ECOTOXICOLOGICAL RISK AND EXPOSURE: A COMPARISON OF WESTERN BURROWING OWLS NESTING IN AGRICULTURAL AND NON-AGRICULTURAL AREAS IN THE MORLEY NELSON SNAKE RIVER BIRDS OF PREY NATIONAL CONSERVATION AREA

by

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The following individuals read and discussed the thesis submitted by student Matthew Stuber, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

I dedicate this thesis to my wife, Jenifer.

Thanks for making the things that are important to me, important to you.

And to my two new sons, Nolan and Daniel.

Thanks for the significantly more smiles you add to my days

($F_{1,82} = 13.61$; $p < 0.001$).

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ABSTRACT

In some portions of their range, western burrowing owls (*Athene cunicularia hypugaea*) nest in higher densities near irrigated agricultural areas when compared to non-agricultural, arid habitat. Previous research suggests that owls may associate with agricultural areas because of more reliable and abundant prey, particularly invertebrates. One potential cost of this association, however, is an increased risk of exposure of owls to pesticides that are applied to agricultural fields. I investigated the exposure to and possible effects on burrowing owls of organophosphate, organochlorine, and carbamate pesticides in the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) located in southern Idaho. I used plasma cholinesterase as a biomarker to investigate *in vivo* organophosphate and carbamate exposure, footwash samples to investigate potential external exposure, and chemical analysis of whole egg contents to investigate organochlorine (*p,p*^{1}-DDE) exposure in nesting adult females. I also compared eggshell thickness in agricultural and non-agricultural areas to determine the potential for thinning caused by pesticide exposure.

Cholinesterase levels and eggshell thickness did not differ between owls nesting at agricultural burrows and non-agricultural burrows. Additionally, there were no pesticide residues detected in footwash samples. Therefore, I found no evidence that owls nesting in agricultural areas were exposed to high levels of pesticides while breeding. However, a metabolite of dichlorodiphenyltrichloroethane (DDT), *p,p*^{1}-DDE, occurred in 27 of 58 eggs sampled. Thus, despite DDT being banned from use in the

United States since 1973, burrowing owls breeding in southern Idaho were exposed to residues of this organochlorine pesticide.

I detected no DDT or metabolites of DDT in the soils that I sampled from areas in which owls bred in the NCA, and presence of *p,p*^{1}-DDE in eggs occurred irrespective of (1) whether owls nested in agricultural or non-agricultural areas, or (2) the distance to the nearest agricultural field. Considering these results, and that organochlorine pesticides are lipid soluble and have long retention in exposed animals, it is possible that owls were exposed to *p,p*^{1}-DDE during migration and/or on their wintering grounds, and not on their breeding grounds in the NCA.

With one exception, *p,p*^{1}-DDE concentrations in eggs in my study were lower than those known to cause reproductive impairment in other avian species. Additionally, *p,p*^{1}-DDE concentrations in eggs were not correlated with eggshell thickness, so there was no evidence of the well-known eggshell thinning effects of DDT and its metabolites. These results suggest that exposure to *p,p*^{1}-DDE in burrowing owls breeding in the NCA was not causing widespread reproductive impairment, regardless of where exposure may have occurred.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Presence of Agriculture

Throughout the last half century, globally increasing human populations have enhanced demand for agricultural services. This demand has, in part, catalyzed the conversion of large portions of naturally arid lands of western North America to irrigated agricultural lands (USDI 1996). Additionally, this demand has given rise to new agricultural practices designed to make existing agricultural land more productive.

Despite the benefits to humans for crop and livestock production, few wildlife species benefit from the conversion of their natural habitat to agricultural lands (Carlson 1985). In fact, populations of many species of wildlife have declined near lands converted to agriculture (Vander Haegen *et al.* 2000, Gaston *et al.* 2003, Murphy 2003, Vander Haegen 2007). For example, erosion from agriculture increases sediment (turbidity), which can influence the fates of contaminants (Cave *et al.* 2003, Warren *et al.* 2003) and nutrients (Catt *et al.* 1998, Collins *et al.* 2005) in nearby streams. These alterations negatively affect aquatic ecosystems, including fish and macroinvertebrate populations (Jahn and Schenck 1991, Vogel and Beauchamp 1999, Kiffney and Bull 2000, Rosemond *et al.* 2000, Heaney *et al.* 2001). Avian species and other wildlife also decline in abundance as a result of agricultural habitat modification (Fuller *et al.* 1995, Matson *et al.* 1997, Green *et al.* 2005, Gregory *et al.* 2005, Vander Haegen 2007). Finally, pesticide exposure in and around agricultural areas has been linked to wildlife mortality (Belisle *et al.* 1972) and reproductive impairment of birds (Fry 1995) through

eggshell thinning (Cade *et al.* 1971, Peakall 1974, Grier 1982, Grubb *et al.* 1990), embryo toxicity and compromised development (Fry 1995), and decreased nervous system function (Yamamoto *et al.* 1996).

Pesticides

Pesticide use by humans dates back to 1200 BC, when salt and ashes were applied to prevent unwanted vegetation growth. From the 18th century into the early 20th century, pesticide use was primitive by today's standards. During that time, pesticides such as kerosene, turpentine, and many lead-, arsenic-, or sulfur-containing compounds were commonly used for killing pests on small plots of land, but large-scale pesticide application was not practiced.

In 1883, however, John Bean invented a pressure sprayer for pesticide application, and by 1921 this technology was employed from airplanes to aerially apply pesticides to large fields. In the early 1940s, synthetic (man-made) chemicals were successfully used for the abatement of insects and, in 1945, dichloro-diphenyl-trichloroethane (DDT) was introduced as an effective way to kill insects with little to no negative effects on humans. By the mid-1940s, there was widespread production of chemical pesticides. Early synthetic chemicals and their application targeted only a few species of insects and plant diseases. Today, however, pesticides and application strategies exist for almost every group of animals and plants.

Three major families of pesticides are: organochlorines (OCs), organophosphates (OPs), and carbamates (CBs). After their introduction in the mid-1940s, OC pesticides were initially heavily used as insecticides, but their use has tapered, largely because of increased regulation from the U.S. government, which began in the 1970s. These

pesticides denature slowly, which improves their effectiveness and reduces frequency of application, but OCs are subject to bioaccumulation in ecological food chains. As such, OCs often harm non-target species (Fry 1995, Han *et al.* 2011).

In contrast to OC pesticides, OP and CB pesticides are relatively short lived in the environment and are often not subject to biological magnification because of their relatively fast detoxification in the liver. These pesticides are still commonly applied to agricultural fields. Though OPs and CBs mostly target insects, they are indeed toxic to other species (Woodbridge *et al.* 1995, Goldstein *et al.* 1997, Grue *et al.* 1997, Goldstein *et al.* 1999). Notably, OP and CB pesticides inhibit cholinesterase, which is an enzyme involved in neurotransmission (Grue *et al.* 1991, Hill 1995). Thus, exposure to these two families of pesticides can result in reduced nervous system function, which can lead to a multitude of negative consequences for wildlife.

The negative effects of the above three families of pesticides – OCs, OPs, and CBs – have been especially well studied in birds. Exposure to these pesticides can occur directly (direct contact with the chemical), indirectly (ingestion of contaminated plants or prey), or both (Gervais *et al.* 2003). Effects of exposure in birds include: decreased immune system function (Grasman and Fox 1999, Sagerup *et al.* 2000, Aggarwal *et al.* 2008), detrimental behavioral alterations (McCarty and Secord 1999, Halldin *et al.* 2003), increased risk of predation (Galindo *et al.* 1985, Buerger *et al.* 1991, Hunt *et al.* 1992), respiratory difficulty or failure (Fildes *et al.* 2009), altered hormone levels (Grue *et al.* 1997), decreased thermoregulatory ability (Rattner and Franson 1983, Maguire and Williams 1987, Grue *et al.* 1991), reduced food consumption (Pope and Ward 1972, Grue *et al.* 1982, Grue *et al.* 1991), disorientation while on migration (Vyas *et al.* 1995),

decreased egg laying (Stromborg 1986, Bennett *et al.* 1991, Halldin *et al.* 2003), and decreased thickness of eggshells (Ratcliffe 1967, Hickey and Anderson 1968, Heath *et al.* 1969, Cooke 1973, Blus *et al.* 1974, Blus 1982, Fry 1995). Generally, the extent of these effects vary and depend on the intensity, amount, and type of pesticide as well as the species exposed. Pesticide exposure can also result in death of the exposed bird (Basili and Temple 1995, Goldstein *et al.* 1999, Mineau *et al.* 1999) and is linked to population declines of some bird species (McLaughlin and Mineau 1995, Mineau and Whiteside 2006, Benton 2007, Mineau and Whiteside 2013). Further, when direct exposure does not occur, but when pesticides have been applied to an area, predatory birds can be affected from a loss of foraging opportunity because of reduced invertebrate or mammal populations (Dechant *et al.* 2003, Klute *et al.* 2003).

One important grassland species that exists in agricultural areas and may therefore be susceptible to pesticide exposure is the western burrowing owl (*Athene cunicularia hypugaea*; hereafter referred to as burrowing owl). Burrowing owls often nest in higher densities in agricultural areas when compared to non-agricultural areas (Conway *et al.* 2006, Moulton *et al.* 2006, Bartok and Conway 2010), and they are among the few raptor species in Idaho that show an association with agricultural areas during the breeding season (Rich 1986, Leptich 1994, Moulton *et al.* 2006). Moreover, burrowing owls may have higher productivity when nesting near agricultural areas when compared to non-agricultural areas, although this difference is not apparent in all years (Belthoff and King 2002).

Moulton *et al.* (2006) examined three potential reasons for the association of burrowing owls with agricultural areas. They rejected hypotheses that the association

was because of a difference in burrow availability or predator abundance between agricultural and non-agricultural areas. Instead, they found that prey consumption differed between agricultural and non-agricultural areas. Specifically, burrowing owls in agricultural areas included invertebrate prey in their diets at higher numbers than owls in non-agricultural areas. Moulton *et al.* (2006) concluded that an increased availability of prey in agricultural areas was a potential reason for the association of burrowing owls with agriculture.

Despite that agricultural areas may increase prey for burrowing owls, I wondered if owls in agricultural areas were at elevated risk of pesticide exposure/poisoning. Mineau *et al.* (1999) concluded that two of the six most significant factors that can lead to raptor poisonings are insectivory and inhabiting agricultural areas. Both of these are characteristic of burrowing owls, which highlights the need to understand if and how pesticides potentially affect this species and to determine whether, in providing a more reliable food source, agricultural areas are increasingly exposing owls to harmful chemicals.

Thus, the goal of my research was to examine whether burrowing owls breeding within portions of the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) in southwestern Idaho are at risk of exposure to pesticides through association with agricultural areas. My research looked for evidence of pesticide exposure and potential effects of that exposure. My hypothesis was that burrowing owls in agricultural areas would be more likely to contact pesticides and pesticide residues than owls nesting in non-agricultural areas. To investigate this, I analyzed (1) footwash samples, (2) blood samples, (3) egg contents and eggshells, and (4) soil samples. These

analyses allowed me to investigate potential OC, OP and/or CB exposure, and possible routes of that exposure, in burrowing owls.

METHODS

Study Area

During 2007-2008, I studied the risk and potential effects of pesticide exposure on burrowing owls that nested in the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) located in southwestern Idaho. This 195,325 ha area 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). It contains one of the densest known populations of nesting birds of prey in North America (USDI 1996). Precipitation averages 31.7 cm annually (NOAA 2002), with 12.1 cm occurring during the burrowing owl breeding season (March through July). The topography in the NCA is mainly flat to rolling with a number of rock outcrops, isolated buttes, and small canyons. The NCA was historically dominated by shrub-steppe (Hironaka et al. 1983), but human disturbances and fires have converted much of the area to disturbed grassland (USDI 2008), which is dominated by invasive annual plants species, such as cheatgrass (*Bromus tectorum*) and tumble mustard (*Sisymbrium altissimum*). Plant communities in areas adjacent to agricultural fields are reasonably similar to those in non-agricultural areas. Cattle and sheep grazing occur in the NCA, primarily during winter (USDI 1996, Moulton *et al.* 2005).

The NCA is partially surrounded by and contains a small number of irrigated agricultural fields within its borders, which creates desirable foraging conditions for burrowing owls (Moulton *et al.* 2006). During my study, the principal crops were alfalfa

(*Medicago sativa*) and corn (*Zea mays*), which were primarily intended for livestock feed. Burrowing owls in the NCA (Belthoff and King 2002, pers. obs.) and elsewhere in southern Idaho (Gleason 1978, Rich 1986) often nest on the outskirts of agricultural fields. American badgers (*Taxidea taxus*), a mammal native to and abundant in the NCA, dig most of the natural burrows that ultimately provide suitable nest sites for burrowing owls.

Researchers have also placed artificial burrow systems at historical burrowing owl nest sites and in areas that are likely to attract owls throughout the NCA. Artificial burrow systems vary in configuration (see Smith and Belthoff 2001) but generally consist of two to three burrows, clustered a few meters apart. Each burrow has a plastic underground chamber and 2 m of 10- or 15-cm diameter irrigation tubing that slopes to the surface. No more than one breeding pair of owls occupies one of these systems at a time; thus, having multiple burrows in system provide a nest burrow and one or more satellite burrows for a nesting pair. There currently are approximately 350 artificial burrow systems available for burrowing owls nesting or roosting within the NCA (Smith and Belthoff 2001, Belthoff and Smith 2003, Moulton *et al.* 2006, Welty 2010), and these burrow systems occur 5 – 13,300 m from the nearest agricultural area. Since 1997, burrowing owl pairs occupied 30 - 60 of the artificial burrows within the NCA each year for nesting (Belthoff and Smith 2003, J. Belthoff, unpublished data).

Study Species: Western Burrowing Owl

Western burrowing owls breed from southern Canada to central Mexico (north to south) and from the eastern edge of the Great Plains to the Pacific coast (east to west). They occupy dry and open habitat, such as deserts, grasslands, prairies and steppes, but

they have declined in abundance in some locations in North America and their range has contracted in recent decades (Gervais *et al.* 2003, Poulin *et al.* 2011). The owls are listed as federally endangered in Canada, threatened in Mexico, and are a species of conservation concern in many western U.S. states (Klute *et al.* 2003).

Burrowing owl breeding usually begins from late March to mid-May depending on latitude. Females typically lay 8-12 eggs in underground burrows previously excavated by fossorial mammals (Poulin *et al.* 2011). Adult females but not males incubate eggs. Onset of incubation generally occurs near the completion of egg laying and lasts approximately 22 d (Conway *et al.* 2012). Despite the large clutch sizes, number of nestlings per nest that survive to fledging typically ranges from 2.9 to 4.9 (Poulin *et al.* 2011). Thus, it is common that some eggs laid do not ultimately produce fledglings. This allowed me to collect one egg per nest to examine contaminants and eggshell thickness (see below) without affecting population reproductive success.

Burrowing owls are food generalists and opportunistic predators. Primary prey items are small mammals, birds, arthropods and other invertebrates, amphibians, and reptiles (Moulton *et al.* 2005, Poulin *et al.* 2011). Owls are primarily crepuscular or nocturnal, but hunting and prey delivery occur at any time of day, with insects hunted throughout the 24-hour day, and vertebrates hunted primarily during morning and evening (Poulin and Todd 2006).

Adult male burrowing owls are the predominant foragers of a burrowing owl pair and travel the greatest distances from nests during the breeding season (Gleason 1978, Thompson and Anderson 1988). Gervais *et al.* (2003) determined that, in California, the mean distance travelled by male burrowing owls from their nest was approximately 400

m, while the maximum distance averaged approximately 1,300 m. In Canada, Haug and Oliphant (1990) found male burrowing owl movements were typically within 600 m of the nest, while the average maximum distance travelled from nests was 1,700 m.

