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Malathion applied at standard rates reduces fledgling condition and adult male survival in a wild lesser kestrel population

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Abstract

The lesser kestrel *Falco naumanni* is a globally threatened colonial bird that has suffered a generalized population decline in Europe. Recent studies have suggested that the land-use changes linked to agriculture intensification are the main factors accounting for the population regression. However, there has been little analysis of the role of pesticide applications. In this study, we examined the consequences of a malathion treatment in a wild population of lesser kestrels, specifically its effects on breeding performance, adult survival and population size. We found that the larger the area treated with malathion around the colony, the lower the size and the body condition of the fledglings, although no effects were found on the number of fledged chicks per pair or their sex ratio. Survival of adult males, but not females, was lower in the malathion-treated areas. These results show that organophosphorus pesticides applied at standard rates might disrupt the lesser kestrel population dynamics by reducing their breeding performance and increasing adult mortality in a sex-biased way. The proportional area treated around the colony did not affect colony size in the following year, indicating that an increased adult male mortality was not enough to lead to a detectable population size reduction and/or that the arrival of immigrants could have masked it. Both the direct malathion toxic effects and the expected reduction in prey availability due to fumigation are likely to underlie the observed effects. Hence, keeping non-treated buffer areas around kestrel colony centers should be a strictly applied conservation measure to avoid the observed negative side effects of malathion applications.

Introduction

After the widespread withdrawal of organochlorine insecticides, organophosphorus (OP) compounds have currently become among the most extensively used pesticides. However, in spite of the advantages of OP pesticides, related to their low environmental persistence and limited bioaccumulation, they have a very high acute toxicity, and thus the potential to affect non-target species (Sanchez-Hernandez, 2001; Story & Cox, 2001). Birds are one of the primarily non-target taxa affected by OP pesticides (Story & Cox, 2001), and several dies-offs have been recorded (Mendelssonh & Paz, 1977; Keith & Bruggers, 1998; Mineau *et al.*, 1999; Fleischli *et al.*, 2004), sometimes accounting for population declines in species of great conservation interest (Mineau *et al.*, 2005).

A number of field and laboratory approaches have evaluated the effects of OP insecticides on birds both at biochemical and organism levels (Hooper *et al.*, 1989; Sanchez-Hernandez, 2001). However, their effect on wild avian populations is still poorly understood (Sanchez-Hernandez, 2001; Story & Cox, 2001). The attempts to extrapolate the results derived from laboratory research to field conditions have usually failed even at the organism level. Environmental matrixes and other chemicals are known to modify OP toxicity under natural conditions (Blus & Henny, 1997), and toxicity data from reference species used in laboratory tests are not usually applicable to other taxa (Schafer & Brunton, 1979; Weimeyer & Sparling, 1991). Thus, laboratory approaches provide little information on the effects of OPs on wild bird populations (Blus & Henny, 1997). While most field studies have investigated the biochemical responses to acute OP exposure (Sanchez-Hernandez, 2001), this is not usually accompanied by additional data at the population level. Hence, the population consequences of pesticide applications, which also depend on several other non-acute and indirect effects, are usually unknown (Howe *et al.*, 1996; Boatman *et al.*, 2004).

Most population-level investigations of OP effects on birds are based on records of mortality events (Mendelssonh & Paz, 1977; Goldstein *et al.*, 1999; Fleischli *et al.*, 2004). However, as most data relate to unmarked individuals, it is often difficult to establish population-level effects (Fleischli *et al.*, 2004). Long-term analyses from well-monitored bird populations could help to fill this gap, and aid in assessing the impacts of pesticide applications on wild populations (Sih, Bell & Kerby, 2004). Long-term monitoring data and toxicological studies can be integrated to develop adequate ecological pesticide risk-assessment policies (Tiebout & Brugger, 1995; Hansen & Johnson, 1999).

The main aim of the present study was to analyze the effects of an aerial application of malathion on breeding performance, adult survival and population size in a wild lesser kestrel *Falco naumanni* population. Malathion is an OP insecticide used in open-country agricultural habitats, which are also the habitat of the lesser kestrel. We carried out the study on lesser kestrel colonies that have been thoroughly monitored for several years, and thus provide a suitable study model to assess the population-level impacts of these chemicals on birds. We discuss the implications of our results for establishing general management strategies for pseudo-steppe bird populations.

Methods

Study area and organism

The study area was a plateau in La Mancha, central Spain (39°20′N, 3°15′W), covering *c.* 1000 km^2. We monitored 16 lesser kestrel colonies (average kestrel pairs per colony = 16, range = 9–64). Climate is meso-Mediterranean, with mean temperatures ranging from 24–26 °C in July to 4–6 °C in January; annual rainfall averages 300–400 mm and is mainly concentrated in spring and autumn. The area is extensively cultivated with barley *Hordeum vulgare*, wheat (*Triticum* spp.) and vineyards *Vitis vinifera*. Other minor habitats include scattered olive groves *Olea europaea*, some recent pine plantations (*Pinus* spp.) and non-cultivated pastoral areas.

