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Author: Stuber, Matthew J.

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EXAMINATION OF PESTICIDE EXPOSURE IN BURROWING OWLS NESTING IN AGRICULTURAL AND NONAGRICULTURAL AREAS IN THE MORLEY NELSON SNAKE RIVER BIRDS OF PREY NATIONAL CONSERVATION AREA, IDAHO

MATTHEW J. STUBER[1]
Department of Biological Sciences and Raptor Research Center, Boise State University, Boise, ID, 83725 USA

MICHAEL J. HOOPER[2]
Environmental Toxicology Department and The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX, 79409 USA

JAMES R. BELTHOFF
Department of Biological Sciences and Raptor Research Center, Boise State University, Boise, ID, 83725 USA

ABSTRACT.—Western Burrowing Owls (*Athene cunicularia hypugaea*) frequently nest near agricultural lands. In southwestern Idaho, greater population density in agricultural landscapes appears to be driven in part by reliable and abundant prey populations. However, these potential benefits may be offset if agricultural land use increases pesticide exposure, especially during the sensitive reproduction period. Thus, we investigated the extent to which Burrowing Owls nesting near croplands within the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA), Idaho, were exposed to organophosphate (OP), carbamate (CB), and/or organochlorine (OC) pesticides. We examined plasma cholinesterase activity, OP and CB residues in foot-wash samples, and OCs in soils and whole egg contents collected from owls and their nests along a distance gradient from agricultural fields. We further measured eggshell thickness to assess potential for thinning from OC exposure. There was no inhibition of cholinesterase activities in adult or nestling owls near agriculture, and foot-wash samples from adults tested negative for OP and CB pesticides. The OC p,p′-DDE, a metabolite of DDT, occurred in eggs at 27 of 58 nests, but there was some evidence that concentrations increased with increasing distance from agriculture. Concentrations of p,p′-DDE in eggs were relatively low compared to harmful levels in other avian species, were not correlated with eggshell thickness, and did not appear to reduce nesting success. Neither DDT, its metabolites, nor any other OCs were detected in soil samples from local owl breeding areas. These results indicate that p,p′-DDE, organophosphate, and carbamate exposure were not causing toxicity or reproductive impairment of Burrowing Owls in the NCA during our study years.

KEY WORDS: *Burrowing Owl*; Athene cunicularia; *cholinesterase*; *DDT*; *eggshell thickness*; *Idaho*; *insecticide*; *pesticide*; *p,p′-DDE*.

ANÁLISIS DE LA EXPOSICIÓN A PESTICIDAS EN INDIVIDUOS DE *ATHENE CUNICULARIA* NIDIFICANDO EN ÁREAS AGRÍCOLAS Y NO AGRÍCOLAS EN EL ÁREA DE CONSERVACIÓN NACIONAL DE AVES DE PRESA MORLEY NELSON SNAKE RIVER, IDAHO

RESUMEN.—*Athene cunicularia hypugaea* nidifica frecuentemente cerca de ambientes agrícolas. En el suroeste de Idaho, una mayor densidad poblacional en paisajes agrícolas parece estar impulsada en parte por poblaciones de presas abundantes y fácilmente accesibles. Sin embargo, estos beneficios potenciales pueden verse contrarestados si en ambientes agrícolas aumenta la exposición a pesticidas, especialmente durante el periodo reproductivo. Por ello, investigamos la magnitud en la que los individuos de *A c. hypugaea* que nidifican cerca de cultivos dentro del Área de Conservación Nacional de Aves de Presa Morley Nelson Snake

[1] Present address: US Fish and Wildlife Service, Pacific Region, 911 NE 11th Avenue, Portland, OR 97232 USA; email address: matthew_stuber@fws.gov
[2] Present address: Columbia Environmental Research Center, US Geological Survey, Columbia, MO 65201 USA.

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River fueron expuestos a pesticidas organofosfatados (OP), carbamatos (CB), y/o organoclorados. Analizamos la actividad colinesterasa, los residuos OP y OB en muestras de lavados de patas, y OCs en suelos y en contenidos completos de huevos recolectados de los búhos y sus nidos a lo largo de un gradiente de distancia desde los campos agrícolas. Medimos el grosor de la cáscara del huevo para evaluar el potencial de adelgazamiento de la cáscara debido a la exposición con OCs. No hubo una inhibición de las actividades de la colinesterasa en búhos adultos o polluelos cerca de sembrados, y las muestras de lavados de patas dieron negativo para pesticidas OP y CB. El OC p,p′-DDE, un metabolito del DDT, se registró en huevos de 27 de 58 nidos, pero hubo evidencia de que las concentraciones aumentaron en función del aumento de la distancia a los ambientes agrícolas. Las concentraciones de p,p′-DDE en los huevos fueron relativamente bajas comparadas con los niveles nocivos en otras especies de aves, no estuvieron correlacionadas con el espesor de la cáscara del huevo y no parecieron disminuir el éxito reproductivo. No se detectó DDT, sus metabolitos, ni otros OCs en muestras de suelo provenientes de áreas de cría locales de los búhos. Estos resultados indican que la exposición a p,p′-DDE, organofosfatos y carbamato no estuvieron causando toxicidad o disfunciones reproductivas en *A. cunicularia* en el Área de Conservación Nacional durante nuestro periodo de estudio.

[Traducción del equipo editorial]

Few wildlife species benefit from large-scale conversion of their natural habitat to agricultural lands (Carlson 1985). In fact, many avian species show population decline near converted lands. Among the factors underlying avian species declines in agricultural areas is pesticide exposure, which can cause reproductive impairment through decreased egg laying (Stromborg 1986, Bennett et al. 1991, Halldin et al. 2003) and decreased eggshell thickness (Ratcliffe 1967, Hickey and Anderson 1968, Heath et al. 1969, Cooke 1973, Blus et al. 1974, Blus 1982, Fry 1995).

At the time of our study (2007 and 2008), organophosphates (OP) and carbamates (CB) were high-use classes of pesticides for agriculture. OP and CB compounds inhibit the cholinesterase (ChE) enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). AChE, found in the brain, peripheral nerves and neuroeffector organs, is an enzyme essential for terminating neuronal transmission. Inhibition of AChE leads to lethal and sublethal effects in birds, and inhibition of its activity in the brain is diagnostic of mortality and sublethal OP and CB exposure. AChE and BChE inhibition in peripheral blood plasma can be an indication of OP or CB exposure. Birds, as a class, tend to be particularly susceptible to negative effects of OP and CB compounds (Mineau and Whiteside 2006, Mitra et al. 2011).

Though rarely encountered in agriculture since their era of heavy use in the 1960s and 1970s and regulatory removal shortly thereafter, residues of some persistent organochlorine (OC) pesticides remain present in the environment. OC compounds, including the infamous pesticide DDT and its metabolite, p,p′-DDE, can bioaccumulate, and cause harm to apex predators such as raptors when concentrations are high (Hickey and Anderson 1968, Porter and Wiemeyer 1969), and can still be detected in the environment and in birds in some areas (Gervais et al. 2000, Yates et al. 2009, Mora et al. 2016). By comparison, OP and CB compounds break down relatively quickly and do not persist in the environment in the same manner as OCs.

Western Burrowing Owls (*Athene cunicularia hypugaea*; hereafter Burrowing Owls) occupy grasslands, steppes, deserts, and prairies throughout western North America. They can be associated with agriculture and nest in higher densities near agriculture in some areas (Rich 1986, Conway et al. 2006, Moulton et al. 2006, Bartok and Conway 2010). Although this may be related to higher burrow availability or decreased predation, prey consumption can also be higher in agricultural areas (Moulton et al. 2006). However, potential foraging advantages in and around agricultural areas may be offset by any increases in risk of exposure to or poisoning by agricultural pesticides. Two factors that can increase risk of raptor poisonings are (1) inhabiting agricultural areas and (2) insectivory (Mineau et al. 1999), both of which are life history characteristics of Burrowing Owls (Poulin et al. 2011). Moreover, the Commission for Environmental Cooperation's North American Conservation Action Plan for Burrowing Owls (Commission for Environmental Cooperation 2005) lists pesticides as an important potential driver of population declines that deserves study.

