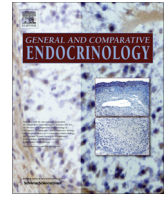




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Physiology of reproductive senescence in Florida scrub-jays: Results from a long-term study and GnRH challenge

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ABSTRACT

In most vertebrates, production of reproductive hormones wanes with age, co-occurring with a decline in reproductive output. Measurement of these hormones can serve as a key marker of the onset of reproductive senescence. Longitudinal studies of physiological parameters in populations of free-living animals are relatively uncommon; however, we have monitored baseline concentrations of hormones for nine years in a population of Florida scrub-jays (*Aphelocoma coerulescens*). We hypothesized that concentrations of circulating reproductive hormones change with age, and predicted declines in reproductive hormones in the oldest jays. We found that baseline levels of luteinizing hormone (LH) and testosterone (T) were relatively low in both young and old male breeders and reach their highest levels in birds aged 4–7 years. Conversely, we found no age-related patterns in baseline levels of LH or estradiol in female jays. In males we determined which component of the hypothalamo-pituitary-gonadal (HPG) axis is responsible for observed age-based differences, by challenging males of different ages with gonadotropin-releasing hormone (GnRH); thereby allowing assessment of pituitary and gonadal responsiveness by measuring plasma concentrations of luteinizing hormone and testosterone, respectively. The magnitude of increase in levels of both LH and T in response to GnRH challenge decreased with age in male breeders. Combined with the baseline levels, the results from the GnRH challenge suggest that younger birds have the capability to produce higher levels of reproductive hormone, whereas the old birds may be constrained by senescence in their ability to produce these hormones.

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1. Introduction

Senescence is a decline in physiological function with age that can affect survival, reproductive performance, or both. In most vertebrates, production of reproductive hormones wanes with age, co-occurring with a decline in reproductive output (Hughes et al., 2002). Accordingly, measurement of these hormones can serve as a key marker of the onset of reproductive senescence. Birds are relatively long-lived, when compared to size-matched mammals (Holmes and Austad, 1995), and therefore they are good models for studying mechanisms of reproductive aging (Holmes and Ottinger, 2004). Although reproductive senescence has been considered extensively in a theoretical framework, it has proven difficult to

study in practical application, particularly in populations of free-living animals (reviewed by Nisbet, 2001). A number of studies of birds have documented an increase in mortality rate in older members of both short- and long-lived species (Holmes et al., 2001; Newton and Rothery, 1997). This type of senescence, termed actuarial senescence (McDonald et al., 1996), needs to be distinguished from reproductive senescence which is characterized by reductions in reproductive success or the function of reproductively-related physiological systems with age. Actuarial senescence does occur in the Florida scrub-jay (*Aphelocoma coerulescens*; McDonald et al., 1996), the focal species of this study.

There are a number of physiological measures that are known to change, sometimes dramatically, with age in birds. These include telomerase activity, thyroid hormone production, response to stressors, immune system response, antioxidant activity, and endocrine signaling (reviewed in Vleck et al., 2007), but few studies have examined these phenomena in free-living species in which the age of each individual is known. The existing studies of free-living birds have provided mixed findings that largely reflect the differences in life spans of the species studied. For example, Nisbet

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et al. (1999) found only slight changes in plasma levels of reproductive hormones with age in long-lived (max longevity 21 years) common terns (*Sterna hirundo*) and concluded that because this occurred in such a small percentage of the population that it could not have much of an impact on reproductive success. This study was a snapshot of different aged birds over a two year period and the authors suggested that a longitudinal study that included samples from more breeding seasons would be more informative. On the opposite end of the pace-of-life spectrum, Ottinger (1992, 2007) studied neuroendocrine changes with age in short-lived (max longevity 5 years) Japanese quail (*Coturnix japonica*) in a laboratory setting and found dramatic decreases in circulating levels of reproductive hormones in the oldest birds. Although reproductive output and success among different aged birds have been studied for many moderately long-lived species of birds (e.g., Møller et al., 2006; Newton and Rothery, 1997; Saino et al., 2002), no research that we are aware of has taken a multi-year longitudinal approach to investigate changes in reproductive hormone physiology among different aged birds.