As burrowing owl movements during nesting appear to be concentrated near their nest burrows, I hypothesized that exposure to most types of pesticides would be influenced by the proximity of nests to agricultural fields. Specifically, I expected that owls nesting near irrigated agricultural areas would be at a higher risk of exposure to pesticides than owls nesting farther from agriculture. To investigate this, I classified nest burrows into *Agricultural*, *Non-Agricultural*, or *Intermediate* categories, based on their distance to irrigated agriculture and the corresponding putative risk of pesticide exposure.

Agricultural Classification of Burrows

Agricultural burrows were those within 600 m of at least one irrigated agricultural field, which was the distance that contained 95 percent of nesting male burrowing owl movements as determined by Haug and Oliphant (1990). Thus, owls nesting in *Agricultural* burrows likely had the highest potential to interact with nearby agriculture and contact pesticides.

Non-Agricultural burrows were those with no agricultural fields within 1,500 m, which is the maximum foraging distance of breeding burrowing owls derived using the average maximum values in Gervais *et al.* (2003) and Haug and Oliphant (1990). Thus, I considered owls at these burrows least likely to interact with agriculture and contact pesticides.

I classified as *Intermediate* burrows those nests located > 600 m and < 1,500 m from irrigated agriculture. I presumed that the chances of foraging adults at *Intermediate*

burrows interacting with agricultural fields and contacting pesticides was lower than at *Agricultural* burrows but higher than at *Non-Agricultural* burrows.

Nest Monitoring

Beginning in mid-March of 2007 and 2008, I visited every artificial burrow site in the NCA at least twice in each year to determine presence of owls. At each visit, I performed visual sweeps of surrounding areas and inspected artificial burrow entrances for owls or signs of occupancy, such as dung, cached prey, droppings, cast pellets, or footprints. If there were signs of occupancy and sufficient time had passed for egg laying to begin, I checked the artificial burrow nest chamber for the presence of eggs. To distinguish eggs laid early in a clutch from eggs laid later, I marked eggs present in the chamber at the first visit with a Sharpie® marker. I visited nests 7 d later and again marked eggs that were present. On several occasions, I visited again 7 d after the second visit to identify the last portion of a clutch.

Owl Capture

Beginning approximately 10 d prior to the projected hatch date of eggs, I captured adult burrowing owls at nests either directly from artificial burrow tunnels or chambers after excavation of the chamber lid, or from a small-gauge wire trap placed at the tunnel entrance that captured the owl in the process of exiting the tunnel. As females spend more time than males in the nest burrow during incubation and brooding, most owls I captured this way were female. If adult owls were outside the burrow when I arrived, I captured them using a one-way door trap placed at the mouth of the nesting burrow or a nearby (satellite) burrow, which I sometimes combined with playback of burrowing owl vocalizations on a small cassette tape recorder placed in the tunnel of the satellite burrow.

Because males were often outside of nest burrows during the breeding season, I captured males more than females using the latter approach.

I captured nestlings by hand either directly from artificial burrow tunnels or chambers after excavation, or from a small-gauge wire trap placed at the tunnel entrance that captured the nestling as it exited the tunnel. I attempted to capture all nestlings in each brood at both 20 d and 30 d after hatching.

Sampling for Pesticide Exposure

In both years the owls that I studied were nesting near agricultural fields where the only crop grown was alfalfa. Mineau and Whiteside (2006) found that, nationwide, alfalfa is high on the list of crops potentially associated with avian mortality. Specifically, alfalfa ranked third, behind corn and cotton respectively, on the list of crops of most concern to birds because of its total planted area in the U.S. and the pesticides that are commonly applied to it (Mineau and Whiteside 2006). Thus, it seems likely that risk to burrowing owls from pesticide exposure is present in the NCA.

Unfortunately, pesticide application records were not available in my study area. All irrigated agriculture is located on private land within the NCA, and private landowners in Idaho are not required to report their pesticide applications to any agency or organizations, nor are they required to keep records of pesticide applications (unless the pesticide is a restricted use pesticide). Although I attempted to acquire information about specific pesticide applications, the landowners did not provide it. Thus, rather than test burrowing owls for residues of a specific pesticide, my analyses focused on multi-residue screens of a suite of pesticides commonly applied to alfalfa and other similar crops (Appendix A).

Footwash Collection and Analysis

Pesticide residues on burrowing owls may be most concentrated and thus most easily detected on their feet, as owl feet are expected to accumulate residues from perching on contaminated surfaces and/or capturing contaminated prey. Residues of OP and/or CB insecticides on the feet of an owl would indicate exposure to these chemicals. The concentration of these residues may also provide more information about the extent or timing of the exposure event(s).

To detect signs of external pesticide exposure (i.e., evidence of pesticides on the bodies of owls), in 2007 I collected footwash samples from adult owls in a manner similar to Gervais *et al.* (2000). I scrubbed owl feet with a toothbrush and rinsed them with 50 ml of 100% ethanol. I collected, with the rinse, any dirt, feathers, or hair/fur present on the talons into a glass funnel, which directed the rinse into 50 ml glass vials. The brush and funnel were cleaned between uses first with water, and then with hexane, and each was allowed to air dry in a cooler.

For comparison, I also collected blank samples in the vicinity of each captured owl on each sampling day by allowing an open sampling vial and funnel to sit uncovered for approximately one minute. I then rinsed the funnel with 100% ethanol and collected the rinse in the sampling vial. Upon collection, footwash and blank samples were stored and transported on ice to a laboratory at Boise State University where they were frozen (-20 °C) until analysis. Chemical analysis for 43 OP and 11 CB insecticides (Appendix A) was performed on footwash samples by the California Animal Health and Food Safety Laboratory at the University of California – Davis in the manner described in Holstege *et al.* (1994).

As cost was prohibitive to analyze all samples that I collected, I ultimately chose a subset of samples and their respective blanks from Agricultural, Non-agricultural, and Intermediate burrows and from a range of dates for analysis. As males do most of the foraging for a pair, I considered male owls more likely to be externally exposed to pesticides and prioritized male samples for analysis.

Blood Collection and Analysis

Exposure to OP and/or CB insecticides can inhibit the production of cholinesterase enzymes (ChEs), which are enzymes created by the body, present in the blood and essential for nervous system function (Rotenberg *et al.* 1995). There are two ChEs – acetylcholinesterase (AChE) and butylcholinesterase (BChE). Both are common biomarkers for OP and CB insecticide exposure. Significantly reduced ChE levels in blood serum may indicate exposure to either of these two families of insecticides (Mineau 1991). As such, reduced activity of AChE or BChE in the plasma of burrowing owls in agricultural areas (compared to non-agricultural areas) could indicate exposure to OP and/or CB insecticides in those areas.

To determine potential internal exposure of adult and nestling burrowing owls to OP and CB insecticides, I collected whole blood via venipuncture of a wing vein with a small lancet. Blood was collected in microhematocrit capillary tubes and immediately transferred to polypropylene microcentrifuge tubes. I captured adults between 7 d prior to and 3 d after the predicted hatch date at their burrow. These captures occurred between 1200 h and 2400 h. I captured nestlings during the daytime at 20 d after hatching and again at 30 d after hatching. These captures generally occurred between

1000 h and 1900 h. Occasionally I recaptured adults during visits to capture nestlings and subsequently collected a second blood sample from those adults.

I collected approximately 200-300 µl of whole blood from each nestling and ultimately pooled blood from all nestlings in a nest to generate samples that contained 1.5 ml of blood per nest. Each nestling contributed an equal amount to the total sample. I was unable to re-sample nestlings at 30 d at n = 4 nests because these nests had either failed or possessed too few nestlings to produce a sample of sufficient size for analysis.

I temporarily stored and transported whole blood on ice until I returned to the laboratory at Boise State University, at which time blood plasma was isolated via centrifugation at 3000 rpm for 12 min. After isolation, plasma was stored at -80° C until laboratory analysis.

I analyzed all adult and nestling plasma samples for ChE activity. I performed these analyses at The Institute of Environmental and Human Health at Texas Tech University. From serum isolated from whole blood, I calculated the activity of AChE and BChE in each sample using the Ellman *et al.* (1961) method, with modifications summarized in Hunt and Hooper (1993).

Egg Collection and Analysis

One common measure of estimating exposure to and effects of OC pesticides in birds is to analyze OC concentrations in eggs. Breeding female birds exposed to contaminants such as OCs can transfer those contaminants into their eggs (Fimreite *et al.* 1982).

I defined **Early** eggs as any of the first three eggs laid in a clutch, while **Late** eggs were eggs that were laid after the fifth egg in a clutch. For pesticide analysis, I ultimately

collected one early or late egg from a clutch at nests in Agricultural, Non-Agricultural, and Intermediate burrows. I assumed that the collection of one egg did not affect fledgling numbers or the burrowing owl population in my study area, as typically owls lay more eggs than the number of young that ultimately fledge. For instance, in a study from 2006 to 2007 in my study area, Riding (2010) found that burrowing owl pairs had 6-11 eggs per clutch (mean eggs per nest = 8.8) and fledged between 1-10 young (mean number fledged per nest was approximately 4.8).

I attempted to collect eggs before incubation began, which I assessed by visual inspection of the eggs (e.g., incubated eggs often have spots or other pigmented portions and lose their pure white appearance) and by temperature (I considered eggs that were warm to the touch to have been incubated). Upon collection, I carefully wrapped eggs in aluminum foil and transported them to a Boise State University laboratory in an egg carton. Eggs were stored in a refrigerator (2.5° C) until processing.

Prior to pesticide analysis, I weighed and measured the length and breadth of each egg. Dimensions were measured using a Fowler digital caliper (accurate to 0.01 mm), and mass was recorded both in air and submerged in water for the purpose of estimating egg volume.

I visited each nest where eggs were sampled at 30 d after hatch to assess nest failure and productivity of each nest. I considered a nest to have failed if no nestlings were discovered in the nest or satellite burrow at this 30 d visit. I considered young to have fledged and the nest successful if nestlings were observed alive at this 30 d visit. I calculated productivity (number of nestlings fledged) for each nest sampled.

Pesticide Analysis

I removed egg contents by cutting away a minimal portion of the shell at the air cell of the egg with surgical scissors and pouring the contents into pre-washed, pre-labeled glass jars. Egg contents were then frozen (-20 °C) until analysis.

Analysis for residues of OC pesticides and their metabolites was performed on all collected eggs by the California Animal Health and Food Safety Laboratory at the University of California – Davis in the manner described in Holstege *et al.* (1994). Appendix A lists chemicals in the multi-residue screen and their quantification limits. The quantification limit for *p,p*^{1}-DDE is 0.1 parts per million (ppm). Thus, any concentration of *p,p*^{1}-DDE greater than or equal to 0.1 ppm is quantifiable, while concentrations of *p,p*^{1}-DDE less than 0.1 ppm are not quantifiable using this analytical method and are termed "trace" concentrations. Trace concentrations tell us that *p,p*^{1}-DDE is present in the sample, but exact concentrations are unknown and are <0.1 ppm.

Eggshell Thickness Analysis

Eggshell thinning has been correlated with DDT and its residues in raptor eggs and is one of the most well-known effects of DDT exposure on raptor reproduction (Johnstone *et al.* 1996, Peakall and Lincer 1996, Blus *et al.* 1997). Thus, I measured the eggshell thickness of all collected eggs and examined the relationship with OC pesticide exposure.

At the time of removal of egg contents, I labeled the exterior of eggshells with a fine-tipped Sharpie® and allowed eggshells to air-dry, along with attached membranes, for approximately six months. Once dry, I measured five randomly selected points along the equator of each eggshell and used the mean of these measurements in analyses

focused on eggshell thickness. I measured eggshell thickness using a Starrett digital micrometer (Model 734MXFL; accurate to 0.001 mm) modified for the concave shape of eggshells with the attachment of a ball bearing to the device's measuring surface. Measurements that I reported include membrane thickness.

Soil Sample Collection and Analysis

To evaluate the presence of DDT, its metabolites, or other OC pesticides in soil from burrowing owl breeding areas, I collected soil samples (15-20 g per sample) from 2-10 cm below the soil surface in 2008 near burrows from which I collected eggs. Upon collection, I placed samples in pre-washed, pre-labeled glass jars and kept them frozen (-20 °C) until analysis.

Chemical analysis of soil samples for residues of OC pesticides and their metabolites was performed by the California Animal Health and Food Safety Laboratory at the University of California – Davis in the manner described in Holstege *et al.* (1994). Appendix A lists chemicals in the multi-residue screen and their quantification limits.

Statistical Analysis

I performed all statistical analyses with SAS (V.9.2; SAS Institute, Cary, NC) and evaluated all statistical tests at an alpha level of 0.05. Means ± 1 SE are reported unless otherwise noted.

Blood Analysis

I used Generalized Estimating Equations (GEE), with observations clustered within nest burrow (i.e., the repeated subject) to compare ChE activity in blood serum from owls among Agricultural Classifications. As ChE levels in birds may differ seasonally, during different stages of breeding (Rattner and Fairbrother 1991), between

sexes (Rattner and Franson 1983, Hill 1989, Rattner and Fairbrother 1991), and by time of day (Rattner and Fairbrother 1991), I used correlations, one-way ANOVAs, and/or paired t-test to determine if Julian date, sex class (male or female), and/or time of sample collection needed to be included as covariates in final models examining effects of Agricultural Classification on AChE and BChE among adult owls. I found that ChE levels were affected by sex (see results), so I included sex and its potential interaction with Agricultural Classification in the final analysis. As I pooled blood samples for nestlings at each nest, I was unable to classify a sample as male or female. And, because nestling samples were all collected during the daytime, I examined only the potential effect of nestling age (20 or 30 d) on AChE and BChE and did so with paired t-tests. Because neither ChE differed between nestling age, I included both 20 d and 30 d samples in subsequent statistical analyses, while accounting for their non-independence by clustering on the variable "nest" in the GEE.

Additionally, I used simple linear regression to examine the relationship between distance of the sample to the nearest agricultural field and ChE activity levels in adults as well as in nestlings. I also examined AChE and BChE values from individual owls in Agricultural and Intermediate burrows for observations that were >2 SD of the mean value for the Non-agricultural classifications according to Hill (1988) and used in Wilson *et al.* (1991). Individual ChE activity values at Agricultural or Intermediate burrows that were below two standard deviations from the mean would be considered to have unusually low ChE activity. Thus, this examination had the potential to uncover even a small number of owl exposures that population level analyses might fail to detect.

Egg Analysis – OC Exposure

Using ordinal logistic regression (dependent variable categories: No detectable *p,p*^{1}-DDE, Trace *p,p*^{1}-DDE, and Quantifiable *p,p*^{1}-DDE), I examined the potential relationship between *p,p*^{1}-DDE concentrations in egg contents from different Agricultural Classifications with year (2007 vs. 2008) and laying order (Early vs. Late) as covariates. I chose this statistical analysis because the laboratory analytical method was not able to quantify *p,p*^{1}-DDE concentrations that were < 0.1 ppm, although such samples either did not have detectable levels or had trace concentrations of *p,p*^{1}-DDE.

Quantifiable p,p^1*-DDE Analyses* - I further used ANOVA to examine the potential effect of Agricultural Classification on quantifiable *p,p*^{1}-DDE (i.e., using only eggs with ≥ 0.1 ppm). Additionally, I used simple linear regression to examine the relationship between quantifiable *p,p*^{1}-DDE concentrations in egg contents and the distance of the sample to the nearest agricultural field. As *p,p*^{1}-DDE concentration data were not normally distributed, I analyzed log transformed as well as non-transformed values for analysis of eggs with quantifiable *p,p*^{1}-DDE. The inferences from each approach did not differ, so I report results from analyses of non-transformed values only.

Egg Analysis – Eggshell Thickness

Using Pearson correlation analyses, I examined potential relationships between eggshell thickness and (1) egg length, (2) breadth, (3) mass, and (4) volume, but found none (see results). I then used ANOVA to investigate if any of these dimensions differed by Agricultural Classification. I further compared eggshell thickness among Agricultural Classifications using ANOVA while including year and laying order as covariates. Following ANOVA, I made pair-wise comparisons between Agricultural Classification

means using the LSMEANS option in SAS. Additionally, I examined the relationship between eggshell thickness and DDE concentration using the Spearman's rank correlation analysis.

