

## GONADAL AND HORMONAL CHARACTERISTICS OF JUVENILE FEMALE MOURNING DOVES IN VIRGINIA

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**Abstract:** Trapped or hunter-harvested juvenile female mourning doves (*Zenaida macroura*) in Virginia were examined for reproductive activity during the late summer and fall of 1975, 1976, and 1977. Body, ovary, and oviduct weights, diameter of the 3 largest follicles in the ovary, presence or absence of eggs in the reproductive tract, and presence or absence of crop gland activity were recorded for all trapped juveniles. Blood samples also were collected for estradiol and progesterin analysis. All pertinent reproductive data, excluding body weights and blood samples, were collected from hunter-harvested juveniles as previously described. Gonadal and crop gland data indicated that a small percentage of female doves attained puberty at approximately 93 days (07 primary feather replacement). Reproductive capabilities of juvenile females appeared to increase with age class. Changes in estradiol and progesterin concentrations were not explicitly diagnostic of puberal changes due to large variability among doves. Approximately 9 percent of the 93 to 131-day old juvenile females sampled during the study were capable of reproduction.

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Proc. Ann. Conf. S.E. Assoc. Fish & Wildl. Agencies 34:415-425

Mourning doves of both sexes have demonstrated breeding capabilities during their first 6 to 12 months of life (Moore 1940, Jenkins 1955, Irby and Blankenship 1966, Armstrong and Noakes 1977). Brown (1967) estimated approximately 8 percent of the juvenile dove population in southern Arizona was breeding during June with some doves nesting at 90 days of age. To our knowledge, however, published papers have neither reported the earliest age at which female mourning doves attain puberty nor the gonadal and hormonal changes associated with puberty attainment. Such information is essential in order to estimate the quantitative contributions hatching-year mourning doves make to the productivity of dove populations during the breeding season. The objectives of this investigation were (1) to delineate changes in reproductive organs and hormone concentrations in immature female mourning doves of various ages that were indicative of puberty attainment and, (2) to determine the proportion of juvenile females in Virginia that achieved reproductive capability during the late summer and early fall.

This research was funded by the Accelerated Research Program for Migratory Shore and Upland Game Birds of the U.S. Fish and Wildlife Service in conjunction with the Virginia Commission of Game and Inland Fisheries. Estradiol antibody was purchased from Dr. G. D. Niswender, Colorado State University. Progesterone antiserum was graciously provided by Dr. R. L. Butcher, West Virginia University. Technical assistance from W. Morehead, D. Aalseth and M. DeSilva is gratefully acknowledged.

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## METHODS

### Trapped Doves

Juvenile female mourning doves were trapped from the first week of August through the first week of October, 1977. Juveniles were transported to the laboratory, held overnight in indoor cages provided with feed and water, and sacrificed at 0900 h the following morning. Blood samples were collected in heparinized vials at sacrifice, centrifuged, and the plasma drawn off and frozen for estradiol and progesterin analysis. All doves were aged in the laboratory using primary feather replacement (Haas and Amend 1976) and bursal inspection (Wight 1956). Body, ovary, and oviduct weights, diameter of the 3 largest follicles in the ovary, the presence or absence of eggs in the reproductive tract, and the presence or absence of crop gland activity were recorded. Juvenile females were classified as successful nesters, potential layers, potential breeders, or non-breeders. Females with any sign of crop gland activity were considered to have successfully nested (Mirarchi and Scanlon 1980). Females that had ovulated (egg in the oviduct), or were likely to ovulate (at least 1 ovarian follicle  $\geq 10$ mm) were classified as potential layers (Cheng 1974). Females whose index of follicular development (IFD = sum of the 3 largest ovarian follicle diameters) was  $\geq 10$  mm were classified as potential breeders. The potential breeder categorization was based on observations made on gonadal activity of adult female mourning doves held in a captive breeding colony (Mirarchi 1978). All doves classified as successful nesters, potential layers, or potential breeders were deemed physiologically capable of reproduction.

