COSTS AND BENEFITS OF VARIABLE NEST DENSITY IN BURROWING OWLS: EFFECTS ON PREDATION, ECTOPARASITES, EGG YOLK HORMONES, AND PRODUCTIVITY

by

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DEDICATION

I dedicate this to my loving wife and daughter, Lauresta and Anise; thank you for all of the love and patience you have provided as I worked to complete this thesis.

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AUTHOR AUTOBIOGRAPHY

I was born in Goshen, IN and moved to La Junta, CO when I was ten. At age 18, I graduated valedictorian of Cheraw High School. I went on to Goshen College, IN where I received a B.A. in Environmental Studies with a minor in Psychology. While there, I maintained a GPA of 3.94, was freshman Chemistry Student of the Year (1998-1999), made the Dean's List (Fall 1999-2000), and tutored students in chemistry, organic chemistry, Spanish, and political science. During the summer of 2000, I worked as a Goshen College Maple Scholar at Merry Lea, the college's 1150ac nature preserve. I conducted surveys of blue bird and thrush boxes, monitored newly created wetlands, and participated in the first Monitoring Avian Productivity and Survivorship song bird banding program at the site. After graduating in May 2002, I spent a year as an environmental education intern at Merry Lea, teaching students of all ages about the earth and the environment. I enrolled in Boise State University's Masters in Raptor Biology program during the fall of 2005. I maintained a 4.0 GPA and also received a Graduate Certificate in Geographic Information Analysis from the university. While attending Boise State University, I conducted my field research in 2006 and 2007. I presented a poster of my research at the 2007 Animal Behavior Society conference, Burlington, VT; and at the American Association for the Advancement of Science Pacific Division (AAASPD, Honorable Mention Award for Excellence) conference in Boise, ID. I also gave an oral presentation of my research at the 2008 Idaho Chapter of the Wildlife

Society conference in Boise, ID. Currently, I am working for the U.S. Geological Survey as the team leader of the Land Treatment Digital Library (LTDL). The LTDL is a database that integrates spatial data (GIS maps), tabular data, original documents, and photos of federal land treatments throughout the west.

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LIST OF ABBREVIATIONS

A4 – Androstenedione

AIL – Avian Insect Liquidator

BCI – Body Condition Index

CORT – Corticosterone

DHT – 5α-Dihydrotestosterone

E2 – 17β-Estradiol

EA/IO - Ethylacetate/Isooctane

NCA – Morley Nelson Snake River Birds of Prey National Conservation Area

PC – Principal Components

RIA - Radioimmunoassay

T – Testosterone

CHAPTER 1: THESIS OVERVIEW

Nesting density in birds is influenced by a number of factors including food availability, predation, and breeding site availability. As a result of a species' nesting density, individuals can incur certain costs and benefits related to predation rates, parasite levels, egg-yolk hormones, and nestling productivity. I evaluated hypotheses related to these costs and benefits in burrowing owls (*Athene cunicularia hypugaea*) that nested within the Morley Nelson Snake River Birds of Prey National Conservation Area in southern Idaho during 2006-2007. I studied the possibility that (1) increasing nest density would attract more predators, but group warning and mutual defense in high-density areas may help to deter predators, (2) increasing density would amplify flea levels in nestlings and these increased flea loads would negatively impact fledgling body condition, (3) increasing nest density would raise egg-yolk hormone levels potentially because of increased aggressive conspecific interactions among nesting female owls, and (4) increasing density would result in a net benefit for burrowing owls resulting in higher productivity at individual nests in these high-density sites. I also examined effects of distance to agriculture, female body condition, and median hatch date (or initial laying date for egg-yolk hormones) as potential covariates on predation, fleas, egg-yolk hormones, and productivity. In addition, I examined the effect of egg order on egg-yolk hormones to determine if early- or late-laid eggs within a clutch had greater hormone levels.

During the 2006-2007 breeding seasons, I observed the effects of density in 107 actual burrowing owl nests. I calculated territory overlap, an index of nesting density, in actual nests by creating a 200m radius buffer around each nest and measuring the percentage that a focal nest buffer was overlapped by neighboring nest buffers. Territory overlap varied from 0% to 100%. I examined predation rates in actual nests (n=105) and within dummy (artificial nests containing quail [*Coturnix* spp.] eggs) nests in high-density (n=16) and low-density (n=16) configurations. In addition, I observed male burrowing owl behavior in both high-density (n=5) and low-density (n=2) nests in response to a mock predator (i.e., a stuffed badger, *Taxidea taxus*) and observed the responses of neighboring owls in high-density areas. Parasites were counted and ranked for each nestling within a nest. To examine effects on fledgling body condition, some nests were sprayed with Avian Insect Liquidator (n=17) to remove parasites, whereas control nests (n=16) were sprayed with water. I examined the relationship between flea abundance on owls and nest density in 52 other nests to examine the prediction that fleas increased in high-density nesting areas. I extracted a small sample of yolk (~75mg) from one randomly selected early- and one late-laid egg in each nest (n=46 nests) to determine the effects of nesting density, laying date, and egg order within a clutch on egg-yolk hormones. Finally, I counted the number of 30-day old nestlings to measure productivity in all nests (n=107).

Burrowing owl nests in artificial burrows (n=107) contained 8.8±0.14 eggs (range: 5-14), 7.6±0.23 hatchlings (range: 0-11), 5.5±0.26 20-day old nestlings (range: 0-10), and 4.8±0.29 30-day old fledglings (range: 0-10). Of the 107 nests, 14 failed to fledge young. When examining the effects of predation, I found an interaction between nest density and agriculture where burrowing owls nesting in high-density and in proximity to agriculture had lower odds of predation. Predators detected high-density and low-density dummy burrowing owl nests with similar frequency, which indicates little if any added cost associated with detection by predators in higher density areas. Trials with the mock predator indicated that male owls in high-density nests may be more aggressive in attacking predators, participate in mutual defense against predators, and behave such that nearby owls are warned of approaching predators. Thus owls in high-density nesting areas appear to benefit from early warning, group defense, and perhaps a higher chance of driving a potential predator away. There was no evidence that any of the variables I examined influenced flea loads in nestlings or that increased flea loads lowered nestling body condition. Many burrowing owl fleas may come from their rodent prey, and this may help explain the lack of a relationship with nest density. Of the five hormones analyzed, only three androgens (testosterone, dihydrotestosterone, and androstenedione) were found in enough eggs and at sufficient quantities to conduct analyses. There was no evidence that egg-yolk androgens increased with increasing nest density, but egg-yolk androgens increased from early- to late-laid eggs within a nest. Egg- yolk androgens were low early and late in the laying season but peaked near the middle of the laying season. Finally, I found that productivity increased with (1) increasing female body condition, (2) decreasing flea loads, and (3) the lack of predation events. There was also a significant interaction between hatch date and nesting density: productivity in higher density nests declined with median hatch date, whereas burrowing owl nests in lower density (0% territory overlap) had relatively consistent productivity throughout the season. However, productivity was generally much higher in high-density nests unless nesting very late in the season, which suggests that burrowing owls benefit from living in higher densities.

In conclusion, burrowing owls appear to gain benefits from living in high-densities, whereas I uncovered few costs. Burrowing owls living in high-density configurations communicated the presence of predators and participated in mutual defense while incurring no apparent costs of added ectoparasites. These results may help to explain why we see a majority of burrowing owls living in high-density areas even at times when ample nest burrows seem to be available elsewhere. There was no effect of nesting density on egg androgens, but egg-yolk androgens exhibited high variation in burrowing owls and appeared to be a product of both the within-nest environment (egg order) and the external environment (laying date). These results increase our understanding of the biology and ecology of burrowing owls in relation to factors driving nesting and the consequences of nesting density.

CHAPTER 2: COSTS AND BENEFITS OF VARIABLE NESTING DENSITY IN BURROWING OWLS: EFFECTS ON PREDATION, ECTOPARASITE LEVELS, AND OVERALL PRODUCTIVITY

Abstract

Nesting density in birds is influenced by a number of factors including food availability, predation, and breeding site availability. As a result of a species' nesting density, individuals incur certain costs and benefits related to predation rates, parasite levels, and productivity. I evaluated hypotheses related to these costs and benefits in burrowing owls (*Athene cunicularia hypugaea*) that nested within the Morley Nelson Snake River Birds of Prey National Conservation Area in southern Idaho. I calculated territory overlap, an index of nesting density, of actual nests by creating a 200m radius buffer around each nest and measuring the percentage that a focal nest buffer was overlapped by neighboring nest buffers. Territory overlap varied from 0% to 100. Nesting density interacted with distance to agriculture to influence predation rates and with hatching date to affect productivity. Lower odds of predation attempts were realized in higher nesting densities, and nests without an observed predation attempt produced nearly twice the number of fledglings. Reduced predation in nests from high-density areas may partially be explained by owls receiving early warning about predators through the actions of neighbors, which I documented in experiments using a mock predator. These same experiments documented a case of mutual defense against predators by

neighboring owls. Increased productivity in higher densities may also be the result of grouping around a localized food source. In addition, I found that increasing fleas reduced productivity but not through reduced nestling body condition. Finally, increased adult female body condition during the nesting season resulted in more fledglings. These results suggest that rather than being forced to live in high-density configurations because of limited resources such as the availability of nest burrows, burrowing owls can benefit from nesting in higher densities under some circumstances.

Introduction

Nesting density in birds can be influenced by a number of factors including food availability (Johnson and Sherry 2001, Christie and Reimchen 2008), predation (Arnold 2000, Roos 2002, Serrano et al. 2005), and breeding site availability (Arambarri and Rodríguez 2000, Cockle et al. 2008). Benefits of nesting in higher densities for individuals include increased vigilance towards predators and the potential for mutual defense (Robinson 1985, Arnold 2000, Olendorf et al. 2003, Williams et al. 2003, Semeniuk and Dill 2005, Watson et al. 2007, Hollen et al. 2008, Krams et al. 2008, Theuerkauf et al. 2009). Increased densities may also provide opportunities for intentional and unintentional information sharing about the location of food or other resources (Brown 1988, Marzluff et al. 1996, Campobello and Hare 2007, Forsman et al. 2007). Costs of increased nesting density include increased vulnerability to predators because of increased detectability (Roos 2002, Krams et al. 2007) and increased disease or parasite transmission via increased contact with neighbors (Brown and Brown 1986, Shields and Crook 1987, Nilsson 2003, Brown and Brown 2004, Simon et al. 2005, Kleindorfer and Dudaniec 2009). High-density groups may also face increased competition for resources that can reduce individual productivity (Sillett et al. 2004, Mallord et al. 2007).

An evaluation of the costs and benefits of variable nesting density in birds could be informed by an examination of a species where some individuals live in higher densities and others nest in much lower densities within the same region. Western burrowing owls (*Athene cunicularia hypugaea*) are such a species in that they nest in both high-density and low-density configurations in many portions of their range (Desmond and Savidge 1996, Desmond et al. 2000, DeSante et al. 2004, Lantz et al. 2007, Tipton et al. 2008). For example, burrowing owl nesting configurations can range from having one or a few breeding pairs within a kilometer radius to situations where more than 20 pairs nest within a relatively small area, e.g., a prairie dog, *Cynomys* spp., colony (DeSante et al. 2004, Lantz et al. 2007, Tipton et al. 2008). Researchers have hypothesized that burrowing owls nest in higher densities around a localized food source (Desmond and Savidge 1996), but it is unclear how burrowing owls select their nest sites or why nesting density can be so variable. However, whether individual burrowing owls incur certain costs or reap benefits by nesting in high- or low-density configurations remains poorly understood. Therefore, I undertook a suite of investigations designed to examine the potential costs and benefits of variable nesting density in this species.

Burrowing owls nest in underground burrows located in prairies, grasslands, steppes, and other open areas (Haug et al. 1993, Poulin et al. 2005, Lantz et al. 2007). Although they frequently nest in well-drained areas, they also show an affinity for nesting near irrigated agriculture in some portions of their range (Rich 1986, Leptich 1994, DeSante et al. 2004, Conway et al. 2006, Moulton et al. 2006, Restani et al. 2008). They nest in burrows previously excavated by other animals such as American badgers (*Taxidea taxus*) or prairie dogs (Gleason and Johnson 1985, Rich 1986, Poulin et al. 2005, Lantz et al. 2007, Tipton et al. 2008). However, burrowing owls also nest in artificial burrows placed by observers (Henny and Blus 1981, Trulio 1995, Smith and Belthoff 2001a, Todd et al. 2003, Smith et al. 2005, Barclay 2008). Artificial burrows typically consist of an underground nesting chamber (e.g., a bucket or tub) with a tunnel leading to the surface (Smith and Belthoff 2001a). Adults generally weigh 120-200g, are 19-25cm in length, and have ~16cm wing chord (Haug et al. 1993). They are opportunistic predators that feed on rodents, small birds, amphibians, reptiles, and a variety of invertebrates (Moulton et al. 2005, Poulin and Todd 2006, Littles et al. 2007, Williford et al. 2009). Burrowing owls are socially monogamous and, in one study in California, rates of extrapair offspring were around 5-10% (Johnson 1997). Females typically lay 8-12 eggs per clutch and incubate while their mates provision them (Haug et al. 1993, Kaufman 1996). Pairs produce, on average, 0.9 to 4.9 nestlings per nesting attempt (Haug et al. 1993, Kaufman 1996, Smith et al. 2005, Wellicome 2005, Conway et al. 2006, Griebel 2007). In northern portions of their range, burrowing owls are annual migrants, whereas they are non-migratory elsewhere (Haug et al. 1993). Migration routes for Idaho burrowing owls remain relatively unknown (Haug et al. 1993, King and Belthoff 2001); however, a small number of band returns indicates that at least some Idaho burrowing owls may overwinter in California (Belthoff, unpublished data).

Study Area

I studied burrowing owls in and around the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) located in southern Idaho. The NCA encompasses 195,325ha, approximately 5% of which is irrigated agriculture (i.e., agricultural areas that require human supplied water to grow crops). The agricultural areas grow primarily alfalfa, corn, sugar beets, and mint. While some native shrub steppe remains, much of the rest of the NCA is disturbed shrub steppe and invasive grasslands upon which some cattle and sheep grazing occurs, primarily during winter (USDI 1996, Moulton et al. 2005). There are approximately 300 artificial burrows in the NCA available for burrowing owls for nesting or roosting (Smith and Belthoff 2001a, Belthoff and Smith 2003, Moulton et al. 2006). These artificial burrows allow easy access for researchers to monitor burrow activity, count, measure and sample eggs, and count, capture, and mark adults and young. From 1997-2007, burrowing owl nesting pairs occupied 30-60 of the artificial burrows in the NCA each year (Belthoff and Smith 2003, Belthoff, unpublished data). As in many portions of their range, the nest distributions of burrowing owls in southern Idaho within both natural and artificial burrows vary from high-density (e.g., 6 neighbors within 400m of a focal nest) to very low-density where kilometers separate nesting pairs (this study and Belthoff, unpublished data).

Hypotheses

My study addressed a suite of hypotheses about potential costs and benefits of variable nesting densities for burrowing owls. These related to predation, ectoparasites, and productivity, as explained below.

Predation

Burrowing owl adults, young, and eggs are prey for a number of species including American badgers (which appear to be their main predator), coyotes (*Canis latrans*), weasels (*Mustela* spp.), snakes, and large raptors (Green and Anthony 1989, Desmond et al. 2000, Leupin and Low 2001, Todd et al. 2003, Chipman et al. 2008). High-density nesting areas likely create more visual (e.g., owls on perches and activities surrounding nests), auditory (owl vocalizations), and scent (defecation, pellets) cues for predators to detect. Therefore, the predation hypothesis predicts that predators have a higher probability of detecting and depredating burrowing owl nests in high-density areas. However, while higher nesting densities may allow greater detection by predators, burrowing owls living in these areas may identify predators earlier because of increased numbers of eyes and potentially take more aggressive actions to avoid or repel them, such as engaging in mutual defense. Therefore, the predator response hypothesis predicts that burrowing owl males in high-density areas respond to predators differently than their counterparts nesting in lower densities (i.e., responses in high-density areas could include more visual cues or audio cues to warn neighbors, focal owls could be more aggressive in defense, and mutual defense by neighboring owls could occur).

Ectoparasites

Another potential cost of nesting in higher densities is increased ectoparasite transmission (Simon et al. 2005, Kleindorfer and Dudaniec 2009). The most common parasites found on burrowing owls in southern Idaho are four different species of fleas (Smith and Belthoff 2001b). Ninety percent of these fleas are the human flea (*Pulex irritans*), and burrowing owls can harbor 0 to >100 fleas (Smith and Belthoff 2001b, pers. observ.), although the costs of fleas on burrowing owls remains mostly unknown (but see Smith 1999). To examine the potential effects of nest density on ectoparasites in burrowing owls, I examined (1) the relationship between nest density and flea abundance on burrowing owl nestlings, and (2) the effects of parasite removal on nestling body condition in both low and high nest density areas. I predicted that flea levels would be higher in high-density nest areas, potentially because of more frequent contact among owls in these areas and the use of the same roosting or satellite burrows that could facilitate transmission of fleas among neighbors. If flea infestations are costly to owls, I predicted that removal of fleas would improve fledgling body condition.

Productivity

Desmond and Savidge (1996) reported that as burrowing owl nest site availability in Nebraska increased from few sites (badger burrows) to many (prairie dog colonies), burrowing owls nested in higher densities. As prairie dog colony size and burrowing owl nest availability continued to increase, burrowing owl density decreased slightly but was denser than in the badger site nesting areas. Their results suggest that burrowing owls have a preferred nesting density that falls between extreme high-density groups and solitary nests. However, the relationship between nesting density and productivity remains relatively poorly studied (but see Lutz and Plumpton 1999). Therefore, I examined a suite of variables including nesting density, distance to agriculture, female body condition, median nestling hatch date, predation attempts, and average flea load in the nest to assess their effects on burrowing owl productivity.

