crops, distance from main roads (≥ 1 km), and seasonal climatic patterns. Sites were approximately > 1.4km apart. Three sites (1, 5 and 8) were chosen within a scrub/revegetation site, as these were the only suitable sites available on each donated property with adequate tree coverage and vehicle access. The rest were selected at the edge of fields amongst eucalyptus or casuarina windbreaks.

Table 1. Site details, including coordinates, distances and directions to the closest field and mouse survey sites, and field usage during the study period.

Site no.	Coordinates	Distance to closest field	Direction to field	Distance from mouse survey site	Direction to mouse survey site	Field type during study
S1	34°58'14.00"S 137°24'24.00"E	152m	SW	160 m	SW	Barley
S2	35° 0'48.00"S 137°26'7.00"E	4m	W	12 m	W	Barley
S3	35° 0'41.89"S 137°27'25.25"E	10m	SWW	30 m	NW	Sheep (Vetch)
S4	35° 1'15.69"S 137°25'48.83"E	25m	NE	80 m	NE	Sheep (Vetch)
S5	34°58'3.00"S 137°19'52.00"E	140 m	NE	135 m	NE	Barley
S6	34°57'42.59"S 137°21'53.44"E	4 m	W	130 m	NE	Barley/Sheep (lentils)
S7	34°59'19.00"S 137°22'40.00"E	27 m	W	30 m	W	Barley
S8	35° 1'42.90"S 137°22'45.31"E	30 m	N	950 m	SWW	Cattle/Barley
S9	35° 2'14.67"S 137°22'22.53"E	14 m	NE	30 m	N	Cattle, Sheep/Barley
S10	34°57'59.26"S 137°23'19.09"E	60 m	SW	250m	SE	Wheat/Barley
S11	35° 2'55.00"S 137°23'32.00"E	5 m	W	160m	NE	Sheep (Vetch)/Barley

2.2. Assessing pre-manipulation barn owl abundance

Known barn owl populations living within Warooka and farm buildings were reported by landholders. However, in November 2017, prior to installing the nest boxes, we performed a preliminary owl census survey to obtain an estimate of pre-manipulation owl densities. The survey was carried out at night by spotlighting from the back of a utility vehicle (ute). Owls were counted within a 180° arc across the front of the vehicle. Vehicle speed was set at 15 km/hr from a set point, covering a distance of 2 km for every 100 ha observed (Bloomfield 1999). For standardisation, these transects were performed across 3 consecutive nights (~28 km / night) to produce an index of owl numbers. Transects were driven within the same hour after sunset and on nights when the weather was clear (Saunders *et al.* 1995). A total of 3 different spot lighting transects were assessed along three roads running east-west in this region, representing 3 different biomes (a, b, c) within the study area. Owl abundance was calculated in owls per hectare.

2.3. Nest box design and orientation

The barn owl nest box used in this study was developed by assessing barn owl nest box designs from published overseas studies under the supervision of a South Australian native fauna nest box manufacturing company, FauNature™. The studies referenced for dimensions, efficacy and suitable pole height are listed in Table 3. The dimensions of natural hollows as reported by McLaughlin (1990) were also considered. Choice of construction material (plywood) was based on findings by Wendt & Johnson (2017) that barn owls were seven times more likely to colonise wooden nest boxes than plastic.

Table 2. A summary of published literature containing details of nest box design, locations, heights from the ground and colonisation rates

Source	Location	Height x width x depth	Entrance hole size	Height from ground	Colonisation rates
Marti <i>et al.</i> (1979)	USA (northern Utah); crops	43 cm x 56 cm x 56 cm	25 cm x 33 cm	900 cm	50% occupancy in the first year and 80% occupancy in the second year.
Taylor <i>et al.</i> (1992)	Scotland (southern region; conifer plantation)	(91 L drums) 46.4 cm x 46.5 cm x 55.25 cm	10 cm x 10 cm	400 – 500 cm	11.5 % (1985) → 50.9% (1988); correlated with vole abundance.
Parker & Castrale (1996)	USA (Indiana); reclaimed grasslands	40 cm x 40 cm x 91 cm	18 cm x 18 cm	Not specified	53%
Klein <i>et al.</i> (2006)	Hungary	70 cm x 50 cm x 80 cm	10 cm x 10 cm	Not specified	(Only used in outdoor aviaries for rescued birds)
Meyrom <i>et al.</i> (2009); Charter <i>et al.</i> (2012)	Israel (Hula Valley); immature palm date plantation	50 cm x 50 cm x 75 cm	25 cm x 15 cm	250 – 300 cm	Fluctuating between 48.1% - 73.5% over four years.
Raid (2012)	USA	45 cm x 96 cm x 31 cm	15 cm x 18 cm	121 cm, 244 cm and 366 cm	90% colonisation in second year. 366 cm boxes colonised first, then 244 cm, then 121 cm.

The selected box design utilised 10mm plywood with dimensions (Figure 2 and 3A) of an internal height of 50 cm, width of 45 cm, and depth of 65 cm. The entrance hole was circular, with a diameter of 12.5 cm, and located centrally 37 cm from the box base. A 22 cm x 20 cm platform, designed by FauNature™, was secured 12.5 cm below the entrance and extended another 20 cm with two sections of c. 20 mm wooden rod (Figure 3C). The rear wall can be opened on hinges to allow access into the box. 1.5 cm ventilation holes sit along the tops of the long sides of the box, protected by a 2 cm overhang of the top, which extends to about 7 cm over the nest box entrance.

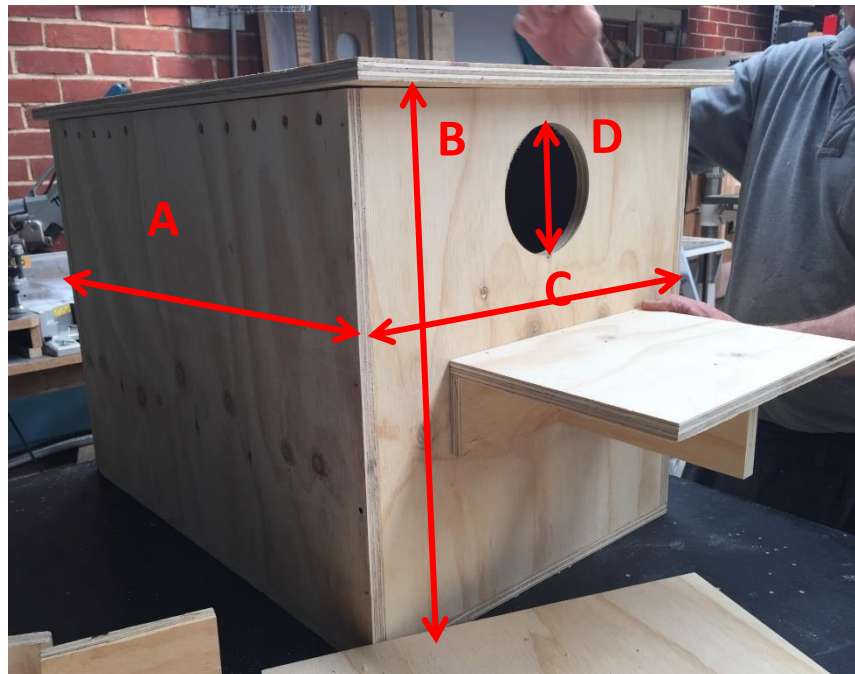


Figure 2. Barn owl nest box dimensions, as indicated by the red arrows. Depth (A) is 65 cm internally / 67 cm externally; Height (B) is 50 cm internally / 53 cm externally; Width (C) is 45 cm internally / 47 cm externally; and entrance hole diameter (D) is 12.5 cm. The roof overhang provides additional protection to the entrance hole. The platform (pictured prior to the affixing of the additional wooden rod perches) is attached 37 cm from the box base and reinforced underneath with a plywood brace.

The boxes were designed to be mounted on a pole where buildings or large trees were absent.

The poles used for mounting the boxes were 400 cm long, 7.5 x 7.5 cm, and 0.25 cm thick galvanised steel, chosen over wooden poles to prevent cats or possums from climbing into the box from below. Square-hollowed posts were chosen over circular poles to ensure structural support of the box during windy weather. The galvanised steel mounting bracket was

constructed from slightly wider sections of post, capped on the top and reinforced along the top and bottom of one side of the box to ensure stability, and fixed to the post by sliding over it like a sleeve then secured by tightening the end of a large bolt onto the smaller post. Boxes were first attached to the pole, then erected using a minimum of two people to gently lever the pole into 75 cm – 100 cm deep holes, as straight as possible with spirit levels (to prevent egg rolling), and set with concrete.



Figure 3 (A to D). A: The internal box dimensions, as described in Figure 2, showing a ladder fixed to the front of the box to allow owls to climb out of the box. B: the back wall of the box can be opened on hinges to allow for access and maintenance of the boxes by landholders or researchers. C: the galvanised steel sleeve bracket, following attachment to the box using nuts and bolts. D: boxes were manually erected using 2-3 people, stabilised according to a spirit level while set into the ground with fast-setting concrete and compacted soil. Holes were either manually dug using shovels + crowbars, or drilled using a portable fence-post digger, to a depth of 1 m.

Boxes were installed near trees for shade and camera placement and oriented north-east, allowing the box to warm in the morning but ensuring that they were not exposed to full sun in summer (Charter *et al.* 2010b). Following installation, a 5-8 cm deep layer of commercially sterilised wood shavings were laid on the floor of each box, with a slight depression in the middle, to prevent eggs from rolling to the edges of the box where incubation would be difficult. Nest box success was represented by time to colonisation, total colonisation rate, time to first fledgling emergence and estimated number of fledglings. A summary of nest box details is shown in Table 3.

Table 3. A summary of box orientation, relationship with surrounds and hunting grounds, and proximity to other species, both wild and domesticated.

Site no.	Box orientation	Box entrance facing	Direct viewing of field from box	Other birds nesting nearby	Land animals det. by camera	Other bird species detected by camera
S1	NE	Open scrub/rock pile	No	Unknown	Kangaroos, fox	Galah, crow, grey butcher bird, European starling, willy wagtail, magpie, brown goshawk
S2	NE	Scrub/trees	Yes/no	Unknown	Sheep	None
S3	NE	Scrub/Field	No	Unknown	Sheep	European starling, galah
S4	NE	Field	Yes	Yes (Black-shouldered kite; magpie)	Sheep	None
S5	NE	Scrub/trees	No	Unknown	Sheep	Magpie
S6	NE	Scrub/Field	Yes/no	Yes (magpie)	Sheep	Magpie
S7	NE	Scrub/Field	Yes	Unknown	Sheep	Magpie
S8	NE	Scrub/trees	No	Unknown	Cattle, fox	European starling
S9	NE	Field	Yes	Unknown	Cattle, sheep	Owlet-nightjar
S10	NE	Scrub/Trees	Yes/No	Unknown	Cattle	Willy wagtail
S11	NE	Scrub/Trees	Yes	Unknown	Sheep	Willy wagtail

2.4. External nest box monitoring system.

Nest boxes were monitored from February 2017 to August 2018 using Scout Guard SG560k-HD trail cameras. These cameras were attached to nearby trees approximately 1.5 – 3 m from the nest box entrance using zip ties or Tek screws through the protective housing box. Limited

by the location of surrounding trees, the cameras were either slightly facing the nest box entrance (Sites 1, 5, 6, 7 and 9), directly side on (Sites 2, 3, 8 and 10) or side-on + upwards towards the entrance (Sites 4 and 11). Cameras were accessed throughout the study period by ladder, where they were then kept overnight for battery charging, settings review and maintenance, before being reinstalled the following morning. The cameras were set to take bursts of 3 photos at 12MP, with PIR trigger sensitivity adjusted to Normal or High based on background movement of foliage. Timer triggering was set to 0; Timer Interval set to OFF and monitoring period set from 17:00 to 8:00. A second camera, set to Video, was also installed next to the photo cameras at 6 sites, however this data was deemed duplicative and excluded from the study. Camera accuracy was assessed by two measures; the percentage of monitored nights per study period in which at least ONE owl + prey event was captured; and the percentage of total owl events per study period containing owl + prey events.



