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Authors: Maute, Kimberly, Story, Paul, Hose, Grant C, Warden, Andrew, Dojchinov, Greg, et al.

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Observations on populations of a small insectivorous bird, *Malurus leucopterus leuconotus* Dumont, after an application of two ultra-low-volume (ULV) insecticides, fenitrothion and fipronil, in arid Australia

Kimberly Maute^{A,*} , Paul Story^B, Grant C Hose^C, Andrew Warden^D, Greg Dojchinov^D and Kristine French^A

For full list of author affiliations and declarations see end of paper

***Correspondence to:**

K. Maute
Centre for Sustainable Ecosystem Solutions,
University of Wollongong, Wollongong,
NSW 2522, Australia
Email: kmaute@uow.edu.au

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ABSTRACT

The use of chemical pesticides to manage locust populations in natural ecosystems is likely to impact non-target arthropods and their predators. However, the relative effects of different locust control applications on Australian birds are unknown. Aerial applications of fipronil and fenitrothion are examples of two pesticides used in locust control in semiarid Australia. To test the relative impacts of pesticides on non-target fauna, pesticides were applied to replicate sites using aerial ultra-low-volume application methods. The body condition and biomarkers of pesticide exposure in resident white-winged fairy wrens (*Malurus leucopterus leuconotus*) at treatment and control sites were measured for two weeks before and after treatments. No measures suggested negative impacts of pesticide applications. However, birds monitored at treatment sites gained mass, possibly due to indirect impacts of pesticides on bird feeding patterns or the availability or behaviour of insect prey. Bird mass measures remained high at fipronil sites, whereas the mass of birds at fenitrothion sites returned to baseline levels within one week. As this study was conducted during dry conditions, when locust plagues are less likely, future insecticide research should also consider the availability of insect prey, its effect on insectivore feeding behaviour and the interaction of rainfall events.

Keywords: Adonis, barrier treatment, drought, Maluridae, insectivore, locust, organophosphate, white-winged fairy wren.

Introduction

Historically, chemical and biological pesticides have been used to manage locust populations across two million square kilometres of eastern Australia (Story and Cox 2001). Two chemical pesticides used for aerial control of locusts are the organophosphate (OP), fenitrothion, and the phenyl pyrazole, fipronil (Story *et al.* 2005). The economic benefits of locust control are well documented (Millist and Abdalla 2011), but the application of pesticides to natural ecosystems undoubtedly impacts non-target biota, particularly arthropods and their predators, and these effects have recently been the topic of increased study (Fildes *et al.* 2006; Story *et al.* 2005, 2011; Kitulagodage *et al.* 2011a; Maute *et al.* 2015a; Walker *et al.* 2016).

The use of insecticides to control locusts also exposes non-target fauna to pesticides, by direct contact or by ingestion of pesticide-exposed prey (Peveling 2001). Pesticide-induced change in prey abundance or composition may also indirectly affect fauna (Peveling *et al.* 2003). Fenitrothion is a cholinesterase (ChE) agent (see Story and Cox (2001) for a review of effects of ChE suppression in vertebrates) and so disrupts proper cholinergic functioning in the vertebrate central and peripheral nervous systems. Exposure to fenitrothion results in significant dose-dependent reductions in flight metabolism and cholinesterase response in captive birds (Fildes *et al.* 2009). Previous surveys using plasma acetylcholinesterase (AChE) as a biomarker of OP exposure, have shown that birds can be exposed

to pesticides during locust control campaigns within the *Mitchell* spp. grass plains of south-western Queensland, Australia (Fildes *et al.* 2006). Although exposure can result from direct overspray from pesticides (Mineau 2002), studies have also documented bird species feeding on pesticide-exposed locusts during spray operations, thus increasing the potential hazard to both individuals and free-living populations (Hunt *et al.* 1992; Fildes *et al.* 2006; Story *et al.* 2013). That exposure studies have documented AChE suppression in several species of partially nomadic granivorous and insectivorous species showed that exposure can occur across trophic levels (Fildes *et al.* 2006; Szabo *et al.* 2009).

Fipronil belongs to a different class of chemical than fenitrothion, namely the phenyl pyrazoles, and exerts effects by impeding the proper functioning of the gamma aminobutyric acid (GABA) neurotransmission pathway (Simon-Delso *et al.* 2014). Little research has been undertaken on avian impacts of fipronil exposure or what the consequences for free-living populations might be (Gibbons *et al.* 2015). Kitulagodage *et al.* (2011a) found that fipronil is maternally transferred in captive zebra finches and that this transfer can result in abnormalities in hatchlings. These findings are in keeping with fipronil being identified as an endocrine-disrupting compound (Gibbons *et al.* 2015) and so further research is needed to better elucidate potential effects on condition, survival and recruitment within wild bird populations resulting from pesticide exposure (Fildes *et al.* 2009; Kitulagodage *et al.* 2011a).