Methods

Data Collection

During the 2006 and 2007 breeding seasons (March through August), I monitored more than 300 artificial burrows located in or near the NCA for nesting by burrowing owls. Once I detected adult owls at an artificial burrow, I revisited at appropriate intervals to confirm egg-laying; determine initial laying date, clutch size, and median hatch date; and capture and measure nestlings at 20 and 30 days post-hatch. I captured adults by hand at nests either directly from artificial burrow tunnels or chambers after excavation, or by using a one-way door trap placed at the mouth of an artificial burrow combined with playback of burrowing owl vocalizations on a small tape recorder placed in the tunnel of an artificial burrow adjacent to the nest burrow (Belthoff, unpublished). Upon capture, I measured the following for each owl: mass (to the nearest gram), wing chord length, tarsus length, exposed culmen length, and tail length (all to the nearest 0.5mm) and abundance of fleas. I quantified flea loads by visually inspecting plumage and skin in the abdominal, wing, head, and back regions. I ranked flea load on a scale of 0-4 with 0=no fleas, 1=low (1-4), 2=medium (5-9), 3=high (10-19), and 4=very high (≥20) flea loads (Smith and Belthoff 2001b). Adult owls with brood patches were classified as females (Martin 1973, Haug et al. 1993), but gender determination of nestlings was not possible because juvenile burrowing owls are sexually monomorphic in size and plumage (Haug et al. 1993). Each captured owl received a U.S. Geological Survey aluminum leg band (size 4) and three colored plastic leg bands arranged in a unique combination for subsequent visual identification in the field. I also obtained blood (100-300ul) from adults and 20-day old juveniles at the time of banding for use in future DNA studies. To do so, I made a small puncture in a wing vein with a Microlance Blood Lancet and collected blood into microhematocrit capillary tubes. I immediately transferred blood into 1.5-ml polypropylene microcentrifuge tubes and then stored the blood frozen at -20 °C.

High-Density Versus Low-Density Nests

While most analyses used a continuous measure of nest density (described below), for certain experiments (flea removal experiment, predator response experiment) I had to establish an operational definition of high-density and low-density nests before the experiments commenced. Moulton et al. (2004) determined that the maximum distance that nesting burrowing owls typically move toward a novel, broadcasted conspecific call is 100m, although they respond vocally beyond this distance. Therefore, 200m represents a distance by which two nests can be separated and be predicted to have high levels of interaction. Therefore, for these experiments, I considered those nests within 200m of a neighboring nest as high-density nests. I considered a nest to be low-density if there were no other burrowing owls nests ≤400m from the focal nest. In low-density situations, interactions between nesting owls are expected to be infrequent, as each of the neighbors would have to travel twice the maximum distance observed in previously conducted controlled field experiments (Moulton et al. 2004).

Predation

To examine the effects of density on predation rates, I inspected nests during each visit for signs that a predator had detected the nest. Looking for visual signs of nest predation likely underestimates the number of predation attempts as predators could be driven from a nesting site by the aggressive behavior of the burrowing owls and, thus, the predators leave no visible sign. However, I had no way to quantify failed predation attempts. Therefore, if digging at the burrow entrance or above the underground tub occurred (typical of badger or coyote predation), or if large numbers of burrowing owl feathers were found around the nesting site (indicating a possible raptor attack), I concluded that the nest had been detected by a predator.

I also evaluated patterns of predation using a field experiment that deployed dummy nests in both low-density (n=16) and high-density (n=16) configurations. I constructed dummy nests (as in Brady 2004) in early to mid-May and monitored them for signs of predation once a week for six weeks. Briefly, these nests were typical artificial burrows containing shredded livestock dung around the tunnel entrance and inside the chamber to imitate the appearance of an actual nest. Each dummy nest also contained eight quail (*Coturnix* spp.) eggs to mimic an actual owl clutch. High-density configurations included four dummy nests arranged in a square with nests on each side separated by 40m. Low-density dummy nests were a single nest placed ≥400m from other dummy nests. Both low- and high-density dummy nests were ≥400m from known actual burrowing owl nests. I scored dummy nests as depredated if digging occurred at the mouth of burrows or above nest chambers, or if I found eggs broken or chewed upon. If one nest within a group was depredated, I marked the entire group as depredated but also recorded status of individual nests within the cluster to determine if a predator was likely to detect more than one nest in that cluster.

To study the behavior of burrowing owls in response to predators, I observed the reaction of focal males whose nests were either in high-density (neighbors within ~200m) or very low-density (no neighbors ≤1km) settings to the presence of a mock predator or to a control treatment. If focal owls were nesting in high-density, I also determined if and how nearby burrowing owls responded to the behavior of the focal male. I conducted mock predator and corresponding control trials when nestlings were between 7 and 30 days old. The mock predator was a stuffed badger mounted on a running board with 100m of monofilament attached (Figure 2.1). I used a dead Russian thistle (*Salsolsa* sp., i.e., tumbleweed, a common element within the natural setting in which burrowing owls nest) with 100m of line attached as the control for this experiment (Figure 2.1).

I conducted all trials between 1700 – 2200h before darkness set in and prevented visual observations of owl behavior. This is a time period when owls are usually at or near their nests and the night's activity period generally begins and overlaps normal badger activity periods. At the start of each trial, I placed the mock predator or control treatment 50m from a focal nest and hid it from view using a camouflaged blanket. I also established a portable observation blind constructed of wood and appropriately colored beige burlap placed 100m from the treatment. In each case, the blind was situated such that the treatment would pass within 2 m of the focal nest upon retrieval (Figure 2.2). In high-density areas, a second observer established an additional blind to record the response of owls at a neighboring nest (Figure 2.2a). Trials did not commence until focal and neighboring owls, in high-density experiments, returned to their nests if they had flushed during the time that treatment or blind was deployed. Once trials commenced, I recorded male burrowing owl behavior during a 5-min. pre-treatment period (used for comparison) followed by a 5-min. treatment period during which time I moved the stuffed badger or control toward the blind at a speed of approximately 20m/min by retrieving the monofilament line. To determine if each focal male behaved differently during both the pre-treatment and treatment periods, I recorded the number of vocalizations uttered, hovers (flying above the treatment in the same location for more than 3 seconds), aerial dives at the treatment (diving towards and swooping over the treatment), head bobs (bobbing the head up and down from a standing position), and any other types of behavior directed toward the treatment for each time period. In high-density areas, a field assistant recorded vocalizations and any other behavior of owls at the neighboring nest to determine if and how they were reacting to the behavior of the focal male in response to the treatment at the focal male's nest.

Ectoparasites

To determine if and how fleas affected burrowing owl nestlings in both low- and high-density nests, I conducted a factorial experiment that involved treating nests/owls with an insecticide to remove fleas or with a control treatment (water). Application of insecticide or control occurred twice at respective nests: once during the late incubation stage 3-5 days before expected hatch, and again when nestlings were 10-20 days old. I sprayed nest tunnel entrances, nest chambers, nestlings, and adults with Avian Insect Liquidator (AIL, VetaFarm, Wagga Wagga, Australia), which is a liquid insecticide approved for birds. Nest tunnels and chambers were sprayed with AIL until there was a thin covering of insecticide. Nestlings and adults had their plumage moistened with AIL before being released, and I was careful to treat all regions of the plumage to kill all fleas currently on owls. Control nest tunnels, chambers, and owls received similar applications but with water. When nestlings were 30-days old, I measured their mass (grams), and wing, tail, and culmen lengths (in mm). From these measurements, I calculated an index of nestling body condition (BCI). To do so, I conducted a principal components (PC) analysis using the size variables and regressed mass on scores along the first PC (index of size). The residuals from this regression were the BCI scores (Jacob et al. 1996, but see Green 2001), with positive residuals indicating owls in better body condition than expected. Ultimately, I examined the effects of insecticide treatment and nest density on the body condition of nestlings.

Nest Variables

I used ArcMap 9.2 and Hawth's Analysis Toolpack for ArcMap (ESRI ArcMap 9.2, Beyer 2004, respectively) to calculate nest density. I calculated multiple density estimates in which each estimate of density included buffers around a focal nest at 100m, 125m (measures of close neighbors), 200m (measure of intermediate neighbors), 400m, and 600m (measures of general area density). The density estimates I calculated were the number of neighbors within the buffer, territory overlap of a focal nest (the total percentage that a focal nest buffer is overlapped by other buffers; 0-100%), and complete overlap of a focal nest (the sum of the percentage of each overlapping neighboring nest buffer on the focal nest; 0->100%; see Figures 2.3 and 2.4) at each buffer distance. I also measured distance to nearest neighbor (Figure 2.3) and the number of neighbors within a buffer zone using Hawth's Analysis Toolpack "Distance Between Points (Within Layer)" tool for ArcMap 9.2. Hawth's Analysis Toolpack "Polygon in Polygon Analysis" tool calculated the territory overlap and complete overlap at each distance.

In addition to measuring nesting density, I included three covariates in my analyses of depredation, ectoparasites, and productivity in actual nests. First, I measured distance to agricultural fields using ArcMap's "Point to Polyline" Tool, because burrowing owls often associate with irrigated agriculture, and this association often results in an increase in productivity (Rich 1986, Leptich 1994, Smith and Belthoff 2001a, Belthoff and Smith 2003, DeSante et al. 2004, Conway et al. 2006, Moulton et al. 2006, Restani et al. 2008, Verboven et al. 2008, Poisbleau et al. 2009) and has the potential to influence other variables. Second, I calculated female BCI in the same manner as nestling BCI (described above), as female BCI can influence productivity in birds (Arroyo et al. 2007, Warner et al. 2007, Love et al. 2008, Robb et al. 2008, Hargitai et al. 2009). Finally, I included hatch date (fitted as median hatch date for that brood) because hatch date can influence productivity through seasonal changes in prey abundance and high quality/older females potentially nesting earlier in the season than low quality/younger females (Spear and Nur 1994, Krapu et al. 2000, Pilz et al. 2003, Michl et al. 2005, Arroyo et al. 2007). Hatch date was the ordinal date (1 representing January 1st to 365 representing December 31st) on which the nestlings hatched.

Data Analysis

Nest Density

I used SAS 9.1 (SAS Institute, Cary, North Carolina) for data analyses and considered effects significant when $p<0.05$. Means ± SE are presented throughout. I determined that all measures of nesting density were highly correlated (Spearman Correlation Analyses: range of r: 0.6-1, all $P < 0.0001$; see Appendix A). Thus, to avoid multicollinearity in linear models I used just one of these measures, i.e., territory overlap of a focal nest with a 200m buffer, henceforth called nest density, when modeling effects of nesting density in burrowing owls.

Predation

For actual burrowing owl nests, I examined potential effects of nest density, distance to agriculture, female body condition, and hatch date on the odds of nest depredation. I used contingency analysis to determine if predation was uniform for low-density and high-density dummy nests. In addition, I wanted to determine the probability of a predator detecting two or more nests given that one nest was already detected in a high-density dummy nest site. To analyze this probability, I calculated the conditional probabilities of detecting 0, 1, 2, 3, or 4 nests in a group along with conservative confidence intervals around those probabilities. To address the predator response hypothesis, I compared aggressive behavior toward the mock predator in focal adult males from high-density and low-density nests; as samples sizes were small for this set of experiments, no statistical analyses were employed.

Ectoparasites

I used mixed model analysis of variance to determine if fleas varied with nest density. Nestlings within a nest are likely to have similar flea loads so I modeled nestlings within a nest as non-independent using the repeated statement in SAS. Using a similar approach, I examined the effect of nest density (high-density or low-density) and insecticide treatment (treatment and control) on the BCI of nestlings that participated in the flea removal experiment. I calculated both the overall means (sum of all fledgling BCIs/n) as well as second order means (mean of nest means/n).

Productivity

I used the number of 30-day old nestlings at each nest as the index for productivity. Using a mixed model regression, I examined the relationship between burrowing owl productivity and nest density, distance to agriculture, hatching date, female BCI, nest depredation (depredated or not), mean flea load in a nest, and treatment with AIL (0=water/no treatment, 1=AIL application).

Results

Burrowing owl nests in artificial burrows (n=107) contained 8.8±0.14 eggs (range: 5-14), 7.6±0.23 hatchlings (range: 0-11), 5.5±0.26 20-day old nestlings (range: 0-10), and 4.8±0.29 30-day old fledglings (range: 0-10). Of the 107 nests, 14 failed to fledge young. Of the nest failures, 10 nests were destroyed by predators, two involved starvation of all nestlings (I suspected, but could not confirm, predation of at least one adult caretaker), and flooding killed all eggs or nestlings within the other two nests (one during the incubation stage and the other when the nestlings were ~10 days old). Average hatch date of nestlings was 20 May±1.20 days (range: 26 April-25 June, n=104 nests with one nest lost due to flooding during the egg stage and two nests where the adult female disappeared, likely depredated, and the male re-nested with another female). Nests were 2.7±0.36km from agriculture (median: 1km; range 0.03-13.0km, n=107 nests). Median female BCI was 0.2±1.97 (range: -59.44-41.01, n=91). Nest density, estimated using the 200m buffer, was 36.0±3.23% (range: 0-100, n=107 nests) with a median nearest neighbor distance of 238m.

Predation

For analyses of actual nests, I removed the two nests that were flooded. Of the remaining 105 nests, 30 experienced a predation attempt, and 75 escaped visible signs of predation. Of the 30 nests that experienced a predation attempt, 10 (33.3%) failed to fledge young, whereas the other nests generally lost some portion of the brood. Only 5.3% (n=4) of the remaining nests that escaped observable predation attempts failed to fledge any nestlings. Nests with an observed predation attempt produced 3.0±0.51 fledglings (range: 0-9), whereas nests that escaped predation attempts produced 5.5±0.31 fledglings (range: 0-10).

The predation hypothesis predicted that the odds of predation would increase with increased nest density. For actual nests, hatching date and female body condition did not affect the odds of predation, but there was a significant interaction between nest density and distance to agriculture (Table 2.1). The odds of a depredation event were lowest for high-density nests irrespective of their distance to agriculture. For lower density nests, odds of predation increased as distance from agriculture increased (Figure 2.5).

Of 16 low-density and 16 high-density dummy nests, nine (56.2%) were depredated in each case. Thus, predators detected low-density nests at the same rate that they detected at least one nest in a high-density group (contingency analysis: χ^2=0.0, p=1.0). When determining if predators would detect more than one dummy nest in a high-density group given that a single dummy nest was already detected, I found that the conservative confidence intervals that were calculated overlapped, indicating no significance. Therefore, there was no need to conduct further analyses that would only increase the size of the confidence intervals making the outcome even less significant. However, sample sizes were likely too small to produce a meaningful result in this instance.

The predator response hypothesis predicted that burrowing owl males in high-density areas respond to predators differently than their low-density counterparts. During initial field experiments to test the predator response hypothesis, male burrowing owls consistently failed to respond to control treatments or during the 5-min. pre-trial observation period. Thus, I removed the control treatment from the experimental protocol for subsequent trials, and focused only on the mock predator treatment preceded by the 5-min. pre-trial observation period.

I attempted the experiment on eight focal males attending nests in high-density areas and eight low-density males. Unfortunately, because owls did not return to their nests before dark or other logistical issues including difficulty moving the mock predator through thick vegetation in some nest areas causing the monofilament line to snap, I was able to collect data from just seven of these nests (n=2 low-density and n=5 high-density).

All but one owl responded to the mock predator, indicating that they recognized it as a potential threat. Focal males in low-density areas (n=2) responded with a small number of calls (n=2) and bobs (n=2; total behavior: 2.0±2.0, range: 0–4) before these males and their putative mates left their nest site as the mock predator approached. In both cases, these owls flew out of view and did not return to the nest during the remainder of the trials. Focal males in the high-density group exhibited 8.8±5.07 (range:

0–12) responses, including bobs (n=6), calls (n=14), hovers (n=13), and dives (n=10). None of the high-density males left the nest site in response to the mock predator, and three of their putative mates also responded to the predator with calls and dives along with the males. One of the high-density males also approached the predator while on the ground with wings fully expanded while within 2m of the predator. One high-density male failed to respond to the mock predator with overt behavior, perhaps because vegetation surrounding its nest was much taller than in other cases (1m tall versus less than 0.25m, typically) and it could not recognize the stimulus as a potential threat. Alternatively, owls may not have wished to advertise the location of their nesting site to a predator with such high vegetation acting to screen the nest from view.

In three of five trials for high-density males, neighboring owls reacted to the focal male. In one instance, nestlings that were outside of a nearby nest chamber immediately fled on foot to their burrows as the focal male began calling in response to the predator. At another nest, the neighboring female (identified by color-band combination) began to call immediately after the focal male called. In the last instance, a neighboring male approached the predator and responded with calls, hovers, and dives when the predator was near the nest of the focal male and while the focal male and its putative mate responded with similar defensive behavior. This resulted in three owls from two nests that were separated by less then 80m responding to the stimulus in apparent mutual nest defense.

Ectoparasites

I predicted that as nesting density increased, flea levels on nestling burrowing owls would also increase, and that increased flea levels would negatively impact fledgling body condition. The flea index for 295 fledglings from 52 untreated nests (i.e., nestlings not treated with AIL or sprayed with water as part of the flea removal experiment) averaged 1.4±0.07 (1.6±0.17 second order mean) on the index of flea abundance (~5-10 fleas per individual; range 0-4 on the ranked index). In contrast to the prediction of the parasite hypothesis, nest density did not affect flea abundance (Table 2.2). Similarly, hatching date, female BCI, and distance to agriculture were not significant predictors of flea levels on burrowing owl fledglings (Table 2.2).

Nests treated with AIL had flea ranks (overall mean=0.2±0.04; second order mean=0.2±0.06; n=17 nests, 103 fledglings) that were significantly lower than control nests (overall mean=1.0±0.11; n=16 nests; second order mean=1.0±0.20; 96 fledglings; $F_{1,31}$=12.11, p<0.001). Thus, AIL was effective in reducing flea loads in nests. Despite this reduction in fleas, there was no indication that fledgling BCI differed in AIL-treated or control nests or in nests from low- or high-density areas, and the two factors did not interact (2-way ANOVA: AIL treatment status x nest density interaction: $F_{1,29}$=2.11, P=0.16 interaction; AIL treatment: $F_{1,29}$=0.76, Figure 2.6a; and P=0.39; nest density: $F_{1,29}$=2.71, P=0.11, Figure 2.6b).