Figure 4. A: Scoutguard camera, as highlighted by the red circle, attached to a nearby tree to monitor a nest box from February 2017 to August 2018. B: Camera being accessed by ladder at 1-3 monthly intervals.

2.5. Photo tagging and data collation

Photos were collected at 1 – 3 monthly intervals over the 6-month monitoring period. These were downloaded directly from the camera SD cards before being sorted and tagged in Exifpro 2.0. Tags were divided into 6 main categories; Number of Owls, Activity, Prey Number, Prey Type, Other Species and Comments. Once a site was tagged for each monitoring period, the tags were converted to a text file for transposing into Excel. Here, tags could be categorised into different owl activity ‘events’ based on their timing, and cleaned for analysis.

Determination of a behavioural event, such as surveillance, mating or feeding young, was done by examining the 3 photos within the burst to identify owl movements, and then comparing these movements and time elapsed from the previous burst. All events containing at least one owl are called ‘owl events.’ Any event containing an owl and a prey item, usually one that had just been hunted and returned to the nest in the owl’s beak or foot, is called an ‘owl + prey’ event. Prey items were classified as mouse, rat, rodent, bat or unknown. Bursts taken within 5 minutes of the last owl + prey burst were considered the same event, with the exception of the following rules:

- i. The previous prey item was of a different identity to the current one
- ii. The previous prey item was now being passed to another owl or into the nest box;
- iii. The previous prey item was now being eaten.

The total monitoring period was divided into five periods (Period 1: 30/01 – 21/02; Period 2: 22/02 – 29/03; Period 3: 30/03 – 26/04, Period 4: 27/04 – 05/06; Period 5: 06/06 - 14/07), separated by camera retrieval/maintenance works and mouse abundance surveys.

2.6. Assessing prey intake, timing and species composition

Three measures were used to assess how well the trail cameras performed as prey intake indicators: average nightly and total prey events per fortnight, timing of prey capture, and prey type/identification. The effects of reproduction on prey intake over time was also assessed.

2.7. Mouse abundance surveys

Two minimally-invasive methods were used to assess mouse abundance throughout the 6-month study period: active burrow transect counts and chew cards. Active burrow counts were estimated by counting all burrows within 4 x 100 m x 1 m survey transects at each site. These transects were marked on a GPS and flagged from start to finish at each time point. The most effective method involved three people; two standing at either end of each transect to mark down the number of mouse holes detected; and one to walk each transect with a 1 m reference stick for gauging transect width. Each mouse hole observed within a metre of the transect line was dusted with corn flour to prevent double counting and to assess mouse activity at each hole observed. Three mouse chew cards, made of plain 10 cm x 10 cm gridded card soaked in canola oil, were also pinned to the ground 50 m from fences at 10 m intervals adjacent to the mouse hole transect lines. Each site was then left overnight and visited the next morning to repeat the transect walk, collect the chew cards and assess the mouse burrow activity. A total of four mouse surveys were undertaken across the study period (February, March, April and August), and active burrows per hectare were calculated as an average of the four transects at each site, at each time point. The presence of grazing animals was noted, and the effect of mouse abundance on prey intake over time was assessed.

2.8. Data analysis

Correlations between total owl + prey events and average nightly prey events or total owl events, as two measures of camera placement accuracy, was determined using Pearson correlation, significant at the 0.01 level (2-tailed) using IMB SPSS statistical software.

Difference in active mouse burrow means between grazed and non-grazed paddocks was analysed using a paired T-test at the 95% confidence level. Histograms describing nest box success were also produced using this program. All other descriptive statistics (camera accuracy, average nightly prey events, total fortnightly prey events, prey type, time of prey events, effect of reproduction on prey intake, and mouse abundance surveys) were calculated and displayed using Microsoft Excel.

2.9. Ethics approval

All activities involving wildlife associated with this project were approved by the University of Adelaide Animal Ethics Committee (Approval Number: S-2017-072, Application ID: 32091).

3. Results

3.1 Pre-manipulation barn owl abundance

Along the 28 km of transects spotlighted, a total of four barn owls were observed on night one. Two owls were observed on night two. Night three was forfeited due to illness and poor weather. The average number of owls therefore was three per 28 km, which represented 3.00 owls per 1400 ha or 2.14 owls per 1000 ha. Owl densities were higher along sections closer to the township of Warooka (Transect 3), at 5 owls per 1000 ha.

3.2. Nest box colonisation rate

Within one month of installation by late December 2017, 5 of the 11 nest boxes (S1, S2, S4, S9 and S10) had been colonised (as detected by the trail cameras). Site 1 was initially colonised by a single male before being joined by a female in February 2018. The other four boxes were colonised by bonded pairs. Site 11 was colonised by a mated pair in February 2018, followed by another two mated pairs at Sites 3 and 8 in March 2018. Site 5 was the last box to be colonised by a single male in July 2018, with several camera events indicating an owl's interest

in the box as early as May 2018. Box 7 was also initially inspected by a single owl in January 2018, however the owl did not inhabit the box. The colonisation rate at February 2018 was 55%, rising to 73% in March and finishing at 82% in August 2018. No other species were recorded living in the boxes at any time, although galahs, magpies (*Gymnorhina tibicen*), grey butcher birds (*Cracticus torquatus*) and russet-tailed thrushes (*Zoothra heinei*) were observed inspecting the boxes throughout the study.

3.3. Breeding behaviour, chick development and reproductive success observed at nest boxes

Mating and courtship behaviours were observed on the perch or roof of the first eight colonised boxes within the first two months of colonisation. These behaviours included mutual preening and regular ‘gifting’ of prey from the male to the female, followed by copulatory posturing of the female and subsequent mating (Figure 5). Following mating, a period of solo hunting was observed, with males entering the box with prey to deliver to the brooding female. One female was photographed on the perch at Site 8 with an exposed brood patch (Figure 6).



Figure 5. Two mating events recorded on the 18th of March at Site 1. During mating, the male balances on the female's back whilst holding onto her neck feathers with his beak. Mating is often preceded by a prey gift from the male, resulting in a 'cache' of prey exceeding the female's appetite. The gifted mouse prey item can be seen on the perch in these photos.



Figure 6. A female barn owl, identified by her larger size and darker colouration, stands outside the nest box entrance at Site 8 with an exposed brood patch. The brood patch is an area of sparsely feathered skin, allowing for greater skin/heat contact between the hen and her eggs during incubation. Fledglings emerged in late May at this site, indicating that this hen was actively incubating eggs or young chicks at the time.

Fledglings were observed emerging around three-to-five months following the initial courtship (between April and July 2018; Figures 7 and 8). The active sites produced an average of four fledglings, with a minimum of one (Site 9) and maximum of six (Site 11). A conservatively estimated total of 32 fledglings were observed during the study, however this number may have been closer to 35 as it was difficult to distinguish adults from fledglings in later photos.





Figure 8. Examples of fledgling events at each site. Site 1 saw the emergence of four fledglings in July 2018. Two fledglings emerged in April 2018 at Site 2 (seen here interacting with a parent) with more potentially present in the box. An estimated four fledglings emerged at Site 3 (possibly five). Five fledglings were present at Site 4 (pictured with a prey exchange between a fledgling and an adult). Between three and four fledglings were present at Site 8 (pictured with an adult returning with prey), while only one fledgling was recorded at Site 9 in May 2018 (again pictured with a returning adult). Site 10 produced between five and six fledglings (pictured with a prey item) in June/July 2018. Site 11 produced the highest number of fledglings, between six and seven, in June 2018. Fledglings were difficult to distinguish from parents as they had reached adult size and plumage before emerging from the nest box, however they were often identified behaving passively/submissively, wing stretching and flapping in preparation for flight, begging for food and squabbling between each other on the perch.

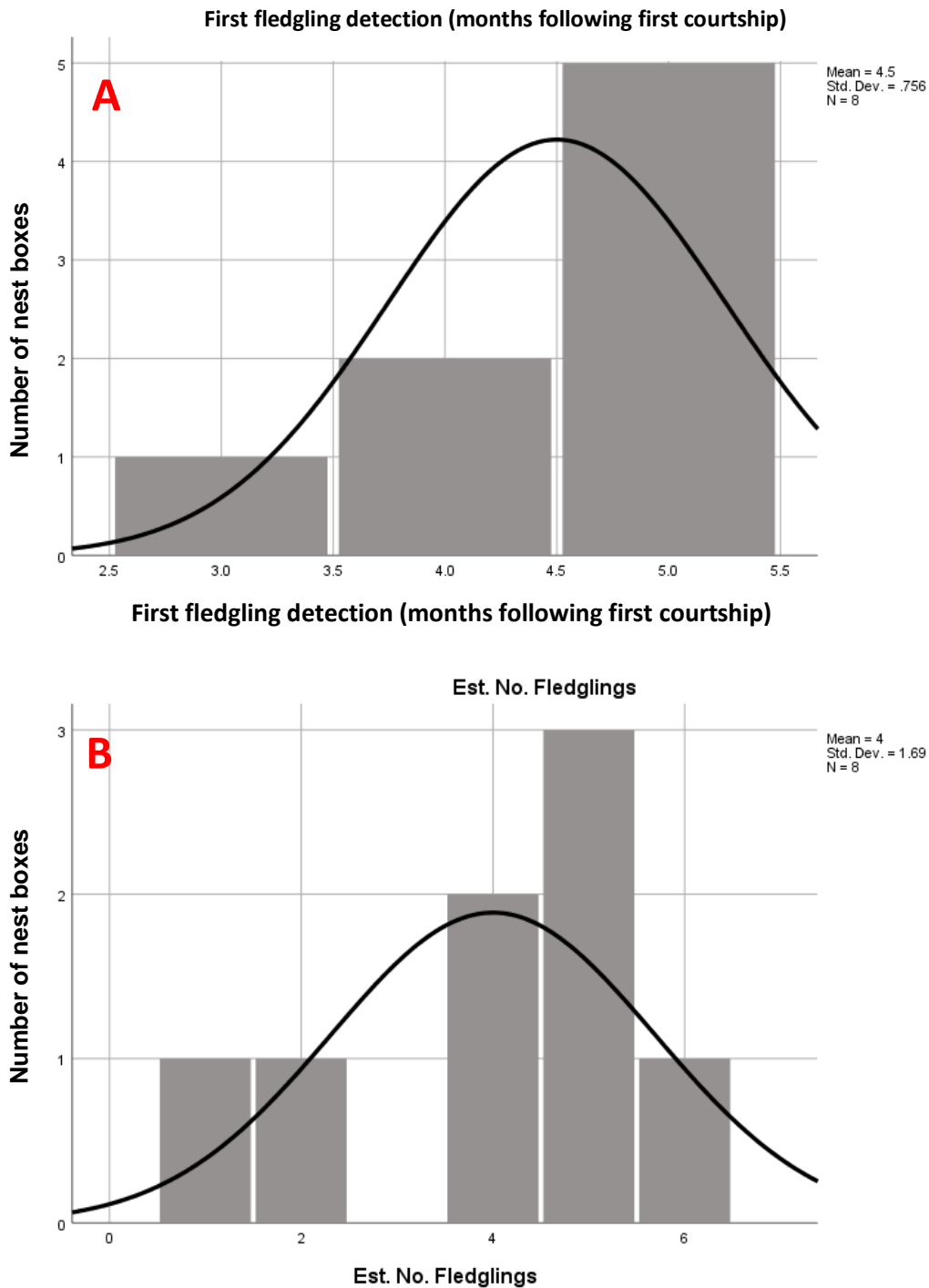


Figure 8. Histograms demonstrating the time following the first indications of courtship when fledglings were first detected (A) and the number of fledglings at each box (B). A. illustrates the variance in time between mating periods and the emergence of fledglings. B. illustrates a conservative estimate of number of fledglings for each nest box, giving a mean of four owlets and a median of five.