We studied Burrowing Owls in the Morley Nelson Snake River Birds of Prey National Conservation

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Area (NCA) in southwestern Idaho to assess potential exposure to OP, CB, and OC pesticides. Burrowing Owls in the NCA (Belthoff and King 2002, Moulton et al. 2005, 2006) and elsewhere in southern Idaho (Gleason 1978, Rich 1986) often nest near croplands, but they also nest in shrub steppe and disturbed grasslands where no farming occurs. This sets the stage for study of pesticide exposure at different distances from croplands, which may result in differential exposure. We hypothesized that exposure would vary as a function of proximity to agricultural operations, because breeding owls typically focus movements to within 600 m of their nest burrow (Haug and Oliphant 1990), although they occasionally move longer distances (Gervais et al. 2003, Marsh et al. 2014). We assessed exposure through collections of footwash samples, blood samples, and eggs from owls and soil samples from areas near nests. We also measured egg characteristics to assess the potential for shell thinning from OC exposure. We predicted that owls nearest to agricultural areas would exhibit decreased cholinesterase enzyme (ChE) activity, increased OP and/or CB residues on their body surfaces, and potentially reduced eggshell thickness from exposure to OC metabolites that may have persisted in their environment or bioaccumulated through prey consumption.

METHODS

The 195,325-ha NCA, located in Ada, Canyon, Elmore, and Owyhee Counties, Idaho, was established in 1993 by Congress (Public Law 103-64) for the conservation, protection, and enhancement of raptor populations and habitats (Sharpe and van Horne 1998) and contains one of the densest populations of nesting birds of prey in North America (US Department of Interior [USDI] 1996). The NCA was historically dominated by shrub steppe (Hironaka et al. 1983), but human disturbances and fires have converted portions of the area to disturbed grassland (USDI 2008), which is dominated by invasive annuals such as cheatgrass (*Bromus tectorum*) and tumble mustard (*Sisymbrium altissimum*). The NCA contains only a small proportion of irrigated croplands within its boundaries (5–6% of the land cover; USDI 1996), though there is extensive agriculture in the region and in areas adjacent to the NCA within private lands. During our study, one principal crop in these fields was alfalfa (*Medicago sativa*), which was typically intended for livestock feed. Alfalfa is third on the list of crops in the US associated with avian mortality because of total planted area in the US and the pesticides commonly applied to it (Mineau and Whiteside 2006). In the NCA, plant communities in areas adjacent to agricultural fields were reasonably similar to those in nonagricultural areas, and Burrowing Owls nested in these rather than directly in the agricultural fields (Moulton et al. 2006).

Because Burrowing Owls typically lay their eggs in underground burrows excavated by fossorial mammals (Poulin et al. 2011), researchers have placed artificial burrow systems (ABS) throughout the NCA to encourage nesting. These ABS generally consist of two or three underground chambers, each with a tunnel to the ground surface, and clustered a few meters apart (Smith and Belthoff 2001, Belthoff and Smith 2003). There were approximately 300 ABS available for Burrowing Owls nesting or roosting within the NCA each year of our study, and these occurred 5–13,300 m from the nearest irrigated cropland. After they return from migration, 60 or more pairs of owls may typically breed (i.e., lay eggs) at the ABS within the NCA each year.

Many of the owls nesting in ABS each year are captured and leg-banded as part of long-term monitoring and research (e.g., Welty et al. 2012, Belthoff et al. 2015, Riding and Belthoff 2015, Wade and Belthoff 2016).

Capture of Owls. During the 2007 and 2008 breeding seasons, we captured adult Burrowing Owls at nests directly from ABS after excavation of the chamber lid, using a small-gauge-wire trap placed at the tunnel entrance that captured an owl as it exited the tunnel, or using a one-way-door trap placed at the nest burrow or a nearby (satellite) burrow entrance accompanied by playback of Burrowing Owl vocalizations (primary call) from a small cassette player placed inside the tunnel of the burrow. Capture of adults generally occurred between 7 d prior to and 3 d after the predicted hatch date for each nest. We captured nestlings by hand directly from artificial burrow tunnels or chambers 20 d and 30 d post-hatch for blood sampling and to assess productivity. We considered a breeding attempt successful if there was $\geq$1 nestling at 30 d and we calculated productivity as the number of nestlings alive 30 d after hatch. All captured owls were banded with USGS aluminum leg bands, weighed, and measured. Sex class of adults was assigned based on presence/absence of a brood patch, which typically occurs only in females.

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Sampling for Pesticide Exposure. The owls we sampled (in the NCA) that occupied agricultural habitat nested near alfalfa fields. Pesticide types and application dates on the study sites were not known, as NCA croplands are located on private property, where pesticide-use reports are required only for restricted-use pesticides. Thus, we performed multi-residue screens for broad suites of the most common and likely OP, CB, and OC compounds applied to agricultural crops in southern Idaho at the time of our study and in the recent past.

Owl feet may accumulate pesticide residues from contaminated surfaces such as perch sites near crops and/or through capture of contaminated prey. Thus, to detect external signs of exposure to OP and/or CB insecticides, we collected foot-wash samples from adult Burrowing Owls using established methods (Gervais et al. 2000), scrubbing owl feet with a toothbrush and rinsing the feet with 50 ml of 100% ethanol. We collected the rinse and any soil, feathers, or hair/fur present on the talons through a funnel and into 50-ml glass vials. The brush and funnel were cleaned with water, rinsed with hexane, and allowed to air dry before reuse. We collected blanks in each area and on each day that sampling occurred by leaving a vial and funnel open to the air during collection of a foot-wash sample. We stored and transported foot-wash samples and blanks on ice until our return to the laboratory at Boise State University each day, at which time we froze samples at –20°C until analysis for OP and CB residues.

We captured adults during both 2007 and 2008 at their nests between 1200–2400 H and collected 0.3–1.0 ml of blood in heparinized capillary tubes following venipuncture of the ulnar vein. We captured and bled nestlings during the daytime (1000–1900 H) at 20 d and 30 d post-hatch to examine temporal changes in pesticide exposure as the nestlings aged. We collected 200–300 µl of blood from each nestling using the same technique as for adults and pooled blood from all nestlings in a nest to generate composite samples that contained approximately 1.5 ml per nest. Each nestling contributed an approximately equal amount to the composite. We were unable to resample nestlings at four nests because the nests either failed before nestlings reached 30 d or contained too few nestlings to produce a sufficient sample for analysis. We stored whole blood on wet ice until processed the same day via centrifugation (3000 rpm, 12 min). Plasma was stored at –80°C until analyzed for ChE activity.

During 2007 and 2008, we collected Burrowing Owl eggs for pesticide analysis and measurement of eggshell thickness. Collection of one egg per nest should not significantly reduce fledgling numbers or the Burrowing Owl population in our study area, as these owls typically lay more eggs than the number of young that ultimately fledge (Riding 2010). We collected eggs before the beginning of incubation, which we assessed by observing adult behavior at nests and by egg temperature (i.e., eggs warm to the touch were considered incubated and not collected). Because we typically trapped the adult female attending a nest after we had collected the egg, we often were not aware of a female's identity at the time of egg collection. This resulted in egg collection in both years for two females and two collections in the same year from one female after she nested in a second burrow. There were seven artificial burrow sites at which we collected an egg in both years but at which different females nested each year. Although sample sizes were small, these collections allowed us to investigate *post hoc* whether contaminant concentrations in eggs were more related to the particular nest site or to the individual female.