Physiological processes and behaviors associated with reproduction are largely regulated by the hypothalamo-pituitary-gonadal (HPG) axis. In a seasonal breeder, the hypothalamus integrates information from the exogenous and endogenous environment to assure the appropriate timing of up-regulation of hormone production and initiation of reproductive preparedness. At a basic level (see Besser and Mortimer, 1974, for a review of the multiple neuroendocrine functions of the hypothalamus), the hypothalamus releases gonadotropin-releasing hormone (GnRH, also called luteinizing hormone releasing hormone, or LHRH), which stimulates the release of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), from the anterior pituitary. True to their names, both LH and FSH are critical for gonadal function in males and females.

We first asked if there was evidence of reproductive senescence, determined by age related fledgling success for male and female breeders from 20 years of nest monitoring in a population of Florida scrub-jays. Our previous research revealed that hatching failure occurs more commonly in older female Florida scrub-jays than middle-aged females (Wilcoxon et al., 2011b), suggesting that Florida scrub-jays undergo reproductive senescence. We therefore hypothesized that the oldest Florida scrub-jays will fledge the fewest young, given that reduced hatching success is likely to lead to fewer fledglings.

With regard to the physiological aspects of reproductive senescence, we predicted that concentrations of circulating reproductive hormones would decrease with age. To test this hypothesis, we collected blood samples for hormone analysis from male and female Florida scrub-jays over nine field seasons (2000–2008, with testosterone measured in each of those years and luteinizing hormone measured from 2000 to 2002). Although natural variation in circulating levels of hormones is informative, challenges of physiological systems can provide additional information about the role and status of specific components of an endocrine axis. We used gonadotropin-releasing hormone (GnRH) challenges of different aged male breeders to determine to what degree the hypothalamus, pituitary, or testes contribute to the observed patterns in baseline levels of hormones among different aged birds. Different outcomes from the GnRH challenge could represent senescence of specific components of the HPG axis. For example, if an old bird has low baseline levels of LH or testosterone (T) prior to reproduction, and when given a GnRH challenge, they respond with a robust increase in LH and T levels, it is likely that senescence has occurred at the level of the hypothalamus or possibly upstream. Further, should an individual with low baseline levels of LH and T show little or no increase in LH levels following GnRH injection, one might conclude that senescence has occurred at the level of the pituitary,

and the low levels of T were due to an insufficient LH signal. Finally, should an individual with low baseline levels of LH and T show a robust LH response to GnRH injection, but an insignificant increase in T levels, senescence has likely occurred at the level of the gonads. Although, these scenarios are slightly oversimplified, this technique provides valuable insight into the mechanisms of reproductive senescence.

2. Material and methods

2.1. Study species and location

The Florida scrub-jay is a federally threatened, non-migratory, cooperatively breeding passerine with a range restricted to xeric oak scrub habitats of peninsular Florida. We monitored a population of Florida scrub-jays at Archbold Biological Station in south central Florida (27° 10' N, 81° 21' W, elevation 38–68 m; see Schoech et al., 1996a, 2007 for further details). The sex and social status (i.e., breeder or non-breeding helper) of each bird in this population are known, as are family group affiliation, and reproductive output from 1989 to present. Each Florida scrub-jay territory has a breeding pair and zero to six non-breeding helpers residing within the territory. The nonbreeders are typically offspring of the breeding pair that are themselves delaying reproduction, often contributing alloparental care to the nestlings and fledglings in the territory (Schoech et al., 1996a). For all breeding jays in our population, we locate all nests and determine laying, hatching, fledging dates, and fledging success; further, we band each nestling prior to fledging, and thus, know the age and pedigree of all birds in the population. Many factors are known to influence fitness and longevity in this population of jays (originally reviewed by Woolfenden and Fitzpatrick, 1984), and some of the more recent findings include habitat quality (Mumme et al., 2000; Schoech and Bowman, 2001), food availability (Reynolds et al., 2003), stress response (Wilcoxon et al., 2011a), variation in immune function (Wilcoxon et al., 2010b), and overall physiological condition (Wilcoxon et al., 2010a). The maximum longevity for a male in this population is 15 years and the maximum longevity for a female in this population is 14 years. Breeding birds from 2 to 13 year-of-age were used in this analysis.