3.4. Camera accuracy and owl only / owl + prey detection rates

The reliability of cameras picking up nightly owl only, or owl + prey events was termed ‘Camera accuracy’ and expressed as a percentage, determined by dividing the number of nights in which at least one owl, or owl with prey, event was captured, by the total number of nights monitored at a site (Measure 1). Camera accuracy varied both between sites and within sites between monitoring periods (Figure 9). Site 1, 3, 8 and 11 consistently performed well throughout the entire study period, with accuracies of 73.5%, 66.5%, 90.1% and 75.3% recorded respectively. At Site 2, camera accuracy varied significantly across the five periods of this study, with very few owl + prey events captured. Sites 4 and 9 however, had the worst performing cameras, with an overall owl-prey detection accuracy of 20.1% and 16.3% respectively.

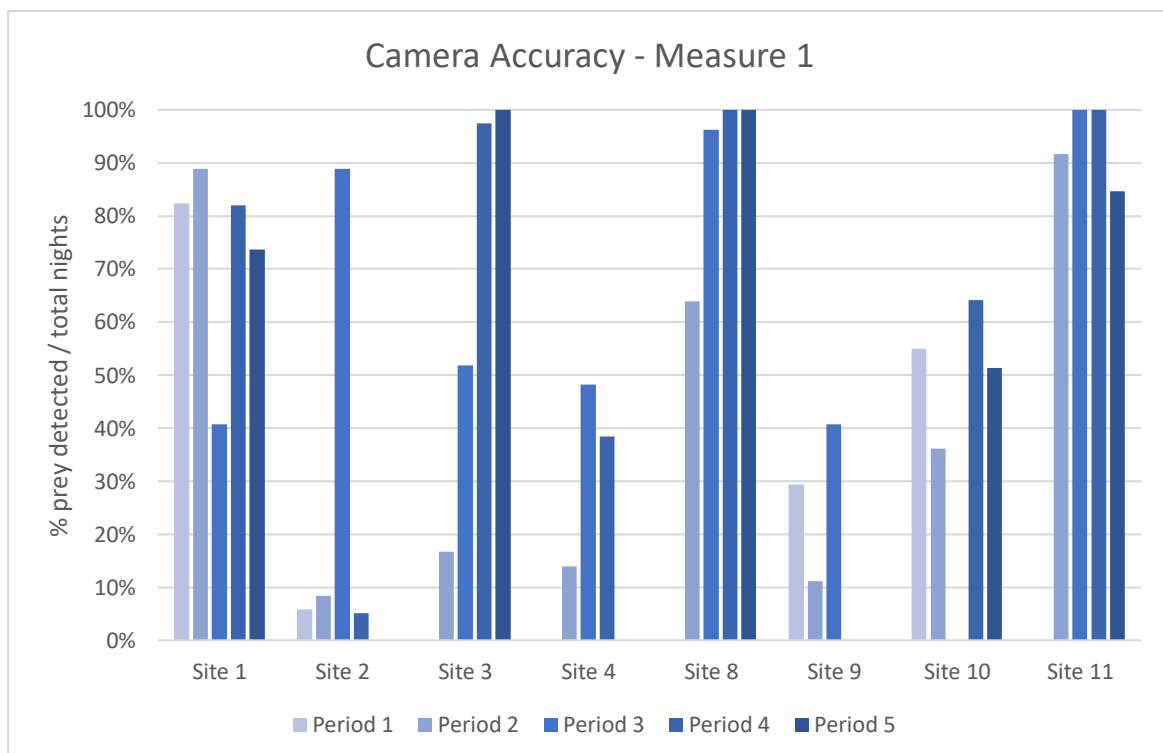


Figure 9. Camera performance according to Measure 1; the percentage of nights in which at least one owl + prey event was captured across all monitoring nights. This measure is a reflection of camera placement, orientation and settings (particularly PIR sensitivity and timer intervals), combined with owl frequency at the box. Period 1: 30/01 – 21/02/18; Period 2: 22/02 – 29/03/18; Period 3: 30/03 – 26/04/18, Period 4: 27/04 – 05/06/18; Period 5: 06/06 - 14/07/18. No settings alterations were made to cameras between Periods 4 and 5 due to inaccessibility to sites, however these time periods were separated for this measure to account for the excessive time between visits due to poor weather and inaccessibility to sites.

As a measure of owl + prey events out of total owl events captured (Measure 2, Figure 10), there was again noticeable variation between sites and monitoring periods. The overall average percentage of owl events containing a prey event across all sites was 18.2%.

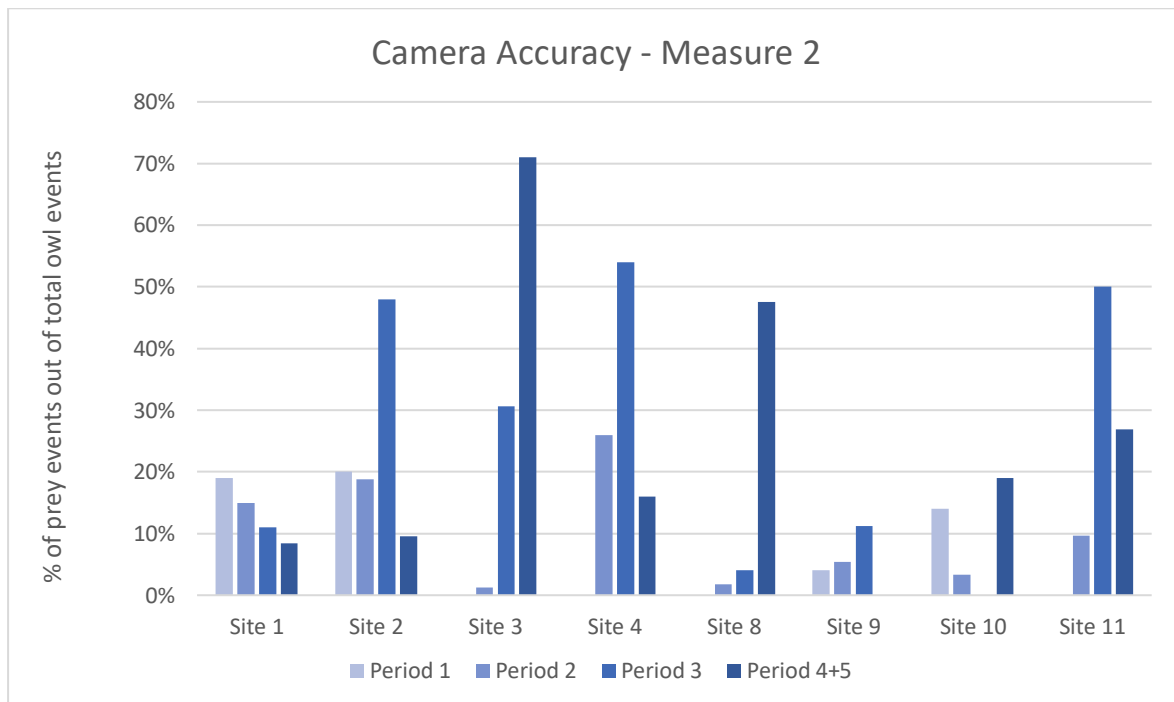


Figure 10. Camera performance according to Measure 2, the percentage of owl + prey events out of total owl events captured. Measure 2 is a reflection of camera orientation in relation to the box, owl behaviour and frequency (changes in owl numbers throughout time, such as the emergence of owlets triggering a higher number of captured owl events). Period 4 + 5 was combined as there were no settings alterations performed to cameras between these periods. Period 1: 30/01 – 21/02/18; Period 2: 22/02 – 29/03/18; Period 3: 30/03 – 26/04/18, Period 4 + 5: 27/04 – 14/07/18.

There was a significant correlation between the percentage of nights in which an owl + prey event was captured during each study period, and the average number of prey events per night for each study period (Pearson correlation: .798**, $P < 0.01$). There was no correlation between the number of owl only events and the number of owl + prey events (Pearson correlation: .333, $P = 0.42$).