We wrapped eggs in aluminum foil, transported them to a Boise State University laboratory, and stored them in a refrigerator (2.5°C) until processing within 1–2 d of collection. In addition to potential differences in pesticide exposure as a function of proximity to agriculture, we were also interested in possible differences that occur with laying order (Custer et al. 2010, Ackerman et al. 2016), as females potentially reduce their body burden of contaminants by sequestering them into eggs. Thus, we noted the number of eggs in the nest at the time of collection and labelled collected eggs as "Early" if they were one of the first three eggs laid in a clutch, and "Late" if they were laid after the fifth egg in a clutch.

We measured length and breadth of each egg using a digital caliper (accurate to 0.01 mm) and determined mass using a digital scale accurate to 0.01 g. Displacement volume (ml) determined by egg submersion in water provided an estimation of egg volume. We cut the eggs along the air cell and poured the contents into pre-cleaned and labelled sample jars and stored them frozen (–20°C) until analysis for OC pesticides.

After removing the egg contents, we measured eggshell thickness after allowing eggshells and their attached membranes to air-dry for a minimum of 6

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mo. Once dry, we broke the shell into at least five fragments that each contained the equator (the egg's largest possible circumference). We used a Starrett digital micrometer (Athol, MA, USA; Model 734MXFL, accurate to 0.001 mm, modified for the concave shape of eggshells with a ball bearing attached to the device's measuring surface, to measure thickness at the equator of each eggshell fragment with its attached membrane. We report the mean of five equatorial eggshell fragment thickness measures for each egg.

We collected soil samples (15–20 g per sample) from 2–10 cm below the soil surface at locations within 200 m of nests where we collected eggs. We collected samples using a small stainless steel trowel that we cleaned between uses with the same method used for the foot-wash equipment. We placed samples in chemically cleaned jars that were stored frozen (–20°C) until analysis for OC pesticides.

Chemical and Biochemical Analyses. Extraction and chemical analysis of OP, CB, and OC pesticides were performed at the California Animal Health and Food Safety Laboratory at University of California, Davis, using a multi-residue screening method that quantified residues of 43 OP, 11 CB, and 22 OC pesticides found in environmental matrices. An initial ethanol:ethyl acetate extraction preceded clean-ups specific to each of the pesticide classes and lipid levels in the specific matrix. Following extraction, OPs were analyzed by gas chromatography (GC) with flame photometric detection, OCs by GC with electron capture detection, and CBs by liquid chromatography followed by post-column hydrolysis and derivatization followed by fluorometric detection. Detailed specifics on these methods are available elsewhere (Holstege et al. 1994). Limits of quantification (LOQs) for OPs were 0.005 μg/g wet wt (or parts per million, ppm), except for azinphos methyl and dioxathion (0.01 and 0.02 ppm, respectively). LOQs for CBs were 0.1 ppm. LOQs for OCs were 0.1 ppm for the DDT family of compounds, and 0.05 ppm for all others except technical chlordane and toxaphene (0.25 and 2 ppm, respectively). Lists of specific pesticide analytes and LOQs are in Stuber (2015).

Ultimately, the only OC detected in sampled eggs was p,p′-DDE. The quantification limit for the p,p′-DDE assay was 0.1 ppm. Thus, any concentration of p,p′-DDE $\geq$ 0.1 ppm was quantified, and those between 0 and 0.1 ppm were considered "trace" concentrations, and estimated to be 0.05 ppm in calculations below. Because we minimized chances for desiccation during processing, p,p′-DDE concentrations were not corrected for desiccation.

Cholinesterase activities were determined at The Institute of Environmental and Human Health at Texas Tech University using the colorometric method in Ellman et al. (1961), modified for use in a 96-well plate reader (Hunt and Hooper 1993). Acetylthiocholine was the substrate, and iso-OMPA (tetraisopropylpyrophosportetraamide), a selective BChE inhibitor, was used to determine AChE activities. BChE was calculated as the difference between total and AChE activity. All ChE activities are expressed as μmoles substrate hydrolyzed/(min*ml plasma) or "units"/ml plasma

Statistical Analysis. We performed all statistical analyses using JMP Pro 13.2 or SAS 9.4 (SAS Institute, Cary, NC, USA) and evaluated tests at an alpha level of 0.05. We report means ± 1 SE unless otherwise noted. For all statistical tests, we analyzed the natural log of distance from agriculture because taking the natural log helped normalize the positively skewed distribution; however, we present figures as a function of untransformed distance throughout.

We used General Linear Models (GLM) for analysis of ChE activity in relation to distance from agriculture for both adults and nestlings. Because ChE levels in birds may differ seasonally, during the different stages of breeding, between sexes, and by time of day (Rattner and Franson 1983, Hill 1989, Rattner and Fairbrother 1991), we also evaluated potential need for Julian date, sex class, and/or time of sample collection to be used as covariates in analyses of relationships between adults and distance to agriculture. We found differences between males and females and a significant correlation between Julian date and AChE, so we included them as covariates where appropriate in final linear models. Because we pooled blood samples at each nest, we were unable to assess the effect of sex on ChE in nestlings. All nestling samples were collected during the daytime, so potential effects of sample time also were not evaluated. We assessed effects of nestling age (20-d and 30-d samples) on ChE in a repeated measures framework using PROC Mixed in SAS and evaluated potential effects of nestling age, distance from agriculture, and their interaction. Finally, according to Hill (1988) and as used in Wilson et al. (1991), we examined AChE and BChE values from adults to locate the number of owls that had values more than 2 SD from a reference population. We used the owls that nested >1500 m from

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agricultural fields as the reference population (calculated mean and SD). We examined a set of owls closest to agriculture (<600 m) and those in an intermediate category (600–1500 m) to identify any samples outside of the reference interval (>2 SD below mean for reference population). Thus, this examination had the potential to uncover even a small number of owl exposures that population-level analyses might have failed to detect.

We used two methods to examine potential relationships between p,p′-DDE concentrations in Burrowing Owl eggs and distance to agriculture. We first used ordinal logistic regression with a categorical dependent variable at three levels (p,p′-DDE concentration: no detectable p,p′-DDE, trace p,p′-DDE, and quantifiable p,p′-DDE). Second, we used a generalized linear model (Poisson distribution, log link) to examine potential effect of distance from agriculture on concentration of p,p′-DDE (which was not normally distributed) in owl eggs, with trace concentrations scored as 0.05 ppm. For each analysis, we incorporated covariates for study year and laying order (Early vs. Late) to account for annual or temporal variability associated with egg order. Because there were three females that each contributed two eggs to our sample, we ran these two analyses with and without the second egg from each female ($n = 58$ eggs and $n = 55$ eggs, respectively). Inferences did not differ for the ordinal logistic regression, so we report results that included all 58 eggs. For the generalized regression analysis, we report results from analysis of the 55 eggs, as the inference surrounding one variable (distance from agriculture) changed. We viewed the latter as the conservative approach because it relied on completely independent observations.

Using Pearson correlation analyses, we examined potential relationships between eggshell thickness and (1) egg length, (2) breadth, (3) mass, and (4) volume. We also used general linear models to examine eggshell thickness as a function of (1) distance from agriculture, and (2) DDE concentration while including egg timing and year as covariates. We conducted these analyses using year and egg order as covariates including all eggs ($n = 58$), and including only the first egg from females if they were resampled ($n = 55$); inferences were the same for each, so we report results including all eggs.

