SECONDARY AND TERTIARY SEX RATIOS IN NESTLING AND HARVESTED
HATCH-YEAR WHITE-WINGED DOVES.

THESIS

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by

Katie A. Moccia, B.S.

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To my Father...

Thank you for everything. I am who I am today because of you.

To my Mother...

Thank you for all your support and love. I miss you more than words can say.

ACKNOWLEDGMENTS

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ABSTRACT

SECONDARY AND TERTIARY SEX RATIOS IN NESTLING AND HARVESTED

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by

Katie Allen Moccia, B.S.

Texas State University-San Marcos

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SUPERVISING PROFESSOR: JOHN T. BACCUS, PH.D.

White-winged Doves (*Zenaida asiatica*) are a popular game bird in Texas.
Historically, the range of White-winged Doves in the United States was limited to the arid
southwest including portions of Texas, New Mexico, Arizona, and California. Currently,
because of a northward range expansion, White-winged Doves occur throughout Texas into
Colorado and Utah. In addition, a population was introduced into Florida in the 1950s and
appears to have spread up the eastern seaboard. The objectives of my study were to
investigate whether sex ratios in wild White-winged Doves is biased in hatch sequence of
eggs, hatchlings, and harvest mortality. Blood samples were collected from 62 hatchlings in

the San Marcos, Texas, area and sex determined by DNA analysis. Additionally, harvest data from 1,016 White-winged Doves was collected from the Anacua Unit of the Las Palomas Wildlife Management Area during the special White-winged Dove hunts in 2009 and 2010. I determined age of harvested doves as either hatching year (HY) or after hatching year (AHY) by examination of morphological characteristics. Further, I determined the sex of harvested birds via internal examination of the gonads. I found that the secondary sex ratio of hatchlings was 1.95:1 and the HY harvest sex ratio was 1.81:1. I concluded that sex ratio is biased, favoring males, in the hatch sequence of eggs, hatchlings, and HYs harvest mortality.

CHAPTER I

INTRODUCTION

Demographic studies of wildlife populations are predicated on parameters which are vital attributes of the past, present, and future of populations (Skalski et al. 2005). Sex ratio is a parameter of interest to demographers because these data often provide an index on the status of a population (Skalski et al. 2005). Sex ratio usually refers to the ratio of males to females in a population (Bolen and Robinson 2003); however, for some applications (i. e., sex-age-kill model of population reconstruction, Skalski and Millspaugh 2002), ratios of females to males is used. In this study I refer to the ratio of males to females in the population, unless stated otherwise.

Bolen and Robinson (2003) defined sex ratios as primary (sex ratio at fertilization), secondary (sex ratio at hatching or birth), tertiary (sex ratio as juveniles), and quaternary (sex ratio as adults). Coupled with species-specific information on life-history strategy, such as mating system, the sex ratio at each life-history stage is important in evaluating the past, present and future of a population (Bolen and Robinson 2003). For example, sex ratio can be used to convert field data (i. e., coo-counts) into population indices (Trautman 1982).

Several factors ultimately affect sex ratios. These include, but are not limited to, evolutionary strategy (Fisher 1930, Charnov 1982, Maynard Smith 1982, Karlin and Lessard 1986, Frank 1998), biasing offspring sex ratios facultatively in response ecological or social environmental cues (Cassinello and Gomedio 1996, Ellegren et al. 1996, Koenig and Dickinson 1996, Komdeur et al. 1997, Westerdahl et al. 1997, Kruuk et al. 1999, Pagliani et

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al. 1999, Sheldon et al. 1999, Radford and Blakey 2000, Kalmbach et al. 2001, Koenig et

al. 2001, Kasumovic et al. 2002), temperature and other climatic or physical factors

(Mysterud et al. 2000, Godfrey and Mrosovsky 2001), population density (Kruuk et al.

1999), competitive ability of the sexes (Oddie 2000), nutritional status of breeding

females (Raedeke et al. 2002, Pike 2005), sex-biased mortality before independence

(Lack 1954, Howe 1977, Dijkstra et al. 1998), predation (Johnson and Sargeant 1977),

and hunter harvest (Raedeke et al. 2002).