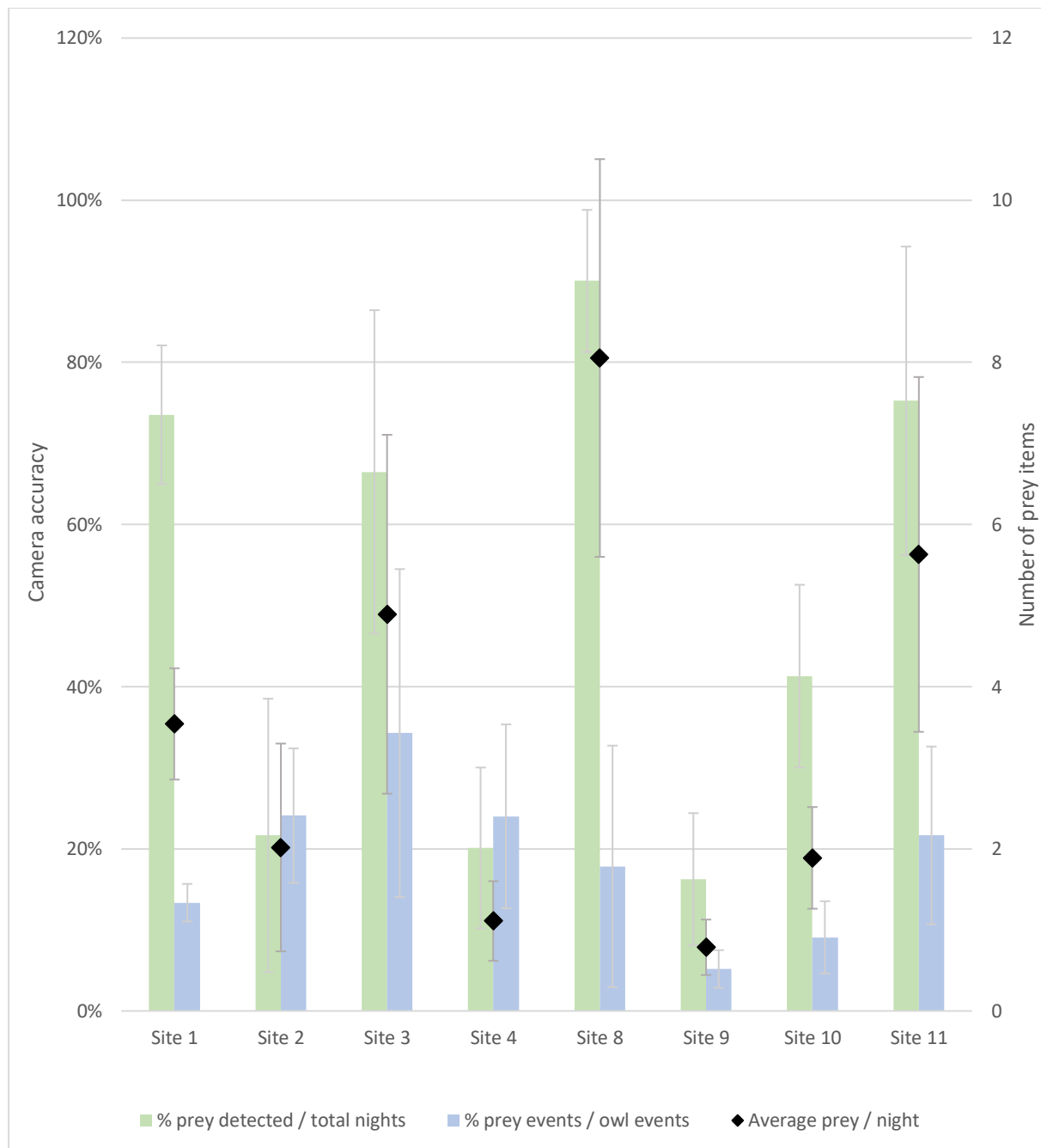


Figure 11. Camera accuracy, described by two unrelated measures, averaged for each site over the entire study period. Measure 1 (green columns) indicates the number of total monitoring nights in which at least one prey item was detected, as a percentage. Measure 2 (blue columns) indicates the proportion of total owl events which included a prey item (labelled ‘owl + prey’ events). The average number of prey taken per night for the entire 6-month study period is shown for each site (black diamonds) and is significantly correlated with Measure 1 (Pearson correlation: .798**, $P < 0.01$) but not Measure 2 (Pearson correlation: .333, $P = 0.42$).

3.5. Prey intake as a fortnightly total

With reference to the effect of camera accuracy on prey event frequency, sites >50% overall accuracy (Measure 1) were used to represent prey intake potential (Figure 12). Average nightly prey intake and fortnightly prey for all sites are shown in Appendix 1. The highest number of prey captured in one night was 27, on the 11th of May at Site 8. Site 11 captured 23 prey items on both the 1st and 9th of May. The highest total number of prey items captured within a fortnight was also at Site 8, reaching 229 prey items, followed by 202 total prey items at Site 11, both around middle-late May. Site 3 reached a fortnightly total of 176 in late June. At Sites 1, 3, 8, 9, 10 and 11, a general trend of increasing prey capture can be observed around May/June preceding the emergence of fledglings, before dropping again into July/August. The same pattern occurred as early as April at Site 2. Patterns of prey intake were closely correlated with camera accuracy at sites 1, 3, 8, 9, 10. This wasn't the case at Site 11, with high camera accuracy but low prey intake around the 18th of April. This was due to the use of end-of-life batteries which were not able to maintain power after being fully charged a few days prior. This was confirmed at the beginning of the next period when the batteries were incapable of charging and needed to be replaced.

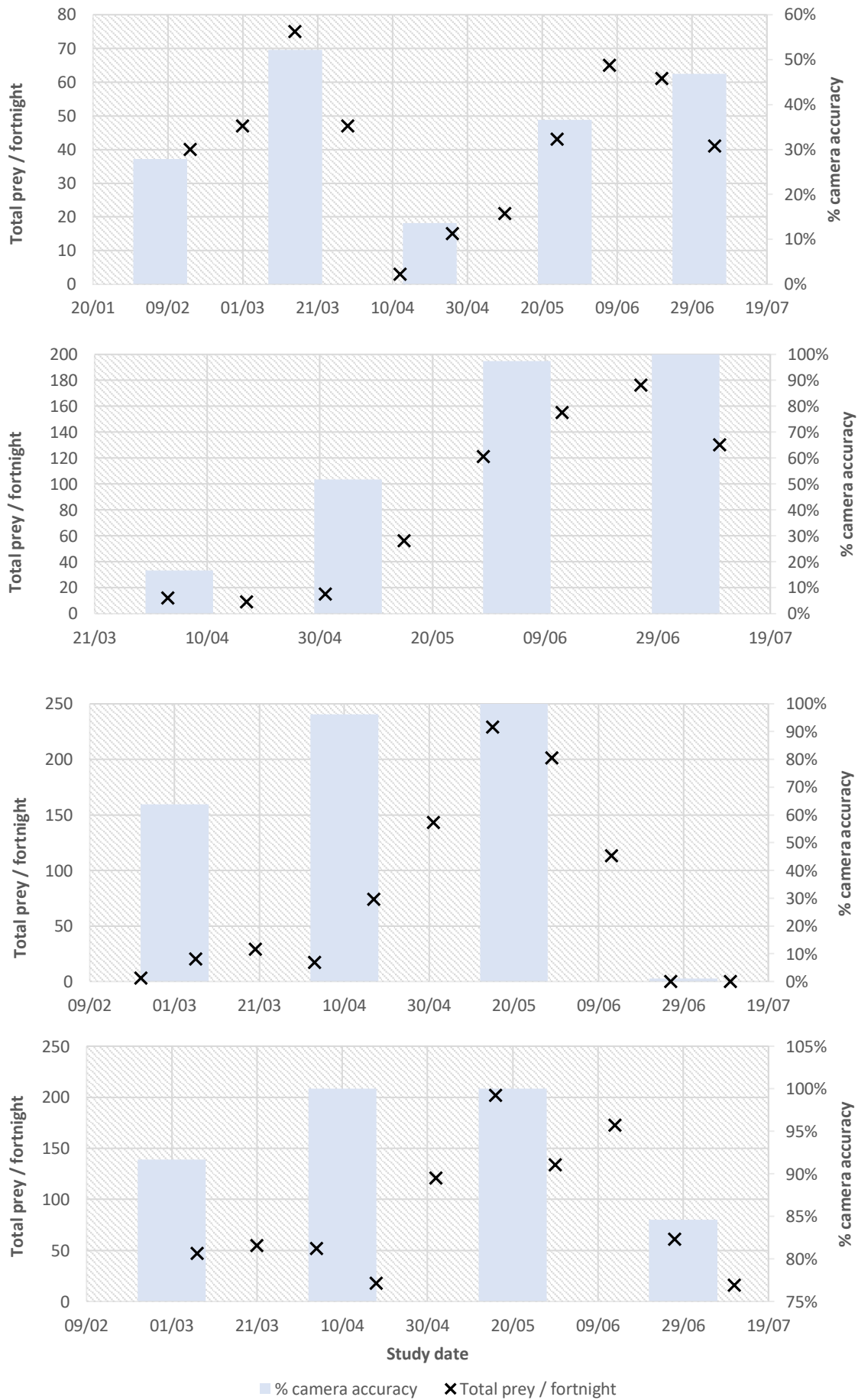


Figure 12. Fortnightly total prey at Sites 1, 3, 8 and 11 (from top to bottom) across the entire study period, plotted against Measure 1 camera accuracy. The black crosses represent fortnightly total prey intake, while the blue columns represent camera accuracy (%). Axes are not standardised between Sites.

3.6. Mouse abundance and relationship with prey intake

Mouse abundance by measure of estimated active burrows varied significantly between sites but followed a general trend of positive growth from February to April 2018, before dropping significantly by the fourth and final survey in July (Table 4). Chew card data was not included in the study as chewing activity was 0 at all sites and time points.

The highest number of active burrows was consistently measured at Site 10, reaching a corrected maximum of 556 active burrows / ha in April. Large amounts of spilled grain and mounds of cut barley were present at this site. Grazing had a significantly negative effect on active mouse burrow numbers in the surveyed paddocks ($P < 0.05$).

Table 4. The estimated number of active mouse burrows per hectare at each study site. Figures are given in Mean Active burrows / ha \pm SEM.

SITE NO.	Mouse census survey date			
	22-Feb	28-Mar	25-Apr	14-Jul
S1	87.50 \pm 0.13	81.25 \pm 0.24	81.25 \pm 0.12	0.00 \pm 0.00
S2	25.00 \pm 0.25†	25.00 \pm 0.18	56.25 \pm 0.16	0.00 \pm 0.00†
S3	12.50 \pm 0.13†	37.50 \pm 0.13†	81.25 \pm 0.24	25.00 \pm 0.25
S4	12.50 \pm 0.13†	25.00 \pm 0.10†	18.75 \pm 0.06†	0.00 \pm 0.00
S5	12.50 \pm 0.13	112.50 \pm 0.22	18.75 \pm 0.12†	25.00 \pm 0.25
S6	12.50 \pm 0.13†	143.75 \pm 0.62	318.75 \pm 0.39	0.00 \pm 0.00†
S7	0.00 \pm 0.00†	31.25 \pm 0.06	43.75 \pm 0.12	0.00 \pm 0.00†
S8	12.50 \pm 0.13	43.75 \pm 0.12†	25.00 \pm 0.10†	0.00 \pm 0.00
S9	62.50 \pm 0.13	50.00 \pm 0.18†	25.00 \pm 0.10†	0.00 \pm 0.00
S10	50.00 \pm 0.00	225.00 \pm 0.60	556.25 \pm 1.08	50.00 \pm 0.50
S11	50.00 \pm 0.25†	37.50 \pm 0.07†	50.00 \pm 0.25†	50.00 \pm 0.29
AVERAGE	30.68 \pm 0.13	73.86 \pm 0.23	115.91 \pm 0.25	13.64 \pm 0.12

† Grazing livestock present

When averaged and plotted against the average nightly prey intake of nest boxes with camera accuracy > 50%, a relationship can be observed (Figure 13). It's estimated that mouse numbers continued to rise in conjunction with prey intake into May and early June before dropping.