In this study, we aimed to improve our understanding of how fipronil and fenitrothion impact on the condition of resident insectivorous birds, *in situ*, during and after chemical pesticide application. Resident populations of white-winged fairy wrens (*Malurus leucopterus leuconotus* Dumont, 1824) were monitored for body condition measures to quantify both exposure to pesticides and ecophysiological effects stemming from this exposure.

Methods

Species and site

White-winged fairy wrens (*M. l. leuconotus*) are small insectivorous birds that live in cooperative breeding groups and defend defined territories throughout the summer breeding season (Rathburn and Montgomerie 2003). The species occurs in the same arid zone habitat types where locust plagues can occur (Szabo *et al.* 2009) following sufficient rainfall. The study was undertaken at Fowlers Gap Arid Zone Research Station, NSW, Australia (31°20'28.50"S, 141°44'33.18"E), a working sheep station and research station. Preceding and during the study period, large numbers of feral goats and an influx of nomadic kangaroos resulted in overgrazing of vegetation across the station (unpubl. data), further exacerbating prevailing drought conditions.

The property contains a mixture of arid woodlands and grasslands, but all sites in the current study were located in arid shrubland habitat, with no trees and a ground layer dominated by perennial grasses and low shrubs. Dominant genera of grasses included *Astrelba*, *Eriachnae* and *Panicum*. The shrub layer was dominated by *Chenopodiaceae* species.

Mean annual rainfall is 242 mm and monthly average maximum temperatures range from 36.4°C to 17.0°C (Australian Bureau of Meteorology 2018). Rainfall was above average in 2015 and 2016 (358 and 391 mm); however, annual rainfall was only 84 and 48 mm in 2017 and 2018, indicating that the experiment occurred during drought.

We used a BACI (before, after, control, impact) experimental design to test the effects of pesticide treatments on physiological measures within *M. l. leuconotus* (Green 1979), employing six sites, each approximately 1.5 km in diameter and spaced at least 2 km apart. Two sites were randomly allocated to each of three treatments: control, fipronil treatment and fenitrothion treatment. We monitored birds at sites during three months in summer 2018, including before treatment in January and early February, and after pesticide spray in mid-February through to mid-March. Birds were captured, measured and sampled at 24 h, one week and two weeks postspray. Individuals were followed for these time periods, as fipronil residue is often retained in the environment for five days (Connelly 2001), and although fenitrothion is largely undetected in the environment after two days (Greenhalgh *et al.* 1975), a longer monitoring period was predicted to allow us to track both sublethal impacts and recovery of birds from exposure to both pesticides.

Aerial spray applications

We used a single pesticide application at each treatment site. The experimental spraying was conducted at a time when there was no locust present and when no other spraying was being undertaken in the region. Although our late summer treatments coincided with the time of year when spraying had historically and intermittently occurred in western New South Wales, the prevalence of prolonged drought in the region had reduced vegetation biomass to such an extent as to no longer reflect reasonable quality locust habitat and therefore the conditions under which pesticides would be applied for locust management. Operational ultra-low-volume (ULV) pesticide application methods differ between fipronil and fenitrothion (Supplementary Table S1), so we used two spray application methodologies in applying the chemical pesticides to the treatment areas.

Fipronil was applied as cross-wind barrier treatments from a Piper Brave (PA36) fixed-wing aircraft equipped with two Micronair AU5000 rotary atomisers (Micron Sprayers). The spray plane was equipped with a Satloc differential global positioning system (Hemisphere GPS) for spray guidance using a constant flow rate. Fipronil (Adonis 3UL formulated at 3 g active ingredient (ai) L⁻¹) treatments involved the

spray plane applying multiple strips of pesticide per site at 90° to the prevailing wind direction (Table S1). The fipronil applications covered a total area of 203.8–223.3 ha per site (Table S1) using 300 m spray swaths and, based on wind speed, each strip of pesticide was approximately 100 m wide with the area in between strips left untreated. The cross-wind drift of pesticide corresponded to an operationally applied dose of 0.25–1.26 g ai ha⁻¹, which has proven effective for the control of locusts in Australia and elsewhere (Balança and de Visscher 1997; Story *et al.* 2005).