The lesser kestrel is a migratory and colonial small bird of prey. This species is highly philopatric; most surviving adults and a third of the yearlings return to the same colony to breed (Serrano *et al.*, 2001). Eggs are laid from the end of April to the first week of June (Aparicio & Bonal, 2002). When feeding nestlings, kestrels forage 3–6 km from the colony (Bonal & Aparicio, 2001; Franco & Sutherland, 2004) but when they are hunting for themselves they forage up to from 10 km from the colony (Donázar, Negro & Hiraldo, 1993). Their diet is mainly insectivorous, primarily grasshoppers and crickets (Orthoptera), with a smaller component of small lizards, micromammals and passerines. Nestlings are mostly fed with grasshoppers (more than 50% biomass intake; R. Bonal & J. M. Aparicio, unpubl. data).

Breeding performance

Kestrel colonies at the study area have been monitored since 1993. In 2003, we located nest sites before the onset of egg laying and each potential nest was regularly examined to determine laying date, clutch size, hatching success, sex and number, mass and condition of fledged young. Adults were trapped with a noose carpet or by hand during incubation, measured and individually marked with metallic and colored plastic rings. Chicks were marked at hatching with nail varnish (claws) or a waterproof felt-tip pen (back down feathers), and they were banded 5–7 days later (Aparicio, 1997). Tarsus length was measured with a digital caliper to the nearest 0.01 mm, and we used this measure as an estimation of size because it reaches an asymptote before fledging. We also used pectoral thickness to estimate fledgling body condition (Aparicio & Cordero, 2001), as it is more reliable than the residuals of body mass on tarsus length (Gosler & Harper, 2000). Pectoral thickness was measured using a portable ultrasonic meter (Krautkrämer USM22F, Hürth, Germany, accuracy 0.1 mm). Both tarsus length and pectoral thickness were recorded when fledglings were 30 days old (all fledglings in the nest were measured). The number of chicks found in the nest was considered the number of fledged young per successful pair, as after that age, most chicks are able to fly. Nestlings were sexed when they were 20–30 days old by checking the color of their tail feathers and upper tail coverts, a method that is 100% reliable (Aparicio & Cordero, 2001).

Malathion application

During the spring of 2003, there was a grasshopper population outbreak in certain parts of the study area, leading the local authorities to carry out a preventive malathion aerial spraying in order to reduce the damage to vineyards. On July 8, 2003, the most affected area (7600 ha) was aerially sprayed with malathion 118% (w/v) at 1 L ha^{-1} ultra-low volume (ULV, without carrier). A non-treatment buffer zone of 500 m around the lesser kestrel colonies and from the lagoons was in place. However, the environmental authorities had incomplete knowledge of the lesser kestrel colonies and spraying took place within the buffer zone of three colonies. We studied the effects of the malathion application in relation to the proportion of treated foraging area around the colony that is within a 6 km radius to study the effects on breeding performance, and a 10 km to investigate the consequences on adult survival and colony size.

Statistical analyses

To analyze the effects of malathion aerial spraying on lesser kestrel breeding performance, breeding pairs were assigned to two groups, ‘before’ and ‘after,’ depending on whether the young fledged before or after spraying (Fig. 1). Tarsus length (normal error and identity link function), pectoral thickness (normal error and identity link function), number of fledglings (Poisson error and log link function) or sex ratio at fledging (binomial error and logit link function)

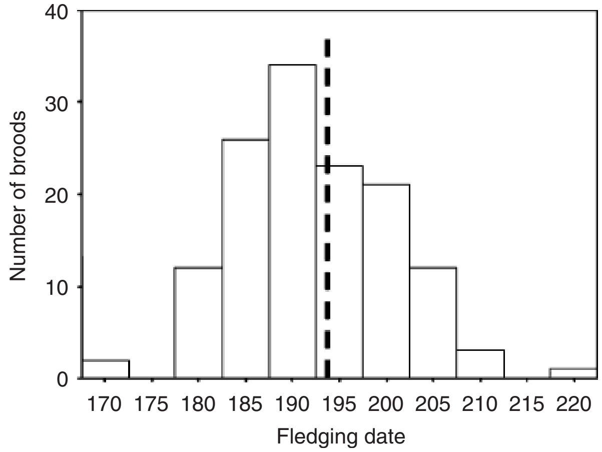

Figure 1 Number of broods when fledglings were 30 days old along the breeding season. The dashed line indicates the fumigation day.

were included as dependent variables in different generalized linear mixed models (GLMMs) using the GLIMIX macro of SAS (SAS Institute, 2004), whereas the period relative to aerial spraying and the proportion of treated area within a 6 km radius of the colonies were included as independent factors and covariates, respectively. We also included laying date as an additional predictor in the analyses to control for potential temporal changes of the breeding parameters considered. As pairs of the same colony could be non-independent, we included colony identity as a random factor in all these analyses. In sex-ratio analysis, we included the number of males as the response variable and brood size as the binomial denominator. Other variables like clutch size and hatching success were not analyzed as all clutches were completed and hatched before the aerial spraying so that the pesticide application could not affect these breeding parameters.