Productivity

I examined the effects of 1) nesting density, 2) distance to agriculture, 3) hatching date, 4) adult female BCI, 5) depredation status, 6) mean flea abundance in a nest, and 7)

AIL treatment application at a nest on the outcome variable nest productivity for 81 of 107 nests in which I had all variables recorded. Neither application of AIL nor the distance from agriculture was significantly related to productivity. Of the remaining variables, female body condition, depredation status, and flea load had significant effects on productivity (Table 2.3). Productivity increased as female BCI increased (Figure 2.7a), as depredation status changed from depredated to not depredated (Figure 2.7b), and as flea load decreased (Figure 2.7c). There was also a significant interaction between hatch date and nest density (Table 2.3). Productivity in both higher density nests declines with median hatch date, whereas lower density nests appear to have more uniform productivity throughout the season (Figure 2.7d). Finally, I noted a negative relationship between female body condition and median hatch date (Figure 2.8).

Discussion

Commonly understood costs of living in higher densities include increased detection by predators and potential increased exposure to parasites while benefits include early warning of predators, cooperative defense, and the potential for information sharing (Krause and Ruxton 2002). My results suggest that burrowing owls nesting in high-density areas gained multiple benefits compared to owls nesting in lower densities. The benefits for high-density nests included decreased odds of predation, warning of approaching predators, and higher productivity for nestlings that hatched early in the season. My study also uncovered instances of apparent mutual defense against predators by burrowing owls in high-density areas. There was no apparent cost of increased detection by predators as the experiment with dummy burrows demonstrated. I found that increased flea loads decreased productivity, but there was no evidence that flea loads increased from low- to high-density areas. One cost of nesting in higher densities was lower productivity if nesting was later in the season. Figure 2.9 summarizes the effects of grouping and other covariates I examined on burrowing owl nesting.

Predation

Predators can have a large influence on productivity in bird populations (Martin 1993, Martin 1995, Wesolowski and Tomialojc 2005, Fontaine and Martin 2006). For instance, black kites (*Milvus migrans*) failed to fledge any nestlings when they nested within one kilometer of an eagle owl (*Bubo bubo*) nest because of predation by the owls (Sergio et al. 2003). Animals existing in high-densities, however, may work in conjunction to mitigate the influence or effectiveness of predators through early warning and cooperative defense (Lazarus 1979, Griesser and Elkman 2005, Krams et al. 2007, Pays et al. 2007). To my knowledge, my observations of the existence of early warning of burrowing owl neighbors is among the first reports of this in relation to anti-predator behavior in burrowing owls. I believe that it is probably not a rare occurrence but instead is just difficult to document unless focal owls and their neighbors are under simultaneous observation. The two cases of early warning that I observed demonstrate that burrowing owls in high-density areas can benefit from a neighbor's reaction to a predator and then take appropriate steps to avoid potential depredation of their nest. Thomsen (1971) noted that adult males and females "chatter" in response to predators and suggested the function of such chatter is to warn nestlings (see also Martin 1973). Whether focal adults are simply calling to warn their nestlings or including warnings specific for neighboring owls as well is unknown, but neighboring owls clearly have the potential to pick up on these cues. My study also demonstrated that burrowing owls nesting in close proximity to one another are also capable of mutual defense. Martin (1973) described similar cooperative defense (or mobbing) when he placed a great horned owl (*Bubo virginianus*) near a nest and observed (1) that the resident pair of owls "mobbed" the great horned owl, and (2) adults from other burrows as far as 300m away joined in. Similarly, Brady (2004) also observed an instance of a neighboring burrowing owl in mutual defense against a mock predator in an area of high nest density where there were five nests within a 1ha area. Mutual defense can occur for a number of reasons including kinship, by-product mutualism, or reciprocal altruism (Maklakov 2002, Olendorf et al. 2003, Krams et al. 2007), all of which are possible in burrowing owls. High relatedness in high-density areas may exist because male nestlings and adult male and female owls are philopatric (Catlin et al. 2005, Riding and Belthoff, unpublished data), which may lead to kin-selected mutual defense. Burrowing owl offspring can be the product of extrapair copulations (Johnson 1997) so the possibility of by-product mutualism (i.e., a male defending a nest where he sired extrapair young) exists. A tit-for-tat strategy whereby burrowing owl males assist each other in nest defense for as long as the males reciprocate is also possible. I also cannot rule out the possibility that the burrowing owl was acting selfishly in an attempt to drive the predator away from its own nest and the area in general. Finally, four of the five focal males in high-density areas (and three of their putative mates) responded aggressively toward the mock predator whereas owls at both solitary nests in this experiment abandoned their nests while displaying few if any aggressive behaviors. While sample sizes are small, burrowing owls nesting in high-density areas may be more aggressive in defending their nests than those in lower densities. Such an effect may help to explain the decrease in the odds of predation in high-density areas detected by my analyses. Alternatively, solitary owls may have left the nest site, not because they were less aggressive, but because it is more beneficial in a solitary environment to leave the nest site and not draw the attention of the approaching predator by making an aggressive display.

In contrast to the benefits of early warning and cooperative defense for burrowing owls nesting in high-density configurations, one potential cost is increased detection by predators. However, given that predators detected dummy nests placed in low- and high-density configurations in equal frequency, it appears that high-density dummy nests were not at increased risk of detection. I have no reason to believe that predator density varied in the locations where I placed high- and low-density dummy nests as I tried to place similar numbers of high- and low-density dummy nests in each location where the experiment occurred. Of course, for these dummy nests, there were no adult burrowing owls tending the nests or nestlings in the area, which could potentially increase detection by predators, particularly in high-density areas.

Data from actual burrowing owl nests indicated an interesting relationship between nest density and distance from agriculture with respect to predation attempts. Irrespective of distance from agriculture, odds of predation were low for higher density nests, and lower density nests farther from agriculture had substantially increased odds of predation (Figure 2.5). I interpret this pattern as follows: First, owls in high-density areas may benefit from a level of protection from predators either through early warning, cooperative defense, or heightened anti-predator behavior, irrespective of their distance from agriculture. Second, lower density nests farther from agriculture probably do not reap any of the benefits mentioned above so they suffer correspondingly higher odds of predation. Why lower density nests in agricultural areas have decreased odds of predation relative to low-density nests elsewhere is not as clear. Moulton et al. (2006) found a trend for greater numbers of badger burrows in non-agricultural areas (7.0±1.1, n=25) compared with plots in agricultural areas (5.4±1.1, n=25), although the difference was not significant. Increasing odds of predation for lower density nests as distance from agriculture increases may reflect this trend in increasing badger (predator) abundance.

Ectoparasites

In addition to the harmful effects of predators, ectoparasites also typically have a negative impact on their hosts both in terms of body condition and survivorship (Brown and Brown 1986, Christe et al. 2000, Fitze 2004, Boughton et al. 2006). In burrowing owls, fleas did not increase with increasing nest density, and removing ectoparasites had no effect on nestling body condition regardless of group (although sample sizes were relatively small for the latter experiment). However, increased ectoparasites were correlated with decreased productivity, which suggests that parasites had an effect on at least some burrowing owl nestlings. The lack of a density effect may be explained by the fact that fleas are probably brought into a nest via the rodent prey captured by owls (Smith and Belthoff 2001b) rather than transferred among owls through close contact. Flea abundance on rodent prey is unknown, so it is not possible to determine if burrows are infested by a small number of rodents that harbor numerous fleas, or if all prey harbor a small number and fleas accumulate with increasing prey brought to a nest. However, the latter seems less likely because more prey typically translate into higher productivity in burrowing owls (Wellicome 1997). The effects of lice and other ectoparasites found on burrowing owls as a function of nest density remain unknown as my study addressed fleas only.

Productivity

I found that burrowing owl productivity was influenced by a number of factors, including nesting density. Burrowing owls nesting in higher densities benefited in terms of greater productivity (Figure 2.7d). These nests fledged greater numbers of young than solitary nests, unless an individual started nesting very late in the season, in which case it appeared beneficial for owls to nest in more solitary situations. Higher productivity in high-density burrowing owl nests may be related to benefits from decreased predation, early warning of predators, mutual defense, and possibly increased aggression towards predators. Another potential explanation is that burrowing owls are nesting in high densities around a localized food source (Desmond and Savidge 1996). Increased food resources have been linked with increased productivity in burrowing owls (Wellicome 1997). Along with decreases in predation, this could account for the increased productivity I observed when owls nested in higher densities. Why late nesters may benefit more from solitary nesting potentially involves (1) avoiding competition in higher density areas (i.e., where numerous young that have a large head start for resources have hatched in other nests), or (2) avoiding losing young to cannibalism, which may occur when adult or recently fledged burrowing owls prey on much smaller nestlings as they emerge from the later nests.

Increased female BCI, decreased flea loads, and low levels of predation are all commonly associated with increases in productivity. A female with a higher BCI suggests that she is in better overall health and may be more likely to produce a larger clutch (Sanz 1995). In addition, I found evidence that female burrowing owls with a higher BCI generally nest earlier in the season, which, based on other studies in birds, may indicate these females are better provisioned (Nilsson 1994), more experienced (i.e., older; Wendeln 1997, Boal 2001), or a combination of these factors. Increased numbers of fleas increase stress and reduce overall survivorship of hosts (Boughton et al. 2006), although specific effects of fleas on burrowing owls remain poorly understood so the mechanism of action on productivity requires further study. Finally, predation has always been recognized as an important factor that decreases productivity (Nordström et al. 2004, Moore 2005, Hoover 2006, Hoover 2009).

Burrowing owls in some areas nest near irrigated agriculture, and this association between owls and agriculture has been linked with higher productivity (Smith and Belthoff 2001a, Belthoff and Smith 2003, DeSante et al. 2004, Moulton et al. 2006, Verboven et al. 2008, Poisbleau et al. 2009). My results indicated that nesting near agriculture was related to reduced odds of predation for all nest densities. I was not able to gather information on the relative abundance of predators in natural and agricultural areas, so perhaps there are fewer predators in agricultural areas because of human activity (e.g., farmers and ranchers frequently kill badgers because they dig holes that disrupt crops or livestock foraging) or other reasons. Additionally, agriculture potentially contributes to productivity by providing a localized food resource, as one common prey species of burrowing owls (*Microtus montanus*) only occurs in irrigated agricultural areas in my study area, and burrowing owls in agricultural areas have a greater number of prey items and larger biomass per pellet than in natural areas (Moulton et al. 2005, 2006). However, it also appears that nesting in higher densities farther from agriculture can carry nearly the same advantages related to avoiding predation, or there may be localized food resources away from agriculture around which owls group. These factors could account for some of the higher productivity that I observed in high-density nests there. It is also possible that burrowing owls nesting in higher densities gain information from conspecifics about the locations of food resources, as they frequently forage outside of their own territorial boundaries, although my study did not address this aspect of group living explicitly.

Other Potential Costs and Benefits

In addition to the costs and benefits of variable nesting density for burrowing owls mentioned above, other factors such as egg-yolk hormones and frequency of extrapair young have the potential to be influenced by nest density. Aggressive conspecific interactions can increase hormone levels in adult female birds. Higher hormones in adult females can result in higher egg-yolk hormone levels within their eggs that can, in turn, potentially influence nestling development and aggression (Schwabl 1993, Reed and Vleck 2001, Whittingham and Schwabl 2002, Pilz and Smith 2004, Love et al. 2008, Tanvez et al. 2008, Hargitai et al. 2009). I found no evidence that increasing nest density raised burrowing owl egg-yolk hormones (Chapter 3). However, there was an increase in androgens (testosterone, dihydrotestosterone, and androstenedione) within a clutch from early- to late-laid eggs as well as a curvilinear pattern where androgens among clutches were lowest early and late in the laying season while peaking in the middle of the laying season (Chapter 3).

In addition to hormones, increased breeding density can also raise the potential for extrapair copulations and extrapair young (Richardson and Burke 2001, Formica et al. 2004, Augustin et al. 2006, Melles et al. 2009). Extrapair copulations (i.e., copulation outside of a pair bond) resulting in increased extrapair young are a benefit to extrapair males but are costly to cuckolded males who raise offspring that do not carry their genes. Each burrowing owl captured in 2006 and 2007 had a small sample of blood removed for DNA analysis (see description in methods). In 2006 and 2007, I collected blood and DNA from 48 families of burrowing owls (i.e., both parents and at least 1 nestling, 386 individuals). In addition to blood collected in 2006 and 2007, I obtained blood and DNA from previous field seasons 1997-2005 (n=74 families, 512 individuals). Some of the DNA from 2006 and 2007 helped us identify and test 18 new microsatellite DNA primers (Faircloth et al. 2010). These microsatellite primers will add to those already in the literature (Korfanta et al. 2002) to provide a sufficient number of markers to facilitate upcoming parentage tests that hopefully will provide not only exclusion of extrapair young but also assignment tests to identify genetic parents. I am currently collaborating with a team of researchers at University of California, Los Angeles to analyze DNA samples (122 families and 898 individuals) to determine if and how grouping affects extrapair mating in burrowing owls.

Conclusions

Burrowing owls appear to gain benefits from living in high densities while incurring but a few costs. By demonstrating the benefits of decreased predation and increased productivity early in the season, I was able to quantify some of the factors involved in the ecology of burrowing owls and help clarify variables that contributed to their productivity. Burrowing owls living in higher density configurations responded more aggressively to a mock predator, potentially communicated the presence of predators to nearby individuals, and participated in mutual defense while incurring no apparent costs of additional parasites. My results may help to explain why we see many burrowing owls living in higher densities even at times when ample nest burrows may be available elsewhere.

Table 2.1. Results of logistic regression to examine effects of distance to agriculture, hatching date, female body condition, and nest density on odds of predation of actual burrowing owls nests (n=105) from the Snake River Birds of Prey National Conservation Area, Idaho in 2006 and 2007.

Effects	**Estimate**	**Standard Error**	**DF**	**Chi-Square**	**P-value**
Intercept	-0.54	4.38	1	0.02	0.90
Distance to Agriculture (km)	1.41	0.46	1	9.59	0.00
Hatching Date	-0.01	0.03	1	0.10	0.75
Female Body Condition	-0.00	0.02	1	0.00	0.96
Nest Density[1]	-0.00	0.00	1	0.00	0.96
Nest Density*Distance to Agriculture (km)	-0.00	0.00	1	8.31	0.00

Table 2.2. Results of linear model using Proc Mixed to assess main variables contributing to variation in parasite levels in burrowing owl fledglings (n=52 nests, 295 fledglings) from the Snake River Birds of Prey National Conservation Area, Idaho in 2006 and 2007.

Effect	**Estimate**	**Standard Error**	**DF**	**t-value**	**P-value**
Intercept	2.41	1.88	44	1.29	0.21
Distance to Agriculture (km)	-0.11	0.06	44	-1.82	0.08
Nest Density[1]	0.09	0.53	44	0.17	0.86
Hatching Date	-0.00	0.01	44	-0.39	0.70
Female Body Condition	-0.01	0.01	44	-1.12	0.27

Table 2.3. Results of linear model using Proc Mixed to assess variables contributing to variation in productivity in 81 burrowing owl nests from the Snake River Birds of Prey National Conservation Area, Idaho in 2006 and 2007.

Fixed Effects	**Estimate**	**Standard Error**	**DF**	**t-value**	**P-value**
Intercept	7.24	3.97	72	1.83	0.07
Distance to Agriculture (km)	-0.07	0.07	72	-1.03	0.31
Hatching Date	-0.01	0.03	72	-0.25	0.80
Female Body Condition	0.03	0.01	72	2.40	0.02
Nest Density[1]	0.20	0.09	72	2.29	0.02
Depredation Status	-1.26	0.63	72	-1.99	0.05
Average Flea Load	-0.61	0.22	72	-2.80	0.01
Flea Treatment[2]	-0.49	0.52	72	-0.94	0.35
Hatching Date * Nest Density	-0.13	0.06	72	-2.19	0.03

[1] Nest density is the territory overlap of a focal burrowing owl nest using a 200m buffer around each nest.

[2] Flea Treatment was categorized as either 0 (No treatment or water) and 1 (AIL treatment application).

Figure 2.1. Photos of the mock badger used to determine response of male burrowing owls to the threat of predation and of the control (Russian thistle) in the Snake River Birds of Prey Area in southern Idaho. Both were presented to focal male burrowing owls in 2006 while only the mock badger was presented in 2007 as the control was eliciting no response.

Figure 2.2. Illustration depicting how mock badger trials were conducted to record male burrowing owl behavior whose nests were in high-density or low-density configurations in the NCA during 2006 and 2007. The mock badger or control was hidden 50m from the focal burrow and pulled toward and past the nest burrow at approximately 20m/min. while the observer(s) recorded the reactions of the focal male a blind (a and b). In high-density areas (a), a second observer measured the reactions of owls at the neighboring nest.

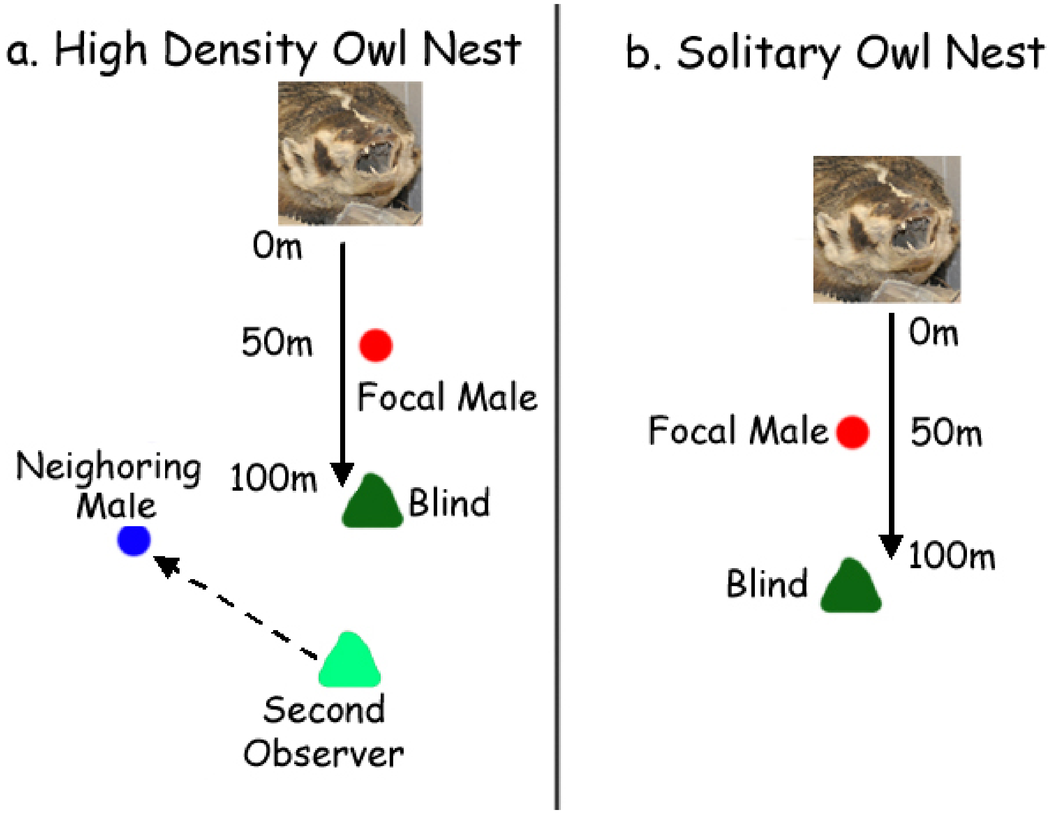


Figure 2.3. Examples of nest density parameters that were measured on burrowing owl nests in 2006 and 2007. Note that for nest B, the closest neighbor is C, but C's closest neighbor is D. Number of neighbors counts the number of owls within a buffer around the nest. Territory overlap determines the total buffered area of a nest that other territories overlap. Complete overlap is the sum of each neighboring nest's overlap with the focal nest. The crosshatched areas indicate where more than one nest overlaps the same area and this area is counted multiple times accordingly. All these nest density parameters were significantly correlated with each other (see Appendix A).