Studies of primary or secondary sex ratios in wild birds are limited to few species

with no particular family having a preponderance of investigations. These species

include Herring Gulls (*Larus argentatus*) (Goethe 1937), Zebra Finches (*Taeniopygia

guttata*) (Bradbury and Blakey 1998, Kilner 1998, Zann and Runciman 2003), European

Kestrels (*Falco tinnunculus*) (Dijkstra et al. 1990), American Kestrels (*Falco sparverius*)

(Wiebe and Bortolotti 1992), Peregrine Falcons (*Falco peregrinus*) (Burnham et al.

2003), Snow Geese (*Chen caerulescens*) (Ankney 1982), Crimson Rosella (*Platycercus

elegans*) (Krebs et al. 2002), Noisy Miners (*Manorina melanocephala*) (Arnold et al.

2001), Red-winged Blackbirds (*Agelaius phoeniceus*) (Blank and Nolan 1983), Tree

Swallows (*Tachycineta bicolor*) (Whittingham and Dunn 2000), Southwestern Willow

Flycatchers (*Empidomax traillii*) (Paxton et al. 2002), Roseate Terns (*Sterna dougallii*)

(Szczys et al. 2001), Blue Tits (*Parus caeruleus*) (Svensson and Nilsson 1996, Sheldon et

al. 1999), Western Bluebirds (*Sialia mexicana*) (Koenig and Dickinson 1996), and

Seychelles Warblers (Komdeur 1996, Komdeur et al. 1997).

White-winged Doves (*Zenaida asiatica*) are a popular game species in Texas.

Currently, the hunting season on White-winged Doves ranges from 70-79 days with a

daily bag limit of 15 birds. Historically, the range of White-winged Doves in the United States was limited to the arid southwest including portions of Texas, New Mexico, Arizona, and California. Currently, because of northward range expansion, White-winged Doves occur throughout Texas and into Colorado and Utah. In addition, a population was introduced into Florida in the 1950s and appears to have spread up the eastern seaboard (Small and Waggerman 1999, Small et al. 2006). Adult White-winged Doves are mainly grayish brown, square-tailed, medium-sized, with a prominent white wing-patch across both outer wing coverts. Adults have bright red feet and bright blue eye rings (Schwertner et al. 2002). Clutch size is usually two eggs. A study conducted in the lower Rio Grande Valley (Cottam and Trefethen 1968) showed only 43 (4.4%) of 987 active nests deviated from the normal two-egg clutch. Usual incubation period is 14 days. Young stay in the nest between 13-18 days and usually start to resemble adults around day 13 (Schwertner et al. 2002).

The objectives of my study were to investigate whether sex ratios in wild White-winged Doves is biased in hatch sequence of eggs, hatchlings, and harvest mortality of hatching year (HY) birds. Sex ratios are among the most important demographic parameters to determine and can provide a suggestion of relative survival of females and males in populations as well as the future breeding potential of the population (Skalski et al. 2005). Estimates like these allow wildlife managers to regulate wildlife harvest to try to maintain desired sex ratios of a population. Conversely, by monitoring sex ratios, wildlife managers can assess how harvest regulations may be influencing the relative mortality rates of the male and female segments of a population (Skalski et al. 2005). Comparison of pre-hunting to post-hunting sex ratios gives information on impacts of

hunting regulations on sex ratios, gender-specific mortality, and survival rates (Bender and Miller 1999, Hatter 1999).

My null hypotheses state there would be no difference in numbers of male and female White-winged Doves in broods; weight of first birds hatched in a brood compared to weight of second birds hatched; sex of first bird hatched in a brood compared to second bird hatched when compared to all other broods; or sex ratio of HY birds in hunters' bags. My alternative hypotheses state there is a difference in numbers of male and female White-winged Doves in broods, weight of first birds hatched in a brood compared to weight of second bird hatched; in the sex of the first bird hatched in a brood compared to the second bird hatched when compared to all other broods; or sex ratio of HY birds in hunters' bags.