We also used GLMMs to study the effects of the treatment on the survival of adults in the following year. In this analysis, we considered a binomial error and a logit link function to study whether the proportion of treated area within a 10 km radius of the colonies affected the recovery rates of marked individual adult lesser kestrels. As an additional predictor, we included a variable that we defined as capture effectiveness (the ratio between the number of captured birds and the total number of individuals at a colony). We did so because the recovery rates in the following year (2004) were expected to be affected by that parameter. Once again, we included the colony as a random factor. We developed separate models for males and females as a sex-biased susceptibility to malathion may occur (Maul & Farris, 2004).

Finally, we studied whether the pesticide treatment influenced the population size measured as the number of pairs breeding at the colonies. For this purpose, we calculated the increment in colony size (CS) between 2003 and 2004 ($\Delta CS = CS_{2004} - CS_{2003}$; this variable was normally distributed, Shapiro–Wilk's *W*-test; $P > 0.05$) and performed a linear regression analysis with the proportion of treated area within a 10 km radius of the colonies as an independent variable.

Results

The proportion of treated area within a 6 km radius of the colony had no effect on chick tarsus length. The period showed a significant effect, indicating that those young that were raised before the treatment grew larger tarsi. However, this temporal effect was not merely due to laying date. The interaction between period and proportion of the treated area significantly affected tarsus length (Table 1). This

Table 1 GLMMs for chick tarsus length and pectoral thickness (normal error and identity link function), number of fledged young (Poisson error and log-link function) and sex ratio at fledgling (binomial error and logit link function) in relation to period (before and after the fumigation treatment, entered as a factor), laying date and proportion of treated area within a 6 km radius of the colonies (entered as covariates)

	d.f.	*F*	*P*
Tarsus length			
Period	1, 90	6.58	0.012
Laying date	1, 90	0.49	0.486
Proportion of treated area[a]	1, 90	0.11	0.739
Period × proportion of treated area	1, 90	9.77	0.002
Covariance parameter estimates:			
	Estimate ± SE	*Z*	*P*
Colony identity	0	–	–
Pectoral thickness			
Period	1, 94	0.03	0.867
Laying date	1, 94	0.81	0.370
Proportion of treated area[a]	1, 94	0.22	0.639
Period × proportion of treated area	1, 94	6.77	0.011
Covariance parameter estimates:			
	Estimate ± SE	*Z*	*P*
Colony identity	0.279 ± 0.163	1.72	0.043
Number of fledglings			
Period	1, 110	1.16	0.284
Laying date	1, 110	6.81	0.010
Proportion of treated area[a]	1, 110	0.14	0.710
Period × proportion of treated area	1, 110	1.10	0.297
Covariance parameter estimates:			
	Estimate ± SE	*Z*	*P*
Colony identity	0.002 ± 0.006	0.34	0.368
Sex ratio at fledgling			
Period	1, 90	0.98	0.326
Laying date	1, 90	0.47	0.495
Proportion of treated area[a]	1, 90	1.38	0.242
Period × proportion of treated area	1, 90	2.09	0.151
Covariance parameter estimates:			
	Estimate ± SE	*Z*	*P*
Colony identity	0.325 ± 0.244	1.33	0.091

[a] Proportion of treated area is arc-sin transformed.
Colony identity was included as random factor.
GLMM, generalized linear mixed models; SE, standard error.

interaction is meaningful because fledgling tarsus length was independent of the proportion of sprayed area before the treatment, whereas there was a negative relationship between tarsus length and the proportion of sprayed area after the malathion application (Fig. 2a). A similar analysis, with pectoral thickness as a dependent variable, gave an identical result for the interaction between the period and proportion of treated area. The proportion of treated area only affected negatively fledgling pectoral thickness in broods remaining in the nests after spraying, but not in broods fledging before the treatment (Fig. 2b). In this case, neither the period nor the laying date had a significant effect on pectoral thickness (Table 1). None of the analyzed variables affected sex ratio at fledging (Table 1).