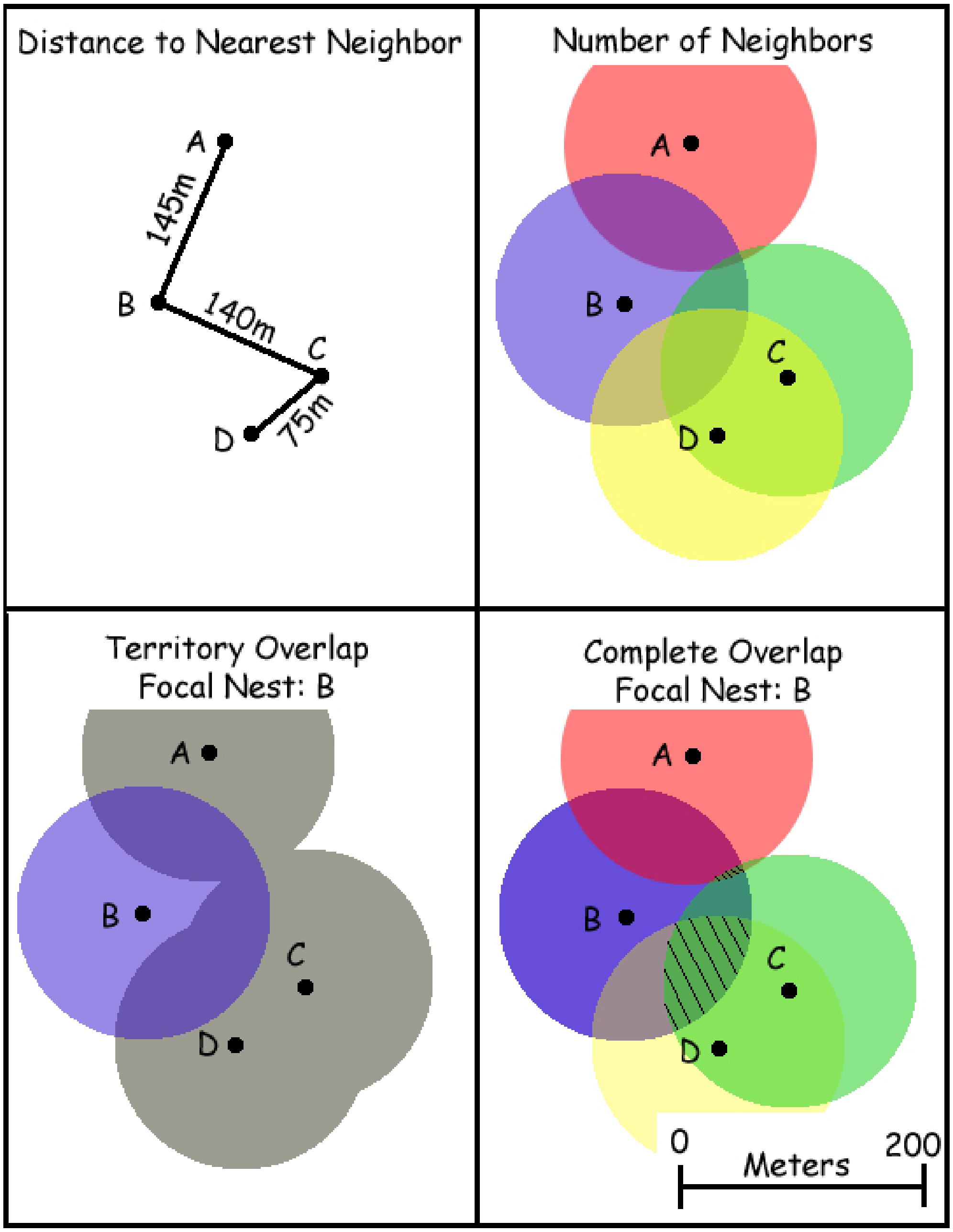


Figure 2.4. Examples of how differing buffer sizes influence territory overlap (top 2 images) using 200m and 400m buffer sizes around a focal burrow which is highlighted in red. Complete overlap (bottom 2 images) with the same buffer configuration is the total summed area of each neighboring burrow that overlapped the focal burrow. In this example, actual burrow locations in 2006 from the NCA are used.

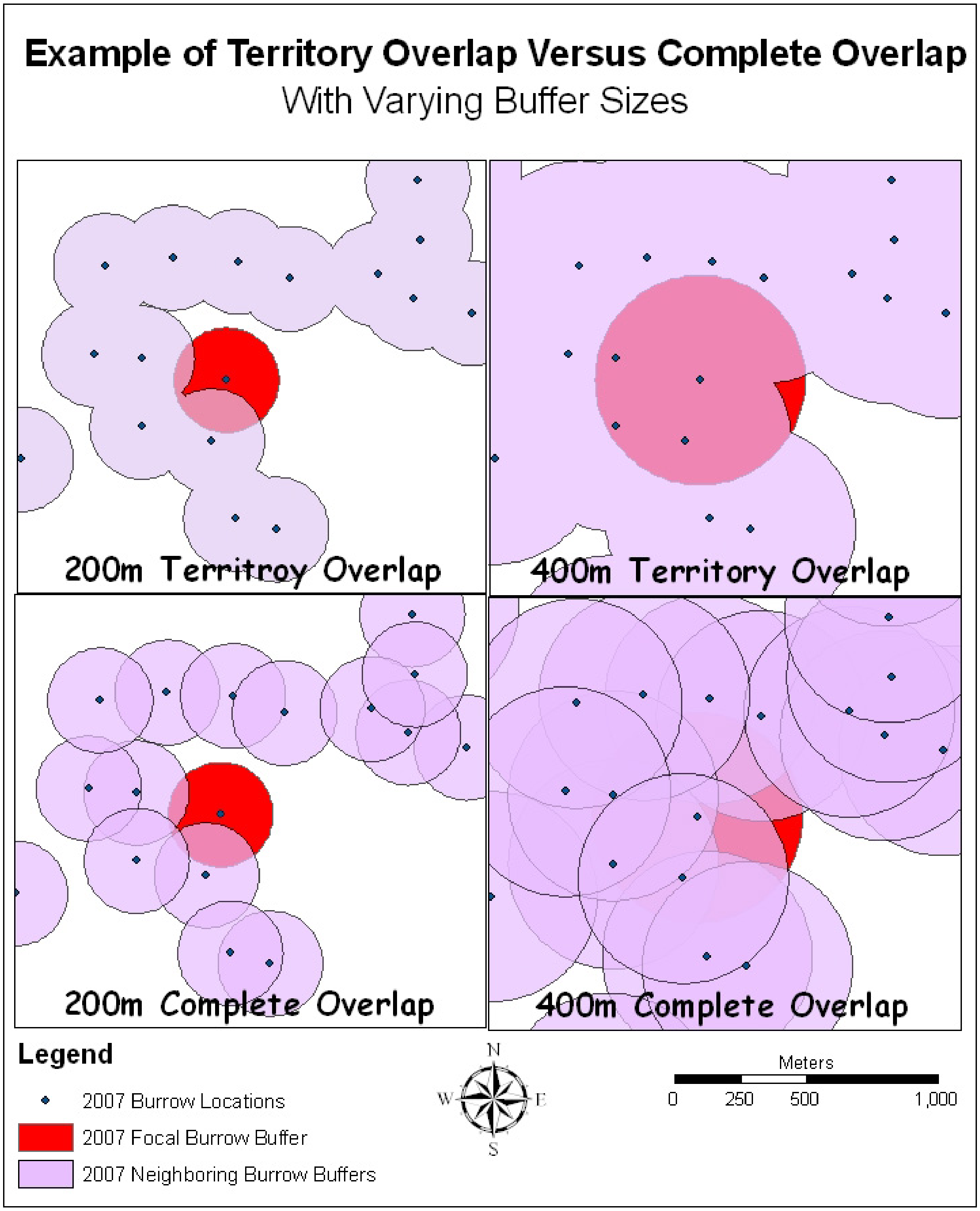


Figure 2.5. Odds of depredation in relation to territory overlap and proximity to agriculture for 105 actual burrowing owl nests from 2006 and 2007 in the NCA located in southern Idaho. Distance from agriculture was analyzed as a continuous variable in the model but is divided into categories in this figure for heuristic purposes to illustrate the interaction.

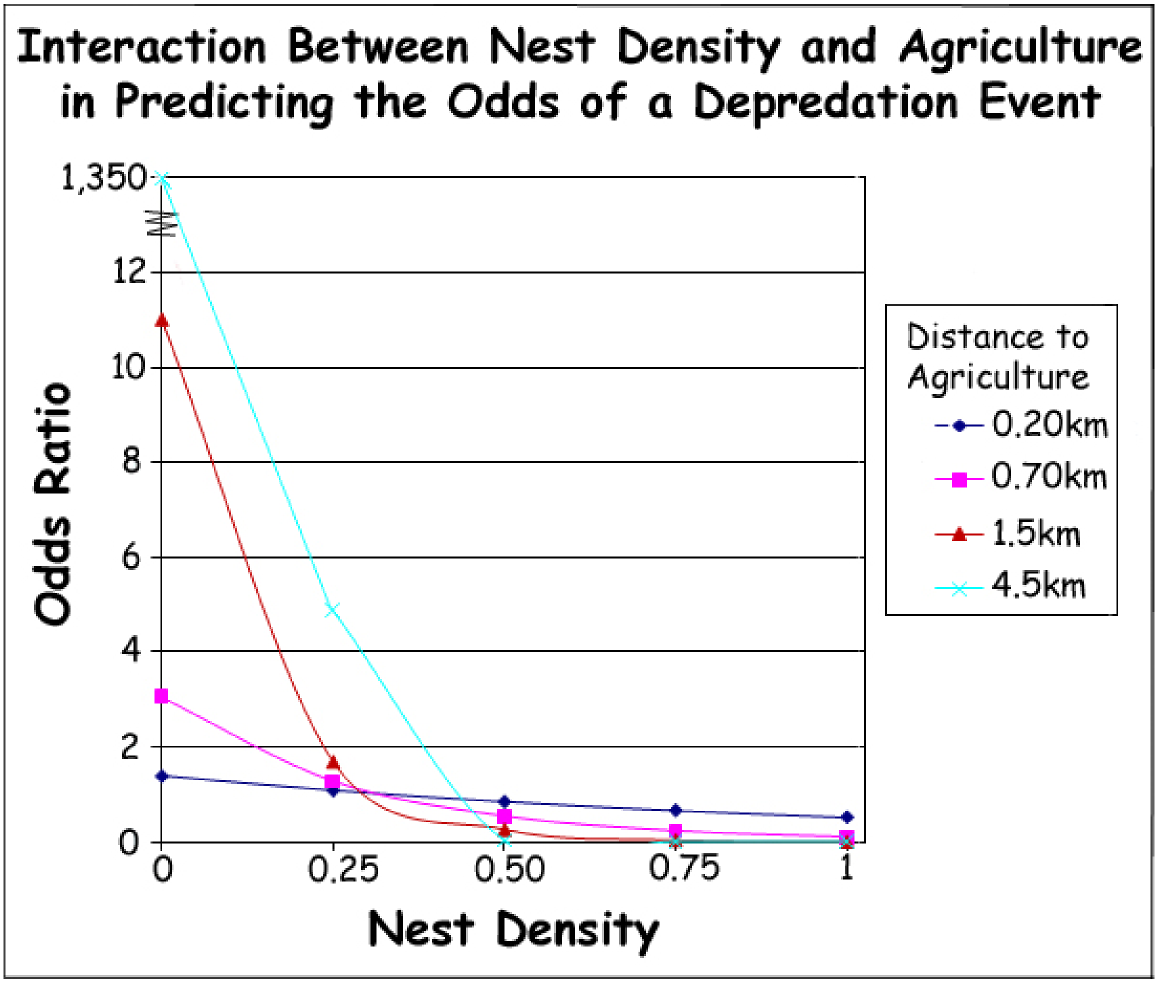


Figure 2.6. Body condition (BCI) of burrowing owl fledglings in the NCA in 2006 and 2007 in relation to (a) AIL treatment (n=17) and control (water-treated, n=16); and (b) nest density (high-density, n=20; low-density, n=13).

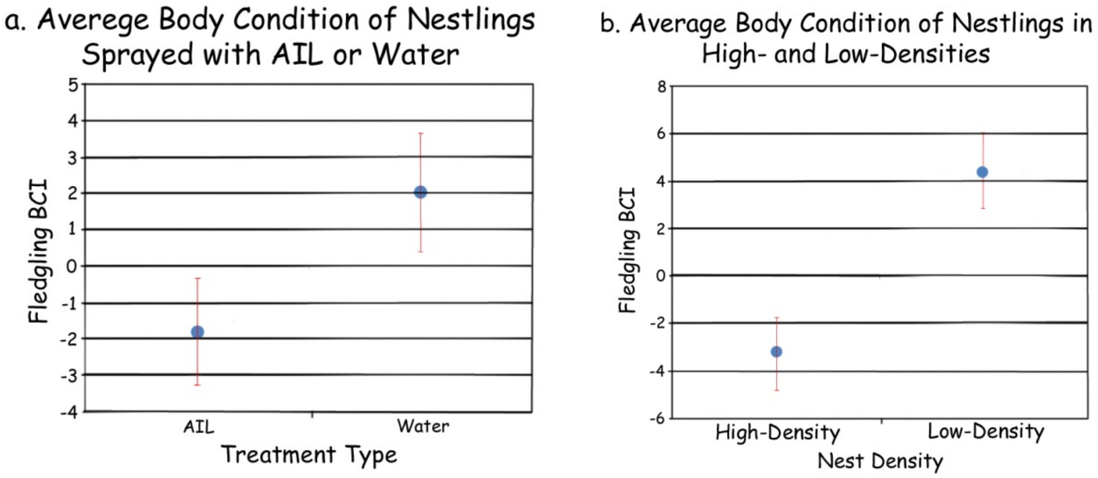
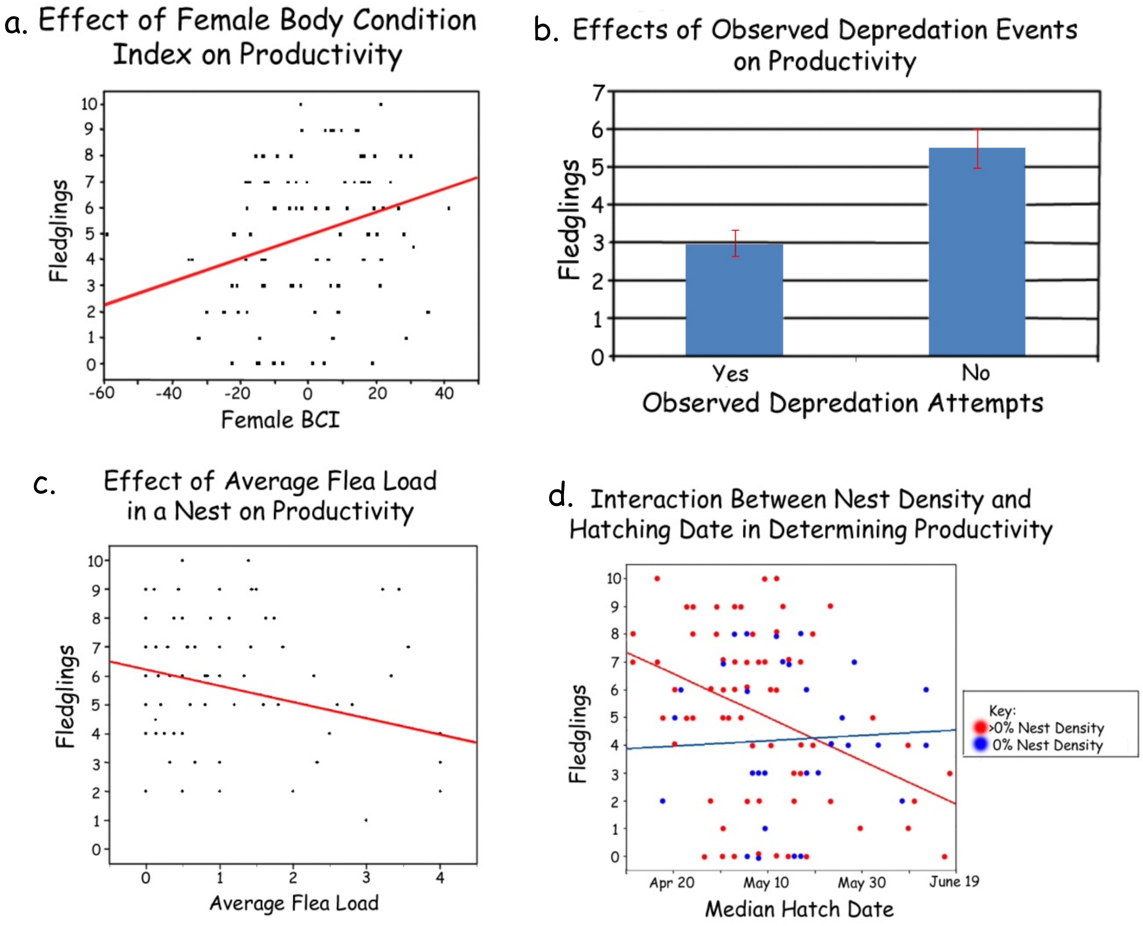