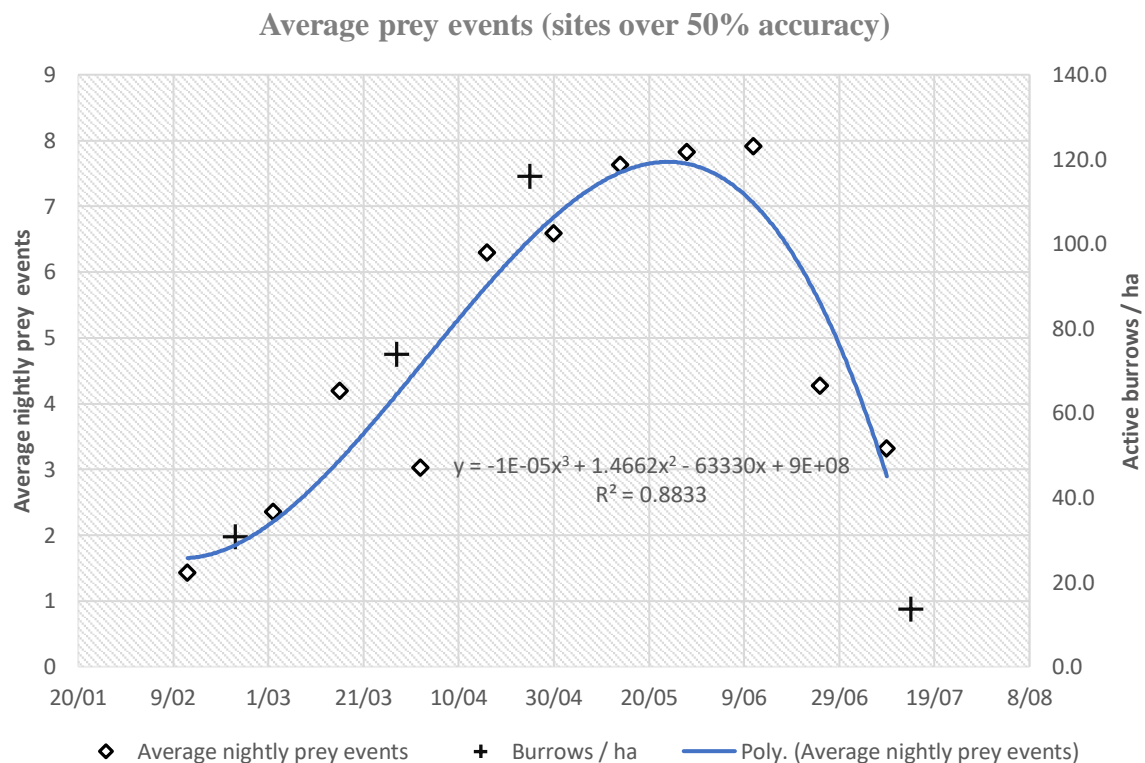


Figure 13. The average number of prey events captured per night, at sites with camera accuracy (Measure 1) > 50 %, plotted against the estimated average number of active mouse burrows across all sites, both grazed and non-grazed. The black diamonds and blue polynomial regression ($R^2 = 0.8833$) represent average nightly prey intake, while the black crosses represent the active mouse burrows. A positive relationship can be seen between prey intake and relative mouse abundance, with numbers of both increasing steadily from February to April 2018. Prey intake continued increasing until peaking in mid-June, before steeply dropping into July. Mouse abundance was lowest in July/August. Sites were chosen based on their Measure 1 accuracy at the end of each study period. Representing period 1 (30/01 – 21/02/18) are Sites 1 and 10; period 2 (22/02 – 29/03/18) are Sites 1, 8 and 11; period 3 (30/03 – 26/04/18) are Sites 2, 8 and 11; periods 4 and 5 (27/04 – 14/07/18) are Sites 1, 3, 8, 10 and 11.

3.7. Reproduction, daily energy requirements and relationship with prey intake

Prey intake also appeared to correlate with the timing of reproduction and fledgling emergence at each nest box. The four nest boxes with high camera accuracy produced a total of 19 fledglings (four owlets at Sites 1 and 8; five owlets at Site 3; and six owlets at Site 11). An

estimation of daily energy requirements of a nest box containing two adults and 4 chicks throughout the study period was produced and plotted against the average nightly prey intake (Figure 15). The average number of detected prey events was around 50 – 60 % lower than the expected mouse (body weight of 15 g (Singleton & Krebs 2007); 330 kJ gross energy (Dierenfeld *et al.* 2002)) consumption for most of the study period. Maximum prey intake was closer to the expected values at these four sites. The nightly average between the 9th and 23rd of May at Site 8 was 19 mice / night, with a maximum of 27 prey items on the 11th. Prey intake at Site 11 was highest between the 27th of April and the 10th of June, with a maximum of 25 prey items on the 22nd of May. The weights and sizes of detected prey items are not known, and included a mixture of larger (up to rat size) and smaller (juvenile rodent) prey items than the standardised 15 g house mouse used in this estimation. The number of prey items consumed away from the nest by adults (at a nearby roost) and prey not detected by the camera in time before the owl entered the nest box is also unknown.

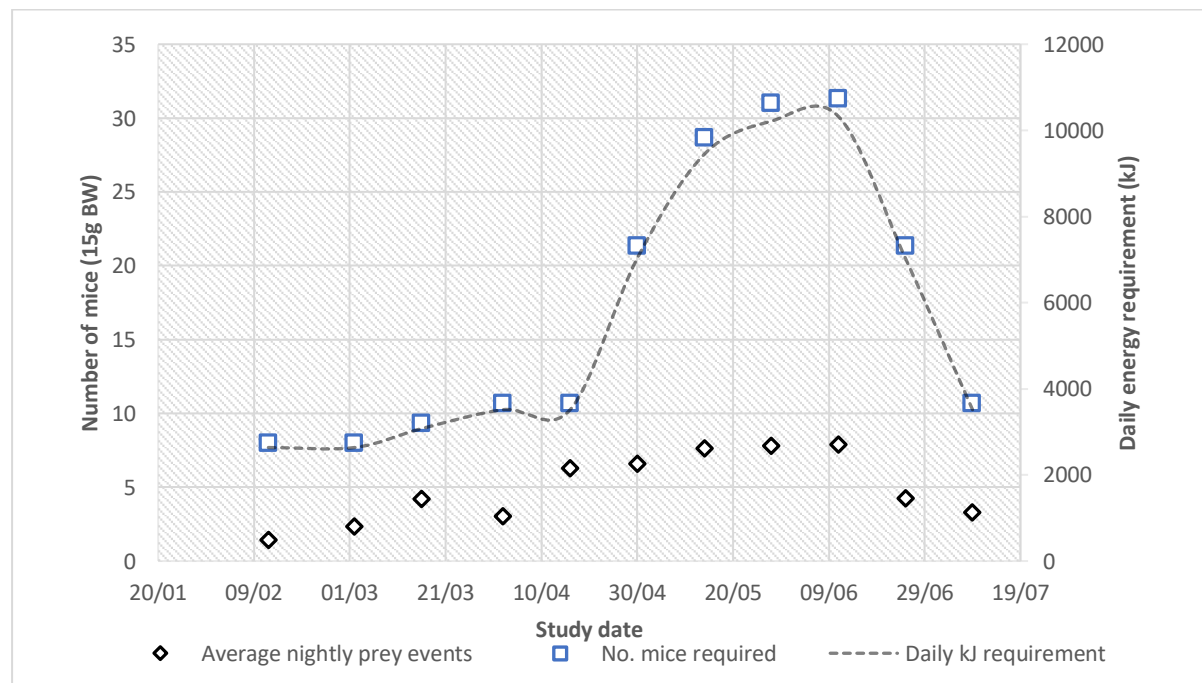
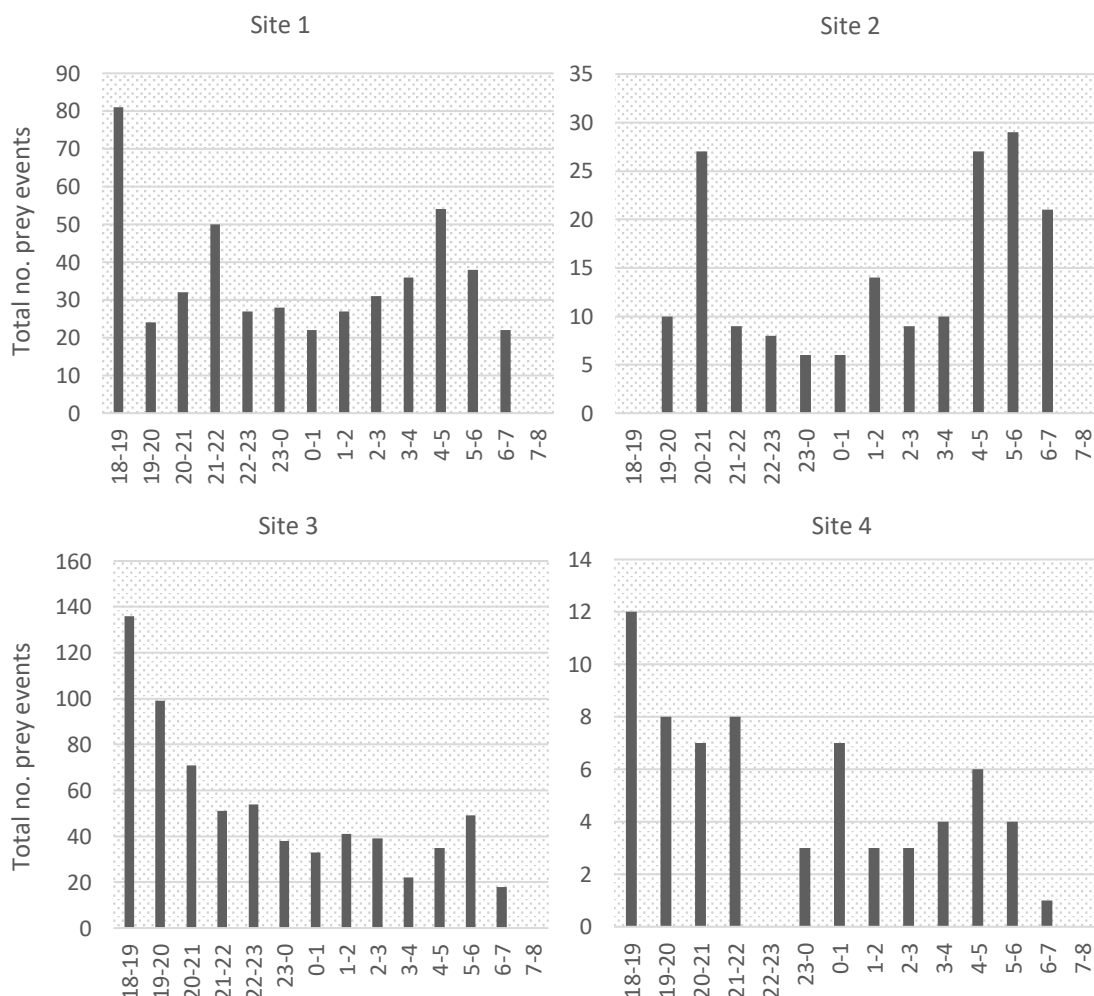


Figure 14. An estimation of daily energy requirements of a nest box containing two adults and 4 chicks throughout the study period (blue squares = number of mice, and dotted line = energy requirements in kJ), plotted against the average nightly prey intake (black diamonds). One 15 g adult house mouse (Dickman *et al.* 1991; Singleton & Krebs 2007) contains about 330 kilojoules (kJ) (Dierenfeld *et al.* 2002). The estimated energy requirements indicated that around 8 mice were required for two pre-

nesting adults during the courtship phase, increasing to 9-11 while the hen is laying and incubating eggs (Hamilton & Neill 1981). From the point at which the first chick hatches around 32 days later in mid-April, energy requirements increase mouse consumption in a linear manner until the eldest owlet is around 40 - 50 days old (early June), peaking at 31 mice / 10324 kJ per box. This number suddenly drops as owlets undergo a period of fasting to reduce to adult bodyweight in preparation for flight. The expected pattern of prey consumption matches that of the detected prey consumption, although observed numbers are much lower.

