Investigating How Factors Experienced During Early Life Affect Adult Behavioral and Physiological Responses in Multiple Species of Anatidae

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Investigating How Factors Experienced During Early Life Affect Adult Behavioral and Physiological Responses in Multiple Species of Anatidae

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Table of Contents

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Abstract

Broadly, the goals of this research were to understand a) how early life differences influence adult phenotype for ducks living entirely in a captive setting and b) how physiological factors provide mechanistic linkages between early and adult phenotypes. Understanding how differences in adult phenotype develop can help us determine which characteristics may contribute to potential reproductive trade-offs and long-term fitness consequences. These questions were explored in two different contexts: domesticated Pekin ducks housed in commercial settings (Chapters 1 & 2) and Wood Ducks reared and living in captivity (Chapter 3). Although seemingly very different, the studies are grounded in a single conceptual framework involving how an animal's early experience, which can be shaped by several factors both internally and externally, may affect their developmental trajectory through certain underlying mechanisms and then have a long-term effect on the way that they respond to their environment.

There are several factors experienced throughout the lifetime of a duck that can influence its phenotype and long-term reproductive success. Factors from the external environment include maternal effects (e.g., incubation efforts as an egg or protection from predators and learning where and when to forage as a duckling), resource availability, weather conditions, and social factors. These factors can act on genetics and physiology (which can also act on one another) to affect phenotype. In adulthood, the responses to any given environment may be fixed due to genome, physiology, early experience, or the interaction between all three factors during development. Alternatively, these three factors may contribute to an animal's potential for flexibility when exposed to new environmental experiences (social, resource, and weather-related). Understanding variation in adult phenotypes as a consequence of variation in early

phenotypes can help reveal the value of particular developmental experiences on future reproductive success.

In the first chapter, I studied domesticated ducks in a commercial setting. This chapter focused on if different social factors in the early rearing environment affect male Pekin duck reproductive behaviors in a commercial environment. Male ducks that had physical access to ducklings of the opposite sex in the early rearing environment were compared to males that did not have physical access to the opposite sex. There was no difference in rate of mounting behavior between groups, but I did find different rates across behavior sampling time points. Furthermore, I observed interesting individual differences in rates of mounting among drakes. Ultimately, this chapter provided novel information on Pekin drake reproductive behaviors in a commercial setting.

The second chapter focused on the same commercial male Pekin ducks. However, this work had direct implications for management practices intended to be readily utilized by the commercial duck industry. The commercial ducks were from the same genetic strain, so there was likely little genotypic variation. Variation in physiological factors was studied to provide links between early environment and adult reproductive success. Testosterone levels in focal males were determined, and fertility was assessed as a more direct measure of reproductive success. There was no evidence of differences in fertility at the pen level between treatment groups, with fertility overall being rather high and no difference in testosterone levels between treatment groups. However, both fertility and testosterone levels varied across sampling time points. Additionally, I observed individual variation in testosterone secretion between drakes at each sampling point, except before birds were reproductively mature. This chapter linked commercial rearing practice with male duck testosterone levels and fertility to provide commercial producers with direct information on flock reproductive success.

The third chapter pivoted away from a commercial setting and instead focused on wild ducks reared and living in captivity. This chapter contributes to behavioral ecology knowledge regarding how early experience affects long-term fitness outcomes. I made connections with life history theory to provide a foundation that can be used for wildlife management strategies related to Wood Duck populations. I assessed adult Wood Duck stress response and compared these measurements to adult body condition. Specifically, I measured adult hormone concentrations related to environmental challenges and energetic regulation that may be relevant during stressful life stages, such as breeding. Though no relationship was found between stress response and body condition, I did reveal repeatable patterns of individual variation in stress response and differences between series taken during and out of the breeding season (in Summer and Fall 2021, respectively). Adult Wood Duck body condition was measured and compared to duckling body condition through previously collected hatchling body morphometric data, and relationships between growth rates and adult body mass were discovered. I then discussed how early phenotype could potentially relate to fitness outcomes. In all, this chapter filled in gaps in our basic knowledge of how variation in early body type can have long-term effects on adult phenotype and the persistence of intra- and inter-individual variation in stress response. In the future, this knowledge of what contributes to individual fitness can be applied to wildlife conservation strategies utilizing artificial housing systems (e.g., captive breeding programs).