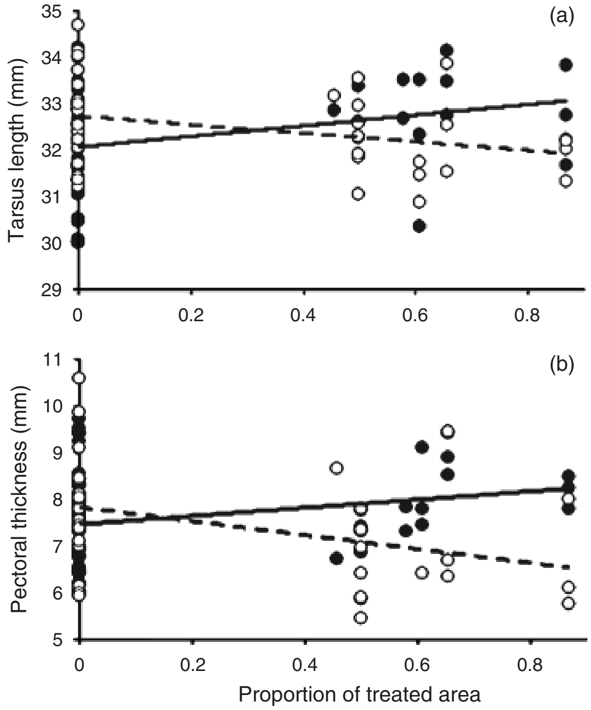

Figure 2 Fledgling tarsus length (a) and pectoral thickness (b) in relation to the proportion of treated area within a 6 km radius of the colony in the period before (filled circles, solid regression lines) and after (open circles, dashed regression lines) the fumigation treatment.

All chicks of three nests, in two different colonies located within the treated area, were found dead in their respective nests within 2 days following malathion treatment. Although we did not measure malathion residues or cholinesterase inhibition in the carcasses, these isolated cases seemed to be a direct consequence of the pesticide because all these chicks were healthy some days before their demise. Nevertheless, neither the proportion of treated area nor its interaction with period had any significant statistical effect on the number of fledged young, which was only negatively affected by laying date (Table 1).

The recovery rate of adult males in the year following the malathion treatment showed a negative relationship with the proportion of treated area and the capture effectiveness in 2004. However, no significant effect was found for adult females (Table 2; Fig. 3). We tested whether the slope of the association between recovering rates and proportion of treated area differed between sexes. For this purpose, we analyzed data for male and female survival all together, including as predictors capture effectiveness and the interaction between the sex and proportion of the treated area. After controlling for the effects of colony identity (included as random factor: $Z = 0.1$; $P = 0.460$), we found that recovering rates increased significantly with capture effectiveness ($F_{1,110} = 5.46$; $P = 0.021$) and the interaction between sex and proportion of treated area was also significant ($F_{1,110} = 4.53$; $P = 0.036$). This interaction indicates that

Table 2 GLMMs with binomial error and logit link function for recovery rates of male and female lesser kestrels *Falco naumanni* in relation with the proportion of treated area within a 10 km radius of the colonies and capture effectiveness

	Estimate[a]	± SE[a]	d.f.	*F*	*P*
Males model					
Intercept	−3.087	1.606			
Proportion of treated area[b]	−3.438	1.483	1, 50	5.38	0.025
Capture effectiveness	4.458	2.311	1, 50	3.72	0.060
Covariance parameter estimates:					
	Estimate ± SE	*Z*	*P*		
Colony identity	0	–	–		
Females model					
Intercept	−0.308	0.266			
Proportion of treated area[b]			1, 59	0.01	0.942
Capture effectiveness			1, 59	2.17	0.146
Covariance parameter estimates:					
	Estimate ± SE	*Z*	*P*		
Colony identity	0	–	–		

[a] Only given for variables which entered the model.

[b] Proportion of treated area is arc-sin transformed.

Colony identity was included as random factor.

GLMM, generalized linear mixed models; SE, standard error.

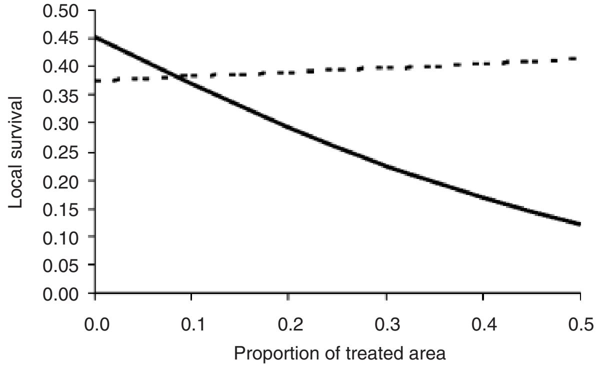

Figure 3 Local survival of adult male (solid line plot) and female (dashed line plot) lesser kestrels *Falco naumanni* predicted from a logistic regression in relation to the proportion of treated area within a 10 km radius of the colony.

the slope of the association between survival and proportion of treated area differed between sexes (Fig. 3). Finally, there was no relationship between the proportion of treated area within a 10 km radius of the colony and the change in colony size from 2003 to 2004 ($F_{1,11} = 0.847$; $P = 0.377$).

Discussion

We found that malathion aerial application affects lesser kestrel breeding performance. The pesticide treatment reduced fledgling quality (size and condition) but no effect was detected on the number of fledged chicks. The aerial spraying could have influenced fledgling quality negatively through direct non-lethal toxic effects (Grue & Shipley, 1981; Day *et al.*, 1995) or by reducing food availability (mainly Orthoptera) along the nestling growth period (Howe *et al.*, 1996; Boatman *et al.*, 2004; Morris *et al.*, 2005); a combination of both factors may have contributed to the observed effects on breeding performance.