Fenitrothion was applied using cross-wind swaths and slightly overlapping tracks, resulting in a continuous or 'blanket' application over the entirety of each site at a dose of 210 mL ai ha⁻¹, similar to previous applications used in Australia (Walker *et al.* 2016).

Capture and measurement

Between January and March 2018, we captured birds using mist nets and banded each individual with one metal Australian Bird and Bat Banding Scheme (ABBBS) band. Birds were herded and flushed into nets, which could not be concealed due to a lack of vegetation in the open chenopod shrubland. We also found song playback ineffective, hence recapture of net-shy banded individuals was rare. Juveniles were rarely captured and were excluded from analyses. We then weighed (± 0.01 g) each bird using a digital scale and took standard morphological measurements (i.e. wing chord, and head to bill length). Birds were inspected for evidence of breeding condition and feather moult. We scored the amount of furcular fat and the shape of the pectoral muscle using ordinal scales as described in Maute *et al.* (2015b). One outer tail feather was collected from each bird, and <75 μ L of blood was collected into a heparinised haematocrit tube by puncture of the brachial vein. About 20 μ L of whole blood samples from birds at fipronil and control treatment sites were placed on 226 Dry Blood Spot sample paper (Perkin-Elmer, USA), dried at room temperature in the field and then frozen at -80°C when samples were returned from the field. Blood in haematocrit tubes was kept cool on ice bricks until the sample could be spun down in a centrifuge and plasma drawn off with a 50 μ L glass and steel syringe (SGE Analytical Science Pty Ltd) within 4 h. Plasma was frozen at -20°C and stored at -80°C when samples were returned from the field until assayed. Bird body, feather and blood measures were assessed at two replicate control and treatment sites before and after treatments. Birds were caught before pesticide spray applications and approximately one day, one week and two weeks after applications (Supplementary Fig. S1).

Feather measures

One or two outer tail feathers were placed on a black paper background and reflectance was measured at three

location along the central part of the feather, starting at approximately one-fourth down from the tip (5 mm) and 1 mm from the rachis, then moving down the feather in 1 mm increments. This corresponds to the most colourful portion of each feather. All *M. l. leuconotus* individuals have blue tail feathers, though full adult males commonly have a darker blue tail (Tidemann 1980). Reflectance was measured by a Jaz spectrometer (Ocean Optics, Inc.) fibre optic probe with a xenon light, held at a 90° angle using a black interior probe holder. All measures were taken in reference to a white reflectance 99% (Sphere Optics GmbH) and black standard (Papermate, Inc. drawing paper). The spectrometer was set to average 20 scans per reading and box car width of five and reflectance was recorded in 0.38 nm intervals.

A measure of feather spectral reflectance was calculated from raw reflectance data. Blue chroma was calculated as the proportion of reflectance between 400 and 495 nm. Blue colour has been found to be influenced by exposure to toxins in other bird species (White and Cristol 2014; McCullagh *et al.* 2015), and was therefore measured as a potential marker of pesticide exposure impacts on physiology. Feather bars are the patterns of light and dark bands on a feather, which correspond to the growth of feathers during the day and night, and is correlated with feather quality and bird condition (Keyser and Hill 1999; Dawson *et al.* 2000). As all *M. l. leuconotus* were moulting in late summer during the experiment, we were able to collect newly growing feathers from birds after the pesticide applications. The length of seven light and dark bands were outlined with pin markers on a foam background for each feather and the total length was measured to the nearest 0.1 mm using callipers. The daily average feather growth length was then calculated by dividing the total length of the bars by seven. Only feathers collected at least one week after spray applications were included in analysis for post-spray treatment sites.

Blood measures

Haematocrit level, the proportion of red blood cells to plasma, was measured using a sliding scale tool (Haematospin) on blood tubes that had been spun down in a centrifuge for 2.5 min at 14 000 RCF (Haematospin 1300, Hawksley and Sons Ltd).