2.2. Blood sampling

Egg laying can begin in early March; however, the population mean first clutch initiation dates display considerable inter-year variance, ranging from mid-March to mid-April (long-term mean of 29 Mar.; see Schoech, 2009). We regard the weeks between mid-January and egg laying as the prebreeding period, during which there are gradual increases in the behaviors and physiology associated with breeding (e.g., territorial defense, courtship feeding, nest building, and gonadal recrudescence with accompanying increases in levels of sex steroid hormones). We collected small blood samples from breeders each year from 2000 to 2008 during the pre-breeding period. Birds were captured in continuously monitored Potter traps baited with peanuts, and blood samples were collected within 3 min of capture, using microhematocrit capillary tubes following venipuncture of the brachial vein. The blood samples were stored on ice in coolers in the field until return to the lab, where the tubes were spun in a microhematocrit centrifuge to separate the cellular and plasma fractions. The plasma was then drawn off with a 100 μ L Hamilton syringe, and stored in plastic vials at -20°C until radioimmunoassay at the University of Memphis. All methods were approved by the University of Memphis IACUC.

2.3. Radioimmunoassays

2.3.1. Luteinizing hormone RIA

Radioimmunoassays were run in duplicate on samples from jays captured during the prebreeding stages of 2000, 2001, and 2002. The LH RIA used was a post-precipitation double antibody RIA that uses purified chicken LH as a standard and rabbit antiserum against LH (Follett et al., 1975). The samples from these three years were run in a single RIA with volumes ranging from 10 to 20 μ L. Intra-assay CV was 5.5%.

All baseline and induced (15 min) samples from the GnRH challenges in 2009 and 2010 were run in duplicate in an RIA for each year. Sample volumes ranged from 10 to 30 μ L and intra-assay CV's were 7.2% and 6.4%, respectively. The inter-assay CV was 8.6%.

2.3.2. Testosterone and estradiol RIA

Plasma samples from 2000 to 2008 were assayed for levels of testosterone and estradiol following chromatographic separation on microcolumns filled with celite (Wingfield and Farner, 1975). For more details on column separation, calculation of recoveries, extraction with diethyl ether, and RIA for each of these hormones see Schoech et al. (1991, 2004). Routine testosterone RIA in the Schoech lab yields intra-assay CVs that range from 4.5% to 10.4% with an inter-assay CV of 14.3%. For E_2 , intra-assay CVs range from 4.5% to 14.4% with an inter-assay CV of 12.6% (see Schoech et al., 2004).

2.4. GnRH challenges

From February 12 to March 8, 2009 and February 13 to March 6, 2010 we delivered a GnRH challenge to 38 known age male breeders: 15 male breeders served as controls (i.e., were injected with saline). Only males were given GnRH challenges because our long-term hormone data (below) showed no age-related differences in baseline LH or E_2 levels in females, and thus, we determined that results from that manipulation would be of little value. Further, the Florida scrub-jay is a threatened species, and we could not justify the risk of conducting a hormonal manipulation on females that showed no evidence of hormonal senescence. Of the 53 males used in this experiment, 9 were captured in both years, and 6 were challenged in both years. In this population of scrub-jays, there are approximately 45 breeding pairs per year, and only males who had established a breeding territory by February were targeted for this study. Due to difficulties in trapping some individuals, it was necessary to use some of the same individuals in both years. To avoid pseudoreplication our statistical model incorporated bird identity as a random factor. Immediately before GnRH injection, we collected an initial blood sample (\sim 200 μ L) to assess basal levels of luteinizing hormone and testosterone. Birds were randomly assigned to receive control or GnRH injections (1.25 μ g of chicken LH-RH [GnRH-I], American Peptide Company, Inc., Sunnyvale, CA, dissolved in 50 μ L of 0.1 M phosphate-buffered saline solution, PBS) in each pectoralis major with a luer-tip Hamilton syringe and a 25 gauge needle (total dose of 2.5 μ g in 100 μ L PBS). Fifteen minutes after the injection, we collected 70 μ L of blood for LH measurement, and at 30 min post-injection, we collected \sim 200 μ L of blood for a testosterone assay. The total volume of blood collected is well below the recommended volume of <1% of total blood volume (McGuill and Rowan, 1989). Between blood samples, birds were held in loosely woven opaque cotton bags that were hung in the shade. Blood samples were transported, prepared, and stored as described above.