3.8. Timing of hunting

At sites 1, 3, 4, 8, 10 and 11, a majority of prey was captured within the first hour of hunting, between 1800 and 1900 hrs, with almost no hunting activity captured before 1800. A slight decrease in hunting activity was seen around midnight. A second peak in hunting activity occurred at all sites in the early morning, between 0500 and 0600 hrs. At Sites 2 and 9 this was the most active time of the night (Figure 15).



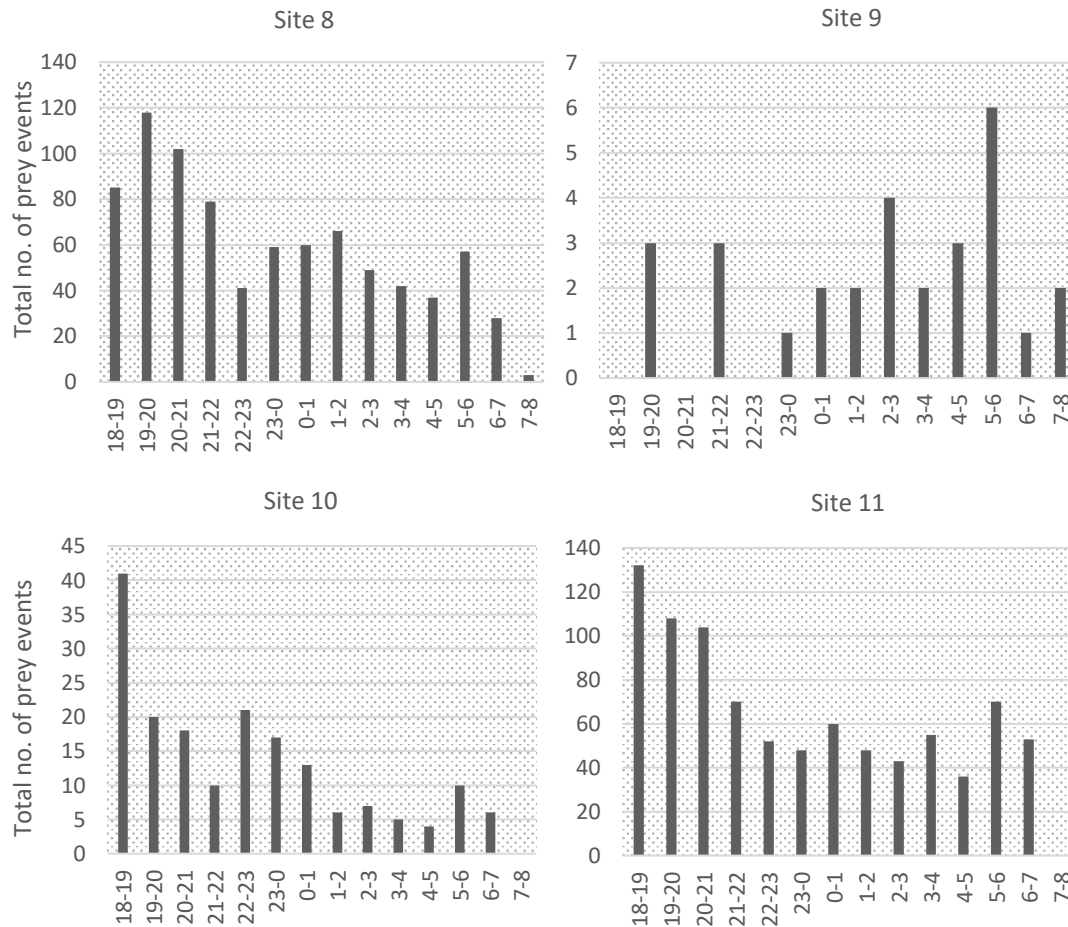


Figure 15. Nightly hunting patterns of barn owls. The Y-axis represents the total number of prey caught over the entire study period at each time point, while the X-axis represents the hour of the night (abbreviated; commencing at 1800 hrs). At sites 1, 3, 4, 8, 10 and 11, most active hunting time was between 18:00 and 20:00. At sites 1, 3, 4 and 11, hunting slowly dropped in frequency from this initial peak before rising fractionally around midnight, then dropping again before making a second peak between 0400 and 0600. Both bimodal and trimodal patterns were observed. Early morning was the most frequent hunting time at Sites 2 and 9.

3.9. Prey type

Prey composition was determined by visually identifying prey species from photos. The most common prey item at all sites was mice. Prey that was too obscured from view to be accurately identified was labelled ‘unknown’. Site 1, with the camera facing into the nest box from above, had the highest proportion of ‘unknown’ prey. Site 11, with the camera facing side-on and slightly upwards towards the nest box, had the highest proportion of prey identified as mice. Other species included rats (Site 1, 2 and 3), and one microbat (Site 2).

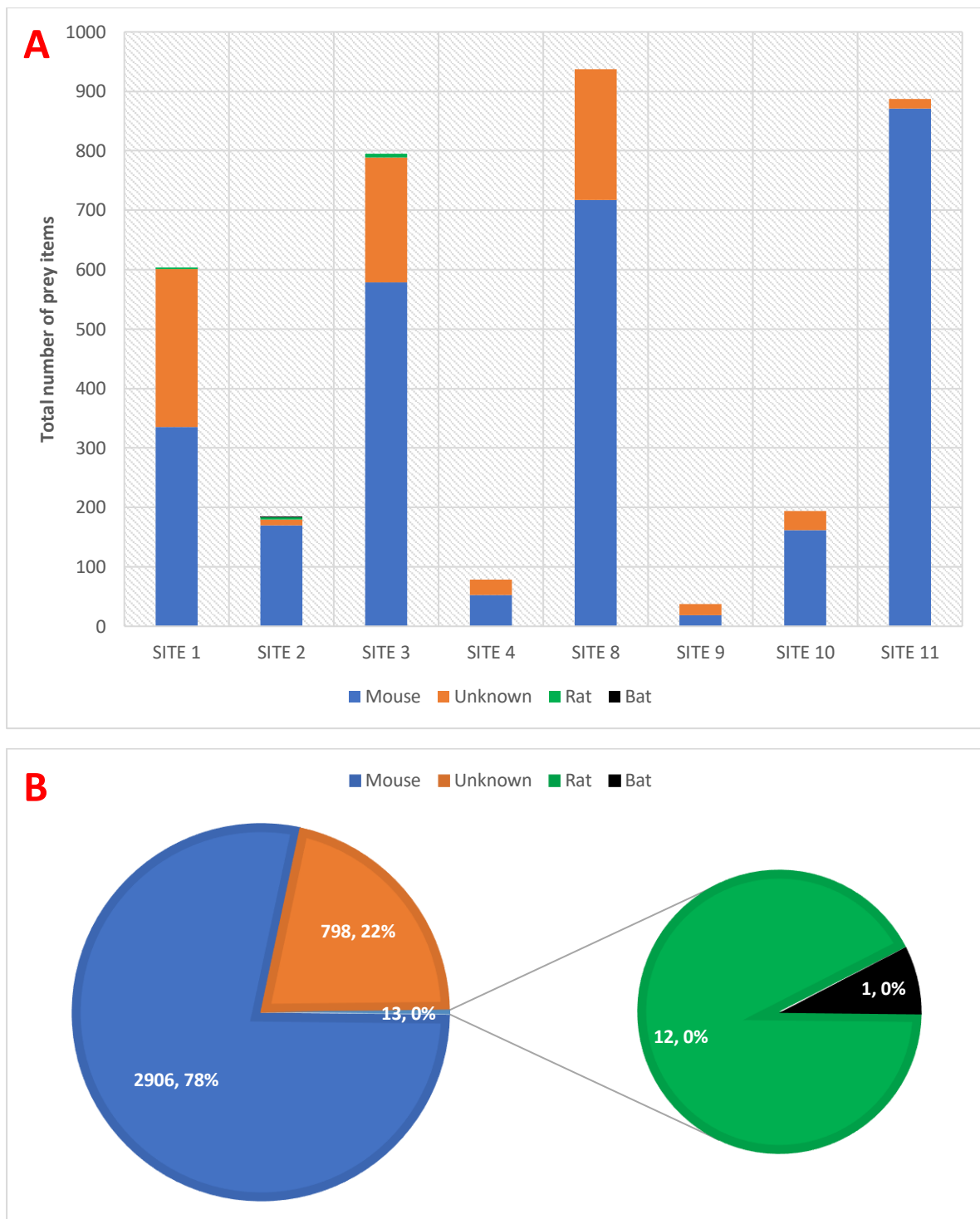


Figure 16. A. Prey composition by site. Prey identification varied between sites, particularly between those of high camera accuracy and low accuracy. The total number of prey captured by each site during the study period is shown. Prey tagged as ‘unknown’ tended to be difficult to see due to being held in the owl’s foot or obscured from view in some way. Site 1 caught the highest number of ‘unknown’ prey whilst Site 11 caught the highest proportion of mice, as identified by size, shape and shading, including the long tail. B. Total prey composition (number, percentage). Mice made up 78% of total prey items identified. 22% of total prey items were not identifiable. Other prey items were 12 rats (identified by large size) and one microbat.

4. Discussion

4.1. Nest box design success and reproduction

The primary aim of this study was to design a pole-mounted nest box suitable for eastern barn owls on the southern Yorke Peninsula (SYP). The lack of suitable nesting cavities for existing barn owl populations in this region was apparent in our study in two ways; the increased density of barn owls near the township during the pre-manipulation survey, and the rapid uptake of nest boxes following manipulation. An owl was detected at 10 out of the 11 nest boxes at least once throughout the period; nine were colonised by August (within 32 weeks), and eight were actively used by mated pairs throughout the study period for reproduction. From these eight nest boxes, the population of SYP barn owls was increased by 32 within six months.

With regard to avoiding off-target species colonisation and optimising barn owl reproduction, the nest box design for this study was very successful. Our first observation was the importance of balancing the internal cavity space, which can positively influence barn owl clutch size (Hattingh pers.comm. 2017), with the retention of a size/weight suitable to be safely and easily mounted onto a pole to withstand wind. FauNature's choice of materials (plywood) is considered a key factor to achieving this.

Secondly, the addition of the front perch, after advice from Dave Irwin at Raptor Domain on Kangaroo Island, proved integral to the study, allowing not only extra space for owls and fledglings to carry out important behaviours such as practicing flight, but also to provide a stage on which to capture these behaviours with an external monitoring system. As a result, we were able to keep track of important behaviours, such as the timing of courtship, markers of hen brooding, and staggered fledgling emergence. It is also likely that we avoided many of the concerns expressed by Klein *et al.* (2007) such as premature fledging, by providing the perch and high placement of the entrance hole. All fledglings were regularly photographed

hopping to-and-from the box roof, stretching their wings and flapping in preparation for flight. The placement of boxes near trees also likely benefitted the survival of owlets, by allowing them to practice short-distance flights and climb to safety if they fell. Two feral foxes were detected looking up at a nest box during the study, indicating the importance of these structures in fledgling survival.