RESULTS

Footwash Sample Analysis – OP and CB Exposure

In 2007, I collected 107 footwash samples from 91 burrowing owls (n = 66 footwash samples from 54 female owls, and n = 41 footwash samples from 37 male owls). Of these, I submitted 15 samples and their associated blanks (controls) for chemical analysis: 12 owl footwash samples were from males (n = 8 from Agricultural burrows, n = 3 from Non-Agricultural burrows, and n = 1 from Intermediate burrows), and three were from females (n = 1 from an Agricultural burrow, Non-Agricultural burrow, and Intermediate burrow, respectively). There were no OP or CB insecticides detected in any of the 15 footwash samples or associated blanks.

Blood Sample Analysis – ChE Activity

In 2007, I collected 96 blood samples from adult burrowing owls (60 samples from 51 females and 36 samples from 33 males); thus, 12 owls were sampled twice (n = 9 females and n = 3 males). I collected 43 pooled blood samples from 20 d old nestlings at each of 43 nest burrows (mean number of nestlings sampled at a nest = 6.30 ± 0.26; Range = 3 to 9 nestlings) and 39 pooled blood samples from 30 d old nestlings at each of 39 nest burrows (mean number of nestlings sampled at a nest = 5.59 ± 0.34; Range = 1 to 9 nestlings).

Adult ChE Analysis

Temporal Variation

I found no correlation between Julian date and AChE activity (Spearman correlation analysis: r = -0.054, p = 0.732) or BChE activity (r = 0.069, p = 0.662). For the 12 adults that I sampled twice, mean AChE activity was significantly higher in the second sample (paired t-test: t_{11} = -3.70, p = 0.004). However, BChE activity was not significantly elevated in the second sample (paired t-test: t_{11} = -0.83, p = 0.423). In the first blood samples, mean AChE and BChE activity was 0.263 ± 0.044 µmoles/(min*ml) and 1.814 ± 0.132 µmoles/(min*ml), respectively. In second blood samples, mean AChE and BChE activity was 0.375 ± 0.061 µmoles/(min*ml) and 1.928 ± 0.139 µmoles/(min*ml), respectively. Because of elevated AChE activity in repeated samples, I used only the first sample collected from each owl in subsequent statistical analysis.

Sexual Variation

AChE activity was significantly greater in adult males than in adult females (ANOVA: $F_{1,82}$ = 11.91; p = 0.001; Figure 1). There was no significant difference in BChE activity between adult males and females (ANOVA: BChE: $F_{1,82}$ = 0.13, p = 0.722; Figure 2).

As ChE activity differed between sexes, I included sex as a covariate in subsequent analyses when appropriate. Additionally, because of likely differential foraging behavior between the sexes at the time of sampling (females were primarily incubating, whereas males were the primary foragers), I examined interactions between sex and Agricultural Classification in subsequent analyses.

Time of Day Variation

I observed no correlation between sample time of day and AChE activity (Spearman correlation analysis: $r = 0.091$, $p = 0.413$) or BChE activity ($r = -0.011$, $p = 0.922$). Therefore, I did not include time of sample as a covariate in subsequent analyses.

Adult ChEs by Agricultural Classification

When I examined the potential effects of Agricultural Classification and sex on AChE and BChE, these factors did not interact (Tables 1 and 2). Moreover, neither mean AChE nor BChE activity differed among the three Agricultural Classifications (Figures 3 and 4). There also was no significant relationship between AChE or BChE and the distance of the nest burrow to the nearest agricultural field (simple linear regression: $AChE = 0.282 + (9.199*10^{-8})*Distance$, $F_{1,82} = 0.00$, $p = 0.984$, $n = 84$; Figure 5a; $BChE = 1.877 + (1.853*10^{-5})*Distance$, $F_{1,82} = 1.14$, $p = 0.289$, $n = 84$; Figure 5b).

Individual Adult ChE Analysis

No plasma AChE or BChE activity levels from individual owls sampled at Agricultural or Intermediate burrows were more than two standard deviations below the mean AChE or BChE from the reference population (Table 3).

Nestling ChE Analysis

Temporal Variation

In the 39 burrows where I sampled nestlings at both 20 d and 30 d of age, mean AChE and BChE activity was 0.361 ± 0.019 μmoles/(min*ml) and 1.827 ± 0.057 μmoles/(min*ml) at 20 d, respectively; and 0.341 ± 0.025 μmoles/(min*ml) and 1.827 ± 0.062 μmoles/(min*ml) at 30 d for AChE and BChE, respectively. Neither AChE nor

BChE differed between the two ages (paired t-test: AChE: $t_{38} = 1.45$, $p = 0.156$; BChE: $t_{38} = -0.01$, $p = 0.996$).

Nestling ChEs by Agricultural Classification

Mean AChE and BChE activity in pooled nestling samples did not differ among the three Agricultural Classifications (Tables 4 and 5; Figures 6 and 7). There also was no significant relationship between any ChE and the distance of the nest burrow to the nearest agricultural field (simple linear regression: AChE = $0.370 – (5.440*10^{-6})$*Distance, $F_{1,42} = 1.17$, $p = 0.287$, $n = 43$; Figure 8a; BChE = $1.861 – (1.62*10^{-5})$*Distance, $F_{1,42} = 0.98$, $p = 0.331$, $n = 43$; Figure 8b).

Individual Burrow (pooled nestling samples) ChE Analysis

No plasma AChE or BChE activity levels from individual burrows sampled at Agricultural or Intermediate burrows were more than two standard deviations below the means of the reference population (Table 6).

Egg Analysis

I collected one egg from each of 29 burrowing owl nests in 2007 and 29 nests in 2008. These 58 eggs were analyzed for OC insecticide residues. Seven eggs collected in 2008 were from individual burrows from which I also collected an egg in 2007, but there was a different female at each between years. Three eggs collected in 2008 were removed from analysis because I subsequently discovered that they were laid by previously sampled females (i.e., I collected the eggs before confirming identification of nesting females at these 2008 nests). Therefore, 55 eggs were used in subsequent statistical analysis.

Of the three resampling events, two females had eggs sampled in 2007 and again in 2008. One of these resampled females did not have *p,p*^{1}-DDE detected in her eggs in each of 2007 and 2008. The other resampled female had 1.6 ppm and 1.3 ppm *p,p*^{1}-DDE in 2007 and 2008, respectively (Note: 1.6 ppm was the third-highest and 1.3 ppm was the fourth-highest value of any egg sampled in both years of my study).

Organochlorine Exposure

p,p^{1}-DDE was the only one of the 19 OC chemicals in the multi-residue screens detected in the 58 burrowing owl eggs submitted for analysis. There were 27 eggs (46.6%; n = 10 in 2007 and n = 17 in 2008) with detectable levels of *p,p*^{1}-DDE. There were 31 (53.4%) eggs where no *p,p*^{1}-DDE was detected. Among nests where *p,p*^{1}-DDE was detected (n = 27), an average of 4.19 ± 0.56 nestlings fledged per nest and five nests (18.5%) failed, while in nests where *p,p*^{1}-DDE was not detected (n = 31), 3.42 ± 0.46 nestlings fledged per nest and seven nests (22.6%) failed. Average productivity for all nests equaled 3.59 ± 0.46 owls per nest.

There was no relationship between *p,p*^{1}-DDE concentrations and Agricultural Classifications (Ordinal Logistic Regression; Table 7). Additionally, there was no significant difference in *p,p*^{1}-DDE concentrations between Agricultural Classifications when analyzing only samples with quantifiable *p,p*^{1}-DDE (samples with *p,p*^{1}-DDE concentrations $\geq$ 0.1 ppm) (ANOVA; Table 8; Figure 9). There also was no significant relationship between *p,p*^{1}-DDE and the distance of the nest burrow to the nearest agricultural field when analyzing only samples with quantifiable *p,p*^{1}-DDE (Simple Linear Regression: *p,p*^{1}-DDE = 0.296 + $(1.15*10^{-4})$*Distance, $F_{1,17} = 3.74$, p = 0.071, n = 18; Figure 10). Although this p-value approached significance, the relationship was in

the opposite direction than predicted; that is *p,p*^{1}-DDE increased with increasing distance from agriculture.

Eggshell Thickness

Egg Size and Eggshell Thickness

Mean egg (n = 58) length, breadth, mass, and volume were 32.35 ± 0.162 mm, 25.92 ± 0.109 mm, 11.54 ± 0.141 mm and 11.13 ± 0.131 mm, respectively. These means did not significantly differ among Agricultural Classifications (length: p = 0.957; breadth: p = 0.652; mass: p = 0.704; volume: p =0.545). Eggshell thickness (n = 58) was not correlated with length, breadth, mass, or volume (Pearson correlation analysis: Length: r = 0.025, p = 0.853; Breadth: r = -0.034, p = 0.803; Mass: r = 0.058, p = 0.667; Volume: r = -0.007, p = 0.958).

Differences in Thickness

Mean eggshell thickness (n = 55) varied among Agricultural Classifications when laying order (Early vs. Late) and year (2007 vs. 2008) were included as covariates (ANOVA: $F_{4,50} = 2.39$, p = 0.102; Table 9; Figure 11). Eggshells were significantly thinner at Agricultural burrows than at Non-agricultural burrows in post-hoc pair-wise comparisons of individual means (Table 10).

There was no significant relationship between eggshell thickness and the distance of the nest burrow to the nearest agricultural field (simple linear regression: Thickness = $0.185 + (2.007*10^{-7})$*Distance, $F_{1,49} = 0.16$, p = 0.687, n = 55; Figure 12).

Eggshell Thickness and DDE

When analyzing all samples regardless of *p,p*^{1}-DDE concentration (n = 55), eggshell thickness was not correlated with *p,p*^{1}-DDE concentrations (Pearson correlation

analysis: r = -0.132, p = 0.357; Figure 13a). There was a similar lack of relationship when I examined only eggs with quantifiable *p,p*^{1}-DDE (samples with *p,p*^{1}-DDE concentrations $\geq$ 0.1 ppm; n = 18; Pearson correlation analysis: r = -0.154, p = 0.541; Figure 13b). Further, there was no difference in eggshell thickness between eggs with *p,p*^{1}-DDE and eggs with none detected (ANOVA: $F_{1,54} = 0.44$, p = 0.509; Figure 14).

Soil Analysis

In 2008, I collected and submitted 25 soil samples for multi-residue screening for OC chemicals. No OCs or their metabolites were detected in these soil samples.

DISCUSSION

My hypothesis was that burrowing owls nesting in agricultural areas would contact pesticides more than owls nesting in non-agricultural areas. Therefore, I predicted that indicators of both external and internal exposure to pesticides would be greater in burrowing owls inhabiting agricultural areas. I also predicted that if exposure occurred, effects such as eggshell thinning would be realized more in agricultural areas compared to non-agricultural areas. Results to the contrary could possibly indicate that (1) there was little or no exposure of burrowing owls to pesticides occurring in my study area even when owls nested in agricultural areas, (2) there was no difference in exposure to pesticides between owls nesting in agricultural and non-agricultural areas because pesticides were pervasive and not restricted to agricultural lands, or (3) exposure occurred away from the owl's breeding grounds rather than when owls inhabited the NCA. My results seem most consistent with explanation 3, which I discuss below.

Footwash Sample Analysis – OP and CB Exposure

The fact that none of the footwash samples analyzed had OP or CB residues is not consistent with my prediction that burrowing owls within the NCA that nest in proximity to agricultural areas are at higher risk of exposure to OP or CB pesticides. However, this result remains tentative because I was only able to analyze 15 of the 91 footwash samples that I collected. Nonetheless, a large percentage of the samples I selected for analysis were from (1) male burrowing owls, which have higher potential exposure than females because of their movement patterns and increased hunting behavior, and (2) samples

from owls that nested nearest current agricultural operations. Therefore, based on the samples that I analyzed, there was no evidence of exposure. It remains possible that the owls that nested near agricultural areas were not foraging within the areas where pesticides had been applied; however, avoiding exposure in this manner seems unlikely and is inconsistent with findings by Gervais et al. (2003) where owls foraged in agricultural areas, and by Woodin *et al.* (2007), where burrowing owl pellets were collected from within agricultural areas, suggesting foraging was occurring in these areas. Importantly, Moulton et al. (2005) found burrowing owls in my study area making use of prey (montane voles, *Microtus montanus*) that occur primarily in irrigated agricultural situations as well as significantly more crickets (*Gryllus* spp.) in agricultural areas compared with diets of birds in non-agricultural areas.

Blood Sample Analysis – ChE Activity

Reduced ChE activity is a biomarker for pesticide exposure in birds and other wildlife (Ellman *et al.* 1961, Mineau 1991, Hunt and Hooper 1993). I found that there was no difference in plasma AChE or BChE activity levels for adult burrowing owls at Agricultural, Intermediate, and Non-agricultural burrows. Similarly, there were no differences in nestling AChE or BChE activity levels. Additionally, no adults or nestlings had unusually low plasma AChE or BChE activity. These results are consistent with the notion that no burrowing owls I studied in the NCA were affected by OP and/or CB insecticides. However, I observed a significant increase in AChE between the first and second samples in 12 adult owls that were re-sampled in the same season, between 17 and 31 days apart. Seasonal differences in natural ChE activities have been documented in other avian species (Rattner and Fairbrother 1991), thus it is possible that

the increase I observed reflects naturally changing ChE activity levels as the breeding season progresses. However, cholinesterase inhibition from some OP insecticides may cause decreased ChE activity for a month or more (Fairbrother *et al.* 1991), and it remains plausible that the increase in AChE activity I found later in the breeding season reflects diminishing effects from exposure of the adult owls to OP or CB insecticides prior to arriving to the NCA.

My findings that burrowing owls nesting in agricultural habitat in the NCA were not exposed to cholinesterase-inhibiting insecticides is contrary to Woodin *et al.* (2007), where burrowing owl pellets collected in and around agricultural areas in southern Texas contained residues of at least one OP and/or CB insecticide. My results are also somewhat surprising considering the findings of Mineau and Whiteside (2013) that pesticide application today still plays a major role in the decline of grassland bird populations. One possible explanation is that OP and CB insecticides were not applied to adjacent agricultural fields, or were applied, but outside of my sampling periods. Alternatively, it is possible that OP of CB insecticides were applied during my study, but the chemicals that were applied only inhibited ChE in sampled owls for brief periods of time, and ChEs had returned or begun to return to normal (i.e., ChEs were no longer inhibited) by the time I collected a sample. The duration of ChE inhibition depends on the insecticide (Fairbrother *et al.* 1991). For example, after exposure to some OP insecticides, ChE activity may take days or even months to measurably increase. Conversely, after exposure to some CB insecticides, ChE activity will naturally return and measurably increase as quickly as 30 minutes after exposure (Fairbrother *et al.* 1991). Unfortunately, I did not have access to pesticide application data in and around

the NCA that might shed some light on this. Nonetheless, my sample results indicate a lack of exposure of burrowing owls to these chemicals in the NCA.

Egg Analysis

Organochlorine Exposure

There was only one OC pesticide residue detected in burrowing owl eggs in my study area – *p,p*^{1}-DDE, which is a metabolite of DDT. This suggests that owls were not exposed to the other OC pesticides in the screen, and only a portion (46%) of the owls I sampled were exposed to *p,p*^{1}-DDE.

As *p,p*^{1}-DDE levels did not differ among Agricultural Classifications, and there was not a relationship between *p,p*^{1}-DDE concentrations in eggs and distance to the nearest agricultural field, proximity to agriculture during nesting was likely not a factor in exposure of a burrowing owl to *p,p*^{1}-DDE. Thus, my results do not support the hypothesis that burrowing owls are at a higher risk of exposure to OC pesticides when nesting in agricultural areas in the NCA.