2.5. GnRH challenge testosterone EIA

We determined T levels using an EIA kit from Assay Designs, Inc. (#901-065; Jawor et al., 2007). Each plasma sample (50 μ L) was combined with 20 μ L of tritiated testosterone (approximately 2000 cpm) to allow for the calculation of recoveries after 3 extractions with diethyl ether. Extracts were then re-suspended in 50 μ L of ethanol. 300 μ L of assay buffer were added for a final volume of 350 μ L. From this, 100 μ L was used to determine recoveries and duplicate 100 μ L aliquots were used in the EIA. Final values were obtained after adjustments for recoveries (average recovery = 76%). Intra-assay coefficients of variation were calculated from 6 standard samples included in each plate yielding CVs of 14.2%, 11.1%, 6.4%, and 6.9%. The inter-assay CV was 13.3%.

2.6. Statistical analyses

2.6.1. General

All statistical analyses were completed with PASW 17.0 unless otherwise noted (SPSS, Inc., 2009). All hormone values below are expressed as means \pm standard error of the mean. Hormone levels for all of the below analyses failed a Kolmogorov-Sminov Test for normal distribution and, therefore, values were normalized with log transformation prior to analysis. For each analysis, age was included as each a linear and quadratic variable. Tests for quadratic effects were included to explore the possibility that reproductive hormone levels were lowest in the young (less developed and experienced) and old (senescing) birds, and that the middle-aged birds may be in 'prime' reproductive condition.

2.6.2. Fledging success

The nests we monitored over the past 20 years included many nests produced by the same birds in multiple years; therefore, to control for non-independence of the data, we used linear mixed models (LMM) with bird identity as a random factor and year as a repeated factor. The number of young fledged was the dependent variable and age of the parent was included as a covariate. Separate LMM's were used for males and females.

2.6.3. Baseline reproductive hormones (2000–2008)

Many individuals were captured in multiple years; therefore, to control for non-independence of data we used LMM's with bird identity as a random factor and year as a repeated factor. The hormone of interest (LH for both sexes, E_2 for females, T for males) was the dependent variable, and age and days before clutch initiation were included as covariates in separate analyses by sex. We also included all two-way interactions, and non-significant interactions ($P > 0.05$) were iteratively removed from the models, beginning with the highest P -value greater than 0.05, followed by a new analysis without the removed variables, and repeated until only statistically significant interactions and main effects were retained in the final models.

2.6.4. GnRH challenge

To assess the endocrine response to the GnRH, we used two-way repeated measures ANOVA with hormone levels at each of the two time points (0 and 15 min for LH, 0 and 30 min for T) as the dependent variable. Treatment (GnRH or control) and year were included as fixed factors.

To test for differences among ages, we used an LMM with male identity as a random factor, the magnitude of the response to the GnRH challenge ($LH_{\max} - LH_{\text{baseline}}$ or $T_{\max} - T_{\text{baseline}}$) as the dependent variable, year as a fixed factor, and age as a covariate. Capture date relative to clutch initiation in an individual's territory (i.e., the number of days before clutch initiation) was also included as a covariate to control for potential differences in HPG axis activity

associated with proximity to reproduction. We also included all two-way interactions. In deriving a final statistical model, non-significant interactions ($P > 0.05$) were iteratively removed from the models; however, all main effects were retained.