*Estrogen Assay.*—Estradiol-17B (E2) in female dove plasma was quantified by radioimmunoassay (RIA) (DeSilva 1978) following column chromatography. Approximately 1500 cpm of radioactive estrogen (6,7- $^3\text{H}$ (N) - estradiol-17B (New England Nuclear) was added to 40 ml glass tubes as an internal standard to correct for procedural losses. Plasma samples (1 ml) were incubated for 10 min with dried recovery isotope at 40°C. Five ml of ethyl ether were added to the mixture, shaken for 60 sec, and frozen for 20 min. The semi-frozen supernatant was poured into a test tube and evaporated to dryness in a water bath (40°C) under air. The dried residue was resolubilized in benzene:methanol (9:1) and eluted through columns of Sephadex LH-20 packed to the 2 cm level in a 1 cm x 3 cm tuberculin syringe. The Sephadex had been soaked previously for at least 6 h in benzene:methanol (9:1) which also was used as the eluting solvent. Estradiol was eluted in the 4.0 to 7.5 ml fraction. Estrone which cross-reacted (8-10%) with the estrogen antibody used in this study was eluted in the 1.5 to 3.5 ml fraction. Following reconstitution of the dried column eluate, a 0.2 ml aliquot was removed to estimate recovery. Aliquots of 0.05 and 0.1 ml, 0.1 and 0.2 ml, or 0.3 and 0.6 ml were dried and assayed for estradiol. Duplicate estimates on each sample satisfied the dose-response relationships. For RIA, dried residues were dissolved in 0.5 ml PBS-Ga, incubated at 45°C for 15 min, and vortexed. One hundred  $\mu\text{l}$  of E2 antibody (1:19,000 dilution) then was added to each tube, vortexed, and incubated for 60 min at room temperature. Following incubation, the samples were placed in a 40°C water bath for 5 min. Eight-tenths ml of dextran-coated charcoal (0.400% dextran and 0.625% charcoal) then was added to all tubes, which were vortexed and centrifuged for 10 min at 1732 x g. A 500  $\mu\text{l}$  aliquot of the supernatant was transferred to a filmware bag (Nalgene) together with 5 ml of Toluene-Triton-X-100 scintillation cocktail. The bags were counted for 5 min each on a Searle Delta-300 beta liquid scintillation counter with a counting efficiency of 42 percent for  $^3\text{H}$ . The sensitivity of the estradiol assay was 5 pg/ml. Mean recovery for radioactive estradiol was 72 percent. Validation of the extraction and assay procedure for E2 was demonstrated by addition of known amounts of nonradioactive E2 to aliquots of pooled dove plasma as described (Table 1).

Table 1. Accuracy and precision of estradiol and progesterone determinations in female mourning dove plasma following Sephadex LH-20 column chromatography and radioimmunoassay.

Estradiol (pg)				Progesterone(ng)			
N <sup>1</sup> Added		Measured		N <sup>1</sup> Added		Measured	
		$\bar{X}$	S.E.			$\bar{X}$	S.E.
4	25.0	30.4	1.6	4	0.30	0.32	0.02
4	100.0	96.0	1.4	4	0.50	0.43	0.06
4	200.0	224.5	23.9	4	1.00	0.98	0.06
4	250.0	260.5	14.6	2	3.00	2.71	0.09

<sup>1</sup>Number of samples assayed.

*Progesterin Assay.*—Progesterin concentrations in female dove plasma also were quantified using RIA (DeSilva 1978). Plasma was extracted with iso-octane, but without column chromatography, using procedures similar to those described previously for E2. Reconstitution, recovery estimations, duplicate estimates, and assay aliquots were as previously described for the E2 assay. For RIA, dried residues were dissolved in 100  $\mu$ l of progesterone antibody (1:1,500 dilution). One hundred  $\mu$ l of labeled progesterone then was added to all tubes, which were vortexed immediately and placed in a 37°C, water bath for 15 min. Following incubation, the samples were placed in a 4°C water bath for 2 h. Eight-tenths ml of dextran-coated charcoal (0.400% dextran and 0.625% charcoal) then was added to all tubes, which were vortexed, and allowed to incubate at 4°C for 10 min. Samples were centrifuged for 10 min at 1732 x g and a 500  $\mu$ l aliquot of the supernatant was transferred to a filmware bag (Nalgene) together with 5 ml of Toluene-Triton-X-100 scintillation cocktail. The bags were counted in the manner previously described for the E2 assay. Cross-reactivity of 17-Hydroxyprogesterone with the progesterone antibody at 100 pg concentrations was 2.9 percent. The sensitivity of the progesterin assay was 20 pg/ml. Mean recovery for radioactive progesterone was 71 percent. Validation of the extraction and assay procedure for progesterone was demonstrated by addition of known amounts of nonradioactive progesterone to aliquots of pooled dove plasma as described (Table 1).