CHAPTER II

METHODS

Study Area and Data Collection

Hatchlings.---I collected White-winged Dove hatchlings from nests in shrubs and trees in and around San Marcos, Texas (N 29°52'46" W 97°56'20") from 1 March to 5 August 2010. Study areas were searched daily for active nests (nests occupied by an adult) for a minimum of 4 h. When I located an active nest, I used a pole with a mirror attached to flush the adult and determine whether eggs or hatchlings were present. Active nests with eggs were revisited every day until the eggs hatched or until the 14-day incubation period ended. I numbered each surviving clutch, removed hatchlings from the nest, collected blood by pricking the leg with a sterile lancet, and placed a drop of blood on an avian blood sample sheet. The collected blood was air dried for 60-90 min. I then applied betadine to the wound and, if necessary, a coagulant (cornstarch), weighed the hatchling using a 100 g Pesola scale (Pesola Ag, Baar, Switzerland), and returned the hatchling to the nest.

I assumed the heaviest hatchling was the first born. My assumption is based on studies of variation in egg size and hatchling weights in Hooded Crows (*Corvus corone cornix*) (Rosdat and Sandvik 1985), Boat-tailed Grackles (*Quiscalus major*) (Brancroft 1984), and Black-headed Gulls (*Larus ridibundus*) (Lez˘alova˘ et al. 2005) where last laid eggs weighed less than first laid eggs and the size of the young at hatching correlated with egg size.

Harvest.---During two special White-winged Dove Hunting Seasons (early September 2009 and 2010), I collected harvest data from both HY and after hatch year (AHY) doves at hunter bag check points at the Anacua Unit of the Las Palomas Wildlife Management Area (N 26°07 W -97°81). Age was determined as either HY or AHY by external examination of morphological characteristics. If the age was not clear or not agreed on by the collectors, that sample was not used in the analysis. Sex was determined by internal examination of the gonads.

Sex Determination

I used molecular methods in the analysis of blood samples to ascertain the sex of nestlings. Blood samples were collected from birds with known sex (determine by examination of gonads) of two AHY males and two AHY females to determine the molecular profile for each sex. DNA analysis was conducted by DCC Veterinary (One DDC Way, Fairfield, OH 45014). DNA was extracted from blood samples following the protocol adapted from Mullenbach et al. (1989) and Fridolfsson and Ellegren (1999). The DNA was amplified using two pairs of primers. The first pair amplifies a 615-bp, female specific (W-linked) marker, *Sco16*, developed by Sabo et al. (1994). The second pair amplifies a 274-bp control autosomal marker, *Igf-1*, that was designed from the insulin-like growth factor receptor *c*DNA sequence (Armstrong and Hogg 1992). In a 25 *u*l reaction, 0.5 *u*l each of forward and reverse primers for the Sco-16 marker and Igf-1 marker were used. The PCR protocol is described by Sabo et al. (1994). Thermal cycling consisted of an initial denaturing step of 120 sec at 94° C, followed by repeated denaturing, annealing, and extension steps for 30 cycles of 30 sec at 94° C, 30 sec at 50° C, and 30 sec at 72° C, with a final extension step of 300 sec at 72° C. The PCR products

were separated on 2% agarose gels, stained with 10 ul ethidium bromide, and electrophoresis was run in 0.5X TBE at 70V for approximately 75 min. Gels were visualized under UV light and photographs were taken of all successful runs. Female sex was assigned if both the CHD-Z and CHD-W bands were present, and male sex was assigned if a single CHD-Z band was present.

Statistical Analyses

I analyzed data using chi-square goodness–of-fit tests to determine whether samples varied from the expected 1:1 ratio (Zar 1999). I used a paired t-test to test for differences in weights of first-hatched (larger chicks) and second-hatched (smaller chicks).

CHAPTER III

RESULTS

Hatchlings

DNA analysis of blood samples collected from 62 hatchlings identified 41 males and 21 females (1.95:1). This sample differed significantly from the expected ($x^2 = 6.45$, df = 60, $P < 0.05$) for the secondary sex ratio of HY White-winged Doves (Table 1). Mean weight of first hatchlings was 35.5 g (SE = 3.37) and mean weight of second hatchlings was 27.7 g (SE = 3.04, Table 1). There was a significant difference between the weight of first-born hatchlings and second-born hatchlings ($t_{30} = 9.54$, $P < 0.001$).