RESULTS

We collected samples from Burrowing Owls and their nests during the nesting seasons of 2007 and 2008. We collected foot-wash and blood samples for anticholinesterase assessments in 2007 only, with foot-wash samples taken from 91 adult owls ($n = 54$ female, and $n = 37$ male) and blood samples taken from 84 adult owls ($n = 51$ female and $n = 33$ male) and nestling families at 43 unique nests. We collected a total of 58 eggs for OC analysis in 2007 and 2008. We collected nest-associated soil samples in 2008 only, a total of 25 samples for OC analysis.

Foot-wash Sample Analysis for OP and CB Exposure. Funding allowed for preliminary screening analysis of only 15 foot-wash samples and their associated blanks (controls). We selected samples for analysis across a range of distances from agriculture: 12 from adult males (1.9 ± 0.91 km from agricultural field, range: 0.03–9.8 km), and three from adult females (2.3 ± 1.9 km from agricultural field, range: 0.1–6.2 km). Irrespective of proximity to agriculture, there were no OP or CB insecticides detected in owl foot-wash samples or their associated blanks. Given these results and the cost of the OP/CB screens, no further foot-wash samples were analyzed.

Blood ChE Activity. In addition to adult samples, we also obtained blood samples from 20-d-old nestlings at 43 nest burrows (6.3 ± 0.3 nestlings sampled per nest; range: 3–9) and from 30-d-old nestlings at 39 of the previously sampled 43 nest burrows (5.6 ± 0.3 nestlings sampled per nest; range: 1–9).

For adults, there was no correlation between Julian date and BChE activity (Spearman correlation: $r_s = 0.11$, $P = 0.331$, $n = 84$); however, sample date and AChE activity had a small but significant correlation ($r_s = 0.27$, $P = 0.01$, $n = 84$) so we included Julian date as a covariate when examining relationships between AChE and distance from agriculture. Sample time of day and AChE (Spearman correlation: $r_s = 0.09$, $P = 0.41$, $n = 84$) or BChE activity ($r_s = -0.01$, $P = 0.92$, $n = 84$) in adult Burrowing Owls were not related.

Plasma AChE activity was 0.28 ± 0.02 units/ml plasma (range: 0.06–0.67) in adult Burrowing Owls ($n = 84$) sampled during 2007. There was no interaction between distance from agriculture and sex of owl when examining potential effects on AChE levels (Generalized Linear Model with Julian date covariate: $F_{1,79} = 0.50$, $P = 0.48$), no effect of sex ($F_{1,79} = 2.27$, $P = 0.14$), although males tended to be higher than females, and no relationship between distance from agriculture and AChE activity ($F_{1,79} = 1.36$, 0.50, $P = 0.25$; Fig. 1). The effect of the covariate Julian date was significant ($F_{1,79} = 4.30$, $P =$

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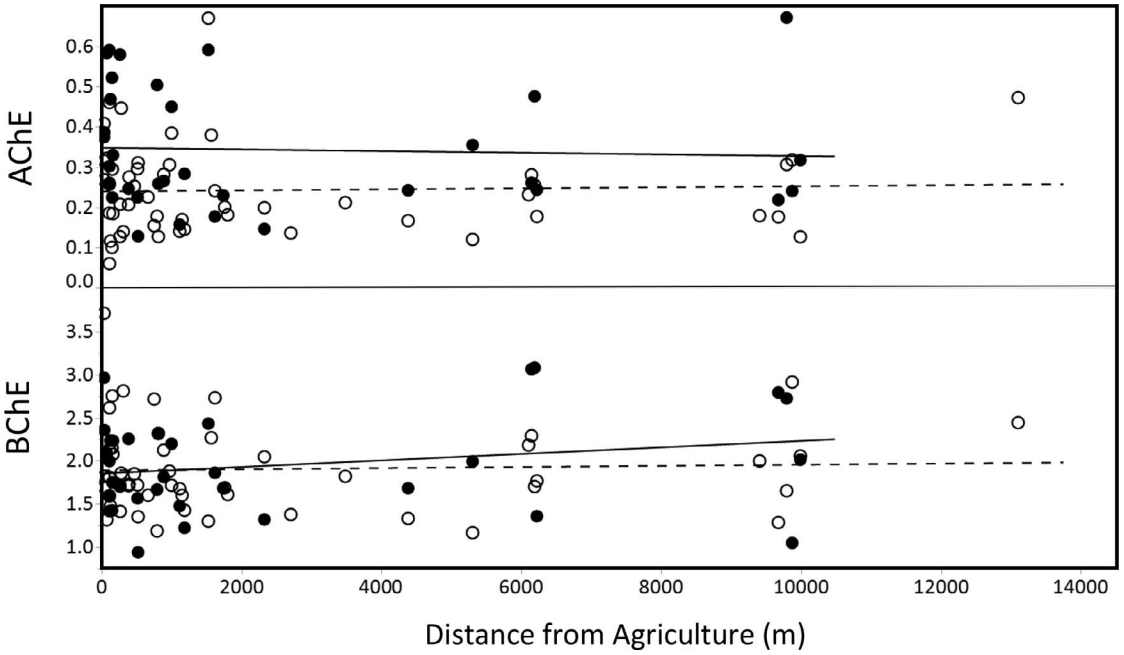


Figure 1. Plasma AChE and BChE levels (units/ml plasma) in adult male (filled symbols) and adult female (open symbols) Burrowing Owls as a function of distance from irrigated agriculture within the Morley Nelson Snake River Birds of Prey National Conservation Area, ID, USA, in 2007. Simple linear regression lines (continuous = males, dashed = females) are provided for illustration.

0.04) and had a positive parameter estimate (0.002 ± 0.001), indicating that AChE increased in adults as the breeding season progressed. For BChE, adults averaged 1.92 ± 0.06 units/ml plasma (range: 0.94–3.71, $n = 84$). Distance from agriculture and sex did not interact ($F_{1,80} = 0.61$, $P = 0.44$), and there was no effect of either sex class ($F_{1,80} = 0.45$, $P = 0.051$) or distance from agriculture ($F_{1,80} = 0.09$, $P = 0.76$; Fig. 1). Finally, when examining plasma AChE and BChE activity levels of adult Burrowing Owls sampled near agriculture in relation to a reference population from farther away, we found none > 2 SD below the mean for the reference population (Table 1).

For pooled nestling samples, neither AChE nor BChE activity was correlated with Julian date at either 20 d or 30 d of age (all $|r_s| < 0.1$, all $P > 0.56$), so we did not include sampling date as a covariate in subsequent analyses. AChE and BChE activity in nestling samples was 0.357 ± 0.017 units/ml plasma and 1.823 ± 0.056 units/ml plasma respectively at 20 d ($n = 43$ nests), and 0.341 ± 0.025 units/ml plasma and 1.827 ± 0.062 units/ml plasma at 30 d ($n = 39$).

There was no interaction between age of nestling and distance from agriculture for either AChE or BChE activity (repeated measures analysis: $F_{1,37} = 0.57$, $P = 0.45$; $F_{1,37} = 1.33$, $P = 0.26$, respectively; Fig. 2). There was also no effect of nestling age (20 or 30 d) on AChE or BChE activity ($F_{1,37} = 0.15$, $P = 0.70$; $F_{1,37} = 1.25$, $P = 0.27$, respectively), suggesting that ChE in nestlings at 20 d of age had reached maturation levels similar to those of 30-d-old nestlings. Finally, AChE and BChE activity levels did not vary with distance from agriculture ($F_{1,41} = 1.75$, $P = 0.19$; $F_{1,41} = 0.44$, $P = 0.51$, respectively; Fig. 2).

Soil OC Analysis. These samples were collected a median distance of 750 m from agricultural fields (2.6 ± 0.8 km, range: 0.03–13.27 km). We detected no OCs or their metabolites in these soil samples irrespective of distance from agriculture.