3. Results

3.1. Fledging success (1989–2008)

There was a significant quadratic relationship between the number of fledglings produced and female age ($F_{2,703} = 6.41$, $P = 0.02$; line of best fit: $y = -0.0303x^2 + 0.4163x + 0.6276$); Fig 1.). For males, there was no significant difference in the number of fledglings produced with age; neither a linear ($F_{1,702} = 0.01$, $P = 0.91$) nor a quadratic ($F_{2,701} = 1.58$, $P = 0.21$) relationship existed. In both LMM's, bird identity (random factor) explained a significant amount of variance in fledglings produced (Wald $Z = 18.59$, $P < 0.01$ for females; Wald $Z = 18.76$, $P < 0.01$ for males).

3.2. Baseline hormone levels – females

There was no significant relationship between baseline LH levels and age in females (linear, $F_{1,139} = 0.22$, $P = 0.64$; quadratic, $F_{2,139} = 0.73$, $P = 0.39$; Fig. 2). Days before clutch initiation was inversely related to baseline LH levels (i.e., levels increased as clutch initiation neared; $F_{1,139} = 15.79$, $P < 0.01$). None of the interaction terms were significant ($P > 0.15$ in all cases). Bird identity explained a significant amount of variance in baseline levels of LH (Wald $Z = 2.44$, $P = 0.02$).

There was no significant relationship between baseline E_2 levels and age in females (linear, $F_{1,197} = 0.66$, $P = 0.42$; quadratic, $F_{2,197} = 1.19$, $P = 0.28$; Fig. 2). Days before clutch initiation was inversely related to baseline E_2 levels ($F_{1,197} = 4.01$, $P = 0.05$). None of the interaction terms were significant ($P > 0.21$ in all cases). Bird identity explained a significant amount of variance in baseline levels of E_2 as well (Wald $Z = 15.15$, $P < 0.01$).

3.3. Baseline hormone levels – males

Neither LH nor T showed a linear relationship with age (LH: $F_{1,188} = 1.82$, $P = 0.14$; T: $F_{1,188} = 0.63$, $P = 0.49$), but there was a significant quadratic relationship in both cases (LH: $F_{2,187} = 9.02$, $P < 0.01$; T: $F_{2,283} = 4.17$, $P = 0.04$; Fig. 3). Finally, as expected, baseline LH and T levels were inversely related to days before clutch initiation (LH: $F_{1,187} = 26.38$, $P < 0.01$; T: $F_{1,283} = 25.59$, $P < 0.01$);

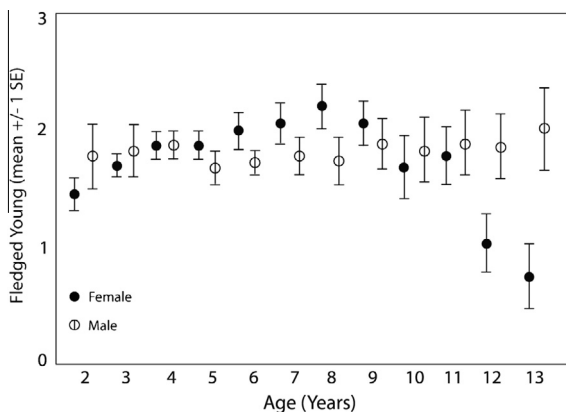


Fig. 1. Relationship between the age of breeding female and male Florida scrub-jays and the average number of fledglings produced. Means are shown with bars representing \pm one standard error of the mean.

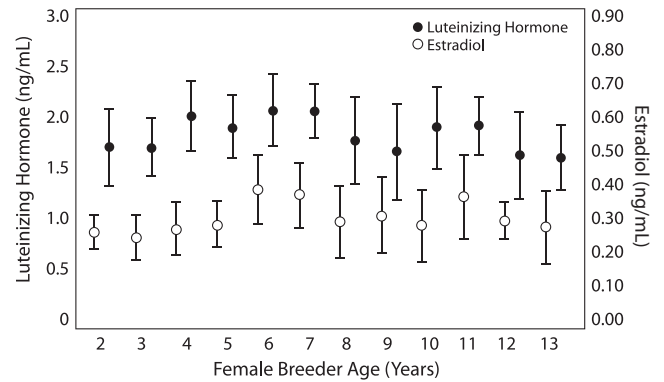


Fig. 2. Relationship between age and baseline luteinizing hormone (solid circles) and estradiol (open circles) in female Florida scrub-jays. Means are shown with bars representing \pm one standard error of the mean.