We did not study the extent of nestling exposure to the insecticide but the presence of sprayed fields within the main foraging areas of some colonies suggests the possibility of negative effects of malathion application due to the consumption of contaminated prey (Story & Cox, 2001). In our study area, many grasshoppers were found dying or with reduced mobility in the malathion-treated fields. These signs are characteristic of OP poisoning (J. M. Aparicio, unpubl. data). These OP-exposed grasshoppers are easier to capture and adults could have preferentially fed on them as well as delivered these insects to the chicks (Hunt *et al.*, 1991, 1992). Therefore, a secondary poisoning could have occurred and may explain the reduced chick size and body condition in those colonies with a higher proportion of treated area in their surroundings. Similar sub-lethal OP effects on chicks have been reported previously for other bird species (Grue & Shipley, 1981). Although malathion has a short environmental half-life and is rapidly eliminated, its pharmacological effects (i.e. cholinesterase inhibition) can accumulate if fledglings have been consuming prey-containing residues over time. In addition, 2 days after aerial spraying, we found that all of the chicks were dead in three nests from two colonies within the treated area. This may have been caused by acute poisoning. Death by starvation is unlikely, as no signs of malnutrition were observed the previous week, and all chicks were found dead inside the nest cavity, whereas hungry chicks usually abandon their own nest to look for adoptive parents (Tella *et al.*, 1997).

For insectivorous species, like the lesser kestrel, indirect pesticide effects on breeding performance can arise as a result of a decrease in prey availability following insecticide application (Howe *et al.*, 1996; Boatman *et al.*, 2004; Morris *et al.*, 2005). Several studies have found that food shortage affects different breeding performance parameters (Tremblay *et al.*, 2003) and this could account for the reduced chick tarsus length and pectoral thickness in the affected colonies. Malathion could also affect adult mortality and behavior, which may in turn affect the chick quality if prey deliveries decrease. The lower adult male recovery rates reported in the treated areas suggest that enhanced male mortality could have reduced chick-feeding rates. Furthermore, adult birds suffering sub-lethal OP effects can be less efficient foragers (Millikin & Smith, 1990), potentially affecting chick growth and body condition (Grue, Powell & McChensney, 1982; Meyers, Cummings & Bennett, 1990).

The lack of effect of malathion on the number of fledged chicks suggests that the effects of malathion treatment were not, in this instance, sufficient to affect this reproductive parameter. The rapid transformation of OP insecticides inside the organism allows those individuals that recover from the acute phase to survive (Story & Cox, 2001). On the other hand, the decrease in prey availability following the malathion spraying could have been strong enough to affect chick growth and condition but not to reduce the number of chicks. As found in other studies, when there is an initial high quantity of prey, they still remain abundant after the pesticide treatment even if their numbers are reduced (Pascual, 1994; Howe *et al.*, 1996). The decrease in prey availability may not have reached the point of limiting brood size, but it may have remained well below the threshold necessary to raise chicks in optimum body condition (Tremblay *et al.*, 2003).

The effects of malathion application on adult survival were evident for males but not for females, suggesting a sex-biased mortality. Colony change in adult lesser kestrels is rare, independent of sex (Aparicio, 1997) or female-biased (Serrano *et al.*, 2001) so that the lower recovery rate for males in relation with treatment is unlikely to be due to sex-biased dispersal. The observed effect could be explained by differences between sexes in the OP sensitivity due to gender differences in the basal levels of cholinesterase activities, metabolic rates and hormones (Rattner & Franson, 1984; Moser, 2000; Wang, Schorer-Apelbaum & Weinstock, 2001; Maul & Farris, 2004). Mortality from acute poisoning could explain the observed male mortality, although other sub-lethal malathion effects might have also played a role, including lower foraging capacity (Story & Cox, 2001), immunotoxicity (Galloway & Handy, 2003), behavioral disturbances (Walker, 2003) and higher predation rates (Galindo *et al.*, 1985; Buerger *et al.*, 1991; Wilson *et al.*, 2001). Sexual differences in survival could also be caused by loss of body condition, which could be equal between sexes, but it could have caused higher mortality in males because adult male survival is more dependent on body condition than female survival in the lesser kestrel (Aparicio & Cordero, 2001).

The change in colony size from 2003 to 2004 was unaffected by the proportion of treated area. Either the higher adult male mortality was insufficient to affect colony size and/or the arrival of immigrants may have masked such mortality. In recent years, the lesser kestrel has shown a positive population trend in our study area and the growth in some colonies is often limited by nest-site availability. Consequently, nest sites released by dead individuals could have been readily occupied by floaters, suggesting that density-dependent compensatory responses may, at least partially, have shaped the population-level pesticide effects (Forbes & Calow, 2002; Schmidt, 2004).