We know that *p,p*^{1}-DDE is lipid soluble and retained in the fat tissues of a bird after exposure (Bernard 1966). Thus, any *p,p*^{1}-DDE excreted and detected in a female owl's egg could be from exposure that occurred months, or possibly even years, earlier. The results from females that I resampled in both years is consistent with this notion because resampled females showed similar *p,p*^{1}-DDE concentrations from year to year. None of the females I sampled in both years exhibited *p,p*^{1}-DDE exposure one year and zero *p,p*^{1}-DDE exposure the next, or vice versa. Thus, *p,p*^{1}-DDE exposure in an adult owl on the NCA could be reflective of that owl's exposure either during a previous nesting attempt, or exposure to *p,p*^{1}-DDE as a nestling. Similarly, *p,p*^{1}-DDE exposure in

an adult owl may also reflect its exposure to *p,p*^{1}-DDE while on migration or in its wintering areas. In contrast, Gervais *et al.* (2003) observed pronounced variation in *p,p*^{1}-DDE among eggs from the same females in different years in their study of burrowing owls in California.

Burrowing owls in Idaho are generally considered to be migratory. There have been a small number of recoveries of individuals banded in the NCA during the non-breeding season. These band returns have been from California (*J. Belthoff, pers. comm.*). Similarly, six of eight burrowing owls tracked using geolocators in southeastern Washington wintered in central or southern California (Washington Dept. of Fish and Wildlife 2013) and three burrowing owls (tracked using either geolocators or PTTs) from the Mountain Home Air Force Base, adjacent to my study area, wintered in Mexico (*C. Rudeen, pers. comm.*). Thus, at least some owls breeding in the NCA likely spend a portion of their winter or migration in California or Mexico. Despite being banned in 1973, DDT persisted for 20 years (Mischke *et al.* 1985, Odermatt *et al.* 1993) and still persists in the food chains of southern California and Mexico (Gervais *et al.* 2000, Yates *et al.* 2009). Gervais *et al*. (2000) studied burrowing owls in the San Joaquin Valley (at the Lemoore Naval Air Station and near the Pixley National Wildlife Refuge) and the Imperial Valley (in the Salton Sea National Wildlife Refuge) of southern California and documented *p,p*^{1}-DDE exposure in owls. In contrast to my results from Idaho where 46% of eggs had *p,p*^{1}-DDE, all but two burrowing owl eggs in Gervais *et al.*'s (2000) study contained *p,p*^{1}-DDE residues. Additionally, burrowing owl eggs from Lemoore Naval Air Station had a mean *p,p*^{1}-DDE concentration of 7.52 ppm, which was more than twice the maximum level I observed (3.50 ppm) for owls breeding in the NCA.

However, eggs in Pixley National Wildlife Refuge had an average *p,p*^{1}-DDE concentration of 1.19 ppm, while concentrations averaged 0.62 ppm at the Salton Sea National Wildlife Refuge (Gervais *et al.* 2000). Concentrations in the latter two areas are similar to those observed in my study area, when I considered only eggs exposed to *p,p*^{1}-DDE (i.e., when eggs with no detected *p,p*^{1}-DDE were left out).

I believe that the most plausible explanation for finding detectable levels of *p,p*^{1}-DDE in eggs from burrowing owls that nested in the NCA is that many of these owls migrated to other regions, such as southern California or Mexico. There, during the non-breeding season, they may have spent time in areas where contaminants are present in the environment and available for uptake into the burrowing owl food chain. As was observed in white-faced ibis migrating from Nevada (Yates *et al.* 2009), perhaps this exposure occurred in agricultural areas of southern California or Mexico where DDT was regularly applied prior to its ban. Owls that wintered in such regions or used them during migration then potentially returned to the NCA to breed, and nested at various distances from agricultural fields, at which time I sampled their eggs.

A second possibility is that the burrowing owl eggs that I collected in the NCA showed exposure to *p,p*^{1}-DDE because, instead of the owls themselves being exposed away from their breeding grounds, their prey were exposed to *p,p*^{1}-DDE away from the NCA. This seems plausible because burrowing owls do occasionally consume prey items that are migratory, such as various birds and various lepidopterans (Moulton *et al.* 2005; Poulin and Todd 2006; Valdez-Gomez *et al.* 2009). Exposure to and consumption of these prey items by burrowing owls may occur irrespective of proximity to agriculture.

A third possibility is that the burrowing owl eggs that I collected in the NCA showed exposure to *p,p*^{1}-DDE because they were layed by females that were exposed in areas outside the NCA and had recently moved into the study area. This is plausible because only nine of the sampled birds were those we had marked previously in the study area. The previously unmarked owls we sampled may have been exposed to *p,p*^{1}-DDE by nesting close to agricultural areas outside the NCA during a previous breeding season, but this would be unknown to me.

A fourth possible explanation for my results is that burrowing owls are being exposed to contaminated prey items during the breeding season.

Eggshell Thickness

I did not detect any differences in eggshell thickness among Agricultural Classifications, and eggshell thickness did not decrease with increased proximity to agricultural fields. This is consistent with the above findings that *p,p*^{1}-DDE did not occur in greater concentrations or at a higher frequency in agricultural areas. However, I noticed during post-hoc contrasts that eggshell thickness was significantly lower in eggs from Agricultural burrows than in eggs from Non-agricultural burrows. Although I predicted that eggshell thickness would be reduced at Agricultural burrows, I expected thickness differences to be a result of increased exposure to *p,p*^{1}-DDE, but that was not the case. Also, I did not detect a correlation between *p,p*^{1}-DDE and eggshell thickness. I conclude from these results that *p,p*^{1}-DDE exposure at Agricultural burrows did not cause a substantial decrease in eggshell thickness in my study area.

There are at least two possible alternative explanations for eggshells being thinner at Agricultural burrows. First, as agricultural habitat provides greater diversity of prey

items (Moulton *et al.* 2006), perhaps territories in agricultural areas are in higher demand and are occupied by the owls with the greatest competitive abilities. If true, it could mean that owls that nested in Agricultural burrows were older and more experienced, which allowed them to occupy the best territories. Independent of pesticide exposure, decreased eggshell thickness could be a result of older females occupying these Agricultural burrows, and laying eggs with thinner shells (Rayan *et al.* 2010). Alternatively, as burrowing owls nesting in agricultural areas within the NCA forage on a greater diversity of invertebrates (Moulton *et al.* 2005), perhaps a different diet at Agricultural burrows influenced the thickness of the burrowing owl eggshells. Diet has been shown to influence eggshell thickness in poultry (Bebout and Hempleman 1994; Jiang *et al.* 2014)

Eggshell Thickness and DDE

Although higher concentrations of *p,p*^{1}-DDE can cause reproductive failure or impairment (Porter and Wiemeyer 1969, Cade *et al.* 1971), there was no indication that concentrations of *p,p*^{1}-DDE I found in burrowing owl eggs in the NCA were causing reproductive harm. Specifically, Table 11, reproduced from Gervais *et al.* (2000), summarizes *p,p*^{1}-DDE concentrations that cause reproductive impairment of other avian species. All but one (3.5 ppm) of the *p,p*^{1}-DDE concentrations in burrowing owl eggs in the NCA were below all values listed in Table 11. However, as illustrated by the studies in Table 11, different avian species have differing levels of susceptibility to *p,p*^{1}-DDE exposure.

Additionally, in California where *p,p*^{1}-DDE concentrations in eggs were slightly higher than what I detected, Gervais and Anthony (2003) found no evidence that *p,p*^{1}-

DDE by itself lowered productivity of burrowing owls or led to the crushing of eggs under incubating females. This is consistent with productivity observations of burrowing owls in my study, where no eggs appear to have been crushed, and where nest failure rates and productivity were lower and higher, respectively, in nests when *p,p*^{1}-DDE was detected. The opposite results (higher failure rate and lower productivity) would be expected if *p,p*^{1}-DDE were negatively affecting reproduction in my studied owl population. Additionally, the nest in my study with the highest concentration of *p,p*^{1}-DDE (3.5 ppm) had concentrations high enough to cause decreased reproduction in prairie falcons (*Falco mexicanus;* Fyfe *et al.* 1976); however, this burrowing owl nest produced five young, which is greater than average in my study (average productivity = 3.6 young). Considering the above, it seems unlikely that *p,p*^{1}-DDE concentrations were impairing productivity in my study area.

Reduced food consumption, however, could act synergistically with *p,p*^{1}-DDE concentrations to reduce reproduction in birds (Keith and Mitchell 1993). Gervais and Anthony (2003) concluded that reduced food consumption in synergy with *p,p*^{1}-DDE concentrations caused some level of reproductive impairment in burrowing owls in southern California. Thus, in certain years of low food availability or when other environmental stressors are present, *p,p*^{1}-DDE may be one of several factors that, in combination, could lead to reproductive impairment. To more precisely determine the extent of reproductive impacts of *p,p*^{1}-DDE, a long-term study that investigates more than *p,p*^{1}-DDE residues in burrowing owls, and includes consideration of interactions with other potential stressors such as prey availability, human disturbance, and/or changes in climate, may be necessary.

Summary and Conclusions

I found no evidence that burrowing owls in the Morley Nelson Snake River Birds of Prey National Conservation Area were regularly exposed to OP or CB insecticides despite nesting near agricultural fields. However, I discovered that a subset of burrowing owls nesting in the NCA were exposed to *p,p*^{1}-DDE, which is a metabolite of DDT. Levels did not appear sufficient to be contributing to declines in eggshell thickness or to reproductive failure, and *p,p*^{1}-DDE in burrowing owl eggs did not differ significantly in agricultural and non-agricultural areas of the NCA. However, even low levels of *p,p*^{1}-DDE may affect reproduction when certain environmental conditions are present (Gervais and Anthony 2003). The pattern of *p,p*^{1}-DDE exposure and the fact that I did not detect *p,p*^{1}-DDE in soil samples near owl nest burrows suggest that *p,p*^{1}-DDE exposure occurred outside of the breeding season, e.g., either when owls were migrating, on their wintering grounds, or both. Albeit limited, available data suggest that owls that breed in the NCA migrate south for winter, and some have been relocated in regions of southern California where other studies (Gervais *et al.* 2000, Gervais and Anthony 2003) demonstrate that a large proportion of resident breeding individuals are exposed to *p,p*^{1}-DDE through their association with agricultural areas. It seems likely that some owls that breed in NCA are migrating to and wintering in southern California, and other areas with a history of DDT use, e.g., Mexico, where they are being exposed to *p,p*^{1}-DDE, which has remained persistent since the ban of DDT.

As I found no evidence of significant exposure of burrowing owls to OP or CB insecticides while nesting in the NCA, and as *p,p*^{1}-DDE concentrations were low relative to endpoints implicated in reproductive impairment for other species, there was no

evidence to support the hypothesis that pesticides were causing harm to burrowing owl populations in the NCA. Thus, in contrast to many other grassland bird species that are experiencing negative effects of agriculture (Mineau and Whiteside 2013), burrowing owls are persisting near agricultural areas in the NCA. We know that agricultural areas in the NCA provide a rich food source for burrowing owls, and owls in agricultural areas do not suffer increased predation or decreased access to nest burrows. By nesting in higher densities near agriculture, owls also have the potential to detect predators better through increased vigilance and to cooperate in defense against predators (Welty 2010). My study provides information about one potential cost of nesting in agricultural areas, e.g., increased pesticide exposure. In the NCA, where irrigated agriculture makes up only 5% of the land cover, there is no evidence that pesticide exposure in the NCA poses a threat to burrowing owls. Of course, any changes in land use or pesticide use in the NCA could alter these relationships. Monitoring for these changes in the NCA should be encouraged. Outside of the NCA, where crop types, percentage of agricultural land, and pesticide application regimes may differ, future investigations may be needed to shed some light on exposure to and impacts of pesticides to burrowing owls in those areas.

Further, there are many potential indirect impacts of pesticide use that could occur, including reduced prey availability, reduced prey diversity, impacts to predators, changes in native vegetation, and others that my study did not investigate. Future investigations of the relationships among these and other indirect factors might paint a more complete picture of potential impacts of pesticides on burrowing owl populations, both within the NCA of southern Idaho and throughout its range. Additionally, future investigations on the mechanisms of pesticide exposure to migrating burrowing owls are

needed during the non-breeding season and in areas where owls are known to migrate and winter.

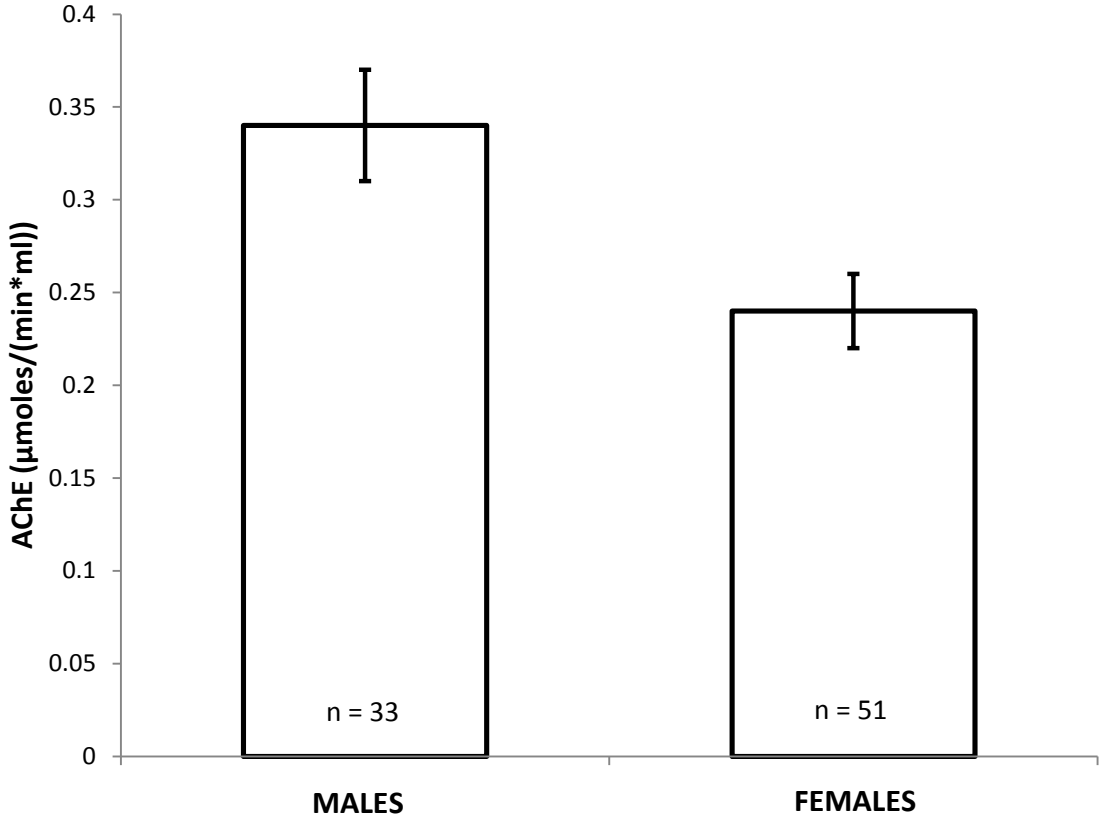

Figure 1. Mean (± SE) plasma AChE levels (µmoles/(min*ml) in adult male and female burrowing owls sampled in southwestern Idaho in 2007. Range for males = 0.128 – 0.672 µmoles/(min*ml). Range for females = 0.060 – 0.671 µmoles/(min*ml). Sample size for each sex is indicated.