Egg OC Analysis. We collected one egg from 29 Burrowing Owl nests in both 2007 and 2008 to help ascertain OC exposure in the adult females that laid them. These included 34 early eggs and 24 late eggs (Table 2). The only OC chemical detected in the multi-residue screen was p,p′-DDE. Of the 58 eggs, 31 (53.4%) had no detected exposure, and 27 (46.6%) had p,p′-DDE concentrations ranging from

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Table 1. Plasma cholinesterase activity levels (± SD) for adult male and female Burrowing Owls breeding in nonagricultural areas (>1500 m from irrigated agriculture; Reference Population), those < 600 m from agriculture (Agricultural Population), and those between 600 and 1500 m from agricultural areas (Intermediate Population) within the Morley Nelson Snake River Birds of Prey National Conservation Area, ID, USA, in 2007. Number of individuals below, within, and above the reference interval are indicated. Individual owls that exhibited ChE activity below the reference interval may have been exposed to cholinesterase-inhibiting pesticides.

		Reference Population					Agricultural Population						Intermediate Population					
					Interval (± 2 SD)[a]				Relative to Reference Interval						Relative to Reference Interval			
Sex	Enzyme	*n*	Mean	SD	Lower	Upper	*n*	Mean	SD	No. Below	No. Within	No. Above	*n*	Mean	SD	No. Below	No. Within	No. Above
Male	**AChE**	13	0.32	0.16	0.00	0.64	14	0.37	0.15	0	14	0	6	0.32	0.13	0	6	0
	BChE	13	2.08	0.68	0.72	3.44	14	1.90	0.51	0	14	0	6	1.78	0.42	0	6	0
Female	**AChE**	20	0.25	0.13	−0.01	0.51	21	0.25	0.11	0	21	0	10	0.21	0.09	0	10	0
	BChE	20	1.88	0.49	0.90	2.86	21	1.97	0.58	0	20	1	10	1.83	0.45	0	10	0

[a]Reference interval established as ± 2 SD from the mean in nonagricultural burrows.

0.05–3.5 ppm (mean = 0.20 ± 0.56 ppm, geometric mean = 0.00 ppm; $n = 58$; Table 2). For the two females that had eggs sampled in both years of study, one had no p,p′-DDE detected in either 2007 or 2008, and the other had 1.6 ppm and 1.3 ppm p,p′-DDE in 2007 and 2008, respectively. These latter concentrations were the third- and fourth-highest in our study (Table 2) and could indicate persistence in the female or re-exposure between years. Eggs from the female sampled twice in the same breeding season (approximately 100 m and 6 d apart) both contained p,p′-DDE (0.1 and 0.2 ppm, respectively). Thus, considering only this small sample of females, there appeared to be some consistency in DDE concentrations from year to year.

Concentration of p,p′-DDE in Burrowing Owl eggs from artificial burrow nest sites that we sampled in both years but were occupied by different females ($n = 7$) was not always consistent (Fig. 3). Disparities were noteworthy at two nest sites. At the first site, p,p′-DDE concentration was below detection limits in the first year of the study (2007), but 3.5 ppm in 2008. At the second site, the egg collected in the first year of study had a concentration 32 times higher than the egg collected in the second year (1.6 ppm vs. 0.05 ppm, respectively; Fig. 3). These differences in egg contaminant concentrations occurred despite no likely local changes in OC use between years, i.e., there was no DDT applied in either year as its use was banned years before our study. Thus, it was probably individual female rather than nest site that explained the patterns.

Our two analyses of the relationship between DDE concentrations and the distance to the nearest agriculture had different outcomes. Distance to agriculture was unrelated to p,p′-DDE category (ordinal logistic regression analysis; likelihood ratio $\chi^2 = 0.004$, $P = 0.95$), although probability of having quantifiable DDE increased from early to late eggs (likelihood ratio $\chi^2 = 4.8$, $P = 0.03$) and in the second year of study (likelihood ratio $\chi^2 = 4.1$, $P = 0.04$, Fig. 4). However, when we analyzed the relationship between p,p′-DDE concentration in eggs and distance from agriculture, we found that p,p′-DDE increased with distance from agriculture (parameter estimate = 0.52 ± 0.24, likelihood ratio $\chi^2 = 6.00$, $P = 0.01$), although there was no relationship with year ($\chi^2 = 2.47$, $P = 0.12$) or egg timing ($\chi^2 = 1.29$, $P = 0.26$).

For 27 breeding attempts where p,p′-DDE was detected in the sampled egg, there were 4.2 ± 0.6 fledglings per attempt ($n = 5$ attempts [18.5%] failed

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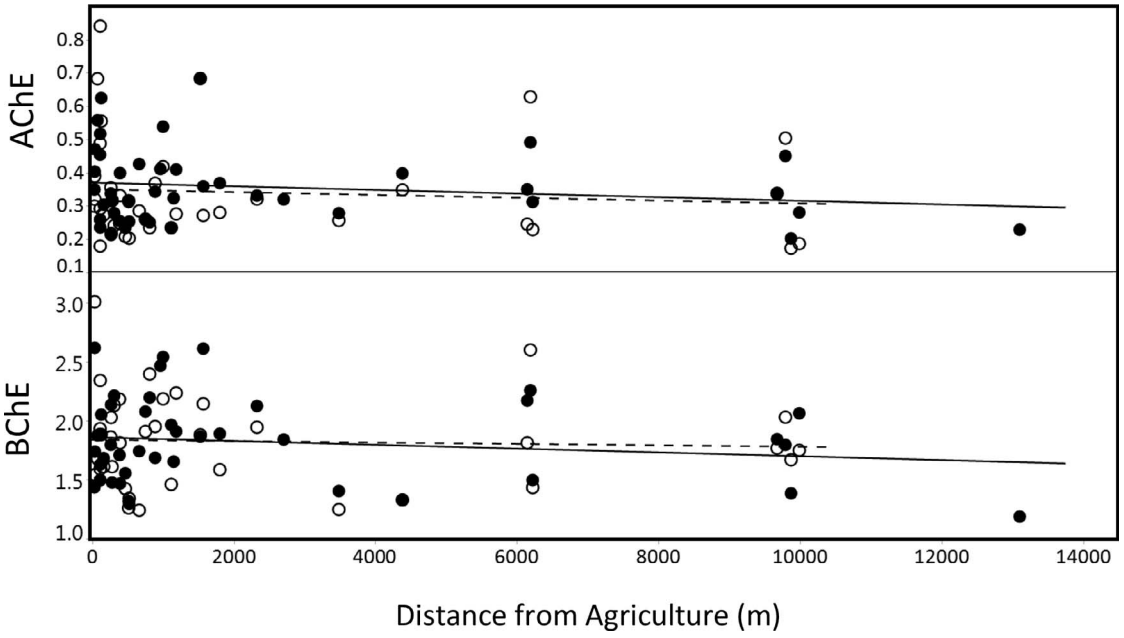


Figure 2. Plasma AChE and BChE levels (units/ml plasma) in pooled samples from nestling Burrowing Owls within broods 20 d (filled symbols) and 30 d (open symbols) after hatch as a function of distance from irrigated agriculture within the Morley Nelson Snake River Birds of Prey National Conservation Area, ID, USA, in 2007. Simple linear regression lines (continuous = 20 d, dashed = 30 d) are for illustration.

to produce any fledglings). In contrast, there were 3.1 ± 0.5 fledglings per breeding attempt and seven failed attempts (22.6%) when p,p′-DDE was not detected in sampled eggs ($n = 31$). Thus, although sample sizes are small, presence of p,p′-DDE did not appear to result in greater nest failures or fewer fledglings. Productivity for all breeding attempts monitored during 2007–2008 irrespective of egg

Table 2. Number of Burrowing Owl eggs containing OC residues of different concentrations during two breeding seasons (2007–2008) in the Morley Nelson Snake River Birds of Prey National Conservation Area, ID, USA. Early eggs were any of the first three eggs laid in a clutch, and Late eggs were those after the fifth in a clutch. One egg per nest from 58 nests was collected and analyzed.