#### Hunter-Harvested Doves

Wild doves were collected at Elm Hill Wildlife Management Area, Mecklenburg County, Virginia. Investigations were conducted on Saturdays during the first 3 weeks of the 1975 hunting season (6, 13, 20 September), and the first 2 weeks of the 1976 hunting season (11 and 18 September). Hunters were asked to donate carcasses of doves after the breast portion was removed. Carcasses were kept chilled until return to the laboratory where they were placed in 10 percent formalin for future detailed examination. All doves were aged in the laboratory using primary feather replacement as previously described. Blood samples and body weights were not taken due to the method of collection. Gonadal and crop data were recorded as previously described.

#### Statistical Analysis

Means were calculated for body weights, gonadal weights and measurements, and hormone concentrations. Non-detectable hormone concentrations were set at the most consistent minimum sensitivity of the appropriate hormone assay. The  $F_{max}$  test was used

to test for homogeneity of variances (Sokal and Rohlf 1969:371). A least squares regression version of analysis of variance (one-way classification) was used for data with unequal sample sizes. Duncan's multiple range test was used to determine the location of significant differences. Appropriate nonparametric statistical procedures (Kruskal-Wallis and multiple comparison tests), as outlined by Hollander and Wolfe (1973), were used when data failed to conform to the assumptions of parametric tests. The analysis of variance and Duncan's test were conducted on the Statistical Analysis System (SAS) of Barr et al. (1976).

## RESULTS

### Trapped Juveniles

*Body Weights.*—Mean body weights of trapped juvenile female mourning doves showed gradual increases in weight from approximately 38 to 131 days of age (Table 2). No extreme increases in weight occurred between any particular age class. No evidence of puberty attainment, such as a sharp rise and/or plateau in body weights, was evident.

*Gonadal and Hormonal Activity.*—Increases ( $P \leq 0.05$ ) in ovary weights of juvenile females did not occur until approximately 93 days of age (Table 2). There were no significant increases in oviduct weights among age classes, although oviduct weights did tend to increase along with ovary weights (Table 2). Follicular development also began at approximately 93 days of age and remained relatively constant thereafter (Table 2). Gonadal weights were quite variable among doves after 93 days of age.

No differences in estradiol or progesterone concentrations were observed between age classes (Table 3). Hormone concentrations were extremely variable among doves.

*Reproductive Capability.*—Three of 22 juvenile females in the 93-131-day age class were deemed capable of reproduction during the late summer and fall of 1977 (Table 4). Two of 22 juveniles capable of reproduction were in the 93-day age class. No females had crop gland activity or gonadal weights and measurements indicative of reproductive capability prior to 93 days of age.

### Hunter-Harvested Juveniles

*Gonadal Activity.*—Mean ovary weights of juvenile doves collected at Elm Hill Wildlife Area were lightest up to approximately 80 to 93 days of age in 1975 and 1976, respectively (Tables 5 and 6). Ovary weights were heaviest from 93 to 131 days of age in 1976. Mean oviduct weights and mean IFD's (Tables 5 and 6) followed essentially the same pattern as ovary weights during both 1975 and 1976, respectively. Considerable variability was evident in both ovary weights and IFD's of doves 93 days and older. Ovary and oviduct weights and IFD's of 93-day old juvenile females appeared to increase from 2 to 4-fold over these same measurements at the previous age class. Juvenile females appeared to reach puberty at a greater age during 1976 than during 1975.

*Reproductive Capability.*—Between 7 and 8 percent of the 93-131-day old female mourning doves harvested during the 1975 and 1976 hunting seasons were deemed capable of reproduction (Table 4). One percent (1/91) of the females capable of reproduction during the 1975 hunting season were in the 93-day age class. No 93-day old females sampled in 1976 were deemed capable of reproduction. Reproductive capability tended to increase with age. No females had crop gland activity or gonadal weights and measurements indicative of reproductive capability prior to 93 days of age.

## DISCUSSION

Changes in ovary and oviduct weights and increases in follicular development in juvenile female mourning doves indicated the possibility of ovulation (attainment of puberty) at approximately 93 days of age during 1975, 1976, and 1977 in Virginia.