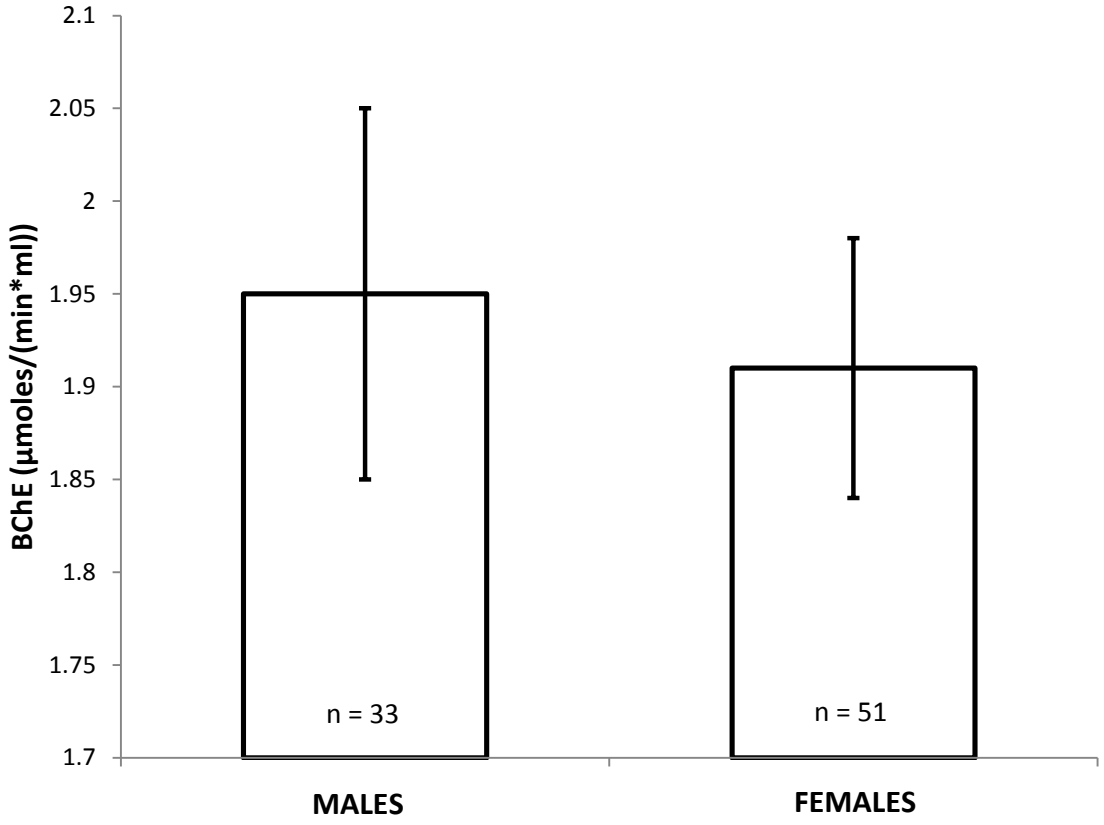

Figure 2. Mean (± SE) plasma BChE levels (µmoles/(min*ml) by sex of adult burrowing owls sampled in southwestern Idaho in 2007. Range for males = 0.941 – 3.081 µmoles/(min*ml). Range for females = 1.168 – 3.712 µmoles/(min*ml). Sample size for each sex is indicated.

Table 1. Results of GEE analysis of plasma AChE (n = 84) as a function of sex (male vs. female), Agricultural Classification (Agricultural [A], Intermediate [I], or Non-agricultural [N]), and their interaction for burrowing owls nesting in southwestern Idaho in 2007. Observations were clustered within nest burrow (n = 52), i.e., samples collected from adults associated with the same burrow were analyzed as repeated measures.

Parameter	Effect	Estimate	SE	95% CI[1]		Z	P
				Lower	Upper		
Intercept		0.3208	0.0412	0.2401	0.4014	7.79	<0.0001
Sex	Female (F)	-0.0705	0.0380	-0.1450	0.0041	-1.85	0.0638
Sex	Male (M)	0.0000	0.0000	0.0000	0.0000	-	-
Ag Classification	Ag (A)	0.0531	0.0577	-0.0600	0.1662	0.92	0.3577
Ag Classification	Intermediate (I)	0.0003	0.0625	-0.1228	0.1221	-0.01	0.9956
Ag Classification	Non-Ag (N)	0.0000	0.0000	0.0000	0.0000	-	-
Sex*Habitat	F * A	-0.0540	0.0637	-0.1789	0.0709	-0.85	0.3968
Sex*Habitat	M * A	0.0000	0.0000	0.0000	0.0000	-	-
Sex*Habitat	F * I	-0.0387	0.0582	-0.1528	0.0754	-0.66	0.5063
Sex*Habitat	M * I	0.0000	0.0000	0.0000	0.0000	-	-
Sex*Habitat	F * N	0.0000	0.0000	0.0000	0.0000	-	-
Sex*Habitat	M * N	0.0000	0.0000	0.0000	0.0000	-	-

[1] 95% confidence interval for the parameter estimate.

Table 2. Results of GEE analysis of plasma BChE (n = 84) as a function of sex (male vs. female) Agricultural Classification (Agricultural [A], Intermediate [I], or Non-agricultural [N]), and their interaction for burrowing owls nesting in southwestern Idaho in 2007. Observations were clustered such that individual nest burrow site (n = 52) was repeated, i.e., samples collected from adults associated with the same burrow were analyzed as repeated measures.

Parameter	Effect	Estimate	SE	95% CI[1]		Z	P
				Lower	Upper		
Intercept		2.0858	0.1842	1.7248	2.4468	11.33	<0.0001
Sex	Female (F)	-0.2053	0.2423	-0.6801	0.2696	-0.85	0.3968
Sex	Male (M)	0.0000	0.0000	0.0000	0.0000	-	-
Ag Classification	Ag (A)	-0.1946	0.2244	-0.6344	0.2452	-0.87	0.3857
Ag Classification	Intermediate (I)	-0.2906	0.2386	-0.7582	0.1771	-1.22	0.2233
Ag Classification	Non-Ag (N)	0.0000	0.0000	0.0000	0.0000	-	-
Sex*Habitat	F * A	0.2823	0.2787	-0.2640	0.8286	1.01	0.3112
Sex*Habitat	M * A	0.0000	0.0000	0.0000	0.0000	-	-
Sex*Habitat	F * I	0.2363	0.2831	-0.3185	0.7912	0.83	0.4038
Sex*Habitat	M * I	0.0000	0.0000	0.0000	0.0000	-	-
Sex*Habitat	F * N	0.0000	0.0000	0.0000	0.0000	-	-
Sex*Habitat	M * N	0.0000	0.0000	0.0000	0.0000	-	-

[1] 95% confidence interval for the parameter estimate.

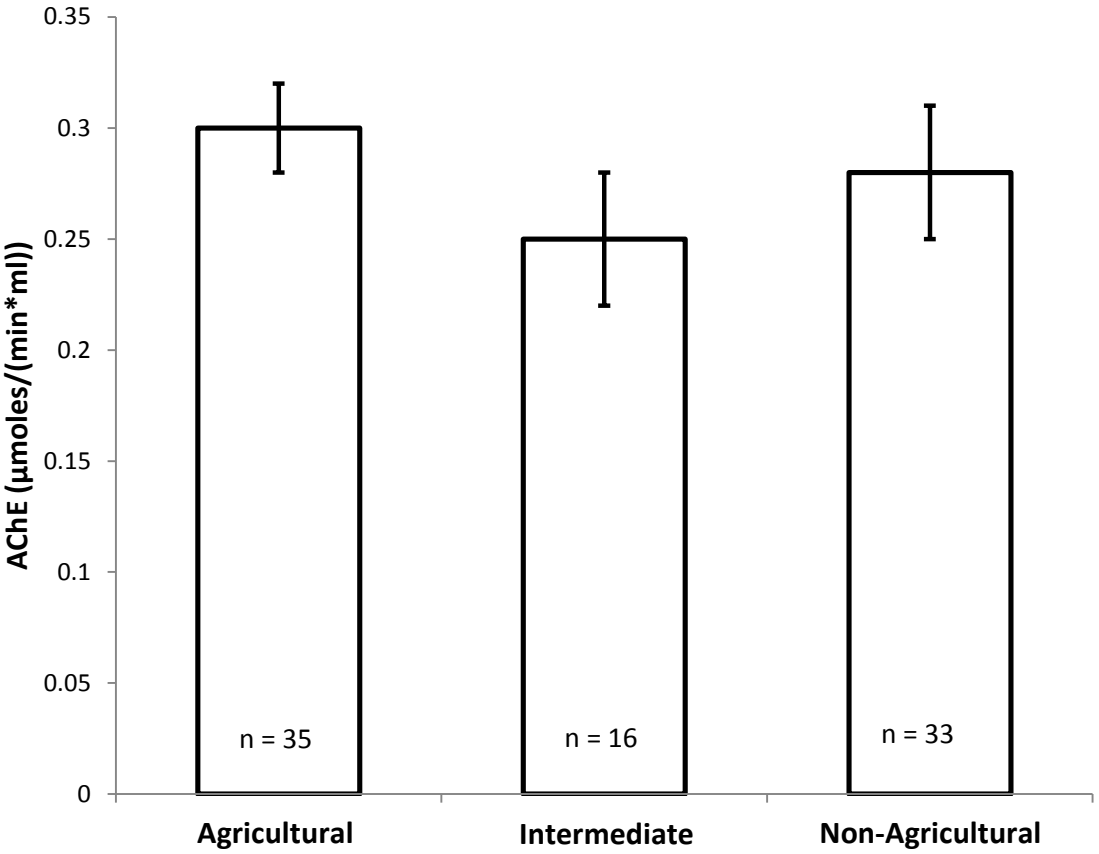

Figure 3. Mean (± SE) plasma AChE levels (µmoles/(min*ml) by Agricultural Classification of adult burrowing owls sampled in southwestern Idaho in 2007. Ranges for Agricultural burrows = 0.060 – 0.591 µmoles/(min*ml), Non-agricultural burrows = 0.120 – 0.672 µmoles/(min*ml), and Intermediate burrows = 0.127 – 0.505 µmoles/(min*ml). Sample size for each Agricultural Classification is indicated.

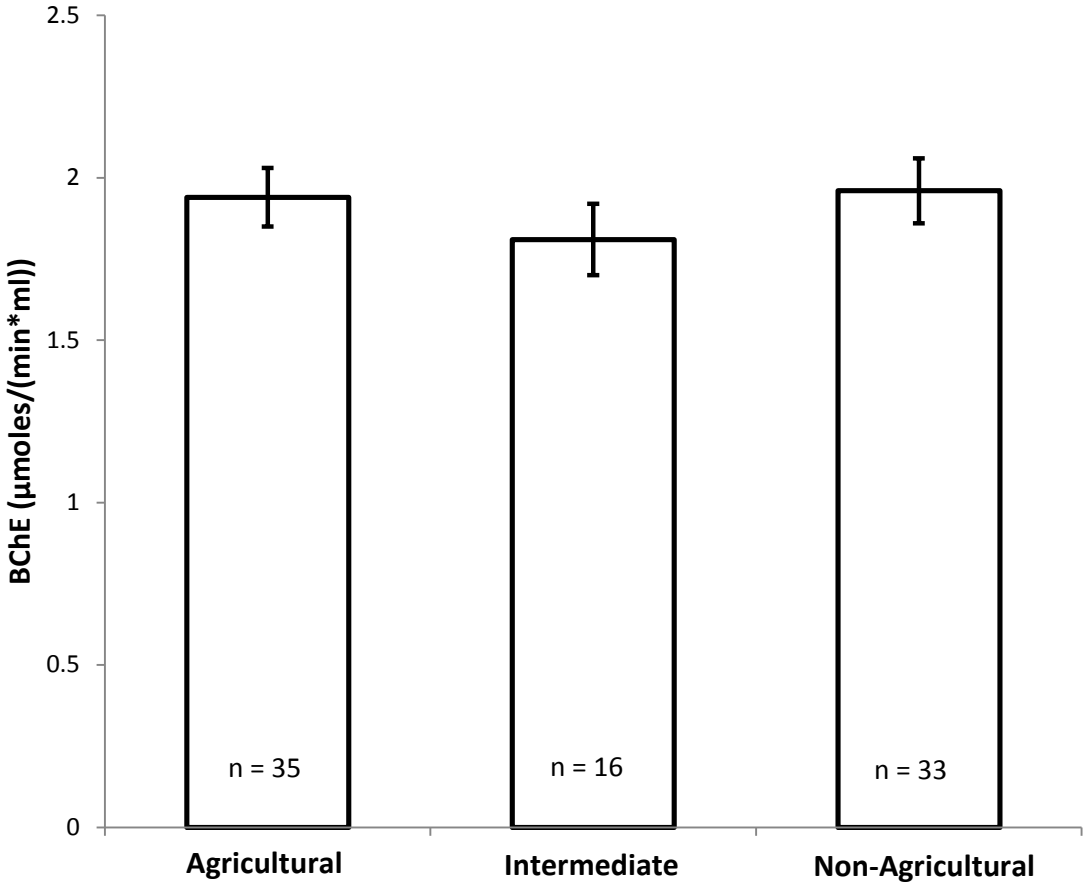

Figure 4. Mean (± SE) plasma BChE levels (µmoles/(min*ml) by Agricultural Classification of adult burrowing owls sampled in southwestern Idaho in 2007. Ranges for Agricultural burrows = 0.941 – 3.712 µmoles/(min*ml), Non-agricultural burrows = 1.050 – 3.081 µmoles/(min*ml), and Intermediate burrows = 1.189 – 2.718 µmoles/(min*ml). Sample size for each Agricultural Classification is indicated.

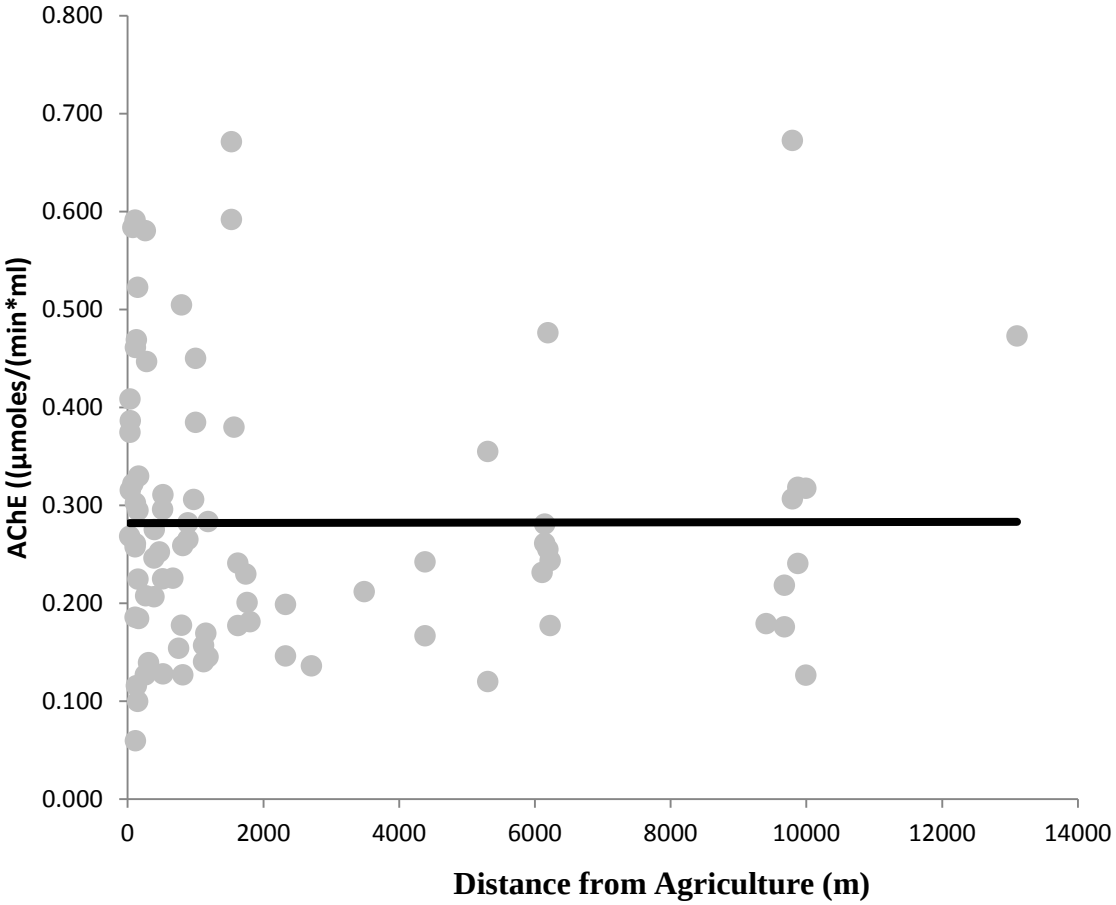

Figure 5a. Relationship between distance to agriculture (m) and plasma AChE levels (µmoles/(min*ml) in adult burrowing owls sampled in southwestern Idaho in 2007. Least squares regression line is shown, but there was no significant relationship detected (AChE = 0.282 + ($9.199*10^{-8}$)*Distance, $F_{1,82} = 0.00$, $p = 0.984$, $n = 84$).