In conclusion, though the studies addressed in my research were different, they both fell under the same central framework that examines how early experiences can shape adult responses in captive duck species. Across both studies, I was able to begin to reveal previously unknown and surprising levels of individual variation. Variation can begin to tell us which traits are important for these ducks in terms of survival and reproduction and what potential evolutionary consequences there may be for differential survival and reproductive success. Together, these chapters laid the groundwork for future longitudinal captive duck studies on sources of individual variation.

CHAPTER 1:

Assessing Pekin drake reproductive behaviors as a consequence of physical access to imprinting hens

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Abstract:

Commercially housed Pekin ducks (*Anas platyrhynchos*) are commonly raised in same sex groups. A few age-matched imprinting hens may be placed in the male pens within the first month after hatch. Early physical contact with hens is assumed to enhance reproductive success in adulthood by promoting successful mating behavior. To evaluate this hypothesis, we reared ducklings in alternating same sex groups (150 hens or 30 drakes/pen; 8 groups/sex). Three females were added to four of the male pens at 12 days of age. Adjacent female and male pens were mixed between 20 and 22 weeks of age. The number of correctly oriented mounts (with cloaca aligned) performed by the focal males in each pen was evaluated over three days (12 hrs/day) at 26, 32, and 45 weeks of age. Resulting count data were analyzed using a Generalized Linear Mixed Model (R 4.0.5). Rearing treatment did not affect male mounting behavior ($P = 0.261$). Correctly oriented mounting behavior was affected by drake age ($P < 0.001$); however, the difference was numerically small (1-2 mounts). A significant effect was not observed for the interaction of treatment and age ($P = 0.056$). We failed to find evidence supporting a positive relationship between physical access to hens during rearing and mounting behavior of mature drakes in this strain of commercial Pekin ducks, suggesting that the practice of providing "imprint hens" may be superfluous, at least for this aspect of reproductive success.

Introduction:

A common practice in breeder Pekin duck commercial settings is to rear ducklings in same sex groups to allow separate feeding regimens. Sometimes, a few female ducklings are mixed with groups of male ducklings in the early rearing environment as a tactic for improving flock reproductive success. Different adult male sexual behaviors may develop between male ducks that had physical access to females in the early environment (as ducklings) and male ducks that did not have physical access to females (Cherry & Morris, 2008). However, the consequences of rearing male ducklings with physical access to females on the development of sexual behavior in Pekin drakes have not been confirmed. We aimed to fill this knowledge gap.

Early social cues can affect how animals behaviorally respond to the opposite sex (Kruijt, 1971; MacFarlane *et al.*, 2007; Rodríguez *et al.*, 2013). Influences from physical exposure to the opposite sex, particularly in the early social environment, can have lasting effects on the expression of social behaviors, including reproductive behaviors (Laviola & Terranova, 1998; Moretz *et al.*, 2007; Naguib *et al.*, 2011). A study on White Leghorn chickens showed that rearing male birds in groups with the opposite sex increased sexual activity. Roosters that had been reared with physical access to female chicks increased the duration and frequency of copulatory (mounting and mating) behaviors toward an adult female compared to males reared without such physical access. However, these differences subsided as the birds aged, with number of mating attempts and successful copulations declining over time for both groups. The decreased copulation frequency was proposed to be due to prolonged exposure to females and increased age (Duncan *et al.*, 1990; Leonard *et al.*, 1993). Therefore, it is possible that male ducklings reared with physical access to female ducklings may have increased average copulatory behaviors in adulthood compared to those that were not reared with physical access. As seen in chickens, average frequency of sexual behaviors may decrease as male ducks age.

This study aimed to determine the effect of social experiences in the early rearing environment (physical or nonphysical access to female ducklings) on the performance of mounting by mature Pekin drakes. We predicted that drakes reared with physical access to female ducklings would have increased average rates of correctly oriented mounts (with cloaca aligned) in adulthood as compared to drakes that were reared in same sex groups. We further expected that sexual activity would be affected by age in the adult social environment, irrespective of early rearing environment. Thus, the average number of mounts per drake would eventually decrease in both treatment groups.