Population consequences and implications for conservation

The lesser kestrel is a globally threatened species that has become rare across much of its range (Peet & Gallo-Orsi, 2000). This appears to be primarily due to a decline in prey populations, linked with land-use changes (Tella *et al.*, 1998; Tella & Forero, 2000). Although the effects of biocide applications on prey availability have been considered previously (Negro *et al.*, 1993; Ursúa, Serrano & Tella, 2005), less attention has been paid to the possible direct consequences of pesticide applications (Negro *et al.*, 1993). Our results show that pesticide applications could be more important in the pseudo-steppe bird population decline than thought previously.

Reduced chick quality in malathion-treated areas could reduce survival during the first year of life, as survival is closely correlated with pectoral thickness (Aparicio & Cordero, 2001). The lesser kestrel is highly philopatric and reduced offspring survival could lead to population declines if adult mortality exceeds juvenile recruitment rates (Hiraldo *et al.*, 1996). In addition, sex-biased effects can intensify the consequences of bird mortality, especially for monogamous species like the lesser kestrel, when the demand for the affected sex exceeds its renewal (Ferrer & Hiraldo, 1992). As sex-biased sensitivity to malathion is one possible explanation of our results, this indicates the need for considering the most sensitive sex in future hazard assessment strategies (Maul & Farris, 2004). While the absence of effect on population size appears to diminish the importance of the fumigation treatment, it should be remembered that population recovery after a pesticide-induced mortality depends on the frequency of pesticide applications, the remaining population size and the immigration and recruitment rates (Story & Cox, 2001; Forbes & Calow, 2002).

Management measures should be focused on keeping adequate non-treated buffer areas around the colony center to avoid reducing prey populations and minimize the impact of secondary poisoning (Burn, 2003). These areas should include the main foraging places throughout the breeding season (Tella *et al.*, 1998; Bonal & Aparicio, 2001; Franco *et al.*, 2004). Our results and available data on the species' home ranges suggest that pesticide applications should not take place within a buffer area of a 10-km radius from the colonies, although this may need to be adjusted in some situations as foraging areas can vary depending on colony size and local prey abundance.

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References

Aparicio, J.M. (1997). Cost and benefits of surplus offspring in the lesser kestrel (*Falco naumanni*). *Behav. Ecol. Sociobiol.* **41**, 129–137.

Aparicio, J.M. & Bonal, R. (2002). Effects of food supplementation and habitat selection on timing of lesser kestrel breeding. *Ecology* **83**, 873–877.

Aparicio, J.M. & Cordero, P.J. (2001). The effects of the minimum threshold condition for breeding on offspring sex-ratio adjustment in the lesser kestrel. *Evolution* **55**, 1188–1197.

Blus, L.J. & Henny, C.J. (1997). Field studies on pesticides and birds: unexpected and unique relations. *Ecol. Appl.* **7**, 1125–1132.

Boatman, N.D., Brickle, N.W., Hart, J.D., Milsom, T.P., Morris, A.J., Murray, A.W.A., Murray, K.A. & Robertson, P.A. (2004). Evidence for the indirect effects of pesticides on farmland birds. *Ibis* **146**, 131–143.

Bonal, R. & Aparicio, J.M. (2001). Utilización del hábitat por el cernícalo primilla (*Falco naumanni*) en La Mancha. In *Biología y conservación del cernícalo primilla*: 53–63. Garces, J.F. & Corroto, M. (Eds). Madrid: Consejería de Medio Ambiente de la Comunidad de Madrid.

Buerger, T.T., Kendall, R.J., Mueller, B.S., DeVos, T. & Williams, B.A. (1991). Effects of methyl parathion on northern bobwhite survivability. *Environ. Toxicol. Chem.* **10**, 527–532.

Burn, A. (2003). Pesticide buffer zones for the protection of wildlife. *Pest Mgmt. Sci.* **59**, 583–590.

Day, B.L., Walser, M.M., Sharma, J.M. & Andersen, D.E. (1995). Immunopathology of 8-week-old ring-necked pheasants (*Phasianus colchicus*) exposed to malathion. *Environ. Toxicol. Chem.* **14**, 1719–1726.

Donázar, J.A., Negro, J.J. & Hiraldo, F. (1993). Foraging habitat selection, land-use changes and population decline in the lesser kestrel *Falco naumanni*. *J. Appl. Ecol.* **30**, 515–522.

Ferrer, M. & Hiraldo, F. (1992). Man-induced sex-biased mortality in the Spanish imperial eagle. *Biol. Conserv.* **60**, 57–60.

Fleischli, M.A., Franson, J.C., Thomas, N.J., Finley, D.L. & Riley, W. (2004). Avian mortality events in the United States caused by anticholinesterase pesticides: a retrospective summary of national wildlife health center records from 1980 to 2000. *Arch. Environ. Contam. Toxicol.* **46**, 542–550.

Forbes, V.E. & Calow, P. (2002). Population growth rate as a basis for ecological risk assessment of toxic chemicals. *Philos. Trans. R. Soc. Lond. B* **357**, 1299–1306.