Figure 2.7. Relationships between the number of fledglings (productivity) per nest and (a) female BCI, (b) depredation status, (c) average flea loads in a nest, and (d) interaction between territory overlap within a 200m buffer and hatching date, from n=81 nests in 2006 and 2007 from southern Idaho. The interaction between hatching date and nest density in relation to productivity shown in figure d. has been broken into two categories for heuristic purposes only to illustrate the interaction. The actual model was run using density as a continuous variable.



a. Effect of Female Body Condition Index on Productivity

b. Effects of Observed Depredation Events on Productivity

c. Effect of Average Flea Load in a Nest on Productivity

d. Interaction Between Nest Density and Hatching Date in Determining Productivity

Figure 2.8. Relationship between female BCI and median hatch date of their nestlings in burrowing owls from the NCA in 2006 and 2007. The two red dots were re-nest attempts and were excluded from the analysis. Female BCI was negatively associated with median hatch date (Simple Linear Regression, *B* = 140.04 - 0.20(Female BCI), $F_{1,88}$=11.03, p<0.00, n=90)

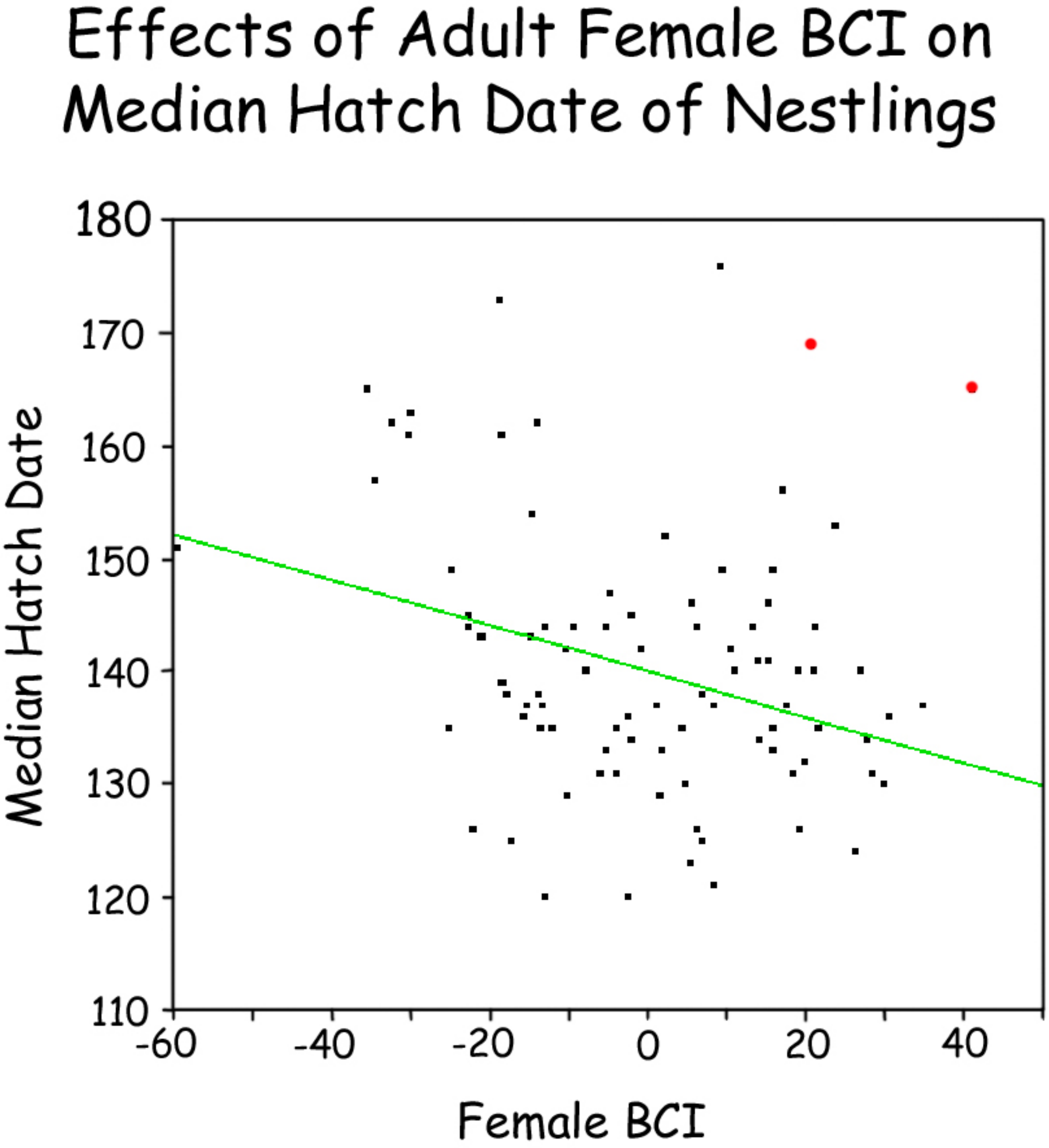
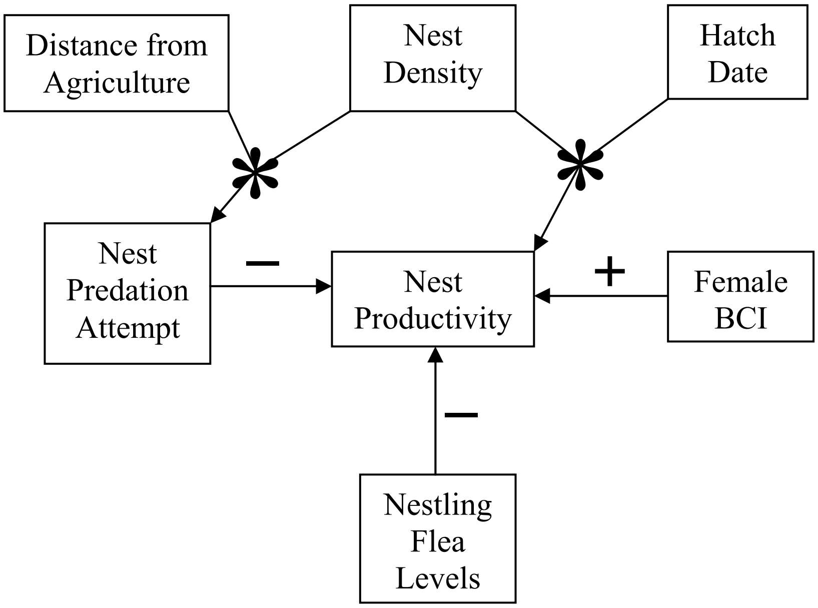


Figure 2.9. Relationships among predation, parasites, and productivity in actual burrowing owl nests in the NCA from 2006 and 2007. The direction of the effect (increase or decrease) is indicated. Interactions are illustrated with merging lines connected with an asterisk (*).

Parameters	Nest Density	Distance from Ag	Hatch Date	Female BCI	Nestling Fleas	Predation Attempt
Predation	Interaction with Ag Distance	Interaction with Nest Density	No	No	N/A	N/A
Parasites	No	No	No	No	N/A	N/A
Productivity	Interaction with Hatch Date	No	Interaction with Nest Density	Yes (Positive)	Yes (Negative)	Yes (Negative)



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CHAPTER 3: EFFECTS OF NEST DENSITY, LAYING DATE, AND EGG ORDER ON YOLK HORMONES IN BURROWING OWL EGGS

Abstract

Avian egg-yolk hormones are of interest to scientists because variation among eggs within and among clutches may be adaptive. Using Radioimmunoassay, I analyzed concentrations of egg-yolk androgens (testosterone, dihydrotestosterone, and androstenedione), estradiol and corticosterone from one early and one late-laid egg in 46 western burrowing owl (*Athene cunicularia hypugaea*) nests located in the Morley Nelson Snake River Birds of Prey National Conservation Area in southern Idaho. I evaluated hypotheses relating hormone levels to density of nesting pairs, laying date of the first egg in a nest, and laying order within a nest. Estradiol and corticosterone concentrations were generally below detection levels of the assay, so my subsequent analyses focused on the three egg-yolk androgens. Nesting density, calculated by measuring territory overlap with a 200m buffer around each nest, varied from 0% to 100% but had no significant effect on yolk androgens. However, egg-yolk androgens varied with laying date, peaking in the middle of the laying season while remaining low early and late in the breeding season. This pattern appears unusual among bird species and may be a result of an interaction between high male quality early in the laying season and a decline in fledgling success as the nesting season progresses. Moreover, within nests, late-laid eggs had higher testosterone, dihydrotestosterone, and androstenedione

than early-laid eggs. This rise in androgens from early to late-laid eggs within a nest is present in many bird species. In burrowing owls, it could reflect a mechanism to assist nestlings from late-laid eggs that hatch one to several days after their siblings to compete for resources within the nest, avoid being cannibalized by older siblings, or promote survival in the presence of larger siblings.

Introduction

Avian egg-yolk hormones are of interest for scientific study because variation among eggs within a single clutch, between different clutches of the same species in the same area, across different species in the same area, and between similar species at different latitudes may be adaptive (Reed and Vleck 2001, Eising and Groothuis 2003, Gorman and Williams 2005, Navara et al. 2006a, Love et al. 2008, Martin and Schwabl 2008). Hormones can function as regulatory signals during the development of genotype into phenotype and mediators of phenotypic responses to environmental changes (reviewed by Groothuis and Schwabl 2007). Although proximate mechanisms associated with egg-yolk hormone variation are only beginning to be understood, it appears that a female's hormone level at the time of laying influences egg-yolk hormone levels (Reed and Vleck 2001, Whittingham and Schwabl 2002, Pilz and Smith 2004, Navara et al. 2006a, Love et al. 2008, Tanvez et al. 2008, Hargitai et al. 2009). In fact, Navara and Mendonça (2008) point to new evidence that suggests, given current environmental conditions, females may be capable of fine tuning hormone content in an egg at the time of laying to maximize reproductive success. The factors that influence female and egg-yolk hormones are relevant because increased egg hormone levels are related to multiple costs and benefits, including accelerated hatching times, increased nestling growth rates and aggression, decreased nestling immune response, decreased body condition in adulthood, and reduced life span (Schwabl 1993, Schwabl 1996a, Schwabl 1996b, Lipar and Ketterson 2000, Sockman and Schwabl 2000, Eising et al. 2001, Lipar 2001, Eising and Groothuis 2003, Pilz et al. 2003, Navara et al. 2005, Navara et al. 2006a, Cucco et al. 2008, Sockman et al. 2008).

One factor that influences circulating hormone levels in adult birds is the level of interaction between conspecifics. Birds that defend territories have to be continually alert for the presence of conspecific intruders who are interested in resources or an extrapair mating. An aggressive display or attack is often required to drive the intruder from the territory, and hormone levels may rise before or as a result of these close encounters (Nephew and Romero 2003, Pilz and Smith 2004, Goymann et al. 2007). This rise can occur in both males and females in high-density areas where interactions are frequent or dominance must be established (Wingfield and Wada 1989, Wingfield 1994, Pilz and Smith 2004, Aubin-Horth et al. 2007). High conspecific neighbor density at the time of laying also is related to increased yolk hormone levels (Reed and Vleck 2001, Groothuis and Schwabl 2002, Whittingham and Schwabl 2002, Pilz and Smith 2004, Navara et al. 2006a, Love et al. 2008). Higher yolk hormone levels may give nestlings in high-density areas a competitive advantage (e.g., increased aggression) while competing for resources as adults in densely populated environments (Pilz and Smith 2004).

Egg hormone levels can also vary with female laying date and with asynchronous hatching. For example, Pilz et al. (2003) found European starling (*Sturnus vulgaris*) clutches laid earlier in the season had higher hormones (but see Michl et al. 2005). In addition to laying date, laying order within a clutch can influence egg-yolk hormones, particularly when there is hatching asynchrony. Hatching asynchrony often results in broods that show a hatching-order dependent size hierarchy (Clark and Wilson 1981).

Younger nestlings may be at both a size and competitive disadvantage against older, larger nestlings. They are also less likely to survive to fledging (O'Conner 1978, Mock et al. 1990, Maddox and Weatherhead 2008). An increase in egg-yolk hormones with laying order may help compensate for this disadvantage (Schwabl 1997, French et al. 2001, Royle et al. 2001, Müller et al. 2004, Tschirren et al. 2004). Increased egg hormones can promote overall growth of an embryo (Schwabl et al. 2007) or growth of the hatching muscle (Eising et al. 2001, Lipar 2001), both of which result in an earlier hatching date. Earlier hatching allows for younger nestlings to be closer in age to their older siblings and, therefore, to more effectively compete with those larger siblings for resources (Schwabl 1993, Eising and Groothuis 2003).

If increasing hormones are beneficial, why wouldn't females maximize hormone levels in every egg? There are also costs associated with increasing yolk hormones beyond a maximum threshold. Sockman and Schwabl (2000) demonstrated that artificially increasing hormones in American kestrels (*Falco sparverius*) reduced the overall body condition of nestlings and resulted in higher mortality. Artificially increased testosterone has also been linked to decreased immune response (Verboven et al. 2003, Navara et al. 2005). In addition, high egg-yolk hormones can increase the metabolic rate without increasing growth rate (Tobler et al. 2007). This requires the nestling to consume more resources to grow at the same rate as its siblings. Thus, natural selection may select for an optimum egg-yolk hormone level for each egg based on multiple factors. However, females may still be able to fine tune yolk hormone levels based on environmental conditions at the time of egg laying to provide a competitive advantage to each nestling while balancing the negative effects that elevated egg-yolk hormones can impose on that nestling.

As previous studies suggest, external factors such as nesting density, laying date, and egg order can affect the amount of hormones deposited in eggs. I examined the potential effects of these factors on egg-yolk hormones in burrowing owls (*Athene cunicularia hypugaea*) that breed in southern Idaho.

Burrowing owls nest in underground burrows located in prairies, grasslands, steppes, and other open areas (Haug et al. 1993, Poulin et al. 2005, Lantz et al. 2007). Although burrowing owls frequently nest in well-drained open areas, they also show an affinity for nesting near irrigated agriculture (Rich 1986, Leptich 1994, DeSante et al. 2004, Conway et al. 2006, Moulton et al. 2006, Restani et al. 2008). Adults typically weigh 120-200g, are 19-25cm in length, and have approximately a 16cm wing chord (Haug et al. 1993). They are opportunistic predators that feed on rodents, small birds, amphibians, reptiles, and a variety of invertebrates (Moulton et al. 2005, Poulin and Todd 2006, Littles et al. 2007, Williford et al. 2009). Burrowing owls are socially monogamous, and females lay, on average, 8-12 eggs per clutch and incubate while their mates provision them (Haug et al. 1993, Kaufman 1996). On average, pairs produce approximately 0.9 to 4.9 nestlings per nesting attempt (Haug et al. 1993, Kaufman 1996, Smith et al. 2005, Wellicome 2005, Conway et al. 2006, Griebel 2007). In northern portions of their range, burrowing owls tend to be migratory. While most burrowing owls that breed in Idaho migrate, their migration routes and wintering areas remain poorly known (Haug et al. 1993, King and Belthoff 2001). However, a small number of band returns indicate that at least some Idaho burrowing owls may overwinter in California (Belthoff, unpublished data).

Burrowing owls nest in underground burrows that have been previously excavated by other animals such as American badgers (*Taxidea taxus*) or prairie dogs (*Cynomys* spp.; Gleason and Johnson 1985, Rich 1986, Poulin et al. 2005, Lantz et al. 2007). However, burrowing owls also nest in artificial burrows placed by researchers (Trulio 1995, Henny and Blus 1981, Smith and Belthoff 2001, Todd et al. 2003, Smith et al. 2005, Barclay 2008). Artificial burrows consist of an underground nesting chamber leading to the surface through a tunnel (Smith and Belthoff 2001).

Study Area

I studied burrowing owls in and around the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) located in southwestern Idaho. The NCA encompasses 195,325ha, approximately 5% of which is irrigated agriculture (agricultural areas that require human supplied water to grow crops). The agricultural areas grow primarily alfalfa, corn, sugar beets, and mint. The remainder is disturbed shrub steppe and grassland upon which some cattle and sheep grazing occurs, primarily during winter (USDI 1996, Moulton et al. 2005). There are approximately 300 artificial burrows in the NCA available for burrowing owls to nest and roost (Smith and Belthoff 2001, Belthoff and Smith 2003, Moulton et al. 2006). The artificial burrows allow easy access for researchers to monitor burrow activity; to count, measure, and sample eggs; and to count, capture, and mark adults and young. From 1997-2007, burrowing owl nesting pairs occupied 30-60 of the artificial burrows in the NCA each year (Belthoff and Smith 2003, Belthoff, unpublished data).

Hypotheses

Burrowing owls are an appropriate species with which to study factors that influence egg hormone levels because (1) nesting density varies (i.e., high-density to low-density nesting distributions), (2) they are partial migrants and can return and begin nesting at different times of the breeding season, and (3) females usually lay between 8-12 eggs per clutch making the time between the laying of early and late eggs quite long, and this long laying period can result in partial asynchronous hatching.

Nest Density

Burrowing owl nesting density can vary from low-density to high-density configurations in many portions of their range and across my study area (e.g., one burrowing owl nest in a square kilometer to 6 neighbors within a 400m radius of a focal nest; Desmond and Savidge 1996, Moulton et al. 2005, Fisher et al. 2007) and both male and female burrowing owls have been observed interacting aggressively with conspecifics (Moulton et al. 2004, pers. observ.). Because of the potential for varying amounts of aggressive interactions among females, the density hypothesis predicts that as neighbor density increases egg-yolk hormone levels will rise.