Total fiprole levels (the residue level associated with the parent chemical, fipronil and any metabolites present) was measured in bird blood samples to determine fipronil exposure in *M. l. leuconotus* individuals sampled. An 8 mm punch of dried blood spots, representing ~ 8 μ L of whole blood was used in a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay (Raju *et al.* 2016). Briefly, each whole blood spot was punched from 226 Five Spot RUO Cards (PerkinElmer Inc.) and transferred to an Eppendorf tube containing 100 μ L of MilliQ water. The Eppendorf tube

was briefly centrifuged to make sure that the DBS disc was completely immersed and then exposed to sonication for 5 min. A 300 μL aliquot of acetonitrile was added to each sample. The tubes were vortexed for 2 min and sonicated again for 5 min. The tubes were centrifuged at 10 000 rpm ($\sim 8944g$) for 10 min and 200 μL of supernatant was transferred into an HPLC vial with 400 μL glass insert for injection. Samples were kept at 4°C during assay. Samples were analysed on an Agilent 6490 Triple Quad LCMS. Solvent A comprised $\text{H}_2\text{O} + 5 \text{ mM ammonium formate} + 0.2\% \text{ formic acid}$; Solvent B comprised 90% methanol + 10% $\text{H}_2\text{O} + 5 \text{ mM ammonium formate} + 0.2\% \text{ formic acid}$. UHPLC Guard Column and Poroshel 120 EC C18 2.7 μm (2.1 mm \times 50 mm) was used for the LC. The elution program was 0.2 mL min^{-1} , 1 min at 70% B, 1–10 min 70–90% B, 10–11 min 90% B. The volume of injected sample was 1 μL . Fipronil desulfinyl (RT 5.2 min), Fipronil (RT 5.4 min), Fipronil sulfide (RT 5.5 min) and Fipronil sulfone (RT 5.7 min) residues were analysed in negative mode and were confirmed by their three most abundant product ions at optimised collision energies.

Exposure to fenitrothion was measured using AChE activity suppression in plasma samples taken from *M. l. leuconotus* and assayed using the Ellman assay (Ellman et al. 1961; Gard and Hooper 1993) as modified by Grad and Hooper (1993) for use in a 96-well spectrophotometric plate reader (BioRad Benchmark Plus, BioRad Laboratories, CA, USA). Assay reagents were obtained from Sigma-Aldrich (Castle Hill, NSW). Samples were assayed singly or in duplicate depending on the amount of plasma provided in each sample, using an optimal dilution ratio of 20:1, for total ChE and AChE activities at 25°C for 3 min (read at 10 s intervals). Assay components were acetylthiocholine iodide (AThCh, 10^{-3} M final concentration; the ChE substrate), 5,5'-dithiobis (2-nitrobenzoic acid; 3.23×10^{-3} M), 0.05 M tris (pH 8.0) buffer and diluted enzyme with a total volume of 250 μL per microplate well. The assay was initiated by the addition of AThCh to all other components. AChE was differentiated from BChE by preincubation (5 min, before AThCh addition) with the specific BChE inhibitor, tetraisopropyl pyrophosphoramidate (iso-OMPA, 10^{-6} M before addition of AThCh). BChE activity was calculated as the difference between total ChE and AChE activities. Mouse serum (Sigma Aldrich Australia) provided a between-assay standard. Blank wells without added enzyme provided background colour formation. The increase in absorbance at 412 nm, corrected for blank, was converted to mmol AThCh hydrolysed per min per mL of plasma using the molar extinction coefficient $13\,600 \text{ M cm}^{-1}$ (as per Ellman et al. 1961). More specific details of the assay are provided in earlier publications from our collaborative research laboratory (Fildes et al. 2006; Buttemer et al. 2008; Story et al. 2016). Samples with sufficient plasma were assayed in duplicate ($n = 6$), but all other samples were run singly. Unfortunately, due to haemolysis of some samples and

others having insufficient plasma, only 26.4% of plasma samples supplied were adequate for inclusion in the AChE activity assay. Therefore all samples taken within 14 days of fenitrothion application were pooled in analysis. Also, due to insufficient volumes of plasma, enzyme reactivation assays were not undertaken.

Statistical analysis

Most wren measurements were normally distributed continuous data, whereas fat, muscle and haematocrit were ordinal data. Feather blue chroma was not normal, and this was resolved with a Johnson Su transformation. Few birds were recaptured between the two periods, so only one capture time for each of these individuals was used in analysis. Linear mixed model analyses (or ordinal logistic models for fat, muscle and haematocrit scores) were used to determine the potential impact of spray applications on wren condition measures. Full factorial models included the pesticide treatments (fipronil, fenitrothion and control), time (prespray and 24 h, one week and two weeks postspray) and sex (male and female) as main and interacting effects, and site nested in treatment (two sites per treatment) as a random factor. All measures included four time periods, except AChE activity, which included only two (pre- and postspray). For scaled mass, the time of day of bird capture was included as an additional factor, as birds tended to gain mass while feeding in the morning. The directions of statistical differences among site and sexes were determined using Tukey–Kramer Highly Significant Difference analysis for normal measures and Wilcoxon tests for all non-parametric measures (all analyses performed in JMP Pro 14.1.0 SAS Institute Inc.).