Franco, A.M.A., Catre, I., Sutherland, W.J. & Palmeirim, J.M. (2004). Do different habitat preference survey methods produce the same conservation recommendations for lesser kestrels? *Anim. Conserv.* **7**, 291–300.

Franco, A.M.A. & Sutherland, W.J. (2004). Modelling the foraging habitat selection of lesser kestrels: conservation implications of European agricultural policies. *Biol. Conserv.* **120**, 63–74.

Galindo, J.C., Kendall, R.J., Driver, C.J. & Lacher, T.E. (1985). The effect of methyl parathion on susceptibility of bobwhite quail *Colinus virginianus* to domestic cat predation. *Behav. Neural Biol.* **43**, 21–36.

Galloway, T. & Handy, R. (2003). Immunotoxicity of organophosphorus insecticides. *Ecotoxicology* **12**, 345–363.

Goldstein, M.I., Lacher, T.E., Woodbridge, B., Bechard, M.J., Canavelli, S.B., Zaccagnini, M.E., Cobb, G.P., Scollon, E.J., Tribolet, R. & Hooper, M.J. (1999). Monocrotophos-induced mass mortality of Swainson's hawks in Argentina, 1995–1996. *Ecotoxicology* **8**, 201–214.

Gosler, A.G. & Harper, D.G.C. (2000). Assessing the heritability of body condition in birds: a challenge exemplified by the great tit *Parus major* L. (Aves). *Biol. J. Linnean Soc.* **71**, 103–117.

Grue, C.E., Powell, V.N. & McChensney, M.J. (1982). Care of nestlings by wild female starlings exposed to an organophosphate pesticide. *J. Appl. Ecol.* **19**, 327–335.

Grue, C.E. & Shipley, B.K. (1981). Interpreting population estimates of birds following pesticide applications – behaviour of male starlings exposed to an organophosphate pesticide. *Stud. Avian Biol.* **6**, 292–296.

Hansen, L.J. & Johnson, M.L. (1999). Conservation and toxicology: integrating the disciplines. *Conserv. Biol.* **13**, 1225–1227.

Hiraldo, F., Negro, J.J., Donázar, J.A. & Gaona, P. (1996). A demographic model for a population of the endangered lesser kestrel in southern Spain. *J. Appl. Ecol.* **33**, 1085–1093.

Hooper, M.J., Detrich, P.J., Weisskopf, C.P. & Wilson, B.W. (1989). Organo-phosphorus insecticide exposure in hawks inhabiting orchards during winter dormant-spraying. *Bull. Environ. Contam. Toxicol.* **42**, 651–659.

Howe, F.P., Knight, R.L., McEwen, L.C. & George, T.L. (1996). Direct and indirect effects of insecticide applications on growth and survival of nestling passerines. *Ecol. Appl.* **6**, 1314–1324.

Hunt, K.A., Bird, D.M., Mineau, P. & Shutt, L. (1991). Secondary poisoning hazard of fenthion to American kestrels. *Arch. Environ. Contam. Toxicol.* **21**, 84–90.

Hunt, K.A., Bird, D.M., Mineau, P. & Shutt, L. (1992). Selective predation of organophosphate-exposed prey by American kestrels. *Anim. Behav.* **43**, 971–976.

Keith, J.O. & Bruggers, R.L. (1998). Review of hazards to raptors from pest control in Sahelian Africa. *J. Raptor Res.* **32**, 151–158.

Maul, J.D. & Farris, J.L. (2004). The effect of sex on avian plasma cholinesterase enzyme activity: a potential source of variation in an avian plasma biomarker endpoint. *Arch. Environ. Contam. Toxicol.* **47**, 253–258.

Mendelssonh, H. & Paz, U. (1977). Mass mortality of birds of prey caused by Azodrin, an organophosphorus insecticide. *Biol. Conserv.* **11**, 163–170.

Meyers, S.M., Cummings, J.L. & Bennett, R.S. (1990). Effects of methyl parathion on red-winged blackbird (*Agelaius phoeniceus*) incubation behaviour and nesting success. *Environ. Toxicol. Chem.* **9**, 807–813.

Millikin, R.L. & Smith, J.N.M. (1990). Sublethal effects of fenitrothion on forest passerines. *J. Appl. Ecol.* **27**, 983–1000.

Mineau, P., Downes, C.M., Kirk, D.A., Bayne, E. & Csizy, M. (2005). Patterns of bird species abundance in relation to granular insecticide use in the Canadian prairies. *Ecoscience* **12**, 267–278.

Mineau, P., Fletcher, M.R., Glaser, L.C., Thomas, N.J., Brassard, C., Wilson, L.K., Elliott, J.E., Lyon, L.A., Henny, C.J., Bollinger, T. & Porter, S.L. (1999). Poisoning of raptors with organophosphorus and carbamate pesticides with emphasis on Canada, US and UK. *J. Raptor Res.* **33**, 1–37.