Table 2. Age (Haas and Amend 1976), mean (+ S.E.) body weights, ovary and oviduct weights (fresh), and indices of follicular development (IFD) of juvenile mourning doves trapped during the summer and fall of 1977.

Age (days)	Primary Feather Replacement	N	Body Weight (g)	Ovary Weight (mg)	Oviduct Weight (mg)	IFD <sup>1</sup> (mm)
< 38	00	4	76.9 ± 3.7 b	11.4 ± 3.9 cd	7.4 ± 1.4 c	0.0 c
38	01	5	82.3 ± 2.2 bc	9.3 ± 2.1 c	3.4 ± 1.1 c	0.0 c
43	02	6	92.1 ± 4.3 cd	10.1 ± 2.1 c	10.1 ± 1.4 c	0.0 c
52	03	5	94.4 ± 3.8 cde	14.8 ± 2.6 cd	8.3 ± 1.0 c	0.0 c
60	04	12	93.5 ± 2.7 cd	16.4 ± 3.0 cd	6.3 ± 0.5 c	0.0 c
70	05	8	97.9 ± 3.0 de	24.2 ± 3.2 cd	8.6 ± 1.1 c	0.79 ± 0.77 de
80	06	6	98.0 ± 2.9 de	14.5 ± 3.0 cd	6.2 ± 0.4 c	0.0 c
93	07	7	101.6 ± 4.4 de	61.3 ± 12.5 d	70.2 ± 49.6 c	2.78 ± 0.95 d
112	08	5	106.1 ± 2.2 ef	66.0 ± 2.4 d	34.5 ± 20.2 c	2.89 ± 0.97 d
122	09	5	105.2 ± 3.3 ef	42.6 ± 10.9 cd	30.7 ± 15.8 c	3.13 ± 0.73 de
131	10	5	114.3 ± 3.3 f	42.3 ± 8.7 cd	14.0 ± 5.7 c	2.13 ± 0.70 de

<sup>1</sup>Follicle diameters less than 0.1 mm could not be measured accurately and were assigned 0. values.  
b,c,d,e,f: Means followed by different letters in the same column are significantly ( $P \leq 0.05$ ) different.

Table 3. Age (Haas and Amend 1976), mean (+S.E.) estradiol and progesterin concentrations of juvenile female mourning doves trapped during the summer and fall of 1977.

Age (days)	Primary feather replacement	N	Estradiol levels <sup>1</sup> (pg/ml)	N	Progesterin levels <sup>1</sup> (ng/ml)
< 38	00	4	468.6 ± 335.5	1	3.34
38	01	3	692.8 ± 383.8	1	1.08
43	02	5	407.1 ± 204.4	2	3.41 ± 1.10
52	03	5	340.7 ± 195.0	3	4.17 ± 2.60
60	04	12	249.1 ± 116.5	9	3.41 ± 1.10
70	05	8	759.5 ± 326.8	5	2.02 ± 0.50
80	06	5	314.1 ± 144.2	4	5.50 ± 3.35
93	07	7	208.7 ± 114.0	7	3.44 ± 0.75
112	08	4	309.0 ± 131.4	3	3.15 ± 0.94
122	09	5	266.4 ± 95.7	2	2.05 ± 1.45
131	10	5	384.3 ± 130.7	4	3.99 ± 1.47

<sup>1</sup>No significant ( $P \leq 0.05$ ) differences among means.

Table 4. Reproductive capability of juvenile female mourning doves in various age classes<sup>1</sup> (Haas and Amend 1976) trapped or shot during the summer and fall of the year.

Collection date	Collection method	Median age (days)	N	Successful nesters (%) <sup>2</sup>	Reproductive Capability Category		
					Potential layers (%) <sup>3</sup>	Potential breeders (%) <sup>4</sup>	Total (%)
1975	Shot	93	33	0	0	1	1
		112	25	1	0	0	1
		122	23	2	0	0	2
		131	10	3	0	0	3
		93-131	91	6(6.6)	0(0.0)	(1.1)	7(7.7)
1976	Shot	93	18	0	0	0	0
		112	21	2	1	0	3
		122	19	1	0	1	2
		131	10	0	0	0	0
		93-131	68	3(4.4)	1(1.5)	1(1.5)	5(7.4)
1977	Trapped	93	7	1	0	1	2
		112	5	0	0	1	1
		122	5	0	0	0	0
		131	5	0	0	0	0
		93-131	22	1(4.5)	0(0.0)	2(9.1)	3(13.6)

<sup>1</sup>No reproductive activity observed prior to reported values.