Laying Date

In northern portions of their range, burrowing owls are generally present only during the breeding season (Haug et al. 1993, Kaufman 1996, King and Belthoff 2001, Davies and Restani 2006); outside of this time, they winter elsewhere. Egg laying in

Idaho generally begins in late March, peaks in April and continues through early May (pers. observ.). Variability in laying date could alter burrowing owl egg hormones. Therefore, the laying date hypothesis predicts that as the laying season progresses egg hormone levels will change.

Asynchrony

I also examined the potential role of hatching asynchrony in altering egg hormones within nests. The laying period for a given female burrowing owl is often quite long and can last at least two weeks (Haug et al. 1993, Wellicome 2005). Females typically begin incubation after most, but not all, eggs have been laid, and some nestlings can hatch 1-7 days behind the rest of the clutch (mode=4 days; Wellicome 2005). This hatching asynchrony is frequently evidenced by some size discrepancies in nestlings after all eggs have hatched. The partial asynchronous hatching pattern may put late-laid burrowing owl nestlings at a competitive disadvantage. Thus, the asynchrony hypothesis predicts that as laying progresses from early- to late-laid eggs, yolk hormones will increase to benefit the younger nestlings in late-laid eggs.

Methods

Yolk Androgen Collection and Assessment

I collected yolk samples in 2006 - 2007 during the laying season (April through May) and analyzed concentrations of egg-yolk hormones. Beginning in late March, I monitored artificial burrows once a week for the presence of adult burrowing owls. When I detected a mated pair, I inspected the nesting chamber in an artificial burrow to determine if the female had initiated egg-laying and to determine initial laying date. I sampled eggs from nests at different densities and from different laying dates to examine both the density and laying date hypotheses. In addition, I wanted to include two possible confounding variables. Burrowing owls often associate with irrigated agriculture, where the possibility of persistent organic pollutants or more frequent contacts with humans could influence egg-yolk hormone levels (Verboven et al. 2008, Poisbleau et al. 2009). Therefore, I measured distance to agricultural fields to assess the relationship with egg hormones. I also calculated a female body condition index (BCI; described in methods below) at the time of capture as female health can influence yolk hormones (Warner et al. 2007, Love et al. 2008, Hargitai et al. 2009).

I was unable to determine exact laying order for eggs within each burrowing owl nest because increased disturbance could have resulted in nest abandonment. Moreover, I limited nest visits to decrease the possibility that my visits resulted in physiological changes in adult females that might alter egg-yolk hormones (Poisbleau et al. 2009). Instead, I visited and inspected nests only with sufficient frequency (2-3 visits/nest) and at appropriate intervals such that I could divide eggs within a clutch into two categories that roughly corresponded with the first and second halves of each clutch. I refer to these as early and late eggs, respectively. I marked early-laid eggs on the shell with a small amount of dark ink to distinguish them from late-laid eggs. I obtained a yolk sample from one randomly selected early and one randomly selected late egg from each clutch to examine the asynchrony hypothesis. I avoided sampling nests that had signs of incubation; in so doing, I ensured that I was measuring hormones that the female had allocated to the eggs and not hormone concentrations altered through embryo growth (Gilbert et al. 2007).

Following Schwabl (1993), I extracted ~75mg of yolk using a 25-gauge, ½-inch butterfly needle to puncture the eggshell and enter the yolk. I did not use a destructive sampling procedure that opened eggs and destroyed the embryo because I wanted to preserve the viability of eggs (but see Lipar et al. 1999a). I swabbed eggs with an alcohol swab both before and after the needle puncture to reduce risk of infection and sealed the puncture with either Loctite Super Glue Gel Control (Henkel Consumer Adhesives, Inc., Avon, OH) or silicone (Window and Door Silicone II, Bioseal GE Sealants and Adhesives, Huntersville, NC). I transferred yolk into a labeled centrifuge tube, and stored the sample at -20°C until analysis using radioimmunoassay techniques.

I assayed burrowing owl egg-yolk samples to determine concentrations of three androgens (androstenedione (A_4), 5α-dihydrotestosterone (DHT), testosterone (T)), 17β-estradiol (E2), and corticosterone (CORT). CORT is an adrenal steroid hormone involved in metabolism, stress response, and immune response. The other hormones are steroids that all have roles in growth, aggression, and sexual behavior. A_4 is metabolized into T and E2, and T is metabolized into DHT (Hadley and Levine 2006). It is important to measure all three androgens (A4, T and DHT) because measuring only one could underestimate androgen levels. For example, if only T is measured and most of the hormones within an egg have already been metabolized in to DHT, then concentrations of egg-yolk androgens would be underestimated.

Hormone Assays

I performed Radioimmunoassay (RIA) in laboratories at Washington State University using the modified Schwabl (1993) yolk hormone protocol. To conduct these assays, I added 20µl of stock titrated A4, DHT, T, E2, and CORT to each sample. This method aids in extraction and purification of recoveries. Samples were covered and placed in a 4°C refrigerator for at least 24 hours for equilibration. Free steroids were extracted twice using 4ml 30:70 (vol/vol) petroleum ether/diethyl ether. Samples were snap frozen and decanted into new tubes and dried in a 37°C water bath using compressed nitrogen. The samples were re-dissolved in 1ml 90% ethanol, vortexed, covered, and placed in a –20°C freezer overnight. Ethanol was separated from proteins and lipids by centrifuging at 1500rpm for 5min at 4°C and decanting the ethanol into a new tube. Ethanol samples were dried using compressed nitrogen in a 50°C water bath. The extract was re-dissolved in 0.5ml 10:90 (vol/vol) ethylacetate/isooctane (EA/IO). The solution was transferred to a microcolumn containing diatomaceous earth, dH_2O, and a 1:1 mixture of propylene glycol:ethylene glycol for hormone extraction. Four milliliters of 2/98 EA/IO was used to extract A4. DHT was extracted with 4.5 ml of 10/90 EA/IO. T was extracted using 4.5 ml of 30:70 EA/IO. E2 was extracted using 4.0 ml of 35:65 EA/IO. Finally, 4.0 ml 45:55 EA/IO was used to extract CORT.

I then dried the fractions under compressed nitrogen in a 45°C water bath. The dried fractions were re-dissolved in 550µl PBSg and 100µl of the appropriate antibody (see below) along with 100ml of the appropriate labeled steroid (see below) were added to the tubes. Of this solution, 100µl was used for recovery and 100µl duplicates (A4 and

T) or 200μl duplicates (DHT, E2, and CORT) were set up for the assays. Antibodies used were B3-163 (Esoterix Endocrinology) for CORT, AR1702 (Biogenesis) for E2, T 3003 (Wien Laboratories) for T and DHT, and A 1707 (Wien Laboratories) for A4. The labeled steroids used were NET-399 (Perkin-Elmer) for CORT, NET-517 for E2, NET-533 for T, NET-544 for DHT, and NET 469 for A4. The samples were vortexed, covered, and refrigerated at 4°C for 12-18 hours. I then added 500μl of charcoal solution (500mg charcoal, 20ml Dextran solution, and 180ml PBSg) to each tube except A4 tubes that contained 300μl of charcoal solution and centrifuged tubes at 2000rpm for 10min at 4°C. I added 5ml of scintillation fluid to each sample. The following day, the samples were counted on a scintillation counter from which hormone levels were calculated.

Nest Variables

I used ArcMap 9.2 and Hawth's Analysis Toolpack for ArcMap (ESRI ArcMap 9.2, Beyer 2004, respectively) to calculate nest density. I calculated multiple density estimates where each estimate of density included buffers around a focal nest at 100m, 125m (measures of close neighbors), 200m (measure of intermediate neighbors), 400m, and 600m (measures of general area density). The density estimates I calculated were the number of neighbors within the buffer, territory overlap of a focal nest (the total percentage that a focal nest buffer is overlapped by other buffers; 0-100%), and complete overlap of a focal nest (the sum of the percentage of each overlapping neighboring nest buffer on the focal nest; 0->100%; see Figures 3.1 and 3.2) at each buffer distance. I also measured distance to the nearest neighbor (Figure 3.1). I measured distance to the nearest neighbor and the number of neighbors within a buffer zone using Hawth's

Analysis Toolpack "Distance Between Points (Within Layer)" tool for ArcMap 9.2. Hawth's Analysis Toolpack "Polygon in Polygon Analysis" tool calculated the territory overlap and complete overlap at each distance. Laying date was the integer day (0 representing January 1st and 365 representing December 31st) on which the female began to lay eggs. I measured the distance to irrigated agriculture using ArcMap's "Point to Polyline" tool. Finally, I calculated female BCI for adult females using weight (grams), wing length, tail length, and culmen length (in mm) of the female at the time of capture (usually 1-3 weeks after incubation started). From these measurements, I calculated female BCI. To do so, I conducted a principal components (PC) analysis using the size variables and regressed mass on scores along the first PC (index of size). The residuals from this regression were the BCI scores (Jacob et al. 1996, but see Green 2001), with positive residuals indicating owls in better body condition than expected.

Data Analysis

The various measures of nesting density that I calculated were highly correlated (see Appendix A). Thus, to avoid multicollinearity in linear models, I chose territory overlap of a focal nest with a 200m buffer as an index of nest density in modeling effects of nest density on egg hormones. Moulton et al. (2004) found that burrowing owls defend their territory in response to simulated territorial intrusion to distances of 100m. Therefore, 200m represents the maximum distance by which two nests can be separated and be predicted to have high levels of interaction. I then analyzed effects of nesting density, laying date, distance to agriculture, female body condition (all random effects among nests), laying order (the fixed effect within a nest), and their interactions on burrowing owl egg-yolk hormone data using a mixed model (PROC MIXED) in SAS SAS 9.1 (SAS Institute, Cary, North Carolina). Laying date had a curvilinear relationship with T, DHT, and A4, so I fit the appropriate exponential terms (Tables 3.1-3.3, Figure 3.3 a-c). No interaction terms were significant for any hormone in the analysis, so I removed them from final models.

Results

I collected 46 yolk samples from 23 nests in 2006 and 46 samples from 23 nests in 2007. I was unable to capture the adult female at four nests so could not calculate body condition for them. Of the five hormones that I analyzed, E2 and CORT did not occur in detectable levels in most egg-yolks (see Appendix B); therefore, I focused subsequent analyses on the three androgens: T, DHT, and A4. Yolk T, DHT, and A4 averaged 10.8±0.81pg/mg (range: 0.4-39.2pg/mg), 8.4±0.56pg/mg yolk (range: 0.6-27.0pg/mg), and 15.2±1.24pg/mg yolk (range: 0.7-62.4pg/mg), respectively (n=92 eggs from n=46 nests in each case).

Nest Density

Most burrowing owl nests had nearest neighbors that were located within 250m (Median=247m, range: 84-3470m, n=46 nests). Thus, there was clear potential for many of these nesting individuals to interact with neighbors. The density hypothesis predicts that increases in burrowing owl nest density will be related to higher egg hormone levels. My index for nest density (i.e., territory overlap within a 200m buffer) averaged 32.0%±3.27% (n=46, range: 0.0-95.9%). Despite substantial territory overlap of burrowing owl territories in many cases, I did not detect an effect of nest density on T, DHT, or A4 levels (Tables 3.1-3.3).

Laying Date

The laying date hypothesis predicts that egg hormone levels will change as the laying season progresses. The average date on which owls laid their first egg was 15 April (integer day 105±1 days; range: 28 March-12 May). The pattern of variation in T, DHT, and A4 across the laying season was similar. Each hormone was low early in the season, peaked in mid-season, and declined during the end of the laying season. As such, all three androgens showed a curvilinear relationship with laying date (Tables 3.1-3.3, Figure 3.3).

Asynchrony

T, DHT, and A4 was 7.6±0.97pg/mg yolk (range: 0.4-39.2pg/mg yolk), 7.1±0.67pg/mg yolk (range: 0.6-25.2pg/mg yolk), and 13.2±1.62pg/mg yolk (range: 0.7-43.1pg/mg yolk) in early laid eggs, and 13.9±1.04pg/mg yolk (range: 2.2-29.36 pg/mg yolk), 9.6±0.83pg/mg yolk (range: 2.7-27.0pg/mg yolk), and 16.9±1.74pg/mg yolk (range: 1.4-62.4pg/mg yolk) in later laid eggs. The asynchrony hypothesis predicts yolk hormones increase as laying progresses from early- to late-laid eggs within a clutch. I found a significant difference between early and late eggs for T and DHT but not A4. T increased by 6.9±1.33pg/mg yolk from early to late eggs (range: -18.2-25.5pg/mg yolk; Table 3.1, Figure 3.4a). DHT increased by 2.4±0.84 pg/mg yolk from early to late eggs (range: -19.1-16.1pg/mg yolk; Table 3.2, Figure 3.4b). There was a 3.6±2.27pg/mg increase in A4 from early to late eggs (range: -29.1-54.1pg/mg yolk; Table 3.3, Figure

3.4c). When examining T, DHT, and A4, there was an absolute increase in egg-yolk androgens from early to late eggs in 42, 35, and 29 of 47 nests, respectively. In only 3 nests were all three androgens higher in the early-laid egg.

Agriculture and Female BCI

Distance to irrigated agricultural fields averaged 3.0±0.41km (Median = 1.0km, n=46 nests, range: 0.1-12.9km). Female body condition averaged 4.9±1.9 (n=42 adult females, range: -34.3-41.0). Neither distance to agriculture nor female body condition affected egg-yolk androgens (Tables 3.1-3.3).

Discussion

My study is one of only a small number of studies of egg-yolk hormones in raptors and perhaps the first report in owls. I found that both E2 and CORT concentrations were low and generally below the level of detection for the assay. I documented the presence of and variability in three egg-yolk androgens in burrowing owl eggs and evaluated three potential explanations for the variability. There was no effect of nest density, but laying date affected burrowing owl egg-yolk T, DHT, and A4. Egg androgens peaked in the middle of the nesting season and were lower near the beginning and end. In addition, T and DHT increased significantly from early to late eggs within a nest, and A4 trended in this direction. Increases in egg androgens were consistent with predictions of the asynchrony hypothesis.

Nest Density

Despite relatively small nearest neighbor distances, substantial density overlap, other known effects of density in burrowing owls (see Chapter 2 of this thesis) and substantial variability in T, DHT, and A4 levels among nests, I detected no effect of nest density on burrowing owl egg-yolk androgens. One possible explanation for the lack of a density effect is that neighboring females did not interact with high enough frequency or aggression to affect hormones in adult females during the egg-laying period. For instance, nesting burrowing owls respond to simulated territorial intrusion with more aggressive approaches when intruders are within 50m, but they respond more frequently with vocal displays when owls are >100m away (Moulton et al. 2004). I found that the median distance to nearest nests was approximately 250m, which means that neighboring owls were generally at least 125m away from one another. Thus, they may have responded simply with vocalizations rather than more aggressive behaviors, while the latter may be needed to substantially affect female hormones. In addition, while aggressive female conspecific interactions have been observed, it is not well understood if and how aggressively female burrowing owls participate in these conspecific interactions. Alternatively, with documented extrapair fertilizations in burrowing owls (Johnson 1997), the potential for extrapair matings may select for behavior on the part of males that protects paternity. Males may have engaged in mate guarding during the laying season, as occurs in other raptors (Mougeot 2004). Mate guarding by males could limit forays of females and males into other territories where aggressive interactions with residents could subsequently alter their hormone milieu. Finally, we do not know if, and for how long, an aggressive encounter may raise female androgen levels, if increases last throughout laying of the entire clutch or for just a single egg, or if they simply do not last long enough to influence egg androgens (see Göth et al. 2008).

Agriculture and Female BCI

Neither distance of the nest to irrigated agricultural fields nor the BCI in females affected egg androgen levels in my study. Theoretically, there is potential for agricultural pesticides or other human activities in agricultural areas to alter hormones in adults and therefore affect their eggs, as pesticides may act as estrogen mimics and reduce androgens. Despite such potential, the literature contains no previous studies that I could find that documented effects of agriculture on egg-yolk androgens. The lack of a BCI effect in burrowing owls that I observed is similar to that of Pilz et al. (2003), who found no relationship between body condition and egg androgens in European starlings, although they found higher androgen levels in eggs from older females.

Laying Date

Burrowing owls in my study area initiated egg-laying as early as late-March and as late as mid-May. T, DHT, and A4 in burrowing owl eggs were lower early in the season, peaked near mid-nesting season, and declined toward the end of the nesting season. To the best of my knowledge, this is the first documentation of such a pattern. In contrast to my study, Verboven et al. (2003) found that egg androgens increased from early to late eggs within a clutch but documented no change with laying date in lesser black-backed gulls (*Larus fuscus*). Pilz et al. (2003) theorized that egg-yolk androgens in European starlings are higher earlier in the season because young that fledge early have a higher success rate, and females invest more by increasing yolk androgens in nestlings for which success is most likely. Michl et al. (2005) found an increase in yolk androgens in clutches laid later in the season in collared flycatchers (*Ficedula albicollis*). They hypothesized that older males, who are better foragers, nest earlier in the season and later nesting females must compensate for younger, inexperienced males by increasing yolk androgens in those eggs. They found that higher androgens increased begging behavior of the chicks and resulted in increased provisioning by the males. Perhaps both of the latter possibilities can be applied to a potential explanation of the pattern of egg-yolk androgen change with laying date that I observed in burrowing owls. Male burrowing owls that nest early in the year may be of higher quality and, as a result, females may not invest as many androgens into these eggs because the likelihood of fledging success is high. Moreover, Belthoff (unpublished data) has documented a significant decline in the number of burrowing owl fledglings per nest as the nesting season progressed. Consequently, females may have deposited fewer androgens in eggs laid later in the season because of the decreased likelihood of success and possible poorer male quality. Androgens may peak near the middle of the breeding season if the pool of available males is of varied quality but chance of productivity remains relatively high if increased begging behavior in the burrowing owl chicks results in an increase in male provisioning.

Asynchrony

My results indicate that different levels of androgens were allocated to burrowing owl eggs in the early and late portions of clutches. Finding increases in egg-yolk androgens within a nest has been common since Schwabl first reported this pattern in canaries (S*erinus canaria,* Schwabl 1993). For example, Sockman and Schwabl (2000) found American kestrels **(***Falco sparverius***)** increase androgens with laying order. Additonally, Schmaltz et al. (2008) reported increases of T within clutches of smooth-billed ani (*Crotophaga ani*), and they found no effect of density on T, which is similar to what I observed in burrowing owls.