To compare body mass of birds to feather measures, we used the scaled body mass index (SBMI) (Peig and Green 2009). The SBMI standardises body mass by taking into account a measure of structural body size. In this study, the scaling exponent of the head to bill length measure relationship with mass using the standardised major axis regression (sma) value was closest to the assumption of isometric growth (closest to 3; $b_{\text{sma}} \text{ head to bill} = 3.01$; $b_{\text{sma}} \text{ wing} = 2.13$), and was chosen as the standard body size score. This allows direct comparisons of body mass without the confounding effects of body size. Ages of females cannot be determined, and therefore we could not analyse the possible influence of age on feathers or condition.

Ethical approval

This study was conducted under NSW National Parks and Wildlife Service Scientific License (SL100109) and UOW animal ethics authority AE1620.

Results

A total of 170 birds were caught and released (Supplementary Fig. S1), and only nine individuals were recaptured, despite observations that banded birds were still present at sites during all weeks of trapping (KM, pers. obs.). All birds were found to be undergoing full postbreeding flight feather moult, but many males still had a cloacal protuberance, suggesting breeding had recently ceased (Tidemann 1980). The proportion of female to male captures was 1.14 ± 0.52 s.d.

Analysis using linear mixed models and ordinal logistic regression suggested that bird haematocrit, fat and muscle scores and feather measures were not impacted by the spray applications (Table 1; full models in Supplementary Table S2). However, although not statistically significant, there was an increase in scaled mass of birds (Table 1), with *post hoc* tests suggesting that birds at treatment sites

gained mass over time, whereas birds at control sites did not (Fig. 1). Specifically, birds at fenitrothion sites showed a 10% increase in scaled mass measurements at one day postspray and return to prespray values afterward. At fipronil treatment sites, bird mass increased by 8% at one day postspray, and was 10% higher than prespray values two weeks later. Changes in scaled mass did not match changes in furcular fat scores, as females had lower fat scores one week postspray, compared to other time periods (Table 1). Muscle scores increased over time for all birds, but only prespray values and Week 2 postspray were significantly different (Table 1). Though the blue colour of tail feathers declined over time, the trend matched a decrease in the proportion of full adult males captured (14% of captures before spray and 5% of captures postspray).

No fipronil or fipronil metabolite residue was detected in wren blood samples collected using blood spot cards,

Table 1. Significant and near-significant factors from statistical models of the impact of pesticide treatments (fenitrothion, fipronil and control) over time (Pre, before treatment; Post, after treatment (Day 1, Week 1 and Weeks 2)) and between the sexes (male and female).

| Condition measure | Key factor(s) | d.f. | χ^2 or <i>F</i> value | <i>P</i> value | Post hoc test results |
|--------------------------------|------------------|------|----------------------------|----------------------|--|
| SBMI (<i>n</i> = 167) | Treatment × time | 6 | 2.15 | 0.05 ^A | Increased SBMI at fenitrothion, fipronil, not control |
| | Capture time | 1 | 12.11 | 0.0007 ^B | Increasing mass over time (hours after dawn each morning) |
| Fat score (<i>n</i> = 153) | Treatment | 2 | 17.20 | 0.0002 ^B | Control < fenitrothion = fipronil |
| | Time × sex | 3 | 10.86 | 0.01 ^A | Females have lower fat scores during Week 1 postspray compared to other times, males show no changes |
| Muscle score (<i>n</i> = 153) | Time | 3 | 18.16 | 0.0004 ^B | Pre < Post Week 2 only |
| Haematocrit (<i>n</i> = 108) | Non-significant | | | >0.08 | |
| Blue chroma (<i>n</i> = 164) | Sex | 1 | 47.13 | <0.0001 ^B | Male > female |
| | Time | 3 | 5.19 | 0.002 ^B | Pre > Post Weeks 1 and 2 |
| Bar length (<i>n</i> = 131) | Treatment × time | 6 | 2.35 | 0.04 | Post hoc tests could not determine any differences |

Site nested in treatment was included as an additional factor in all models, and capture time was included as an interacting factor for Scaled Body Mass Index (SBMI) only. Haematocrit model only included control and fenitrothion sites. Direction of significant differences were determined using *t*-tests for two-means comparisons and Tukey–Kramer HSD three-means comparisons of normal data, or Wilcoxon tests for ordinal data.

^ANon-significant trend with significant differences among groups based on *post hoc* tests.

^BSignificant trend, based on Bonferroni adjustment ($P < 0.008$).