	Year			
	2007		2008	
Concentration of DDE (ppm)	Early	Late	Early	Late
0.0	12	7	9	3
0.05[a]	1	1	4	1
0.1	0	3	1	4
0.2	2	1	1	0
0.3	0	0	1	1
0.6	0	1	1	0
1.3	0	0	1	0
1.6	0	1	0	0
1.7	0	0	0	1
3.5	0	0	1	0

[a] Trace concentration. Estimated to be 0.05 ppm for all samples with trace but below quantifiable levels (see text).

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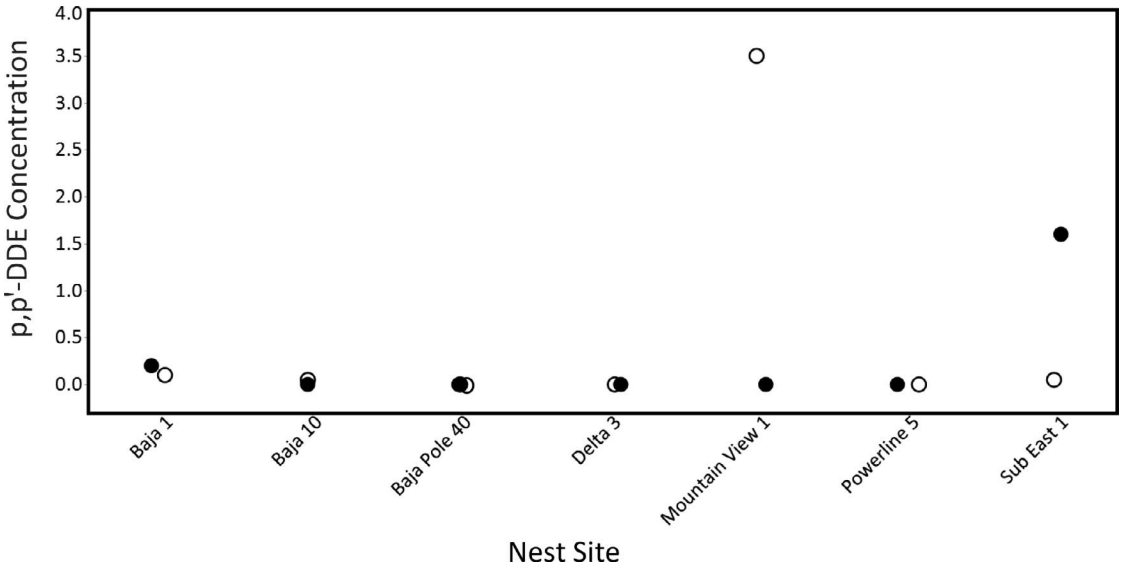


Figure 3. Concentrations of p,p′-DDE (ppm) in Burrowing Owl eggs at seven nest sites sampled both in 2007 (filled symbols) and 2008 (open symbols) within the Morley Nelson Snake River Birds of Prey National Conservation Area, ID, USA. Some points are jittered for clarity.

collection was 3.6 ± 0.5 (n = 82) fledglings per attempt.

Egg Dimensions and Shell Thickness. Mean egg length, breadth, mass, and volume were 32.35 ± 0.16 mm (n = 58), 25.92 ± 0.11 mm (n = 58), 11.54 ± 0.14 g (n = 57), and 11.13 ± 0.13 cm^3 (n = 57), respectively. Eggshell thickness was not strongly related to an egg's length, breadth, mass, or volume (Fig. 5). Thickness was greater in early eggs (0.189 ± 0.002, n = 34) compared with late eggs (0.180 ± 0.002, n = 24, General Linear Model: $F_{1,54} = 8.12$, P = 0.01, Fig. 6a), and there was no effect of year ($F_{1,54}$ = 1.54, P = 0.22). Eggshell thickness was not related to either distance from agriculture ($F_{1,54}$ = 1.55, P = 0.22, Fig. 6b) or p,p′-DDE concentration ($F_{1,54}$ = 1.20, P = 0.28, Fig. 6c). Eggshell thickness in eggs with and without p,p′-DDE was 0.184 ± 0.003 mm (range: 0.160–0.207, n = 27), and 0.186 ± 0.002 mm (range: 0.157–0.214, n = 31), respectively.

DISCUSSION

Our hypothesis was that Burrowing Owls nesting in agricultural areas within the NCA may be more exposed to pesticides than owls nesting in nonagricultural areas. We predicted that this would be reflected in residue analyses in relation to proximity to agriculture. We also envisioned that contaminants such as OC compounds might result in eggshell thinning, and this too might covary with proximity to agriculture. But, despite examining a number of external (e.g., foot-wash) and internal (e.g., ChE activity) indicators, we did not detect evidence of OP or CB pesticide exposure in NCA Burrowing Owls. However, nearly one-half of all Burrowing Owl eggs we examined were contaminated with p,p′-DDE, a metabolite of DDT. In contrast to the prediction that pesticide concentrations would be higher nearer to agriculture, we observed that p,p′-DDE concentration in eggs was higher farther from agriculture in one analysis and was unrelated to distance from agriculture in another analysis. Local soil samples tested negative for p,p′-DDE and, for individual females who moved between nesting sites but who had eggs sampled in both years of our study, individual owl rather than nest site appeared to better explain egg p,p′-DDE concentration. Thus, we posit that these contaminants may not have been derived from local sources and perhaps stemmed from sources encountered while on migration or in the owls' wintering areas.

There was no evidence of exposure to OP or CB compounds in foot-wash samples, and plasma AChE and BChE activity did not covary with proximity to agriculture in either adult or nestling Burrowing Owls as we had hypothesized. Moreover, no owls in agricultural areas had plasma AChE or BChE activity

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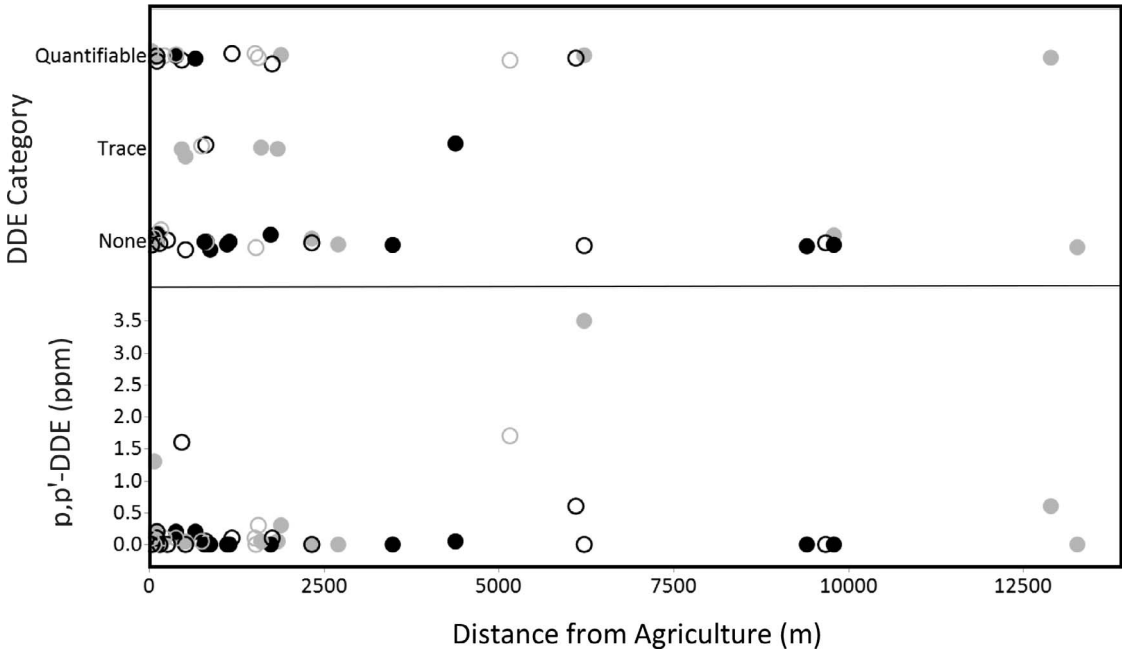