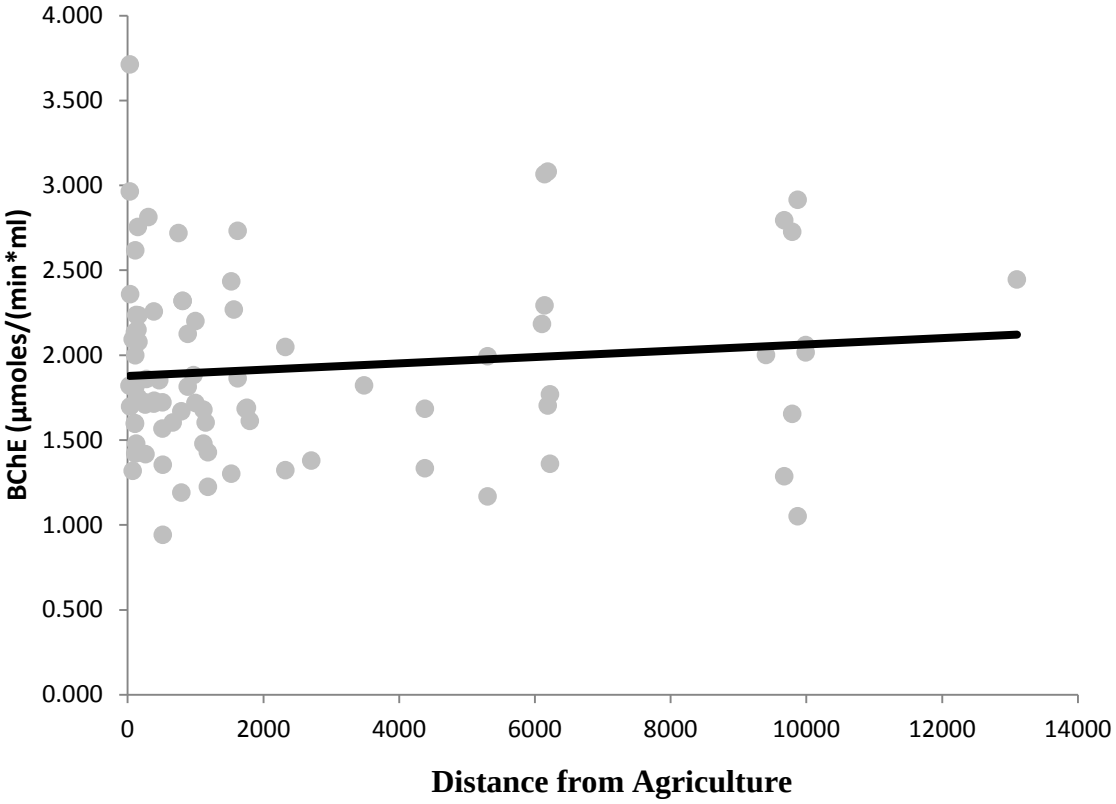

Figure 5b. Relationship between distance to agriculture (m) and plasma BChE levels (µmoles/(min*ml) in adult burrowing owls sampled in southwestern Idaho in 2007. Least squared regression line is shown, but there was no significant relationship detected (BChE = 1.877 + ($1.853*10^{-5}$)*Distance, $F_{1,82} = 1.14$, $p = 0.289$, $n = 84$).

Table 3. Plasma cholinesterase activity levels ($\bar{x}$ ± SD) for adult male and female burrowing owls breeding in non-agricultural burrows (reference population) and in Agricultural and Intermediate burrows in southwestern Idaho in 2007. Table shows the number of individuals below, within, and above the reference interval[1]. Individual owls at Agricultural and Intermediate burrows that exhibited ChE activity outside and below the reference interval may have been exposed to cholinesterase-inhibiting pesticides.

		Reference Population					Agricultural Population						Intermediate Population					
					Interval (± 2SD)[1]					Relative to Reference Interval						Relative to Reference Interval		
		N	Mean	SD	Lower	Upper	N	Mean	SD	# Below	# Within	# Above	N	Mean	SD	# Below	# Within	# 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

[1] Reference interval = ± 2SD from the mean in non-agricultural burrows.

Table 4. Results of GEE modeling for plasma AChE (n = 82) in nestling burrowing owls as a function of Agricultural Classification in southwestern Idaho in 2007. Individual burrows (n = 43) were treated as clusters; burrows sampled at both 20d and 30d were analyzed as repeated measures.

Parameter	DF	Estimate	SE	95% CI[1]		Z	P
				Lower	Upper		
Intercept	1	0.3476	0.0332	0.2825	0.4128	10.47	<0.0001
Ag Classification – Ag	1	0.0086	0.0457	-0.0810	0.0982	0.19	0.8512
Ag Classification - Intermediate	1	-0.0143	0.0419	-0.0963	0.0677	-0.34	0.7328
Ag Classification – Non-Ag	0	0.0000	0.0000	0.0000	0.0000	-	-

[1] 95% confidence interval for the parameter estimate.

Table 5. Results of GEE modeling for plasma BChE (n = 82) in nestling burrowing owls as a function of Agricultural Classification in southwestern Idaho in 2007. Individual burrows (n = 43) were treated as clusters; burrows sampled at both 20d and 30d were analyzed as repeated measures.

Parameter	DF	Estimate	SE	95% CI[1]		Z	P
				Lower	Upper		
Intercept	1	1.7885	0.0903	1.6116	1.9655	19.81	<0.0001
Ag Classification - Ag	1	-0.0107	0.1196	-0.2452	0.2237	-0.09	0.9286
Ag Classification - Intermediate	1	0.1945	0.1388	-0.0774	0.4665	1.40	0.1609
Ag Classification – Non-Ag	0	0.0000	0.0000	0.0000	0.0000	-	-

[1] 95% confidence interval for the parameter estimate.

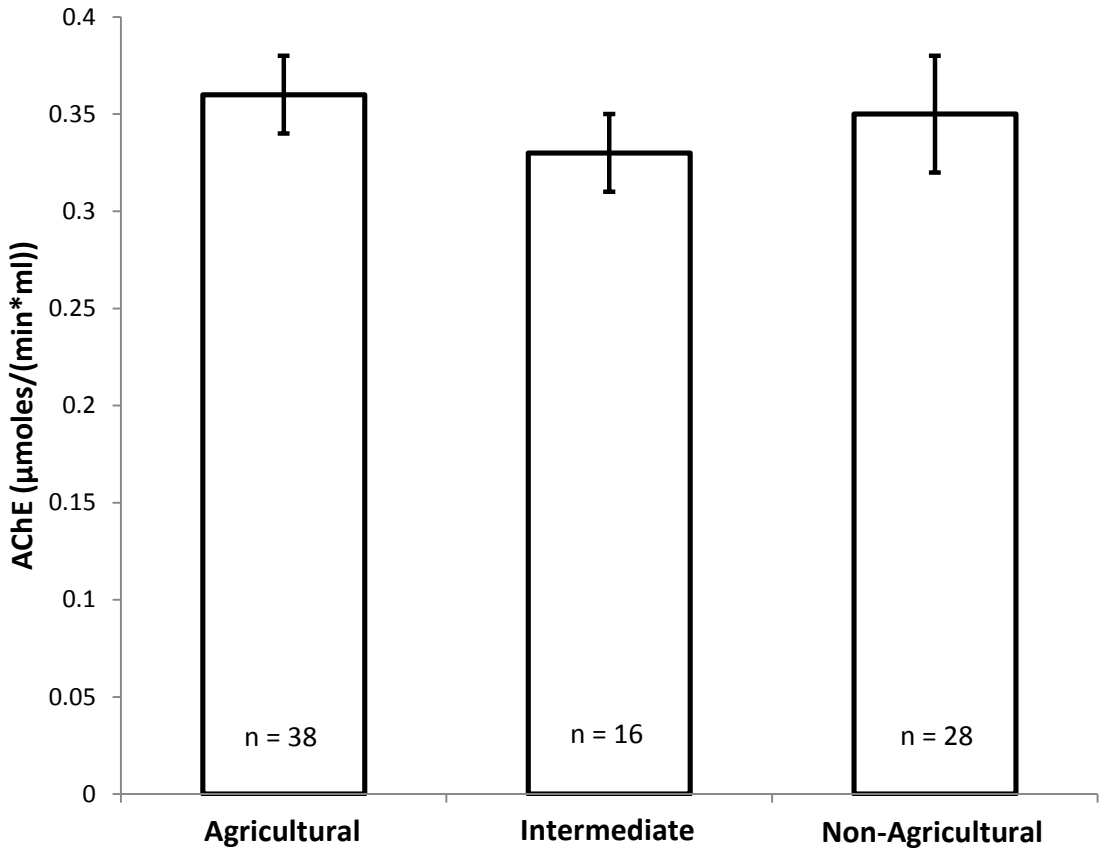

Figure 6. Mean (± SE) plasma AChE levels (µmoles/(min*ml) by Agricultural Classification of nestling burrowing owl samples (pooled within each nest) in southwestern Idaho in 2007. Ranges for Agricultural burrows = 0.179 – 0.841 µmoles/(min*ml), Non-agricultural burrows = 0.173 – 0.683 µmoles/(min*ml), and Intermediate burrows = 0.234 – 0.538 µmoles/(min*ml). Sample size (number of nests) for each Agricultural Classification is indicated.

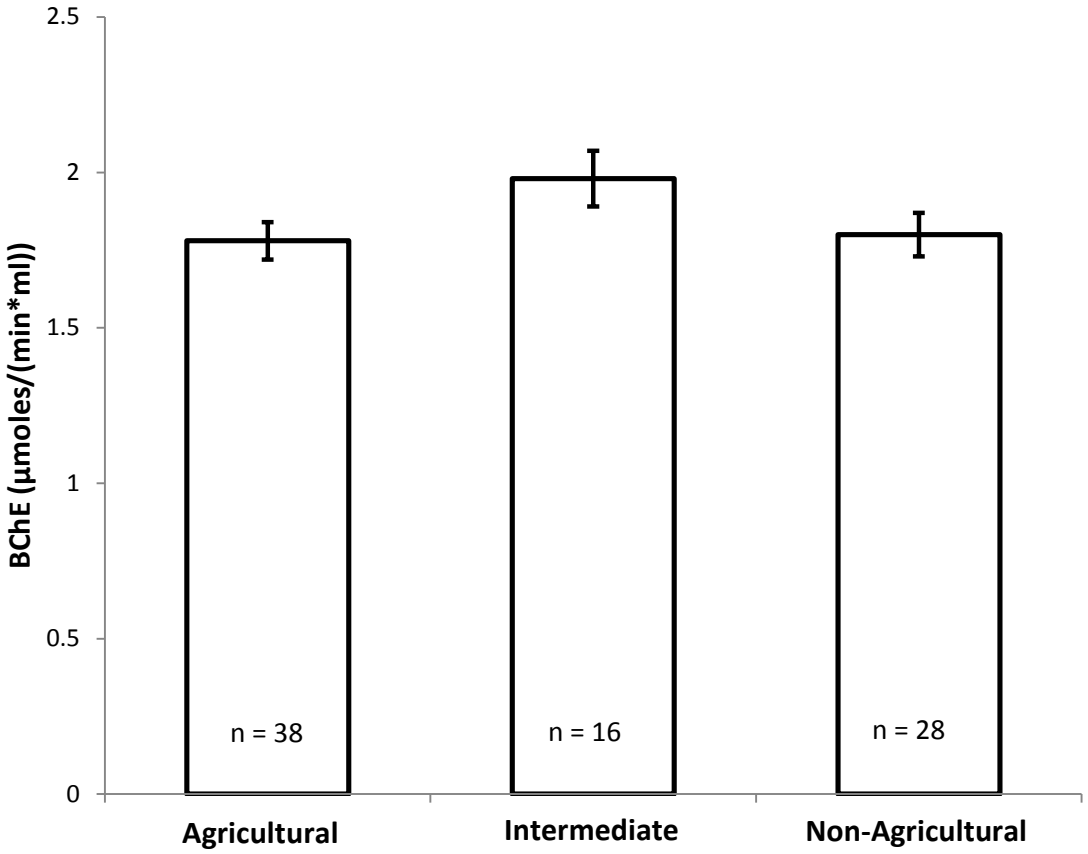

Figure 7. Mean (± SE) plasma BChE levels (µmoles/(min*ml) by Agricultural Classification of nestling burrowing owl samples (pooled within each nest) in southwestern Idaho in 2007. Ranges for Agricultural burrows = 1.261 – 3.009 µmoles/(min*ml), Non-agricultural burrows = 1.188 – 2.612 µmoles/(min*ml), and Intermediate burrows = 1.242 – 2.541 µmoles/(min*ml). Sample size (number of nests) for each Agricultural Classification is indicated.

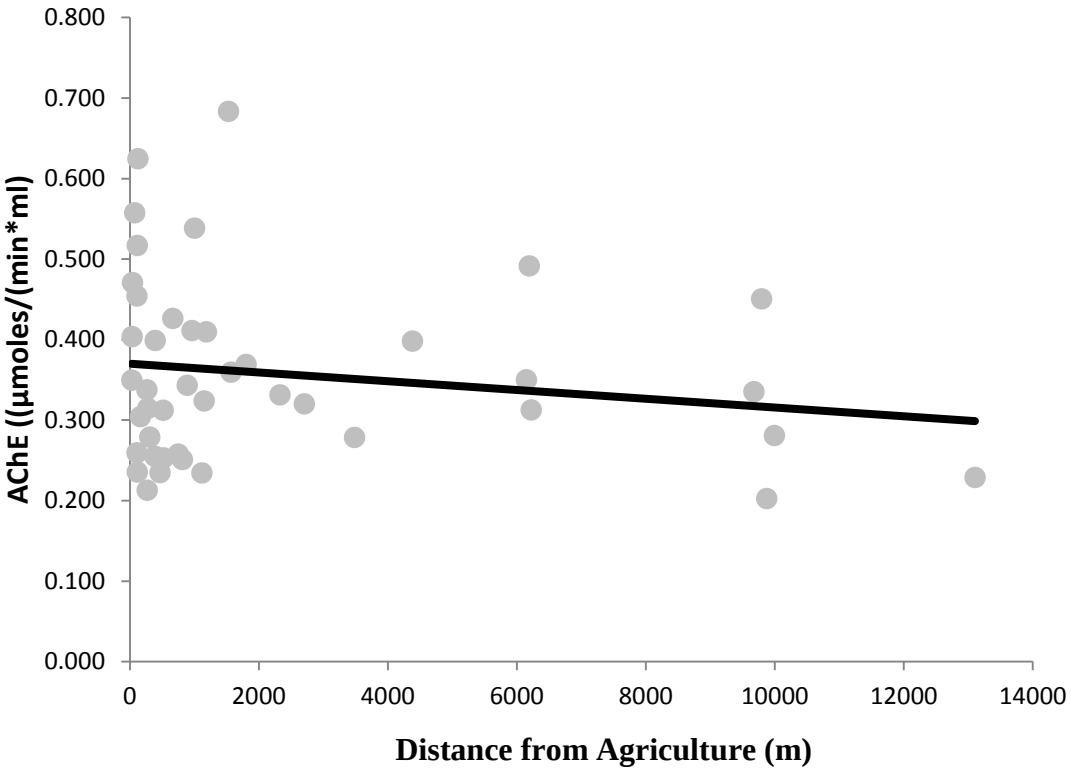

Figure 8a. Relationship between distance to agriculture (m) and pooled nestling AChE levels (µmoles/(min*ml) in nestling burrowing owls sampled in southwestern Idaho in 2007. No significant relationship was detected (AChE = 0.370 – ($5.440*10^{-6}$)*Distance, $F_{1,42} = 1.17$, $p = 0.287$, $n = 43$).