When comparing sex ratio and sequence of egg hatch, four variations of egg hatch sequence are possible (M:M, M:F, F:F, F:M). Of the 31 nests, fifteen were M:M, seven were M:F, five were F:F, and four were F:M. There was a significant difference in sex ratio and sequence of egg hatch ($x^2 = 9.65$, df= 30, $P < 0.05$).

Table 1. White-winged Dove hatchlings examined by date, nest number, sex and weight of larger chick, sex and weight of smaller chick, and difference in weights of the larger and smaller chicks.

Date	Nest	Sex	Weight (g)	Sex	Weight (g)	Weight difference
3/21/10	1	F	24	F	23	1
3/25/10	2	M	33	M	31	2

Table 1- Continued. White-winged Dove hatchlings examined by date, nest number, sex and weight of larger chick, sex and weight of smaller chick, and difference in the weights of the larger and smaller chicks.

3/25/10	3	M	70	M	59	11
5/27/10	4	M	21	M	16	5
6/4/10	5	M	46	M	31	15
6/8/10	6	M	27	M	13	14
6/8/10	7	M	13	M	11	2
6/8/10	9	M	28	M	17	11
6/8/10	10	F	15	F	7	8
6/30/10	14	F	51	M	40	11
7/5/10	15	M	17	M	11	6
7/7/10	16	M	54	F	51	3
7/7/10	17	F	12	F	7	5
7/7/10	18	F	71	F	59	12
7/7/10	19	M	44	F	37	7
7/6/10	20	F	56	M	41	15
7/20/10	21	F	72	M	69	3
7/20/10	22	F	47	F	38	9
7/19/10	23	M	17	F	13	4
7/27/10	24	M	16	F	12	4
7/27/10	25	M	28	M	22	6
7/27/10	26	M	11	M	7	4

Table 1- Continued. White-winged Dove hatchlings examined by date, nest number, sex and weight of larger chick, sex and weight of smaller chick, and difference in the weights of the larger and smaller chicks.

7/29/10	27	M	14	F	11	3
7/19/10	28	M	54	F	39	15
7/25/10	29	M	19	M	11	8
7/29/10	30	M	52	M	37	15
8/2/10	31	M	29	M	24	5

Harvest

A total of 1,016 harvested White-winged Doves (433 HYs and 583 AHYs) were examined and sex and age determined. The HY sex ratio was 1.81:1 (279 males and 154 females) in the harvest which differed significantly from the expected ($x^2 = 36.1$, df=432, $P < 0.05$). There were 583 AHYs (304 males and 279 females) in the harvest. The AHY sex ratio was 1.09:1. There was no significant difference in the AHY sex ratio from the expected ($x^2 = 1.072$, df=583, $P = 0.30$).

CHAPTER IV

DISSCUSSION

Change in sex ratio can have a profound effect on population dynamics of monogamous species compared to polygamous species (Leopold 1933). In monogamous breeders, such as White-winged Doves (Cottam and Trefethen 1968), productivity is maximized when sex ratio is maintained at an exact 1:1 ratio; any disparity decreases productivity. The importance of adult (quaternary) sex ratio has long been recognized in human populations (Mayr 1939). However, in wild birds, sex ratios have received little attention (Mayr 1939, McIlhenny 1940, Ewen et al. 2004), especially as applied to population and harvest management of game birds, in contrast to a literature replete with information on sex ratios in domestic fowl.

Regulations often exist to manage the adult (quaternary) sex ratio, but often little attention is given to the secondary and tertiary sex ratio of most game birds (Skalski et al. 2005). Sex ratios of nestlings and harvested HY birds are pertinent information needed for the development of realistic models of productivity or adaptive harvest management. It is difficult to sex most monochromic avian species by external morphology. White-winged Doves do not display a high degree of sexual dimorphism either in size or plumage characters as adults or nestlings (Schwertner et al. 2002). Because male birds have two identical sex chromosomes (ZZ) and females are heterogametic (ZW), sex identification can be made by detection of the W chromosome or DNA sequences present on the W chromosome (Tone et al. 1984). Studies of secondary sex ratio in columbids in