<sup>2</sup>Doves with active or developing/regressing crop phases.

<sup>3</sup>Doves with eggs in the oviduct or at least 1 ovarian follicle diameter  $\geq$  10mm; based on Cheng (1974).

<sup>4</sup>Sum of three largest ovarian follicle diameters  $\geq$  10 mm.

Table 5. Age (Haas and Amend 1976), mean ( $\pm$  S.E.) ovary and oviduct weights (preserved), and indices of follicular development (IFD) of juvenile mourning doves collected during the 1975 hunting season.

Age (days)	Primary feather replacement		Ovary		Oviduct		IFD <sup>1</sup> (mm)
	N	Weight (mg)	N	Weight (mg)	N	Weight (mg)	
< 38	18	7.4 $\pm$ 3.4 b	18	4.7 $\pm$ 1.2 bc	14	0.0 c	
38	5	6.2 $\pm$ 3.9 b	5	3.6 $\pm$ 1.2 bcdf	4	0.0 c	
43	5	4.4 $\pm$ 1.7 b	5	2.6 $\pm$ 0.5 bcdf	4	0.0 c	
52	11	8.6 $\pm$ 2.1 b	11	4.0 $\pm$ 0.6 bcdf	11	0.0 c	
60	12	9.9 $\pm$ 2.3 b	12	3.1 $\pm$ 0.6 b	10	0.0 c	
70	22	19.2 $\pm$ 3.2 bcdf	22	5.3 $\pm$ 0.8 bcdf	22	0.12 $\pm$ 0.12 c	
80	23	26.9 $\pm$ 3.9 bcdef	21	4.9 $\pm$ 0.6 bcdf	23	0.62 $\pm$ 0.26 cd	
93	33	40.0 $\pm$ 6.4 cdef	33	46.2 $\pm$ 25.8 cdef	33	1.44 $\pm$ 0.42 d	
112	26	42.6 $\pm$ 5.1 def	25	45.9 $\pm$ 14.9 cdf	25	3.25 $\pm$ 0.47 e	
122	23	58.0 $\pm$ 6.1 ef	23	126.7 $\pm$ 33.4 ef	22	4.83 $\pm$ 0.55 f	
131	10	75.7 $\pm$ 20.2 f	10	62.8 $\pm$ 28.4 f	9	4.91 $\pm$ 0.64 f	

<sup>1</sup>Follicle diameters less than 0.1 mm could not be measured accurately and were assigned 0. values.  
b,c,d,e,f: Means with different superscripts in the same column are significantly ( $P \leq 0.05$ ) different.



Table 6. Age (Haas and Amend 1976), mean ( $\pm$  S.E.) ovary and oviduct weights (preserved), and indices of follicular development (IFD) of juvenile mourning doves collected during the 1976 hunting season.

Age (days)	Primary feather replacement		Ovary		Oviduct		IFD <sup>1</sup> (mm)
	N	Weight (mg)	N	Weight (mg)	N	Weight (mg)	
< 38	17	11.9 $\pm$ 1.9 b	16	4.0 $\pm$ 0.6 b	16	0.0 c	0.0 c
38	3	5.0 $\pm$ 1.7 b	3	3.5 $\pm$ 0.9 bcd	3	0.0 c	0.0 c
43	6	10.2 $\pm$ 2.2 b	5	4.4 $\pm$ 1.3 bc	6	0.0 c	0.0 c
52	14	11.8 $\pm$ 2.2 b	15	4.0 $\pm$ 0.5 b	14	0.0 c	0.0 c
60	10	16.6 $\pm$ 2.3 b	10	3.6 $\pm$ 0.6 b	9	0.0 c	0.0 c
70	7	19.7 $\pm$ 4.1 b	5	5.4 $\pm$ 0.8 bcd	7	0.0 c	0.0 c
80	8	14.0 $\pm$ 3.6 b	9	4.2 $\pm$ 0.6 bc	8	0.0 c	0.0 c
93	18	24.1 $\pm$ 2.6 bc	18	6.0 $\pm$ 1.0 bc	18	0.48 $\pm$ 0.27 c	0.48 $\pm$ 0.27 c
112	21	45.9 $\pm$ 9.5 cd	21	114.4 $\pm$ 75.6 cd	21	2.41 $\pm$ 0.55 d	2.41 $\pm$ 0.55 d
122	19	53.7 $\pm$ 11.7 cd	19	129.7 $\pm$ 57.1 cd	19	4.36 $\pm$ 0.74 e	4.36 $\pm$ 0.74 e
131	10	34.5 $\pm$ 5.1 bd	10	95.2 $\pm$ 28.2 d	10	3.09 $\pm$ 0.56 de	3.09 $\pm$ 0.56 de