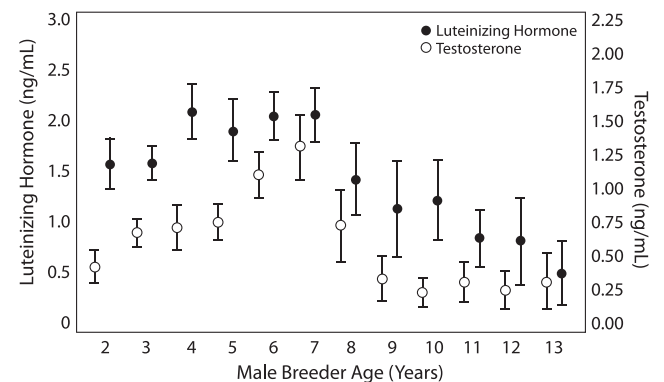


Fig. 3. Relationship between age and baseline luteinizing hormone (solid circles) and testosterone (open circles) levels in male Florida scrub-jays. Means are shown with bars representing \pm one standard error of the mean.

i.e., hormone levels were higher in birds sampled closer to clutch initiation. None of the interaction terms were significant ($P > 0.14$ in all cases). Bird identity explained a significant amount of variance in baseline levels of both LH and T (LH: Wald $Z = 2.57$, $P = 0.01$; T: Wald $Z = 2.42$, $P = 0.02$).

3.4. GnRH challenges

There was a significant interaction between time and treatment (GnRH vs. saline) relative to LH levels (repeated measures ANOVA; $F_{1,52} = 13.59$, $P < 0.01$) and T levels (repeated measures ANOVA; $F_{1,52} = 13.748$, $P = 0.001$). Birds challenged with GnRH had LH levels that increased significantly from time 0 to 15 min post-injection ($F_{1,37} = 11.89$, $P < 0.01$), whereas controls exhibited no change in LH levels ($F_{1,14} = 0.65$, $P = 0.43$; Fig. 4a). In addition, birds challenged with GnRH had T levels that increased significantly from time 0 to 30 min post-injection ($F_{1,36} = 14.44$, $P < 0.01$), whereas controls exhibited no change in T levels ($F_{1,15} = 0.40$, $P = 0.87$; Fig. 4b). Bird identity explained a significant amount of variance in both LH response (Wald $Z = 3.85$, $P < 0.01$) and T response (Wald $Z = 4.00$, $P < 0.01$) to GnRH challenge.

The magnitude of each the LH and T response to GnRH challenge decreased with age (LH: $F_{1,37} = 6.90$, $P = 0.01$, r^2 linear = 0.16; Fig. 5; T: $F_{1,37} = 28.65$, $P < 0.01$, r^2 linear = 0.45; Fig. 5). The LH response did not significantly covary with days before clutch initiation ($F_{1,37} = 2.12$, $P = 0.16$). No other interaction terms were significant in the LH or the T analyses ($P > 0.12$ in all cases) and T-responsiveness also did not vary with days before clutch initiation ($F_{1,37} = 0.59$, $P = 0.49$).

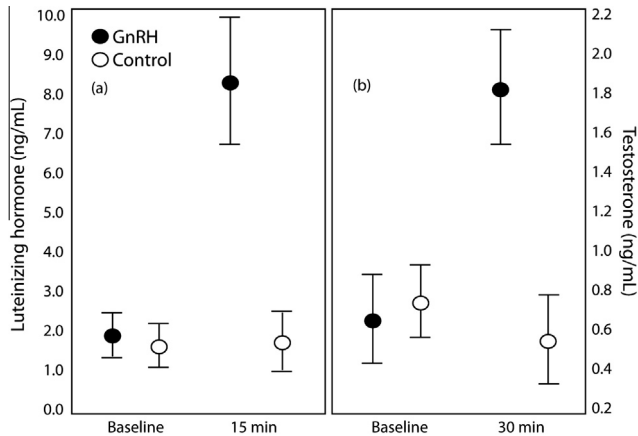


Fig. 4. Response to GnRH injection (filled circles) and control injection (open circles) for LH (a) and T (b). Means are shown with bars representing \pm one standard error of the mean.