Additionally, the inclusion of nesting material in the form of wood shavings, chips, carpet or another substrate, was equally important. Barn owls are secondary cavity nesters and do not line their nests with materials before egg laying, instead relying on pre-existing nesting materials (Lambrechts *et al.* 2012). The base of the nest boxes are smooth, flat and usually on an angle due to the imperfect nature of installation on a pole. The prevention of egg rolling is crucial for proper incubation.

The uptake of boxes by only barn owls indicated an attraction to specific cavity features, particularly the small entrance hole. In overseas studies, there is evidence that smaller entrance holes can prevent the predation of smaller owl species nesting in these cavities by larger owl species (Hakkarainen & Korpamaki 1996), prevent premature fledging, lower human-induced stress (Roulin *et al.* 2010), and potentially lower the incidence of owl ectoparasite incidence by reducing contact with intruding species (Lambrechts *et al.* 2012). However, 3 months following the end of the study, one farmer noted that one of their boxes (Site 9) had been abandoned by the owls and re-colonised by European honey bees. This issue was expected before the study, and has been controlled in the past by FauNature™ and Raid (pers. comm. 2015) by fixing a permethrin-infused tag into the nest cavity and providing alternative hives nearby.

Two of our boxes, Sites 6 and 7, failed to colonise. We speculate that the presence of nesting magpies < 3 m from the box likely prevented the colonisation of Site 6. Australian magpies are notoriously territorial and will aggressively defend nesting sites from other species,

including humans (Morgan *et al.* 2005). The failure of Site 7 to colonise is harder to explain, as it was located in a revegetated paddock, close to a grain crop with ideal mouse abundance. However, this box was exposed to western sun. Full sun exposure to nest boxes has been associated with lower colonisation rates and lower reproduction rates (Charter *et al.* 2010b). Whether magpies or another territorial competitor was present at this site is unknown. Nesting magpies were present at other sites, including Site 4, which was also located beneath an active black-shouldered kite nest, however this had no negative impact on colonisation. A component to nest box design that was not investigated during this study is thermoregulation and insulation. Nest boxes have historically struggled to replicate the insulative properties of natural cavities (Goldingay & Stevens 2009; Amat-Valero *et al.* 2012; Wendt & Johnson 2017). In southern Australia, where winter temperatures can reach zero and summer temperatures over 45°C, it's likely that the internal temperatures of exposed, pole-mounted boxes would reach dangerous temperatures without some intervention (Meyrom *et al.* 2009). The plywood used for our boxes was 10 mm thick, thus we relied on the shade of trees and orientation of the box entrance to lessen the impact of harsh weather events. The use of an internal temperature logger and experimentation with materials of increased thermal mass may be ideal for future studies of this nature.

4.2. Camera accuracy and effect on prey intake

Optimization of the external camera monitoring system to monitor and assess barn owl behaviour and prey intake took several weeks. Owl monitoring began in early January, with all cameras standardised, but the initial results performed so poorly that this data was only used to assess early colonisation of the nest boxes. Passive infrared sensor (PIR) sensitivity was particularly difficult to optimise for each site due to the unstandardized vegetation movement in the background. Some sites were able to capture high specificity despite a high amount of background vegetation (e.g. Site 8) while others (e.g. Site 2 and 9) captured almost

no owl photos, despite filling up SD cards with non-target photos. Cameras set to Low PIR sensitivity resulted in almost no owl detections. The successful cameras were optimised on the settings of Sites 3 and 8, which managed to capture high numbers of owl events while maintaining adequate battery charge and SD card space for each monitoring period. Setting the cameras to be most sensitive between dusk and dawn eliminated unwanted daytime non-target images, thus saving energy. Setting all timer intervals to OFF prevented cameras from triggering at intervals when owls were not present on the box. PIR sensitivity was eventually set to HIGH for all sites when included with other energy-saving interventions, thus maximising camera accuracy towards the end of the study.

The most impactful error to camera setup was the use of a 10 minute timer interval, which caused the cameras to trigger every 10 minutes over the entire study period. Simultaneous disablement of PIR movement triggering resulted in the failure of monitoring for Period 3 at Site 10. Similar scenarios occurred at Site 2 and 9. Additionally, the use of old rechargeable batteries resulted in failure to monitor from the 5th of May onwards at Site 2, and from the 11th of June onwards at Site 8, and nine days into Period 3 at Site 11, despite batteries being fully charged.

The two measures used for camera accuracy described different scenarios. Measure 1 (proportion of total monitoring nights in which at least one prey item was detected) was likely a reflection of camera positioning and setup, as well as overall owl activity at the nest. Cameras which failed to capture nights with prey events also failed to detect more than a few prey events on even the most active hunting nights. A good example of this is the difference between Site 8 and Site 9, which were situated on the same property. During the busiest period, prey events were captured every night at Site 8, with over 20 prey events for a number of these nights. Contrastingly, a prey item was detected on only a handful of monitoring nights at Site 9, with a maximum of only one or two prey items detected on each

of these nights. However, it's possible that adult birds at Site 9 were hunting and roosting away from the box, returning occasionally to feed only one owlet.

In contrast, Measure 2 focused particularly on the proportion of owl events containing a prey item, and also provided more detailed information on the change in owl activities and behaviours throughout the breeding season. High levels of non-prey-related activities, such as courtship, surveillance from the perch, and owlets jostling for space on the box, tended to decrease the proportion of total prey events captured.

The camera at Site 1 was subject to a large amount of infrared-flash-induced overexposure. This resulted in a majority of photos being significantly washed-out, making it difficult to categorise the activities or prey items in the photos. Camera positioning further away from the box, as with most other sites, seemed to eliminate this issue. The use of an extendable trail-camera attachment arm is advised for future studies, to standardise camera positioning.

In terms of minimal disturbance to owls, the external camera system was relatively successful. The owls did not appear to be negatively disturbed by the cameras, with photos indicating curiosity from the owls, possibly in response to the flashing photo indicator or the sound of the camera shutter. Adult owls (and fledglings, at the end of the study) typically left the nest boxes for a short period when we arrived, to either watch from a nearby tree or circle the area, while we were servicing the cameras. They then returned shortly after we left, as captured on camera.

4.3. Effect of mouse abundance and owl reproduction on prey intake

There was a clear relationship between changes in prey intake over time, owl reproduction and relative mouse abundance. We speculate that this was down to the timing of the study; with nest boxes provided just prior to the barn owl autumn breeding season, whilst mouse numbers were predicted to rise towards April 2018 (<https://www.pestsmart.org.au/pest->

animal-species/mouse/), coinciding with the highest energy demands of growing owlets and working adults. This trend was apparent during the study.

The effect of grazing livestock on mouse abundance was also highly apparent and could be assessed anecdotally when we visited the mouse survey sites. Several un-grazed paddocks, particularly Site 10, experienced plague-like mouse numbers throughout the study. These numbers were likely associated with the spilt grain (food availability) and abundant refugia in the form of straw piles or tall stubble (Singleton 2007). The sharp drop in mouse numbers seen at the end of the study aligns with the seasonal population dynamics noted by Singleton (2007), most likely associated with the decline in food availability, low temperatures and disease associated with high population densities during winter. This coincided with the voluntary period of fasting observed in fledgling barn owls (Durant & Handrich 1998) and subsequent departure from the nest upon reaching adulthood.

It has been well established that rodent abundance can positively influence barn owl fecundity (McLaughlin 1994). The mean of four and median of five fledglings aligns with the clutch size observations of McLaughlin (1994) on wild eastern barn owls in natural cavities. To predict how clutch size would affect prey intake and nest box energy requirements throughout time, several overseas and Australian studies were reviewed (Hamilton & Neill 1981; Hamilton 1985; McLaughlin 1994; Durant & Handrich 1998; Dierenfeld *et al.* 2002). In Dickman *et al.* (1991) the average weight of consumed mice was around 15 g. The expected nightly prey was far higher than the recorded average nightly prey intake. This could be explained in a number of ways. For example, a number of prey items may not have been detected, either due to the adult owls consuming prey on a nearby roost, or entering the nest box too quickly for a single camera to capture. Or perhaps, the numerous bursts of photos showing an adult owl either entering or looking into a nest box, with their heads and any carried items hidden inside the box, may have been prey events counted as false

negatives. Evidence of this occurring can be seen in February at Site 1, where the ritualistic gifting of prey to the female by the male resulted in ‘caching’ of multiple uneaten prey items on the perch (Bunn 1977). The events in which these prey items were returned were not photographed, but the evidence of their capture was there nonetheless. Second, some occasional prey items may have been larger than the assumed 15 g mouse, especially if the prey item was a small rat or pregnant mouse (Singleton & Krebs 2007), thus providing more energy per catch. In addition, our estimates of barn owl energy requirements were based on studies on European and North American sub species (*T. alba guttata*; *T. alba furcata*) whose energy requirements have been shown to increase in colder conditions by around 125 kJ for every decrease in 10°C below 25°C (Hamilton 1985; Klein *et al.* 2006; Roulin *et al.* 2009). It has not yet been determined whether subspecies from warmer climates, such as our Eastern barn owl, also have different energy requirements or thermoregulatory abilities than the North American subspecies used in our estimated energy requirement model. This could be investigated, along with thermoprotective properties of the nest box itself, with the aforementioned temperature logger in future studies.

4.4. Timing of prey intake

As an additional measure of owl hunting behaviour, we assessed the frequency of prey events throughout each hunting night. A similar study has been performed on Boullanger Island, Western Australia, by Dickman *et al.* (1991) and compared the hunting activity of barn owls with mouse activity. Male mice were most active at around 2200hrs and again at around 0500hrs, while females were most active (in lower numbers) at around 1900hrs and again from 0400-0500hrs. This correlates with the bimodal hunting activity observed at Sites 1, 2, 3, 8 and 11, but differs slightly to the trimodal pattern observed by Dickman *et al.* (1991), in which barn owl hunting also peaked between 0000hrs and 0100hrs. However, their other two peaks aligned with those observed in our study, and suggests that increased mouse activity

correlates with increased owl hunting activity. Hunger following a period of fasting may also be the driving force behind the huge spike in hunting immediately after sundown, especially if adults are stimulated by vocalisations and begging calls of hungry chicks (Bunn 1977).

4.5. Effect of camera placement on prey identification

The introduced house mouse made up nearly 80% of the total prey identified. We believe that the positioning of the camera in relation to the nest box entrance had a significant impact on prey identification. An important example of this is the difference between Site 1, where the camera faced downwards towards the front of the box, and Site 11, where the camera was facing side-on and upwards towards the box. Site 1 had a higher proportion of ‘unknown’ prey, most likely unidentified mice, due to the fact that the faces of returning owls were obscured as they were facing into the box. This allowed only parts of the prey to be seen by the camera, especially if the prey was being held in the owl’s foot. Cameras facing side-on tended to identify prey more clearly, as the whole prey animal was usually visible as it was being carried into the box. We suspect that, based on previous diet composition studies (Mortin & Martin 1979; Baker-Gabb 1984; McLaughlin 1994; Tores *et al.* 2005; Avery *et al.* 2005; Kitowski 2013) and prey species present at the study area, unknown prey may have occasionally consisted of juvenile mice or rats, rabbit/hare kittens, altricial nestling birds, small reptiles or terrestrial arthropods. One suspected bat, based on the wing-shape, was photographed at Site 2, which is not unusual given the findings of Santos-Moreno & Alfaro (2009) where microbats made a significant proportion of barn owl diet in Chile. In the interest of identifying off-target impacts, prey composition assessment involving the dissection of pellets would be ideal for future studies, however we noted that pellets were not present beneath the box as expected, and would require retrieval from inside the box or from an associated roost.