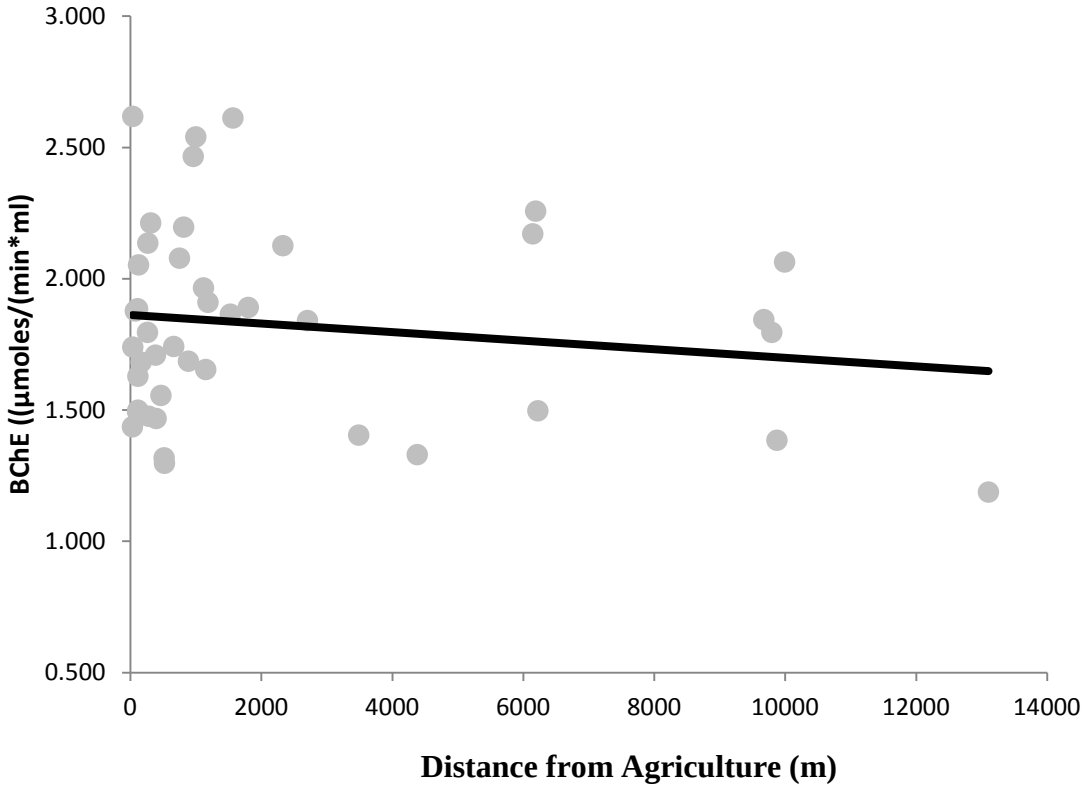

Figure 8b. Relationship between distance to agriculture (m) and pooled nestling BChE levels (µmoles/(min*ml) in nestling burrowing owls sampled in southwestern Idaho in 2007. No significant relationship was detected (BChE = 1.861 – $(1.62*10^{-5})$*Distance, $F_{1,42}$ = 0.98, p = 0.331, n = 43).

Table 6. Plasma cholinesterase activity levels ($\bar{x}$ ± SD) for pooled samples of burrowing owl nestlings from Non-Agricultural burrows (reference population) and from Agricultural and Intermediate burrows in southwest Idaho in 2007. Table shows the number of pooled samples below, within, and above the reference interval[1]. Individual pooled nestling samples from Agricultural and Intermediate burrows that exhibit cholinesterase activity outside and below the reference interval may have been exposed to cholinesterase-inhibiting insecticides.

	Reference Population					Agricultural Population						Intermediate Population					
				Interval (±2SD)[1]					Relative to Reference Interval						Relative to Reference Interval		
	N	Mean	SD	Lower	Upper	N	Mean	SD	# Below	# Within	# Above	N	Mean	SD	# Below	# Within	# Above
AChE	28	0.35	0.14	0.07	0.63	38	0.36	0.15	0	36	2	16	0.33	0.09	0	16	0
BChE	28	1.80	0.37	1.06	2.54	38	1.78	0.37	0	36	2	16	1.98	0.36	0	15	1

[1] Reference interval = ± 2SD from the mean in non-agricultural burrows.

Table 7. Results of ordinal logistic regression model of p,p^1-DDE (cumulative logit model; n = 55) as a function of Agricultural Classification, year (2007 vs. 2008), and laying order (Early vs. Late) in southwestern Idaho in 2007 and 2008.

Parameter		**DF**	**Estimate**	**SE**	**Wald Chi-Square**	**Pr > ChiSq**
Intercept	3	1	-2.0924	0.8544	5.9980	0.0143
Intercept	2	1	-1.4801	0.8299	3.1807	0.0745
Ag Classification		1	0.1073	0.3042	0.1244	0.7243
Year	2008	1	0.0142	0.5671	3.1979	0.0737
Laying Order	Late	1	1.3456	0.5760	5.4566	0.0195

Table 8. Analysis of variance for *p,p*[1]-DDE (n = 18) as a function of Agricultural Classification, year (2007 vs. 2008), and laying order (Early vs. Late) of burrowing owl egg samples with quantifiable *p,p*[1]-DDE concentrations (greater than or equal to 0.1 ppm) in southwestern Idaho in 2007 and 2008.

Source	**DF**	**SS**	**Mean Square**	**F Value**	**Pr > F**
Ag Classification	2	0.5424	0.2712	0.26	0.7748
Year	1	0.0221	0.0221	0.02	0.8870
Laying Order	1	0.1268	0.1268	0.12	0.7342
Ag Classification * Year	1	0.5202	0.5202	0.50	0.4959
Ag Classification * Laying Order	2	0.3792	0.1896	0.18	0.8354
Year * Laying Order	1	0.0704	0.0704	0.07	0.7999
Error	9	9.2983	1.0331		
Total	17	13.2444			

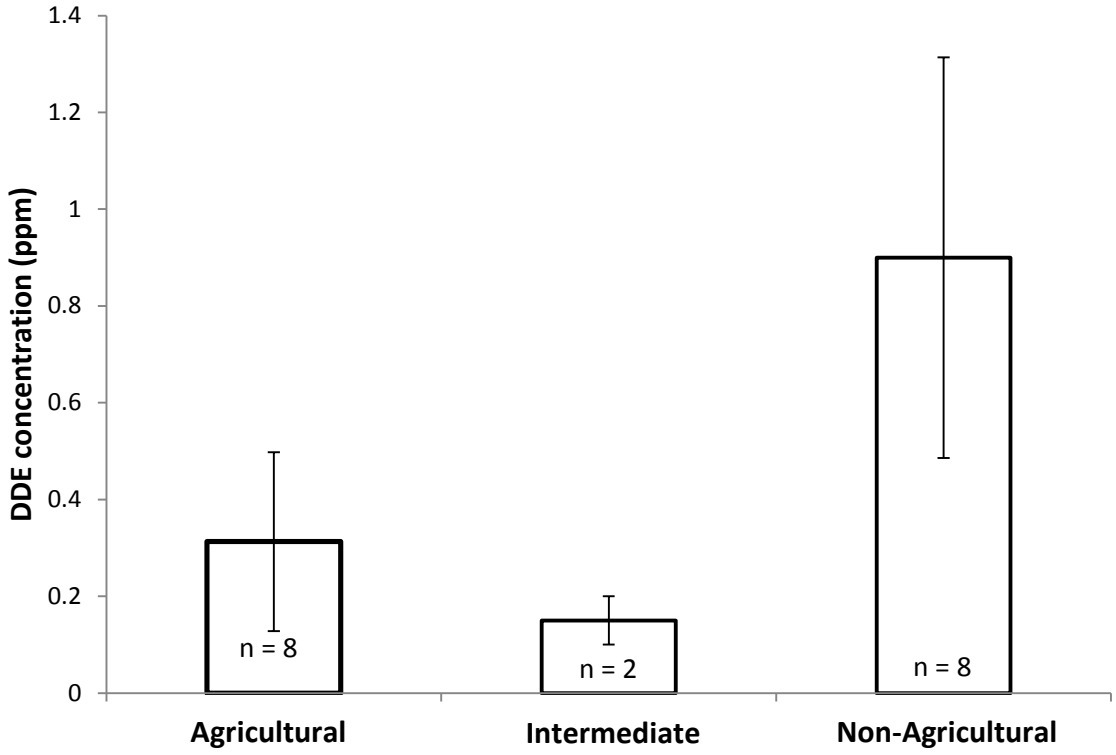

Figure 9. Mean (± SE) *p,p*[1]-DDE (ppm) by Agricultural Classification of burrowing owl egg samples with quantifiable *p,p*[1]-DDE concentrations (e.g., greater than or equal to 0.1 ppm; see text for explanation) in southwestern Idaho in 2007 and 2008. Ranges for Agricultural burrows = 0.1 – 1.6 ppm, Non-agricultural burrows= 0.1 – 3.5 ppm, and Intermediate burrows = 0.1 – 0.20 ppm. Sample size for each Agricultural Classification is indicated.

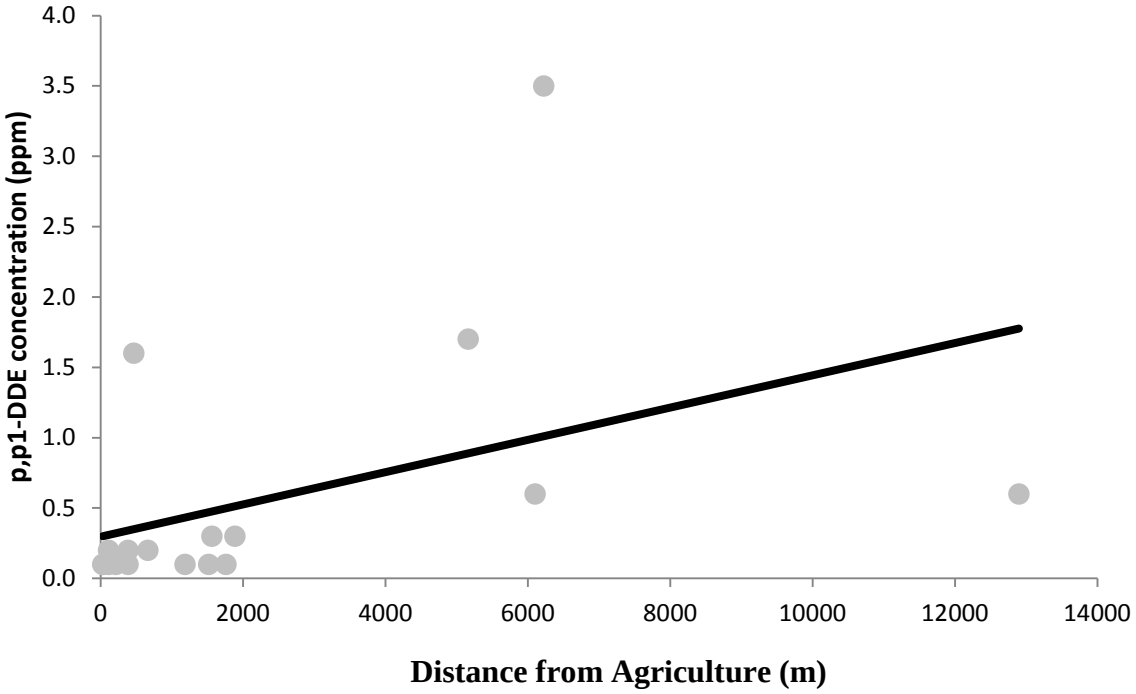

Figure 10. Relationship between distance to agriculture (m) and *p,p*^{1}-DDE concentration (ppm) in burrowing owl eggs in southwestern Idaho in 2007 and 2008. No significant relationship was detected (*p,p*^{1}-DDE = 0.296 + ($1.15*10^{-4}$)*Distance, $F_{1,17}$ = 3.74, p = 0.071, n = 18).

Table 9. Results of analysis of variance (ANOVA) for eggshell thickness (n = 55) as a function of Agricultural Classification, laying order (Early vs. Late) and year (2007 vs. 2008) in southwestern Idaho in 2007 and 2008.

Source	**DF**	**SS**	**Mean Square**	**F Value**	**Pr > F**
Laying Order	1	0.00079	0.00079	6.22	0.0160
Year	1	0.00021	0.00021	1.65	0.2043
Ag Classification	2	0.00060	0.00030	2.39	0.1024
Error	50	0.0063	0.00013		
Total	54	0.0082			

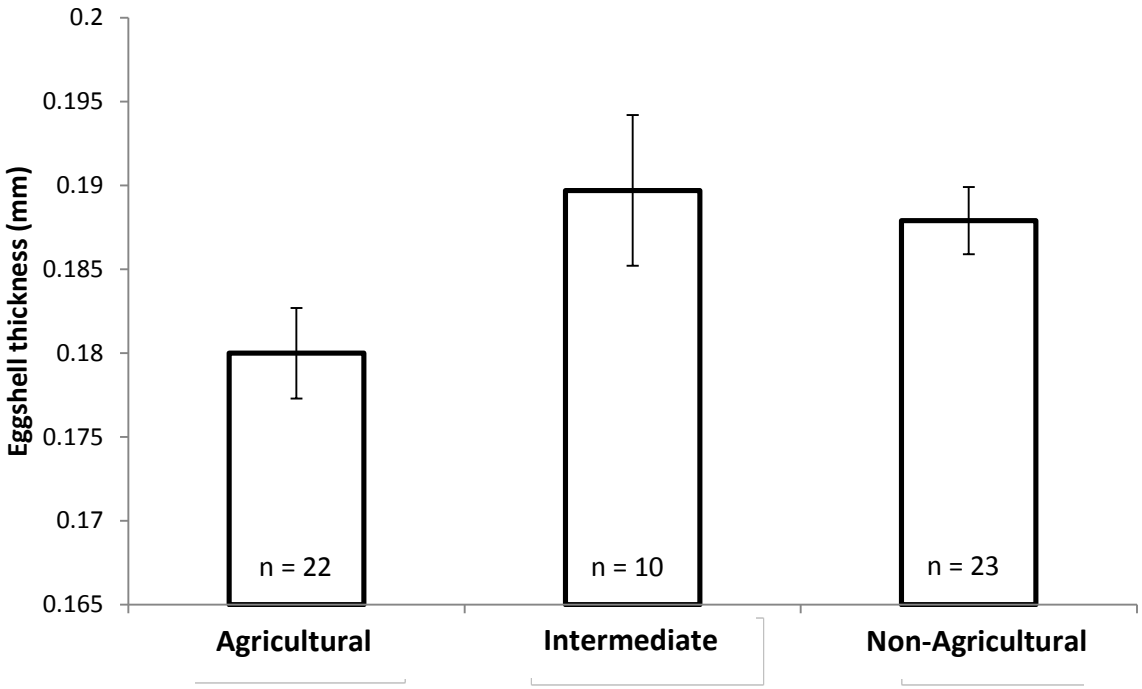

Figure 11. Eggshell thickness ($\bar{x}$ ± SE mm) by Agricultural Classification of burrowing owl egg samples in southwestern Idaho in 2007 and 2008. Ranges for Agricultural burrows = 0.157 – 0.207 mm, Non-agricultural burrows = 0.164 – 0.207 mm, and Intermediate burrows = 0.168 – 0.215 mm. Sample size for each Agricultural Classification is indicated.

Table 10. Results of post-hoc contrasts between Agricultural Classifications for eggshell thickness (n = 55). All possible contrasts were performed – Agricultural vs. Intermediate, Agricultural vs. Non-Agricultural, and Intermediate vs. Non-Agricultural.