Fig. 5. Relationship between age and magnitude of luteinizing hormone (solid circles and solid line) and testosterone (open circles and broken line) response to gonadotropin-releasing hormone challenge in male Florida scrub-jays (luteinizing hormone $r^2 = 0.16$; testosterone $r^2 = 0.45$).

4. Discussion

4.1. Fledging success

The number of fledglings produced per year by females followed an inverted U quadratic pattern across ages, with relatively low fledging success in young females, highest levels in middle-aged birds, followed by a steady decline in older birds. The decline in fledging success among older females supports our prediction that older birds would express reproductive senescence; however, this did not appear to be the case with males as there was no significant trend in fledging success for males across ages – male age was not significantly correlated with hatching failure in this population either (Wilcoxon et al., 2011b). Lower fledging production in young females is likely attributable to inexperience, as has been shown in many other species (e.g., Angelier et al., 2007; Forslund and Part, 1995; Komdeur, 1996; Mauck et al., 2004). In addition, the lower fledging productivity in the youngest and oldest females is almost certainly influenced by greater rates of hatching failure as was noted in an earlier study of this same Florida scrub-jay population (see Wilcoxon et al., 2011b).

4.2. Baseline T and LH – males

Our predictions of declines in plasma levels of LH and T in the oldest male breeders were also met. However, the young birds also

had relatively low LH and T levels, creating a quadratic relationship between age and levels of these hormones. In both Japanese quail (Ottinger, 1992) and Common terns (Nisbet et al., 1999) in breeding condition, there is a gradual decline in plasma androgen concentrations during aging, but only the oldest individuals have significantly reduced androgen levels, and each are consistent with the endocrine patterns observed in this study of Florida scrub-jays. Although these patterns are of interest and suggest age-based changes in the HPG axis, the use of a GnRH challenge allows determination of the level of the HPG axis at which the observed decrement in levels of reproductive hormones occurs (see Introduction). Further, this direct test also allowed testing of the younger birds to determine whether they were capable of higher levels of these hormones.

4.3. Females

Because of the observed patterns of increased hatching failure in old female Florida scrub-jays (Wilcoxon et al., 2011b), we predicted declines in levels of LH or E_2 in female breeders. However, we found no evidence of declines in circulating levels of LH or E_2 in female Florida scrub-jays. In other avian studies, estradiol levels did not decrease significantly in older female common terns (Ottinger et al., 1995) and estradiol levels only declined significantly when ovarian cycles became sporadic in Japanese quail (Ottinger et al., 1992). There are certainly many other physiological processes that may undergo significant changes as individual females age that could serve as the underlying mechanism of reduced reproductive success in the oldest females, we simply have no data that address what those processes might be for the Florida scrub-jays in this population.

4.4. GnRH challenge

There was an inverse relationship between age and LH levels in response to a GnRH challenge, although the relationship explained only 16% of the variation. There was a similar inverse relationship in the T response to GnRH injection; however, the linear relationship was much stronger, with 45% of the variation explained by age. Together, the LH and T data in response to GnRH challenge suggest that low baseline levels of LH and T in the oldest male breeders is due to senescence at either the anterior pituitary or upstream of the pituitary. In general, low baseline levels of LH and T in the oldest birds seems to be a product of constraint (Desrochers, 1992) – for they cannot produce increased reproductive hormone levels, even with a GnRH-induced increase in levels of tropic hormones. In a captive study of Japanese quail, males that showed no sexual behavior increased their LH release over baseline levels in response to a GnRH challenge, indicating, for that species, that pituitary stores of LH are present in senescent birds, but not necessarily secreted (Ottinger, 1998; Ottinger et al., 2003). Unlike short-lived Japanese quail, where senescent males undergo total loss of sexual behavior (Ottinger et al., 1997), male Florida scrub-jays retain their territorial and reproductive behavior despite maintaining low levels of testosterone. In the quail studies, senescent males were found to have substantially decreased aromatase activity, which likely prevents production of sufficient E_2 to stimulate sexual behavior (Ottinger et al., 1997). Perhaps, then, aromatase activity is sustained to a sufficient degree throughout the lifetime of male Florida Scrub-jays despite their low levels of testosterone, effectively permitting the sustained reproductive behavior and function, although we have no data on aromatase activity to specifically address this.