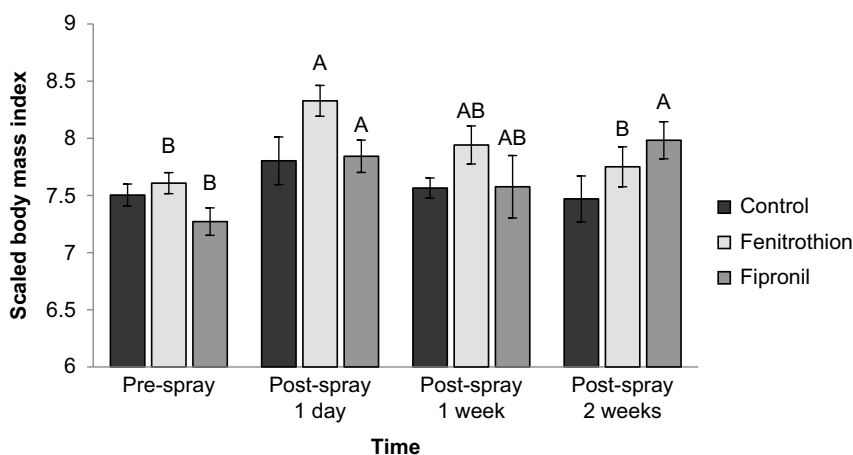


Fig. 1. Mean scaled body mass indices (SBMI) \pm s.d. of birds sampled before and after experimental spray applications of fipronil and fenitrothion. Different letters show significant differences among time comparisons within pesticide treatments, as determined by Tukey–Kramer Highly Significant Differences tests. No differences were found at control sites.

indicating that the birds sampled were not sufficiently exposed to the fipronil pesticide treatments. Similarly, analysis of bird plasma samples for AChE activity revealed no statistically significant effect of treatment, although it should be noted that analysis was restricted to 26.4% of samples considered suitable (non-haemolysed samples or those with sufficient plasma to accommodate the assay, $n = 36$; Supplementary Table S3; Fig. S2).

Discussion

There was no evidence of exposure of *M. l. leuconotus* populations to either fipronil, using fiprole residues from whole blood samples collected on DBS papers, or fenitrothion, using plasma AChE activity, as biomarkers of chemical exposure at our treatment sites. All physiological measures used in the current study suggest there were no negative impacts on bird body condition and additional measures (haematocrit, feather colour and growth measures) were found to be similar among treatment and control sites. Pesticide applications and chemical contamination in the environment can reduce bird body condition and feather quality, and cause anaemia in some circumstances (Dawson et al. 2000; Kitulagodage et al. 2011b; Lopez-Antia et al. 2013; McCullagh et al. 2015). In contrast, the applications used in this study did not represent a significant risk to resident *M. l. leuconotus*. These results should be interpreted with care, as our experiment may not accurately describe the full risk posed by locust control campaigns due to a lack of superabundant prey (in this case locusts) and drought conditions that prevailed during the study. In addition, repeated sampling of individuals, as opposed to population level monitoring, would give our results more confidence. Improvements could be made by working with a species that is more easily recaptured.

Application methods and bird behaviour influence pesticide exposure risk

The lack of evidence of exposure experienced by birds in this study may be due to the use of lower dose ULV spray application methods. For example, 420 g ai ha⁻¹ fenitrothion aerial spray applications resulted in a 60% reduction in the breeding success of white-crowned sparrows (*Zonotrichia albicollis* Gmelin 1789) in Canadian forest (Busby et al. 1990), whereas ULV 300 g ai ha⁻¹ fenitrothion applications did not impact coal tits (*Parus ater* L.1758) nesting in Scottish pine plantations (Spray et al. 1987). In our experiment, our application rate of 267 g ai ha⁻¹ fenitrothion is well below the 560 g ai ha⁻¹ threshold above which avian mortality has been shown to increase (Story and Cox 2001). Similarly, high fipronil application rates of 3–5 g ai ha⁻¹ using blanket spray application methods had significant impacts on termites and their predators in Madagascar and