Contrast	**DF**	**Contrast SS**	**Mean Square**	**F Value**	**Pr > F**
Ag vs. Intermediate	1	0.000273	0.000273	2.16	0.1481
Ag vs. Non-Ag	1	0.000539	0.000539	4.26	0.0443
Intermediate vs. Non-Ag	1	0.000001	0.000001	0.01	0.9264

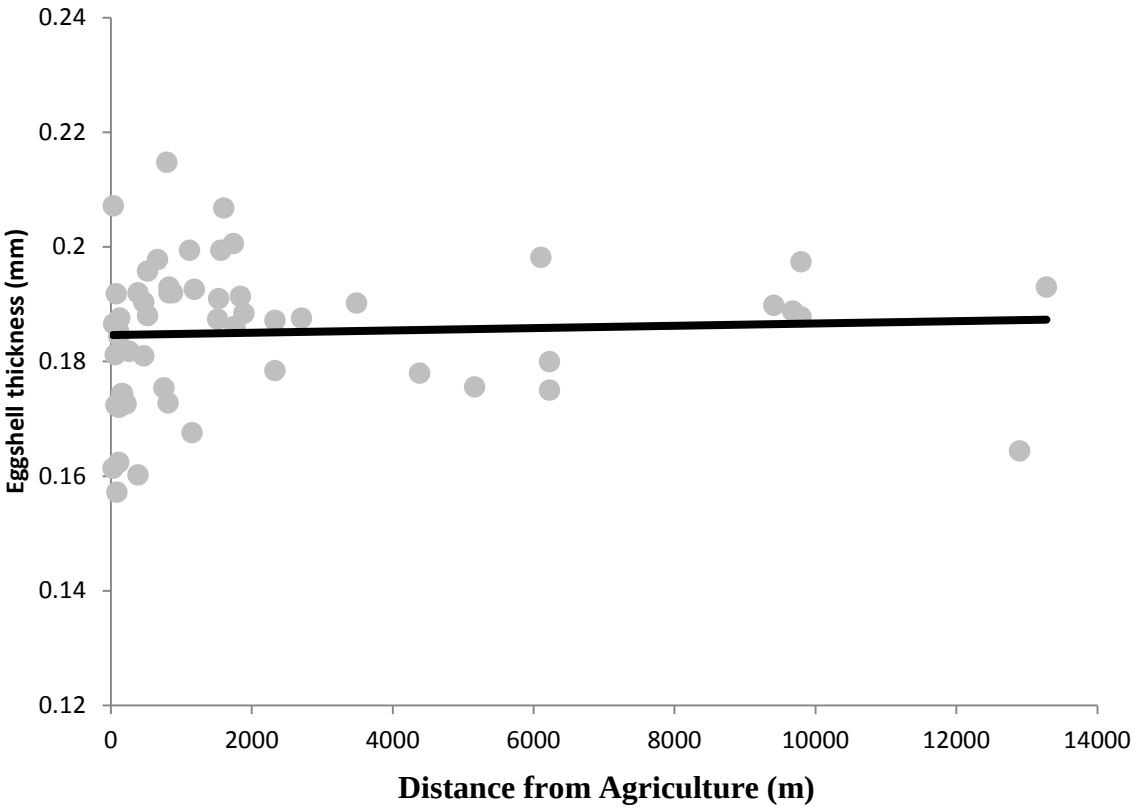

Figure 12. Relationship between distance to agriculture (m) and eggshell thickness (mm) in burrowing owl eggs in southwestern Idaho in 2007 and 2008. No significant relationship was detected (Thickness = 0.185 + ($2.007*10^{-7}$)*Distance, $F_{1,49} = 0.16$, $p = 0.687$, $n = 55$).

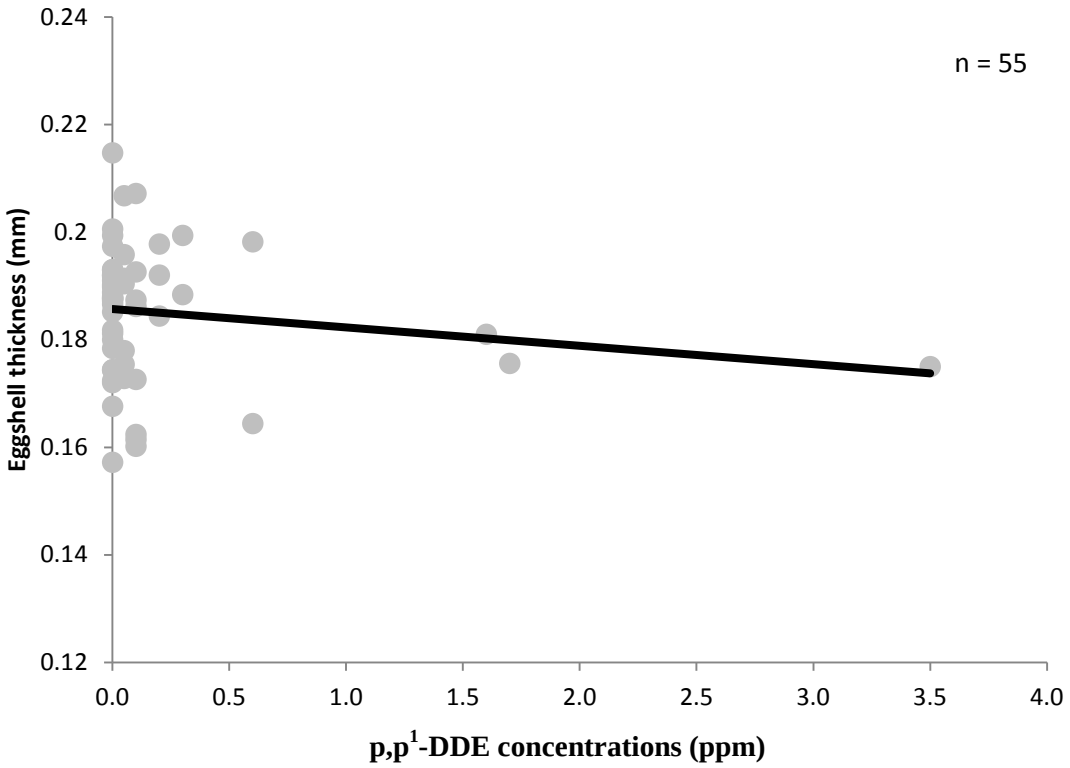

Figure 13a. Relationship between eggshell thickness (mm) and *p,p*[1]-DDE concentrations (ppm) in burrowing owl eggs in southwestern Idaho in 2007 and 2008. No significant relationship was detected.

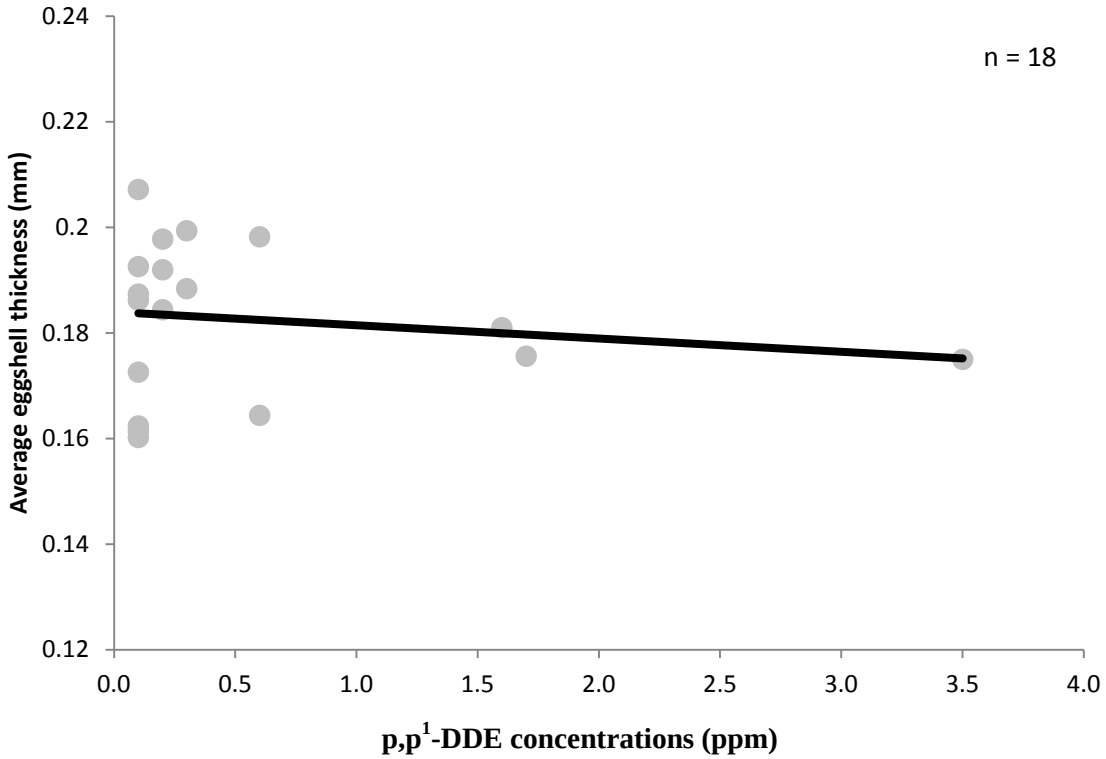

Figure 13b. Relationship between eggshell thickness (mm) and *p,p*^{1}-DDE concentrations (ppm) in burrowing owl eggs in southwestern Idaho in 2007 and 2008. Only eggs with quantifiable *p,p*^{1}-DDE (samples with $\geq$ 0.10 ppm) were use in this analysis. No significant relationship was detected.

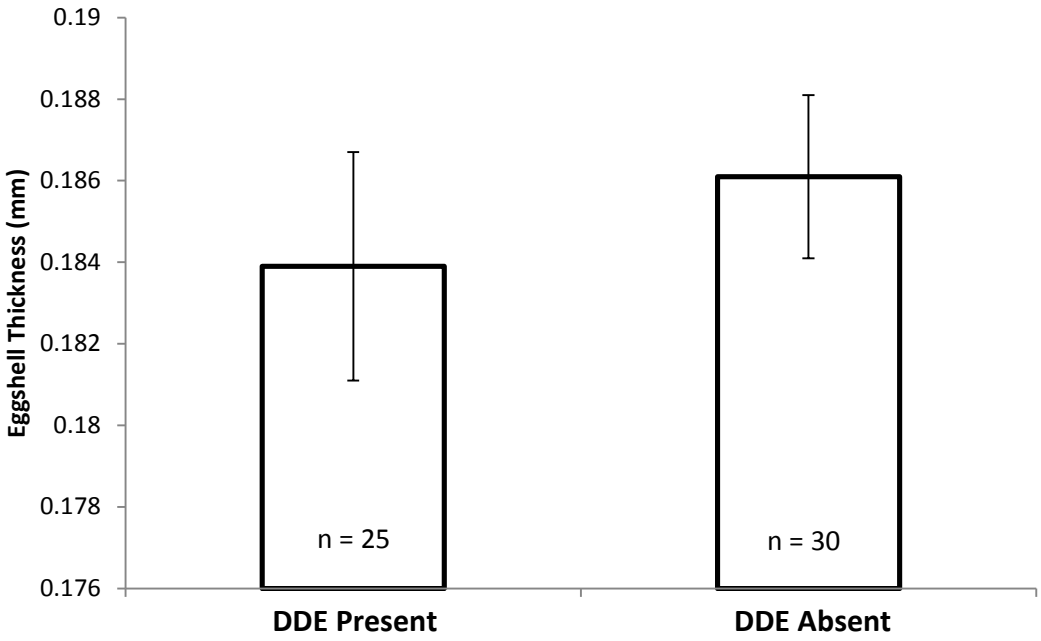

Figure 14. Mean (± SE) eggshell thickness (mm) by p,p^1-DDE category (p,p^1-DDE present vs. p,p^1-DDE absent) of burrowing owl eggs in southwestern Idaho in 2007 and 2008. Range for p,p^1-DDE present = 0.160 – 0.207mm. Range for p,p^1-DDE absent = 0.157 – 0.215 mm. Sample size for each p,p^1-DDE category is indicated.

Table 11 Levels of *p,p*[1]-DDE in eggs of other avian species that have been implicated in reproductive impairment (information from Table 3 in Gervais *et al.* 2000).

Species	**p,p[1]-DDE concentration (ppm)**	**Comments**	**Source**
Bald Eagle (*Haliaeetus leucocephalus*)	5	Decreased reproduction at 5 ppm	Krantz *et al.* (1970); Wiemeyer *et al.* (1993)
Barn Owl (*Tyto alba*)	16	Nest failure at 16 ppm; 5 ppm no-effects limit suggested	Klass *et al.* (1978)
Black Duck (*Anas rubripes*)	6	Decreased reproduction at 6 ppm; thinner eggshells	Longcore and Stendell (1977)
Black-crowned Night Heron (*Nycticorax nycticorax*)	8	Decreased reproduction at 8 ppm; broken eggshells	Henny *et al.* (1984); Hothem *et al.* (1995)
Brown Pelican (*Pelecanus occidentalis*)	3	Total reproductive failure at 4 ppm	Blus (1982)
Merlin (*Falco columbarius*)	6	Decreased reproduction at 6 ppm	Fyfe *et al.* (1976)
Peregrine Falcon (*Falco peregrinus*)	20	18% eggshell thinning at 20 ppm; declining reproduction	Enderson *et al.* (1982)
Prairie Falcon (*Falco mexicanus*)	2	Decreased reproduction at 2 ppm	Fyfe *et al.* (1976)
Osprey (*Pandion haliaetus*)	14	Addled egg samples at 14 ppm; decreased reproduction	Henny *et al.* (1977)
White-faced Ibis (*Plegadis chihi*)	4	Decreased reproduction at 4 ppm	Henny and Herron (1989)

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APPENDIX

Analytes and Their Detection Limits

Carbamate Multiresidue Insecticide Screen

Analyte	Detection Limit (ppm)
3-Hydroxycarbofuran	0.1
Aldicarb	0.1
Aldicarb Sulfone	0.1
Bendiocarb	0.1
Carbaryl	0.1
Carbofuran	0.1
Methicarb	0.1
Methomyl	0.1
Mexacarbate	0.1
Oxamyl	0.1
Propoxur	0.1

Organochlorine Multiresidue Insecticide Screen

Analyte	Detection Limit (ppm)
Aldrin	0.05
BHC alpha	0.05
Gamma Chlordane	0.05
DDE-p.p	0.1
DDD-p.p	0.1
DDT-p.p	0.1
DDE-o.p	0.1
DDD-o.p	0.1
DDT-o.p	0.1
Dicofol	0.1
Dieldrin	0.05
Endosulfan I	0.05
Endosulfan II	0.05
Endin	0.05
HCB	0.05
Heptachlor	0.05
Heptachlor Epoxide	0.05
Lindane	0.05
Methoxychlor	0.05
Mirex	0.05
Technical Chlordane	0.25
Toxaphene	2

Organophosphate Multiresidue Insecticide Screen

Analyte	Detection Limit (ppm)
Acephate	0.0050
Azinphos methyl	0.0100
Carbophenothion	0.0050
Chlorfenvinphos	0.0050
Chlorpyrifos	0.0050
Coumaphos	0.0050
Crotoxyphos	0.0050
Crufomate	0.0050
DDVP	0.0050
Demeton-O	0.0050
DEF	0.0050
Demeton-S	0.0050
Diazinon	0.0050
Dicrotophos	0.0050
Dimethoate	0.0050
Dioxathion	0.02
Disulfoton	0.0050
EPN	0.0050
Ethion	0.0050
Ethoprop	0.0050
Famphur	0.0050
Fenamiphos	0.0050
Fensulfothion	0.0050
Fenthion	0.0050
Fonofos	0.0050
Isofenphos	0.0050
Malathion	0.0050
Methamidophos	0.0050
Methidathion	0.0050
Methyl Parathion	0.0050
Mevinphos	0.0050
Monocrotophos	0.0050
Naled	0.0050
Parathion	0.0050
Phorate	0.0050
Phosalone	0.0050
Phosphamidon	0.0050
Profenophos	0.0050
Propetamphos	0.0050
Ronnel	0.0050
Terbufos	0.0050
Tetrachlorvinphos	0.0050
Triazophos	0.0050