Asynchronous hatching appears to be a common element in many of the species for which late-laid eggs have higher egg-yolk androgens (Schwabl 1993, Schwabl 1996b, Lipar et al. 1999b, Sockman and Schwabl 2000, Eising et al. 2001, Royle et al. 2001, Groothuis and Schwabl 2002, Eising and Groothuis 2003, Groothuis et al. 2005, Schmaltz et al. 2008, Tanvez et al. 2008). In burrowing owls, Wellicome (2005) found age disparities between first- and last-hatched siblings (i.e., hatching spans) varied considerably (ranging between 1 and 7 days) with a mode of 4 days. Thus, asynchronous hatching occurs in burrowing owls and may be related to the pattern of increased androgens in late-laid eggs that I observed.

One of the most competitive periods in a bird's life may be the nestling period (Ros 2008). Increasing androgens decrease incubation time those eggs and increase aggression and begging behaviors within the nestlings that hatch from these eggs. This, in turn, can allow younger nestlings who receive higher androgen levels to more effectively compete for the resources available within a nest (Schwabl 1993, Schwabl 1996b, Lipar and Ketterson 2000, Eising et al. 2001, Lipar 2001, Eising and Groothuis 2003, Goodship and Buchanan 2007, Groothuis and Schwabl 2007, Ros 2008, Müller and Eens 2009). For burrowing owls and other raptors, competition within a nest for resources can be great depending on environmental conditions. For instance, Smith and Johnson (1985), in a 7-year study of Townsend ground squirrel (*Spermophilus townsendi*) in southern Idaho, found that in one drought year grass cover decreased from 14.9% to

>1%, and this resulted in a 50% decline in the ground squirrel population. Declines in prey density can have a direct negative effect on raptor density, breeding success, and fledgling success (Grant et al. 1991, Rutz and Bijlsma 2006, Weins et al. 2006, Sergio et al. 2008). In times such as these, younger burrowing owl nestlings may gain some advantage from the physiological results of greater egg-yolk androgens that can include increased aggression and competition for resources within the nest. On the other hand, later-hatched nestlings may act as a final food source for their older, stronger siblings. Buchanan et al. (2001) found that testosterone raises the metabolic rate in birds, so individual burrowing owl nestlings with higher testosterone levels may be at a metabolic and size disadvantage in poor food environments, starve, and be consumed by siblings after death. Older nestlings may also attack and kill their smaller, undernourished siblings to increase provisioning of food resources to the remaining nestlings as is found in other raptors that practice siblicide (Simmons 2002). When food is plentiful, higher androgen levels in later eggs may help these nestlings compete with their older siblings for food within the nest and allow these nestlings to become highly competitive as adults. However, despite these benefits, increased androgens in younger nestlings may result in costs including decreased immune response and reduced lifespan (Sockman and Schwabl 2000, Navara et al. 2005). Increasing egg androgens within a burrowing owl clutch may be one measure by which adults help to mediate the variable environmental conditions found in southern Idaho and fledge an optimum number of nestlings each year.

Further Research

There are several other potential explanations for variation in egg-yolk androgens among nests that remain to be studied in burrowing owls. Individual aggression and social status among females has been linked to changes in androgen levels (Whittingham and Schwabl 2002, Tanvez et al. 2008). Gil et al. (2004, 2006) found greater amounts of testosterone in zebra finch (*Taeniopygia guttata*eggs) eggs when the female was exposed to preferred male songs and in barn swallow (*Hirundo rustica*) eggs when mates had experimentally elongated tails (also see Loyau et al. 2007). However, Navara et al. (2006b) found female house finches (*Carpodacus mexicanus*) increased egg-yolk androgen levels in eggs sired by less attractive males, and Garamszegi et al. (2007) found that song duration and syllable repertoire size were significantly negatively related to testosterone levels in the egg across 36 passerine species. Kingma et al. (2008) determined that experimentally altering a male's appearance to a more or less dominant state affected androgens in the subsequently laid eggs of their mates. Göth et al. (2008) found egg androgen levels varied among single egg clutches with no parental care depending on the size and depth of the incubation mound and size of the egg within the mound. Their results suggest that even in the absence of siblings and adults, egg androgens may be important in embryo and nestling growth. Addison et al. (2008) demonstrated high egg androgen variability, in two single-egg-laying seabird species, that was independent of the constantly changing marine environment. Finally, only little is known about the role that hormone receptors and binding globulins play in regulating hormone levels in birds and other species. I do not know what effects these two factors may have had on the amount of androgens used by cells and how this compares to the circulating levels that I measured in the egg-yolk.

Conclusions

My study indicates that E2 and CORT concentrations were low in burrowing owl egg-yolks and that many samples had concentrations below the level of detectability of the assay. Thus, reasons for any variability in E2 and CORT concentrations and their respective role in altering burrowing owl nestling behavior or physiology remain unknown. Yolk androgens exhibited high variation in burrowing owls but appeared to be a product of both the within-nest environment (egg order) and the external environment (laying date) in which the eggs were laid. That is, yolk androgens peaked near the middle of the nesting season while remaining low early and late in the season, and androgens increased from early- to late-laid eggs within a nest. Possible benefits of increasing androgens may include more rapid growth of embryos and nestlings, increased aggression of nestlings, potential aggressive characteristics post-fledging, and increased male provisioning through enhanced begging behavior in nestlings. Possible costs including decreased immune system response, adult body condition, and lifespan.

Table 3.1. Results of linear model using Proc Mixed to assess main variables contributing to variation in egg-yolk T in 84 burrowing owl eggs (n=42 nests) from the NCA, Idaho.

Fixed Effects	**Estimate**	**Standard Error**	**DF**	**t Value**	**P-value**
Intercept	-254.19	74.94	37	-3.39	<0.00
Distance to Agriculture (km)	0.05	0.21	40	0.23	0.82
Laying Date	4.81	1.37	40	3.51	<0.00
Laying Date^2	-0.02	0.01	40	-3.44	<0.00
200m Territory Overlap	0.04	0.02	40	1.83	0.08
Body Condition Index	0.06	0.05	40	1.29	0.20
Laying Order (1st egg relative to 2nd)	-6.90	1.33	40	-5.18	<0.00
Random Effects					
Within-nest covariance	4.48	6.48			
Residual (overall error)	37.29	8.14			

Table 3.2. Results of linear model using Proc Mixed to assess main variables contributing to variation in egg-yolk DHT in 84 burrowing owl eggs (n=42 nests) in the NCA, Idaho.

Fixed Effects	**Estimate**	**Standard Error**	**DF**	**t Value**	**P-value**
Intercept	-157.85	65.87	37	-2.40	0.0217
Distance to Agriculture (km)	0.01	0.19	40	0.03	0.98
Laying Date	3.13	1.21	40	2.60	0.01
Laying Date^2	-0.01	0.01	40	-2.66	0.01
200m Territory Overlap (m)	0.01	0.02	40	0.39	0.70
Body Condition Index	0.02	0.04	40	0.39	0.70
Laying Order (1st egg relative to 2nd)	-2.46	0.84	40	-2.93	0.01
Random Effects					
Within-nest covariance	10.52	4.22			
Residual (overall error)	14.70	3.21			

Table 3.3. Results of linear model using Proc Mixed to assess main variables contributing to variation in egg-yolk A4 in 84 burrowing owl eggs (n=42 nests) from the NCA, Idaho.

Fixed Effects	**Estimate**	**Standard Error**	**DF**	**t Value**	**P-value**
Intercept	-294.97	145.56	37	-2.03	0.05
Distance to Agriculture (km)	0.16	0.42	40	0.38	0.71
Laying Date	5.77	2.67	40	2.17	0.04
Laying Date^2	-0.03	0.01	40	-2.18	0.04
200m Territory Overlap (m)	0.00	0.047	40	0.10	0.92
Body Condition Index	-0.14	0.10	40	-1.50	0.14
Laying Order (1st egg relative to 2nd)	-3.67	2.27	40	-1.61	0.11
Random Effects					
Within-nest covariance	33.04	23.80			
Residual (overall error)	108.43	23.94			

Figure 3.1. Examples of the different nesting density parameters that were measured on burrowing owl nests in 2006 and 2007. Note that for nest B, the closest neighbor is C, but C's closest neighbor is D. Number of neighbors counts the number of owls within a buffer around the nest. Territory overlap determines the total proportion of a nesting territory that is overlapped by other territories. Complete overlap is the sum of each neighboring nest's overlap with the focal nest. The crosshatched areas indicate where more than one nest overlaps the same area. All of these parameters were significantly correlated with each other (see Appendix A).

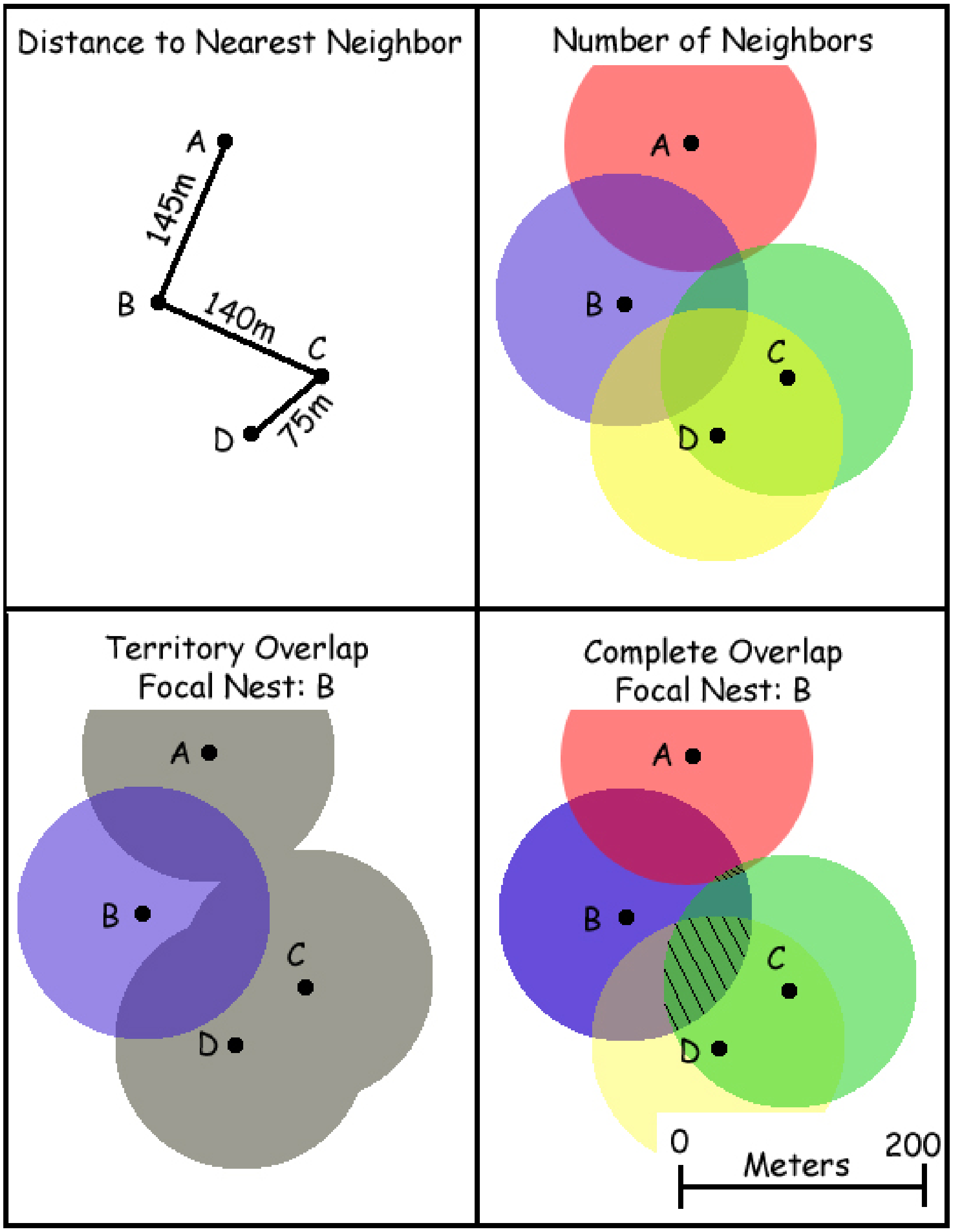


Figure 3.2. Examples of how differing buffer sizes influence territory overlap (top two images) using 200m and 400m buffer sizes around a focal burrow (highlighted in red). Complete overlap (bottom 2 images) with the same buffer configuration is the total summed area of each neighboring burrow that overlapped the focal burrow. In this example, actual burrowing owl nest locations from the NCA in southern Idaho are used.

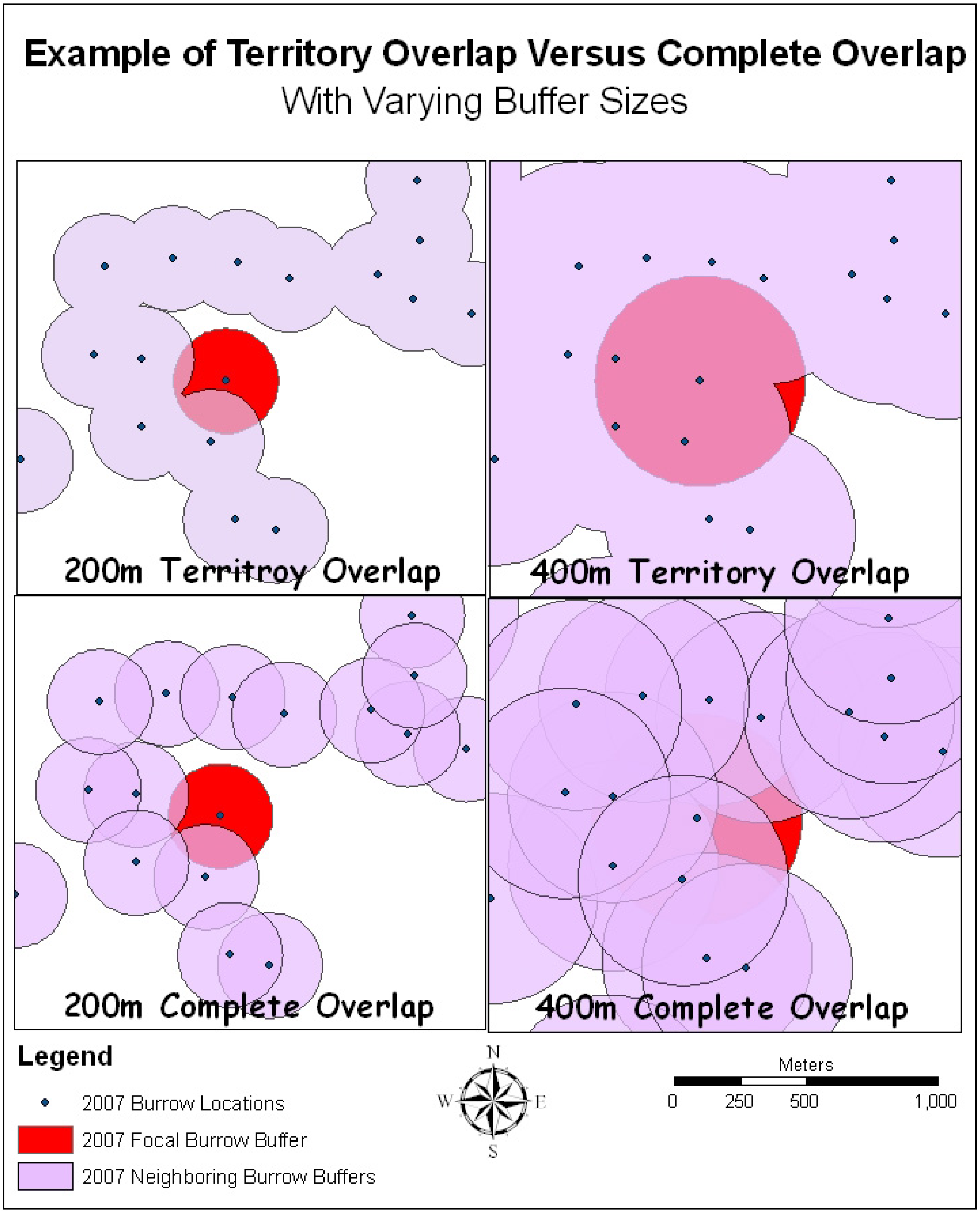


Figure 3.3. Best-fit curve for T, DHT, and A4 levels in relationship to burrowing owl laying date in southwestern Idaho (n=92 during 2006 and 2007).