northern Australia (Peveling et al. 2003; Steinbauer and Peveling 2011), and ULV barrier spray application rates <1.25 g ai ha⁻¹ were not found to impact termites and predators in arid Australia (Maute et al. 2015a, 2016, 2017a). Fipronil and metabolites can dissipate faster under hot arid conditions than in temperate conditions, due to increased photometabolism of residues (Bobé et al. 1998). Australian locust control commonly occurs during hot weather in late summer (Hunter 2004; Szabo et al. 2009). In the current study, it is possible that a combination of factors resulted in the lack of detectable impacts of pesticide applications on *M. l. leuconotus*, including ULV applications under arid conditions, a shortage of pesticide-exposed prey and beneficial bird behaviours that facilitated low exposure. The consumption of pesticide-exposed prey and/or direct contact with residues in the air, soil or vegetation can result in pesticide exposure (Driver et al. 1991). *M. l. leuconotus* spends most of the day foraging on open soil or the outer branches of shrubs, with limited body contact with thick grass vegetation (Tidemann 1980). Soil and shrub pesticide residue levels were much lower than those recorded on grasses (unpubl. data), suggesting that birds foraging in the open would be less likely to come in contact with pesticide residue. Alternatively, birds were able to avoid oral exposure due to a lack of exposed superabundant prey, as large numbers of locusts were not present during our experiment. Moreover, below average rainfall during our experiment would have reduced insect prey availability more broadly. Acute exposure may also not occur if this species was unlikely to eat large volumes of prey (gorge feed), was able to develop a feeding aversion to dosed prey, or simply preferred more active prey that are less likely to carry high pesticide burdens (Forsyth et al. 1994; Nicolaus and Lee 1999; Stafford et al. 2003). However, research on the feeding behaviour of *M. l. leuconotus* would be needed to confirm its propensity to gorge feed on dosed prey, or physical contact with environmental residue, to determine which behaviours were likely to lead to reduced exposure levels.

Fenitrothion exposure

Reduced feeding behaviour and plasma ChE activity in birds subject to subacute exposure to OPs can return to levels similar to controls within 8 h (Holmes and Boag 1990; Holmes and Sundaram 1992; Hart 1993). In our study, only two of the birds captured within 24 h of spray applications at fenitrothion sites were sampled appropriately for use in ChE assays, and only seven samples were collected over two weeks. Therefore we were not able to detect significant depression in total ChE activity, as most samples were collected many days after pesticide applications (1–14 days). Due to the small size (<9 g) and small family groups of birds at our sites, it was not possible to collect large numbers of high quality blood samples within 24 h of fenitrothion

applications. Although other research has found significant depressions in AChE for birds sampled up to 5 days postspray (Fildes *et al.* 2006), our postfenitrothion application sample pool contained five out of seven samples taken two weeks postspray, suggesting this null result could be due to the extremely low power of our analysis to detect enzyme activity depression, which would not be expected to occur this long after exposure. Collection of blood from the brachial vein during hot, arid conditions caused cell lysis in more samples than expected from using the methodology on larger species (>15 g, pers. obs.). Therefore the detection of OP exposure would have required that larger sized species and different blood collection methods be utilised to make certain that a population-level depression in AChE activity could be detected.

Fipronil exposure

Unpublished data from previous research within our laboratory shows that central bearded dragons (*Pogona vitticeps* Ahl, 1927) had up to 2.4 ng mL⁻¹ fipronil and 7.4 ng mL⁻¹ fipronil sulfone residue in whole blood from the same experimental insecticide applications, suggesting low levels of exposure to fipronil, but above the limits of detection (0.1 ng mL⁻¹). These lizards were monitored at the same sites and at the same time as birds used in this study. Though we would expect birds to be able to metabolise and eliminate pesticides faster than ectothermic lizards that have lower metabolic rates and capacity than birds and mammals (Else and Hulbert 1981; Talent 2005), it is unlikely that this would occur before our 24 h sampling period (Hamernik 1997; Kitulagodage *et al.* 2011b). In addition, omnivorous and ground dwelling lizards are likely to ingest or contact more vegetation and soil than insectivorous birds (Weir *et al.* 2010). Although oral exposure is considered to be the most important exposure pathway for pesticides (Hamernik 1997; European Food Safety Authority 2006), the increased risk of both oral and dermal exposure in lizards could help explain why dragon blood samples were found to contain low levels of fiprole residues. This again suggests that birds did not consume large enough amounts of pesticide dosed prey to show evidence of exposure, but research on feeding behaviour is needed to confirm these hypotheses.