Morris, A.J., Wilson, J.D., Whittingham, M.J. & Bradbury, R.B. (2005). Indirect effects of pesticides on breeding yellowhammer (*Emberiza citrinella*). *Agric. Ecosys. Environ.* **106**, 1–16.

Moser, V.C. (2000). Dose-response and time-course of neurobehavioral changes following oral chlorpyrifos in rats of different ages. *Neurotoxicol. Teratol.* **22**, 713–723.

Negro, J.J., Donázar, J.A., Hiraldo, F., Hernández, L.M. & Fernández, M.A. (1993). Organochlorine and heavy-metal contamination in nonviable eggs and its relation to breeding success in a Spanish population of lesser kestrels (*Falco naumanni*). *Environ. Poll.* **82**, 201–205.

Pascual, J.A. (1994). No effects of a forest spraying of malathion on breeding blue tits (*Parus caereuleus*). *Environ. Toxicol. Chem.* **13**, 1127–1131.

Peet, N.B. & Gallo-Orsi, U. (2000). *Action plan for the lesser kestrel Falco naumanni*. Cambridge: Council of Europe and BirdLife International.

Rattner, B.A. & Franson, J.C. (1984). Methyl parathion and fenvalerate toxicity in American kestrels – acute physiological-responses and effects of cold. *Can. J. Physiol. Pharmacol.* **62**, 787–792.

Sanchez-Hernandez, J.C. (2001). Wildlife exposure to organophosphorus insecticides. *Rev. Environ. Contam. Toxicol.* **172**, 21–63.

SAS Institute (2004). *SAS/STAT 9.1 user's guide*. Cary: SAS Institute Inc.

Schafer, E.W. & Brunton, R.B. (1979). Indicator species for toxicity determinations: is the technique usable in test method development? In *Vertebrate pest control and management*: 158–168. Beck, J.R. (Ed.). Philadelphia: American Society of Testing and Materials.

Schmidt, B.R. (2004). Pesticides, mortality and population growth rate. *Trends Ecol. Evol.* **19**, 459–450.

Serrano, D., Tella, J.L., Forero, M.G. & Donázar, J.A. (2001). Factors affecting breeding dispersal in the facultatively colonial lesser kestrel: individual experience vs. conspecific cues. *J. Anim. Ecol.* **70**, 568–578.

Sih, A., Bell, A.M. & Kerby, J.L. (2004). Two stressors are far deadlier than one. *Trends Ecol. Evol.* **19**, 274–276.

Story, P. & Cox, M. (2001). Review of the effects of organophosphorus and carbamate insecticides onvertebrates. Are there implications for locust management in Australia? *Wildl. Res.* **28**, 179–193.

Tella, J.L. & Forero, M.G. (2000). Farmland habitat selection of wintering lesser kestrels in a Spanish pseudosteppe: implications for conservation strategies. *Biodivers. Conserv.* **9**, 433–441.

Tella, J.L., Forero, M.G., Donázar, J.A., Negro, J.J. & Hiraldo, F. (1997). Non-adaptive adoptions of nestlings in the colonial lesser kestrel: proximate causes and fitness consequences. *Behav. Ecol. Sociobiol.* **40**, 253–260.

Tella, J.L., Forero, M.G., Hiraldo, F. & Donázar, J.A. (1998). Conflicts between lesser kestrel conservation and European Agricultural Policies as identified by habitat use analysis. *Conserv. Biol.* **12**, 593–604.

Tiebout, H.M. & Brugger, K.E. (1995). Ecological risk assessment of pesticides for terrestrial vertebrates: evaluation and application of the U.S. environmental protection agency's quotient model. *Conserv. Biol.* **9**, 1605–1618.

Tremblay, I., Thomas, D.W., Lambrechts, M.M., Blondel, J. & Perret, P. (2003). Variation in blue tit breeding performance across gradients in habitat richness. *Ecology* **84**, 3033–3043.

Ursúa, E., Serrano, D. & Tella, J.L. (2005). Does land irrigation actually reduce foraging habitat for breeding lesser kestrels? The role of crop types. *Biol. Conserv.* **122**, 643–648.

Walker, C.H. (2003). Neurotoxic pesticides and behavioural effects upon birds. *Ecotoxicology* **12**, 307–316.

Wang, R., Schorer-Apelbaum, D. & Weinstock, M. (2001). Testosterone mediates sex difference in hypothermia and cholinesterase inhibition by rivastigmine. *Eur. J. Pharmacol.* **433**, 73–79.

Weimeyer, S.N. & Sparling, D.W. (1991). Acute toxicity of four anticholinesterase insecticides to American kestrels, Eastern screech owls and Northern bobwhites. *Environ. Toxicol. Chem.* **10**, 1139–1148.

Wilson, L., Martin, P.A., Elliott, J.E., Mineau, P. & Cheng, K.M. (2001). Exposure of California quail to organophosphorus insecticides in apple orchards in the Okanagan Valley, British Columbia. *Ecotoxicology* **10**, 70–90.