Figure 4. Top: DDE category as a function of distance from agriculture for Burrowing Owl eggs from the Morley Nelson Snake River Birds of Prey National Conservation Area, ID, USA (2007–2008). Categories of DDE concentrations: Quantifiable = ≥0.1 ppm; Trace: <0.1 ppm but greater than zero; None: not detected. Bottom: p,p′-DDE concentration as a function of distance from agriculture. 2007: Black, 2008: gray, Early eggs: filled symbols, Late eggs: open symbols for both top and bottom panels.

< 2 SD below the mean of a reference population. Although it is possible that owls nesting near agricultural areas in our study did not forage in areas where pesticides had been applied, which could explain our negative results, avoiding exposure in this manner seems unlikely and is not consistent with Gervais et al. (2003) and Marsh et al. (2014), who found that owls foraged commonly in agricultural areas. Woodin et al. (2007) also found the regurgitated pellets of Burrowing Owls within agricultural areas and Moulton et al. (2005) reported Burrowing Owls in the NCA preying extensively on montane voles (*Microtus montanus*), which occur predominately in irrigated agricultural lands rather than in surrounding arid grasslands. We also observed Burrowing Owls perching on fence posts adjacent to croplands in the NCA, so it is unlikely that exposure could have been avoided if the environment contained extensive OP and CB contaminants during our study. It remains possible, however, that either (a) due to the short half-lives of OP and CB contaminants, we did not capture owls and collect samples at the appropriate time to detect exposure to these chemicals, or (b) OP and CB chemicals in our multi-residue screen were not applied to the agricultural fields in question during our study. In the case of the former, exposure and associated negative effects may have occurred but were not detected in our study.

Although there was only one OC analyte, p,p′-DDE, detected in Burrowing Owl eggs in our study area, 46% of sampled eggs tested positive for this chemical. Occurrence of this p,p′-DDE was not restricted to a small number of nest sites or to one particular portion of our study area, and there was some evidence that p,p′-DDE concentration in eggs may increase with distance to the nearest agricultural field, further suggesting that current NCA agriculture was not the source of this p,p′-DDE. Soil samples collected near owl nest sites were negative for OCs, which also supported this conclusion. However, as a caveat, soil concentrations of contaminants are typically low, much lower than concentrations in prey species.

Because p,p′-DDE is lipid soluble and retained in adipose tissues of a bird after exposure (Bernard

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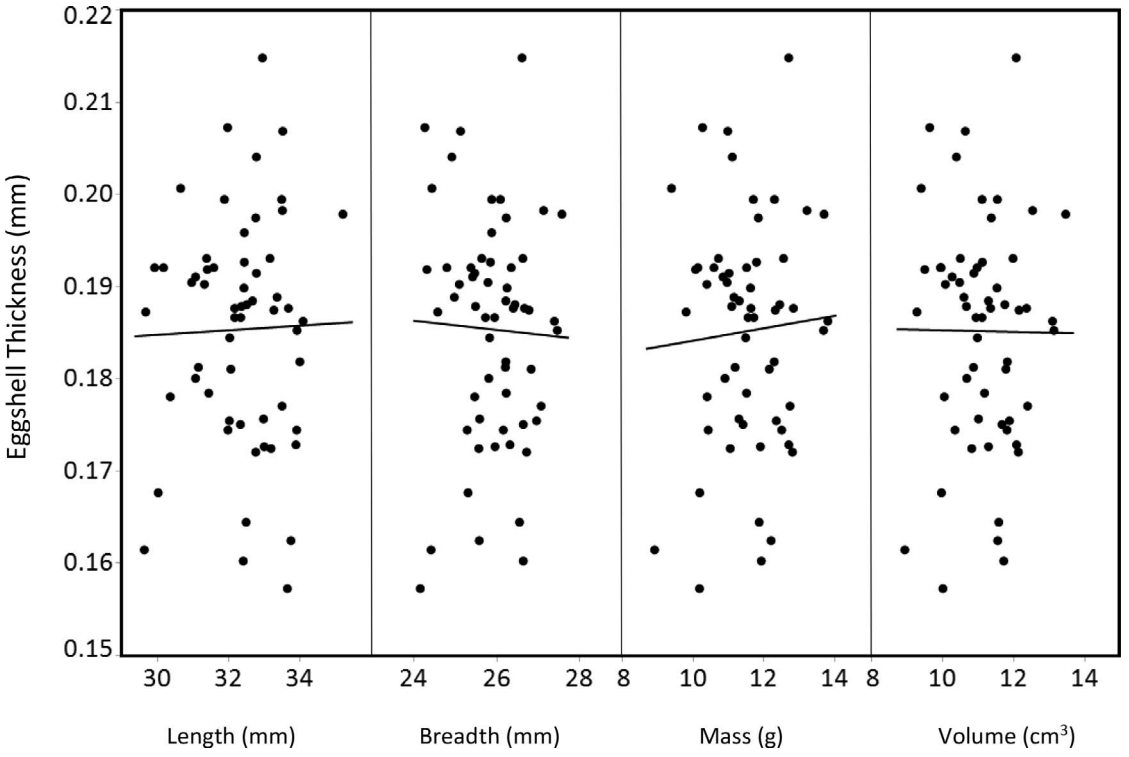


Figure 5. Thickness of eggshells for Burrowing Owl eggs collected in 2007–2008 in the Morley Nelson Snake River Birds of Prey National Conservation Area, ID, USA, as a function of egg length, breadth, mass and volume. Pearson correlation analysis ($n=57^a$ or 58^b) with shell thickness: Length[a]: $r=-0.025$, $P=0.853$; Breadth[a]: $r=-0.034$, $P=0.803$; Mass[b]: $r=0.059$, $P=0.664$; Volume[b]: $r=-0.007$, $P=0.960$).

1966), p,p′-DDE excreted and detected in a female's egg could be derived from exposure that occurred long before arriving on nesting grounds. Burrowing Owls in Idaho are generally annual migrants and move from breeding grounds to wintering grounds and back each year. Of the five sightings, recaptures, or recoveries of leg-banded owls from the NCA outside of the breeding season, all occurred in California (J. Belthoff unpubl. data). Regionally, six of eight Burrowing Owls tracked from breeding grounds in southeastern Washington wintered in central or southern California (Washington Department of Fish and Wildlife [WDFW] 2013), and Burrowing Owls tagged at the Mountain Home Air Force Base (Elmore County, Idaho, near our study area) wintered in southern California or northern Mexico (D. Johnson pers. comm.). Thus, Burrowing Owls that breed in the NCA likely migrate through or spend their winters in California and/or northern Mexico, where DDT and its metabolites still persist in higher levels in the food chain (Gervais et al. 2000, Yates et al. 2009).