Condition changes at treatment sites

Unexpectedly, *M. l. leuconotus* showed increased mass after the application of fenitrothion and fipronil at treatment sites, but not at control sites. Subtle changes in bird feeding behaviour may explain how pesticide applications could contribute to *M. l. leuconotus* mass gains. For example, small songbirds are known to forage more and gain fat or mass when faced with unpredictable access to food (Anselme *et al.* 2017). Although all sites may have experienced some reduction

in insect prey due to drought conditions, we would expect that reductions at treatment sites would be greater and more rapid after insecticide applications (Balança and de Visscher 1997; Story *et al.* 2013). Such unexpected reductions in insect prey could trigger increased foraging and weight gain. Alternatively, birds simply may have gained mass due to increased foraging efficiency, a result of the debilitated state of exposed insects. Other bird species have been found to increase feeding rates on dosed prey (Forsyth *et al.* 1994), as pesticide-affected prey could be more attractive due to erratic behaviour or are easier to capture (Hunt *et al.* 1992). Future research on *M. l. leuconotus* feeding behaviour and prey population dynamics in response to pesticide applications would be needed to test these potential drivers of mass change.

Significant increases in mass occurred directly after both pesticide applications, but mass measures remained 10% higher than baseline for two weeks at fipronil treated sites and returned to baseline within a week at fenitrothion sites. For a small (<9 g) arid zone insectivore, the 10% change in mass was likely biologically significant and not due to changes in water balance. For example, arid zone insectivores easily regain the 3–4% normal overnight water mass loss through feeding (du Plessis *et al.* 2012), but fluctuations of more than 5% body weight through water loss is abnormal and stressful (Wolf and Walsberg 1996). Capture time-of-day explained a significant proportion of mass differences in our study, but did not differ between treatments over time, confirming that this was not a confounding effect. Therefore our findings most likely represent an effect of increased feeding behaviour of birds at treatment sites. A lack of similar changes in fat scores further supports increased food intake as the likely cause of increases in mass, particularly directly after spray applications. It is possible that the longer half-life of fipronil in the environment meant that impacts on the prey species of *M. l. leuconotus* were persistent (Walker *et al.* 2016; Maute *et al.* 2017b), leading to longer-term changes in bird feeding behaviour at these sites.

Other field-based studies reinforce our findings that lower doses of pesticides can result in increased feeding behaviour in birds (Forsyth and Martin 1993; Forsyth *et al.* 1994). Additionally, laboratory investigations into birds exposed to ecologically realistic doses of fenitrothion (based on residue levels on sprayed locusts: Story *et al.* 2013) also showed no changes in body mass despite dose-dependent reduced peak metabolic rates and postexposure suppressions in plasma AChE activities (Fildes *et al.* 2009). The persistence of higher scaled mass for *M. l. leuconotus* at fipronil sites over two weeks is not likely to be driven by increases to metabolism due to pesticide exposure, as we did not detect fipronil or metabolites in blood samples at any time. Instead, previous research suggests that exposure is more likely to cause decreases in metabolism (20–60%) and mass (>15%) for birds given acute doses of OPs or fipronil (Mineau *et al.* 1990; Grue *et al.* 1997;

Kitulagodage et al. 2011b; Lopez-Antia et al. 2015; Narváez et al. 2016). Consequently, it is very unlikely that changes in metabolism resulted in the mass gain observed in wild insectivorous *M. l. leuconotus*.

Conclusions

Fipronil and fenitrothion applications were not found to negatively impact any measures of *M. l. leuconotus* condition, and blood measures failed to detect evidence of bird exposure to these locust control pesticides. Instead, *M. l. leuconotus* at treatment sites gained mass, possibly due to changes in the availability or behaviour of insect prey. While these findings suggest that the applications used in this study did not represent a significant risk to resident insectivorous birds, our experiment may not accurately describe the typical risk posed by locust control campaigns. Firstly, there were no large locust populations recorded at the site, and the presence of superabundant prey may increase the risk of pesticide exposure for resident birds. Secondly, locust plagues and control operations do not occur during drought conditions, therefore pesticide applications may have different effects on bird populations under improved climatic conditions. Despite dry conditions and the potential for pesticide exposure, birds remained in good health throughout the experiment and at all sites. In the future, the results of this study could be compared to measures taken from birds exposed to pesticide applications during locust plagues, to confirm the low risk to bird health suggested by our findings. This discrepancy highlights the importance of the timing of pesticide applications to the potential impacts on non-target fauna populations.

Supplementary material

Supplementary material is available [online](#).

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Data availability. Data can be provided upon request. Contact first author.

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Author affiliations

^ACentre for Sustainable Ecosystem Solutions, University of Wollongong, Wollongong, NSW 2522, Australia.

^BAustralian Plague Locust Commission, GPO Box 858, Canberra, ACT 2601, Australia.

^CDepartment of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia.

^DCSIRO Black Mountain Laboratories, Acton, ACT 2600, Australia.