<sup>1</sup>Follicle diameters less than 0.1 mm could not be measured accurately and were assigned 0. values. b,c,d,e,f: Means with different superscripts in the same column are significantly ( $P \leq 0.05$ ) different.

Reproductive capability, particularly the presence of active crop glands and eggs in the oviduct, provided conclusive evidence of juvenile females breeding and nesting after 93 days of age (Table 4). Ninety-three days (07 primary feather replacement) was considered the *minimal* age of puberty attainment during this period of decreasing day length since no doves sampled at earlier ages during the 3 years of study exhibited reproductive capability. Only 5.2 percent (3/58) of the 93-day old females sampled manifested breeding capability during that period as compared to 9.8 (5/51), 8.5 (4/47), and 12 percent (3/25) of the females sampled in the 112, 122, and 131-day age classes, respectively. The age of puberty attainment and the percentages of hunter-harvested juvenile females 93-131 days old that reached puberty (7.7% and 7.4%) in 1975 and 1976, respectively, compared favorably with similar data collected in southern Arizona (Brown 1967).

Several explanations are possible for the large variability in gonadal weights and measurements among female doves 93 days of age and older. These relate primarily to the coincidence between the end of the breeding season and the attainment of the probable age of puberty in this region. Out of necessity the juvenile population was sampled from August through September. Consequently, some juvenile females that had surpassed 93 days of age may not have been reproductively active due to the lateness of the breeding season. Even though a large number of female doves may reach 93 days of age there is no certainty that they will attain puberty due to photoperiodic constraints (i.e., day length may be too short at the time they reach 93 days of age to stimulate reproductive activity). Thus, the sampling regimen may have been partially responsible for the small percentage of females that attained puberty and subsequently may have added to the variability among doves. Additionally, differences in nutritional intake due to food, climate, genetic composition, the amount of exposure of juvenile females to the breeding behavior of males and/or other unknown factors may have influenced the extent to which juvenile females bred within a given year. These factors also could have been responsible for the apparent differences in the age of puberty attainment between years.

Concentrations (pg/ml) of estradiol observed in juvenile female mourning doves compared favorably with concentrations observed in prepuberal chicken (Senior 1974, Yu et al. 1974) and turkey hens (Wineland and Wentworth 1975), but were much higher than those observed in breeding adult female ring doves (Silver 1978). Progesterone concentrations were comparable to levels observed in breeding adult female ring doves but were quite variable among doves (Silver 1978). The great variability in estradiol and progesterone concentrations in the female doves most probably resulted from a combination of small sample sizes, pulsatile patterns of hormone secretion and photoperiodic constraints discussed previously. Future studies of hormone concentrations in this species should involve a captive flock where larger sample sizes, frequent blood collections throughout the day, and control of photoperiod could be used to minimize variability and enhance diagnostic capability.

In summary, the earliest age of puberty attainment for female mourning doves in Virginia was approximately 93 days. Five to 12 percent of the juvenile female doves sampled in Virginia attained puberty between 93-131 days of age and *could* have contributed to the productivity of the mourning dove population during 1975, 1976, and 1977. Based on the minimal age of puberty attainment it is unlikely that many juvenile females born after 30 June of each season in Virginia become reproductively active during their first summer or fall. More research is needed to investigate the effects of age, photoperiod, nutrition, and climatic factors on the attainment of puberty in mourning doves. Additionally, an exhaustive study of the quantitative contribution immature mourning doves make to the productivity of dove populations throughout the breeding season at various latitudes in the northern hemisphere is recommended.

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