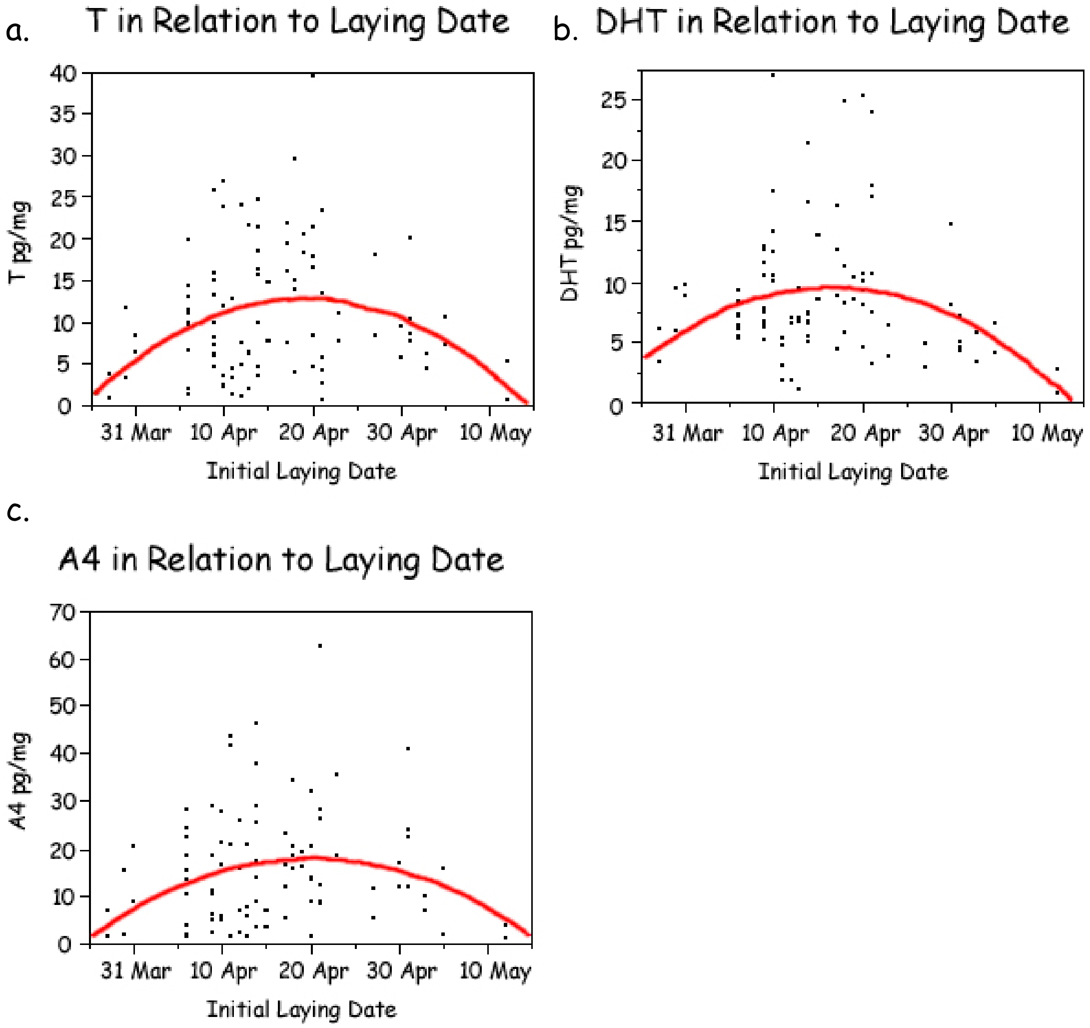
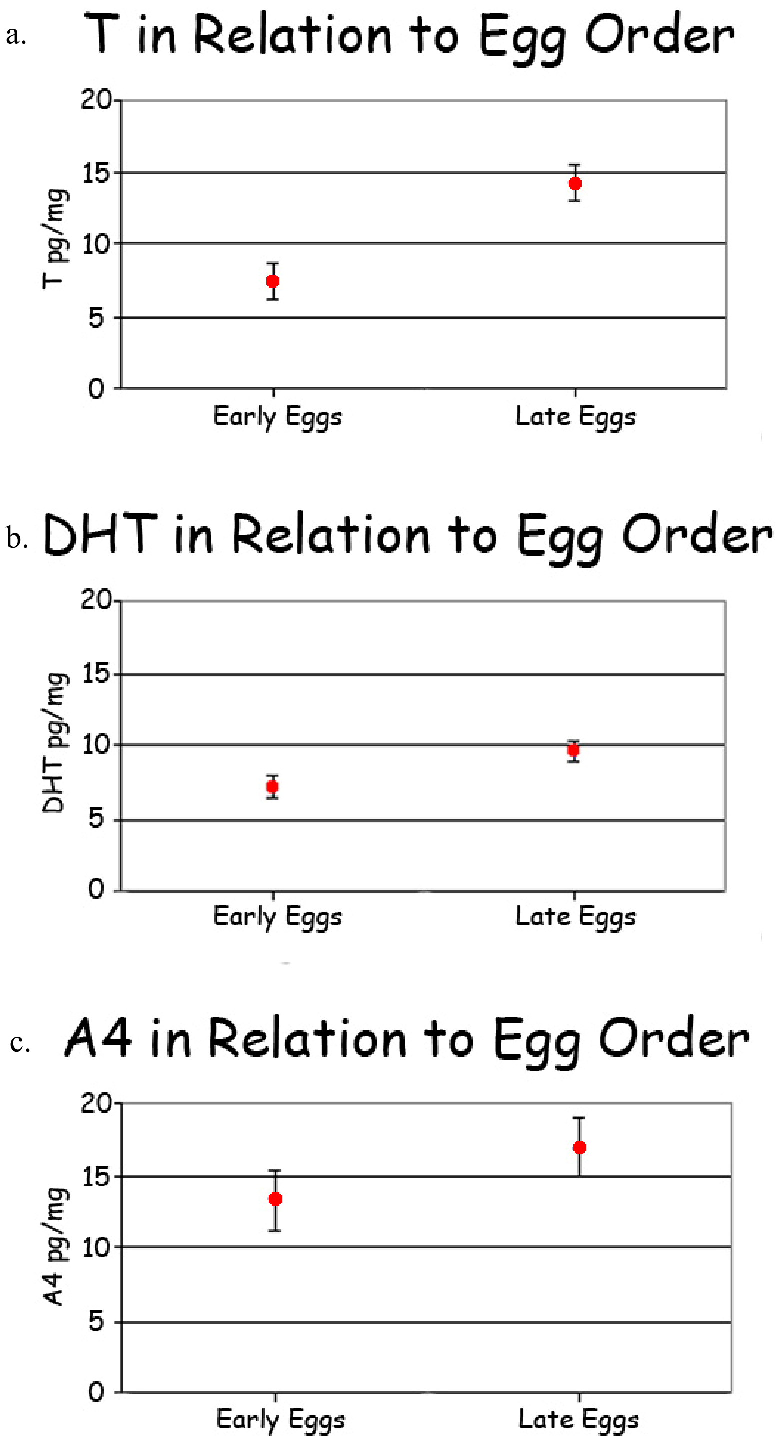


Figure 3.4. Mean (±SE) T, DHT, and A4 in one early and one later-laid burrowing owl eggs in 46 clutches during 2006 and 2007 in southwestern Idaho.



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APPENDIX A

Spearman correlations of the different burrowing owl nesting density variables from 2006 and 2007 in NCA showing the high correlation among all nest density variables.

Variable	**By Variable**	**Spearman p**	**Prob>|p|**
# Neighbors within 400m	# Neighbors within 200m	0.65485997	<.0001
# Neighbors within 600m	# Neighbors within 200m	0.62318857	<.0001
# Neighbors within 600m	# Neighbors within 400m	0.83945143	<.0001
100m Territory Overlap	# Neighbors within 200m	0.96995166	<.0001
100m Territory Overlap	# Neighbors within 400m	0.60085796	<.0001
100m Territory Overlap	# Neighbors within 600m	0.61157442	<.0001
125m Territory Overlap	# Neighbors within 200m	0.89108008	<.0001
125m Territory Overlap	# Neighbors within 400m	0.7359781	<.0001
125m Territory Overlap	# Neighbors within 600m	0.67389375	<.0001
125m Territory Overlap	100m Territory Overlap	0.91497912	<.0001
200m Territory Overlap	# Neighbors within 200m	0.86628391	<.0001
200m Territory Overlap	# Neighbors within 400m	0.84302617	<.0001
200m Territory Overlap	# Neighbors within 600m	0.77464305	<.0001
200m Territory Overlap	100m Territory Overlap	0.8676226	<.0001
200m Territory Overlap	125m Territory Overlap	0.94003619	<.0001
400m Territory Overlap	# Neighbors within 200m	0.72542242	<.0001
400m Territory Overlap	# Neighbors within 400m	0.8275633	<.0001
400m Territory Overlap	# Neighbors within 600m	0.88675654	<.0001
400m Territory Overlap	100m Territory Overlap	0.73098969	<.0001
400m Territory Overlap	125m Territory Overlap	0.76746673	<.0001
400m Territory Overlap	200m Territory Overlap	0.88687249	<.0001
600m Territory Overlap	# Neighbors within 200m	0.65364507	<.0001
600m Territory Overlap	# Neighbors within 400m	0.76889836	<.0001
600m Territory Overlap	# Neighbors within 600m	0.85578501	<.0001
600m Territory Overlap	100m Territory Overlap	0.65315641	<.0001
600m Territory Overlap	125m Territory Overlap	0.69658699	<.0001
600m Territory Overlap	200m Territory Overlap	0.79376683	<.0001
600m Territory Overlap	400m Territory Overlap	0.95971845	<.0001
125m Total Overlap	# Neighbors within 200m	0.78404977	<.0001
125m Total Overlap	# Neighbors within 400m	0.63996318	<.0001
125m Total Overlap	# Neighbors within 600m	0.68006706	<.0001
125m Total Overlap	100m Territory Overlap	0.80941554	<.0001
125m Total Overlap	125m Territory Overlap	0.7765944	<.0001
125m Total Overlap	200m Territory Overlap	0.77178905	<.0001
125m Total Overlap	400m Territory Overlap	0.70876127	<.0001
125m Total Overlap	600m Territory Overlap	0.61503532	<.0001
200m Total Overlap	# Neighbors within 200m	0.83473664	<.0001
200m Total Overlap	# Neighbors within 400m	0.88137434	<.0001
200m Total Overlap	# Neighbors within 600m	0.78459586	<.0001
200m Total Overlap	100m Territory Overlap	0.82260333	<.0001
200m Total Overlap	125m Territory Overlap	0.92091429	<.0001
200m Total Overlap	200m Territory Overlap	0.97218989	<.0001
200m Total Overlap	400m Territory Overlap	0.85710842	<.0001
200m Total Overlap	600m Territory Overlap	0.76668129	<.0001
200m Total Overlap	125m Total Overlap	0.79433196	<.0001

400m Total Overlap	# Neighbors within 200m	0.7274674	<.0001
400m Total Overlap	# Neighbors within 400m	0.91042185	<.0001
400m Total Overlap	# Neighbors within 600m	0.9661526	<.0001
400m Total Overlap	100m Territory Overlap	0.70848527	<.0001
400m Total Overlap	125m Territory Overlap	0.78060638	<.0001
400m Total Overlap	200m Territory Overlap	0.87219569	<.0001
400m Total Overlap	400m Territory Overlap	0.92783266	<.0001
400m Total Overlap	600m Territory Overlap	0.88315442	<.0001
400m Total Overlap	125m Total Overlap	0.73955293	<.0001
400m Total Overlap	200m Total Overlap	0.88986073	<.0001
600m Total Overlap	# Neighbors within 200m	0.63533531	<.0001
600m Total Overlap	# Neighbors within 400m	0.82492462	<.0001
600m Total Overlap	# Neighbors within 600m	0.96627757	<.0001
600m Total Overlap	100m Territory Overlap	0.61098125	<.0001
600m Total Overlap	125m Territory Overlap	0.65685375	<.0001
600m Total Overlap	200m Territory Overlap	0.77158209	<.0001
600m Total Overlap	400m Territory Overlap	0.89395548	<.0001
600m Total Overlap	600m Territory Overlap	0.88108609	<.0001
600m Total Overlap	125m Total Overlap	0.64337389	<.0001
600m Total Overlap	200m Total Overlap	0.77469075	<.0001
600m Total Overlap	400m Total Overlap	0.95740347	<.0001
Distance to Nearest Neighbor	# Neighbors within 200m	-0.8295908	<.0001
Distance to Nearest Neighbor	# Neighbors within 400m	-0.7899537	<.0001
Distance to Nearest Neighbor	# Neighbors within 600m	-0.7886792	<.0001
Distance to Nearest Neighbor	100m Territory Overlap	-0.8500257	<.0001
Distance to Nearest Neighbor	125m Territory Overlap	-0.930062	<.0001
Distance to Nearest Neighbor	200m Territory Overlap	-0.9427844	<.0001
Distance to Nearest Neighbor	400m Territory Overlap	-0.8733208	<.0001
Distance to Nearest Neighbor	600m Territory Overlap	-0.8128021	<.0001
Distance to Nearest Neighbor	125m Total Overlap	-0.770653	<.0001
Distance to Nearest Neighbor	200m Total Overlap	-0.9199278	<.0001
Distance to Nearest Neighbor	400m Total Overlap	-0.8703639	<.0001
Distance to Nearest Neighbor	600m Total Overlap	-0.7913545	<.0001

APPENDIX B

Original data for all variables analyzed in burrowing owl egg yolk androgens from 2006 and 2007 in the Snake NCA. Cells highlighted in yellow had only 1 duplicate on the curve (above 3.91gp/tube) while empty cells had neither duplicate on the curve.

Burrow	Year	200m Territory Overlap	Laying Date	Distance (meters) to Agriculture	T pg/mg Early	T pg/mg Late	DHT pg/mg Early	DHT pg/mg Late	A4 pg/mg Early	A4 pg/mg Late	CORT pg/mg Early	CORT pg/mg Late	E2 pg/mg Early	E2 pg/mg Late
398-3	2007	65.30%	96	1186	1.71	9.47	7.14	5.94	1.65	15.06	0.99	0.85	.	.
Backyard 9	2007	0%	110	167	4.48	17.51	4.5	10	1.09	13.53	.	.	.	.
Baja 10	2007	52%	96	586	12.79	19.61	8.22	9.23	3.43	13.13	.	.	.	.
Baja 2	2007	49.80%	121	131	8.55	10.13	4.09	4.88	23.87	22.27	.	.	.	.
Baja Pole 19	2007	51%	102	124	4.6	23.75	1.67	6.43	6.74	25.54	.	.	.	.
Canyon 4	2007	0%	123	304	4.3	6.01	3.19	5.7	9.73	6.57	.	.	.	.
Corner 3	2007	0%	104	1523	18.12	15.58	6.61	4.93	37.36	45.96	.	.	.	.
Delta 2	2007	52.30%	103	9483	1.69	6.11	0.94	6.56	0.97	7.38	.	.	.	.
Delta 3	2007	49.30%	96	9519	6.46	14.13	5.4	8.12	10.02	24	.	0.76	.	.
Dirtmound 5	2007	58%	90	889	6.23	8.19	8.78	9.61	8.74	20.06	0.66	0.93	.	.
Dorsey East 6	2007	0%	113	4384	10.99	7.59	6.28	3.65	18.15	35.08	.	.	.	.
Highway 2	2007	0%	102	1069	0.78	7.42	1.66	6.9	1.86	15.59	.	.	.	.
Hilltop 1	2007	0%	87	3518	0.66	3.56	3.26	6.03	1.21	6.42	.	.	.	.
Level 6	2007	18.80%	103	1669	6.02	21.25	6.88	9.33	5.38	20.44	.	.	.	.
Mountain View 3	2007	73.70%	101	6152	1.03	12.73	1.66	4.61	1.36	20.7	.	.	.	.
Powerline 3	2007	95.90%	96	1001	1.03	9.84	5.29	6.96	1.09	27.98	0.71	.	.	.
Powerline 4	2007	86.70%	89	1117	3.14	11.63	5.85	9.32	1.57	15.18	.	.	1.38	.
South Cinder 1	2007	0%	132	523	0.39	5.14	0.57	2.67	0.7	3.65	.	.	.	.
South Cinder 4	2007	0%	104	166	4.55	16.12	5.34	7.14	3.26	8.61	.	.	.	.
Tadpole 2	2007	17.90%	111	9180	18.02	20.24	10.21	8.45	19.13	16.14	.	.	.	.
Trailerview 4	2007	32.10%	99	110	9.7	14.84	10.43	12.43	10.92	5.71	1.34	0.717	.	.
Valley 1	2007	26.40%	99	1803	13.03	15.74	7.58	6.25	10.93	28.78	.	.	.	.
View 1	2007	0%	99	2571	4.43	7.15	7.39	12.75	1.92	4.83	.	.	.	.
Backyard 4	2006	0%	101	122	3.02	4.24	5.2	2.97	43.09	41.09	.	.	.	.
Bennett 4	2006	0%	111	9130	2.36	23.03	10.56	23.77	8.17	62.36	.	3.69	.	.

Canyon 4	2006	0%	121	280	7.48	19.84	4.44	7.02	40.62	11.51	1.67	1.46	.	.
Curlew 2	2006	87.20%	110	1237	39.23	21	25.24	9.13	31.43	13.04	.	.	.	.
Delta 1	2006	36.90%	107	9890	7.37	21.45	4.28	8.69	22.81	16.52	1.61	1.1	.	.
Delta 3	2006	36.90%	100	9750	11.74	26.64	17.24	13.98	16.16	5.62	.	.	.	.
Dirtmound 2	2006	73.50%	117	959	17.89	8.25	4.75	2.7	11.3	5.11	1.79	1.61	.	0.47
Dorsey East 6	2006	0%	111	4367	0.46	3.98	3.06	7.31	8.58	12.05	1.36	1.81	.	.
East Cinder 1	2006	0%	105	47	7.49	14.72	8.44	13.54	6.64	2.97	.	.	.	.
Grandview 1	2006	73.50%	99	52	6.11	25.59	6.5	11.5	10.51	18.41	1.47	2.67	.	.
Grandview 3	2006	73.50%	104	96	9.74	24.5	16.32	21.23	13.68	17.3	2.21	2.81	1.75	.
Missile 3	2006	63.50%	104	12744	3.26	21.01	6.37	7.26	28.9	25.2	1.44	1.01	.	0.71
Missile 5	2006	31.80%	122	12870	5.61	9.35	7.95	14.5	11.65	16.55	1.05	1.89	.	.
Powerline 4	2006	79.50%	100	1066	2.1	2.16	12.25	10.39	27.59	4.64	.	.	.	.
Powerline 5	2006	54.80%	97	797	10.96	11.26	5.18	6.33	18.19	22.12	.	.	.	.
Shadscale West 2	2006	0%	107	5258	16.02	19.18	12.39	15.97	4.88	11.85	1.22	0.85	0.46	.
South Cinder 2	2006	56.60%	108	415	13.78	14.9	8.16	11.16	33.77	15.36	2.38	3.59	.	.
South Cinder 8	2006	0%	111	94	5.61	13.36	16.81	17.75	27.87	26.01	1.23	1.7	.	0.36
Sub East 1	2006	0%	99	412	5.83	7.9	6.08	4.99	9.97	5.87	.	.	.	.
Tadpole 1	2006	0%	108	9509	3.84	29.36	5.62	24.74	18.16	20.35	6.52	.	.	.
Trailer 1	2006	43.30%	100	68	3.4	23.66	9.91	26.96	5.26	20.81	1.37	1.48	.	.
Trailerview 4	2006	32.10%	125	79	7.09	10.4	3.98	6.37	15.59	1.38	0.97	0.71	.	0.37
Valley 2	2006	1.50%	110	1845	16.43	8.19	10.57	7.98	20.25	8.59	1.57	1.86	.	.
Average		33.40%	105.28	2959.906	7.4892	13.934	7.1293	9.5559	13.173	16.946	1.1154	1.0689	1.1967	0.4775