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North America have been limited to two species; captive populations of Rock Pigeon (*Columba livia* f. *domestica*) (Riddle 1917, Pike 2005) and Mourning Doves (*Zenaida macroura*) (Kossack and Hanson 1957, MacGregor 1958, Hanson and Kosack 1963, Edmunds and Ankney 1987). Pike (2005) found the sex ratio of hatchlings was skewed toward females in a small experimental sample (< 25 females) of Rock Pigeon, however, control hatchlings indicated no sex ratio bias. MacGregor (1958) and Kossack and Hanson (1957) showed a skewed sex ratio in unisexual broods of pen-raised Mourning Doves. Edmunds and Ankney (1987) studied a larger sample of 306 Mourning Dove hatchlings and found a near 1:1 sex ratio. No information, until now, was available on the sex ratio of hatchling White-winged Doves. My analyses shows a significant difference in M:F sex ratio of White-winged Dove hatchlings and harvested HYs. I expected a 1:1 sex ratio which was actually 1.95:1 and 1:81:1, respectively. I rejected my hypothesis, H_1, which stated no difference in number of males and females in White-winged Dove broods. I also rejected my hypothesis, H_4, which stated no difference in the sex ratio of HY birds in hunters' bags. The sex ratio of AHYs was closer to 1:1 with a ratio of 1.09:1.

The tertiary sex ratio of White-winged Doves in my study was substantially greater than that of other game and nongame birds. Stoddard (1932) reported a 1.14:1 sex ratio (53.3% males) for 19,423 harvested Northern Bobwhites (*Colinus virginianus*). Black-bellied Whistling Ducks (*Dendro-cygna autumnalis*) showed a 1.2:1 tertiary sex ratio (Bolen 1970). The tertiary sex ratio for Southerwestern Willow Flycatchers (*Empidonax traillii extimus*) did not vary from the expected 1:1 (Paxton et al. 2002). The

White-winged Dove population in San Marcos, Texas is skewed toward males. However, the sample size in my study was not large.

I rejected my hypothesis, H_2, which stated no difference in the weight of the first bird hatched in a brood compared to the weight of the second bird hatched. Each set of hatchlings showed a difference in weight, ranging from 1-15 g with a mean weight difference of 7.8 g. I rejected my hypothesis, H_3, of no difference in the sex of the first bird hatched in a brood compared to the second bird hatched. From 31 nests, one would expect 25% of nests M:M, 25% of nests M:F, 25% of nests F:F, and 25% of nests F:M. My results showed a 48% significant deviation from this ratio.

Animals are harvested for many reasons from controlling pests to reduce their damage to other resources to providing food and recreational hunting opportunities (Caughley and Sinclair 1994). The effect of differential harvest on productivity is greater for monogamous species than those with other mating strategies. For this reason, harvest management often focuses on maintenance of appropriate sex ratios to ensure maximum reproduction (Raedeke et al. 2002). In monogamous breeders, such as the Northern Bobwhite, productivity is maximized at an exact 1:1 sex ratio; any disparity decreases productivity (Skalski et al. 2005). The quaternary sex ratio in the harvest of AHY White-winged Doves at 1:09:1 indicates the current fecundity of this species is maximized. My study suggests the sex ratio in wild White-winged Doves is biased in hatchlings, hatch sequence of eggs, and harvest mortality of HY birds. The observed sex ratio near 1:1 in the harvest of AHYs is probably a consequence of natural selection on males and females and any anthropogenic effects of harvest (Skalski et al. 2005).

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VITA

Katie Allen Moccia was born in Houston, TX to Charles and Nancy Mallett in 1985. After graduating from Jack C. Hays High School in 2003, she enrolled at Southwest Texas State University. During her undergraduate career she was vice president of the biochemistry society and was also involved in The Wildlife Society. After completing her B.S. degree in Biology with a minor in Chemistry in Criminal Justice she enrolled in the M.S. Wildlife Ecology program at Texas State University-San Marcos. She developed a project with Dr. Baccus as her advisor.

Permanent Address: 12706 Topper Lane

Manchaca, Texas 78652

This thesis was typed by Katie Allen Moccia