Another interesting finding was that although young birds had significantly lower levels of baseline LH and T than the middle-aged birds, they had the greatest LH and T responses to GnRH

challenge. It appears that low baseline levels of luteinizing hormone and testosterone in the young breeders may be a product of restraint (Desrochers, 1992) – for although they can produce more testosterone, they simply do not. Alternatively, young male breeders may exhibit low baseline levels of LH and T because of limited stimulation (Schoech et al., 1996b). When Florida scrub-jay males first become breeders, they commonly ‘bud’ a territory from their natal territory, although some disperse from their natal territory to pair with an opposite sex conspecific whose mate has died. Because the majority of young males form a territory adjacent to their father’s territory, they are unlikely to face the stimulation of regular territory intrusion and challenges by nearby male breeders that may be common in species that do not form territories in close proximity to family. It would also seem beneficial to avoid challenging the father as well and, by producing enough T to successfully mate while minimizing aggressive behaviors, this end might be achieved. Selection may favor restraint independently of any behavioral effects of reproductive hormones but may act solely on a physiological basis in young male breeders (McGlothlin et al., 2010). Because production of steroid hormones can be energetically costly (Ketterson et al., 1991) and production and clearance of these hormones can increase damage from free radicals (Barja, 1998), maintaining relatively low levels of T early in life may increase longevity, and thus, increase lifetime reproductive success (Torres and Velando, 2007; Peron et al., 2010). Alternatively, high levels of damage accumulated with age may prevent the production of higher levels of hormones. For instance, steroidogenic production was delayed in an experiment on Norway Rats (*Rattus norvegicus*), which in turn delayed free radical damage to Leydig cells, and after the delay, period Leydig cell production of testosterone returned to the level of a young individual before the delay rather than the level associated with their current age (Chen and Zirkin, 1999).

4.5. Conclusions

Overall, our findings of changes in LH and T levels with age in male Florida scrub-jays correspond in many ways with other avian species with very different life histories, both in the field and in captivity, suggesting highly conserved mechanisms of neuroendocrine aging (reviewed by Ottinger, 2007).

One of the most intriguing observations from this species is that older males on average exhibit reproductive success similar to younger birds, and are almost never ‘usurped’ from their breeding territory (Woolfenden and Fitzpatrick, 1984). The findings from the current study suggest that the ability to hold a territory is to a degree independent of T levels. Further, the lack of a drop-off in the production of offspring with age noted in this study makes it clear that the observed low levels of T in older jays are sufficient to maintain sperm production and reproductive behaviors in this socially and genetically monogamous species (see Quinn et al., 1999; Townsend et al., 2011). Higher T levels may occur in the younger birds as they establish territories and engage in agonistic interactions to enforce their boundaries against encroachment from neighbors: relatively elevated T levels may either facilitate or result from such interactions (Jawor, 2007; Soma, 2006; Wingfield et al., 1990). In contrast, the oldest birds may have already established their status and rely less on physiological contributions of T to territorial behavior and more on ‘rules’ established from extensive interactions with neighbors, leaving the older, more experienced males largely unchallenged (Wingfield et al., 1990). Indeed, other data from male breeders in this population suggest that males that survive to old age are typically high quality individuals that contribute more food to nestlings, have strong immune systems, robust stress responses, and generally above-average body condition (Wilcoxon et al., 2010a,b). It would seem that if

these individuals were regularly challenged and fighting to preserve their territory while also caring for young, they would not be able to maintain such good condition.

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