4.6. Implications for future study and use in integrated management systems

The data obtained from this pilot study provided an ideal platform on which to develop further manipulative barn owl studies in southern Australia. We identified a number of limitations, including those already mentioned in detail. One challenge not yet mentioned was the methods used for the mouse abundance surveys. These were initially performed by only one or two people, marking transects using coloured tape, tied to existing foliage in the study paddocks. These markers were almost impossible to find in tall stubble or undulated paddocks. The result was a significant loss of time spent searching for each transect, and a possible doubling up of mouse counts if the ends of each transect couldn't be accurately located, so we made significant changes to the methods to address this, including the use of a 60 cm paddock marker + flagging tape and a third person to perform transects. The second issue was the accidental counting of 'blind' mouse burrows, or diggings, which was accounted for by assessing the number of diggings against actual active burrows and applying a correction factor to mouse abundance results up to that point. The third challenge was overall standardisation of sites and camera placement, particularly with orientation and crop type. This was difficult to attain as we were limited to land offered by farmers, and the focus for this study was more on finding appropriate trees and accessible sites for monitoring. Future studies will also need to account for the substantial time required to tag photos.

The main areas of interest for this study, aside from nest box efficacy, were prey intake rates, composition and relationship with reproduction, mouse population dynamics and seasonal changes. Between the eight active nest boxes, a total of 3,717 prey items, of which most were the target prey, were captured within six months. We have not yet manipulated owl densities enough to impact overall mouse dynamics or assess the impact of predation pressure on mouse behaviour, but it's likely that our owls had a localised impact. The effect of grazing,

combined with mouse predation by barn owls, offers a small hint to the impacts that these strategies could have for southern Australian farmers in the future.

This study also opens up a number of questions for further investigation. Two major concerns have been noted. The first is whether barn owls are affected by secondary zinc phosphide poisoning. The non-target impacts of anticoagulant rodenticides on predators has been assessed by a number of studies (Mendenhall & Pank, 1980; Naim *et al.* 2010; Salim *et al.* 2014; Lohr & Davis 2018), but there is little to no evidence of this occurring with zinc phosphide due to its rapid metabolism (Joermann 1998). The second concern is what happens to the owls after a significant drop in mouse abundance, which we were not able to assess after August 2018. The use of GPS trackers could be used to determine how mouse abundance alters the hunting ranges of barn owls. Migration to areas of higher food abundance, increased mortality, or off-target predation are also potential outcomes, to which we do not know the extent.

4.7. Conclusion

The findings of this pilot study have indicated that the eastern barn owl is an excellent candidate for use as an avian predator on feral mouse-affected properties of southern Australia. Our nest box design was sound and provided a safe and very effective nesting cavity for breeding barn owls. The addition of a perch and installation of the box near trees was beneficial to both owls and to researchers, by providing a target space to monitor owls from an external camera. The barn owls were capable of hunting large numbers of the target prey species, with little off-target impacts. Longitudinal-style research will hopefully carry on from this study at a larger scale, to further refine monitoring and standardisation techniques, assess owl movements, nest box design, prey intake and potential impacts of zinc phosphide baiting, as well as identifying an appropriate owl density for mouse control.

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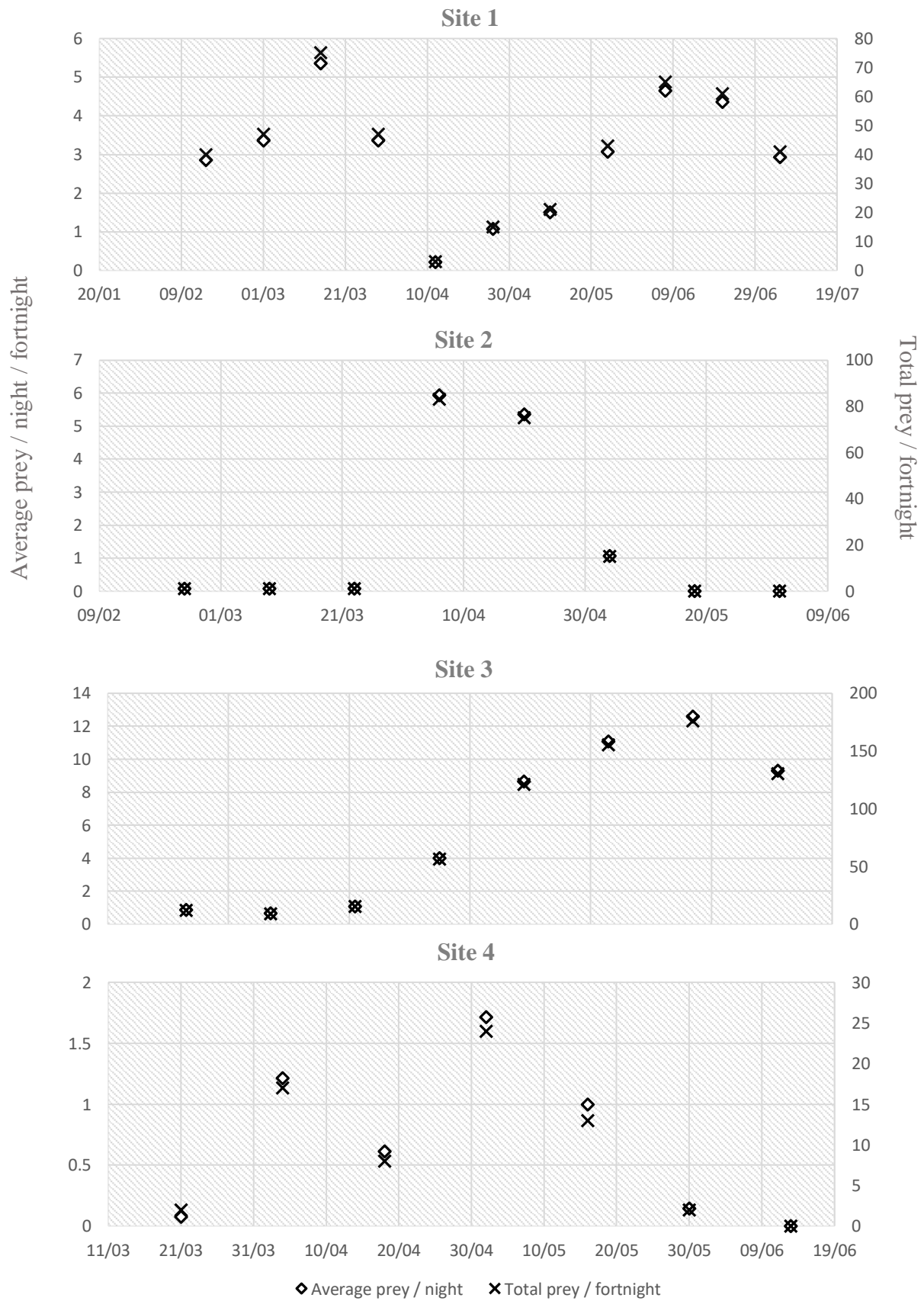
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Appendix 1.



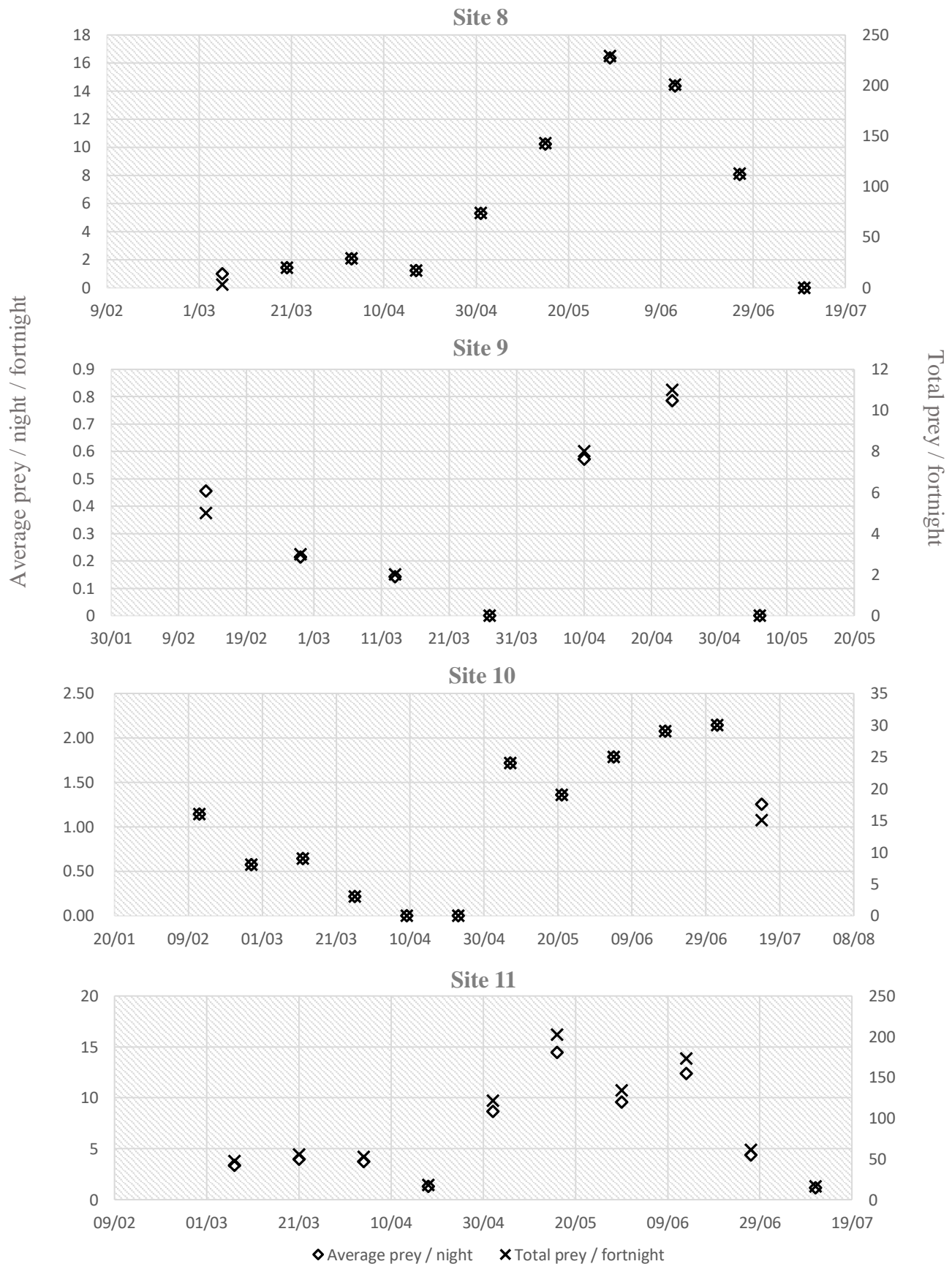


Figure 18. Average nightly prey intake (black diamonds) plotted against the total number of prey captured within each fortnight of monitoring (black crosses). Dates and axes are not standardised. Dates are in day/month format, all from 2018.