Gervais et al. (2000) studied Burrowing Owls in the San Joaquin Valley (Lemoore Naval Air Station and near Pixley National Wildlife Refuge) and the Imperial Valley (Salton Sea National Wildlife Refuge) of southern California and found all but two eggs in their sample contained p,p′-DDE residues. Eggs from Lemoore Naval Air Station had geometric mean p,p′-DDE concentration of 7.52 ppm, more than twice the highest egg concentration we observed for Burrowing Owls breeding in the NCA. In contrast, eggs at Pixley National Wildlife Refuge and Salton Sea National Wildlife Refuge had a geometric mean of 1.10 ppm and 0.62 ppm p,p′-DDE, respectively (Gervais et al. 2000, Gervais and Catlin 2004). Concentrations in our study (NCA), were lower. Moreover, concentrations of p,p′-DDE in Burrowing Owl eggs in the NCA were almost all less than concentrations reported in Blus (2011) for other raptor species, including Peregrine Falcons

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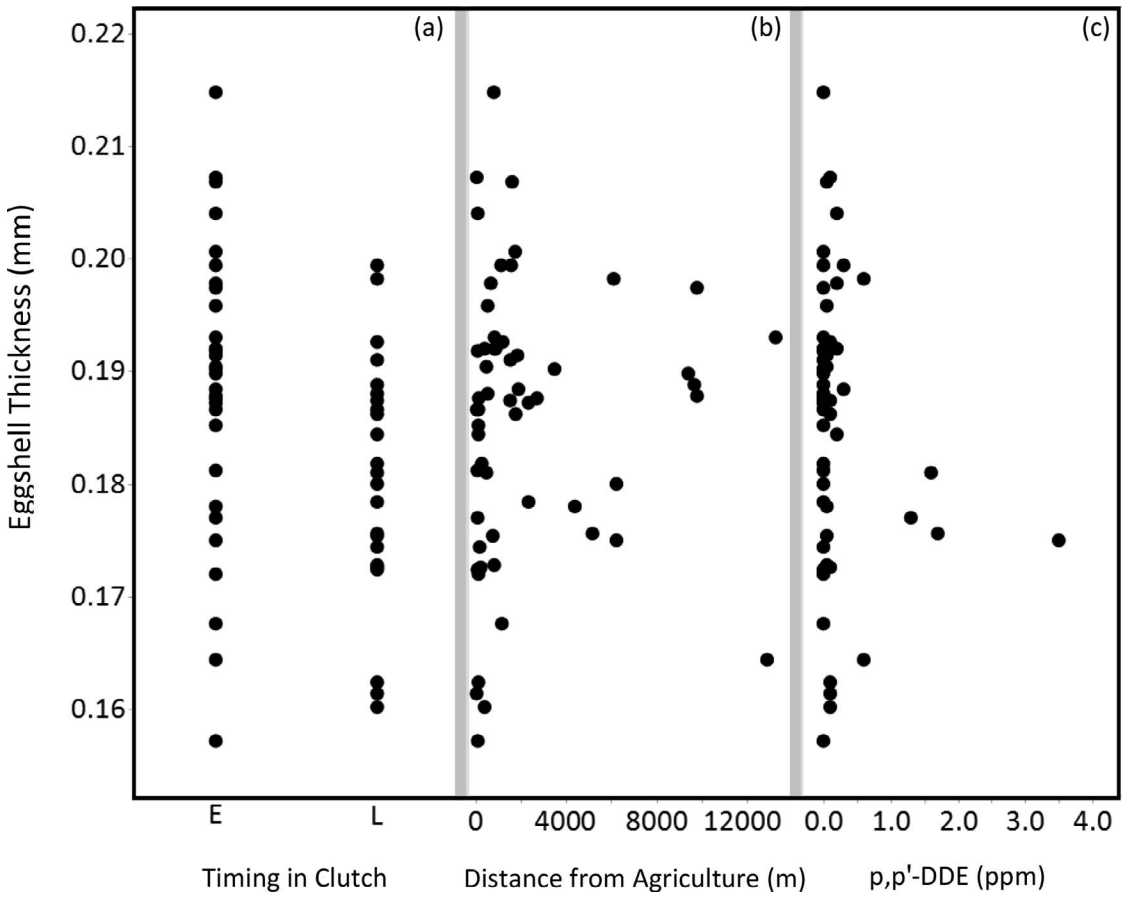


Figure 6. Burrowing Owl eggshell thickness as a function of (a) relative timing of egg (E = early, L = late) within a clutch, (b) distance from agricultural fields, and (c) DDE concentration in the Morley Nelson Snake River Birds of Prey National Conservation Area, ID, USA, 2007–2008. For (a), early eggs ($n = 34$) were any of the first three eggs laid in a clutch, whereas late eggs ($n = 24$) were after the fifth egg.

(*Falco peregrinus*; means ranged from 2–44 ppm; Cade et al. [1971], White et al. [1973]), White-tailed Eagles (*Haliaeetus albicilla*; 30 ppm; Koivusaari et al. [1980]), Eurasian Sparrowhawks (*Accipiter nisus*; 7 ppm; Newton and Bogan [1974]), Ospreys (*Pandion haliaetus*; means ranged from 2–9 ppm; Wiemeyer et al. [1975]), Bald Eagles (*Haliaeetus leucocephalus*; 10 ppm; Anthony et al. [1993]), and Golden Eagles (*Aquila chrysaetos*; means ranged from 0.1–0.3 ppm; Newton and Galbraith [1991]). We observed approximately 93% of all sampled eggs contained <1.0 ppm p,p′-DDE, which suggests that concentrations of p,p′-DDE in Burrowing Owl eggs in the NCA are not greater than expected in North American raptors in the post-DDT era.

There also was no indication that the concentrations of p,p′-DDE we observed in Burrowing Owl eggs in the NCA caused thin-shelled eggs or nest failures. Despite detection of p,p′-DDE in Burrowing Owl eggs from the NCA, we did not observe any strong relationship between eggshell thickness and p,p′-DDE concentration or eggshell thickness and proximity to agricultural fields. These findings suggest that Burrowing Owls in the NCA, which have lower concentrations of p,p′-DDE in eggs than owls in some other agricultural areas of western North America, escaped significant thinning. We did, however, detect an effect of egg order on shell thickness in that early eggs had thicker shells on average, which is a pattern that occurs in some species (e.g., Romanoff and Romanoff 1949, Bitman

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et al. 1969) but is reversed in others (Massaro and Davis 2005).

Conclusions. Our study provided insight into one potential cost of Burrowing Owls nesting in agricultural areas, i.e., pesticide exposure and associated effects. Our study is encouraging in that we found little evidence that owls in the NCA were exposed to OP and CB pesticides during nesting, although our sampling scheme was limited. Our findings suggest that anti-cholinesterase pesticides likely did not pose a threat to nesting Burrowing Owls at the time of our study. Of course, significant changes in land or pesticide use in the NCA could alter these relationships. Indeed, assessing the potential for direct or indirect effects of newer insecticides such as neonicotinoids, which are in increasing use within the range of Burrowing Owls, or the role of rodenticides, are likely topics for new investigations of the effects of contaminants on Burrowing Owls.

A pattern of p,p′-DDE exposure covarying more with individual than with a particular nest site, and the fact that we did not detect p,p′-DDE in soil near owl breeding areas in the NCA, suggest that p,p′-DDE exposure probably occurred outside of the breeding season, e.g., either when owls were migrating, when on their wintering grounds, or both. Though nearly half of the Burrowing Owl nests had detectable residues of p,p′-DDE in their eggs, levels did not appear sufficient to contribute to decreases in eggshell thickness or to reduce productivity. Concentrations of p,p′-DDE are still detectable in many other avian species, but they continue to decrease with time (Mora et al. 2016). Burrowing Owls in the NCA may be following this trend toward background levels that do not, to the best of our knowledge, pose a threat to species health.

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