Methods:

Ethical Statement

Procedures performed in this study were approved by the University of California, Davis Animal Care and Use Committee (Protocol #20583).

Animals and Housing

One day old Pekin ducklings (N = 1,440) were placed in a commercial developer barn (Maple Leaf Farms, Inc. West, Tranquility, California, USA). The ducklings were housed in eight two-pen units within the barn. The units were separated from one another by an empty pen and served as treatment replicates. Within each unit, 150 female ducklings were placed into the larger pen (15.24 m x 6.10 m), and 30 male ducklings were placed into the smaller pen (15.24 m x 3.05 m). All birds had visual access to ducklings of the opposite sex through the mesh fence that separated the adjacent pens. At 12 days of age, within four of the units, three female ducklings (hens) were moved from the same sex female pen into the previously all-male pen (mixed sex treatment). Ducklings were not redistributed within the remaining four units (same sex pen treatment). Ten focal male ducklings from each mixed and same sex pen were marked to facilitate later behavioral observations. All ducks were remarked as markings began to fade.

At 20 weeks of age (woa), ducks were moved with their respective units into a breeder barn located on the same property. Males were mixed with females from the same rearing unit into new pens (15.24 m x 11.5 m). One pen of each treatment was moved at 22 woa due to staffing constraints. Barns had windows allowing for natural light on both sides, along with supplemented lighting. Wood shavings were used as bedding and covered the floor, with more shavings added to all pens as needed. Round feeders and nipple drinkers were provided. Nest boxes were provided for nesting and egg laying use. All procedures were carried out according to standard operating procedures and Maple Leaf Farms, Inc. guidelines (Maple Leaf Farms, Inc., 2012). In the breeder barn, four Lorex security cameras were arranged to cover each pen (Lorex Technology Inc., Markham, Ontario, Canada).

Behavioral Analysis

Video was logged by Network Video Recorder (NVR) systems and recorded to a hard drive for later review (Lorex Technology Inc., Markham, Ontario, Canada). All video in the breeder barn was recorded when ducks were 26, 32, and 45 woa with three days at each time point. All observations focused on individually marked males (initially 80 focal males total across eight pens). Across the study period, a few ducks were removed due to injury or mortality, which impacted the number of focal drakes available. At 26 woa, when fertile eggs were first collected, 78 ducks were observed (one duck had been removed per treatment), at 32 woa, during peak egg production, 38 ducks from the same sex treatment and 37 ducks for the mixed sex treatment were observed, and at 45 woa, when production starts to plateau, 36 ducks from the same sex treatment and 35 ducks for the mixed sex treatment were observed. Rates of correctly oriented mounts on females by each focal male were collected from video data for 12 hours per day from 0600 – 1800 for each of the three days at each time point. Correctly oriented mounts were defined as mounts with parallel head to head and tail to tail orientation that lasted for at least one second (Fig. 1.1). This mounting orientation opens the potential for copulation to occur due to the ballistic nature of the male penis. To determine if males mounted females, zoom capabilities on 4K HD footage were utilized. In this strain of Pekin ducks, males had distinctly larger bodies and blockier heads than females, and a curled tail feather similar to the male Mallard ancestor. As multiple observers collected data, observer inter-rater reliability was continuously confirmed at every time point based on two hours of continuous video watching of the same footage with 90% accuracy between all viewers.

Statistical Analysis

At the individual level, a Generalized Linear Mixed Model (GLMM) was used to assess the effect of the rearing treatment (mixed or same sex) on correctly oriented mounts (response variable) in R 4.0.5 (glmmTMB package; Brooks *et al.*, 2017; R Core Team, 2020). The response variable was compared to the same sex and mixed rearing treatments (explanatory variables-fixed effects). In addition to treatment, age (26, 32, or 45 woa) and interactions between both were considered fixed effects. Pen was accounted for as a random effect with individual nested in pen. Backwards selection was used on the model to determine best fit, and the most parsimonious model was selected (Table 1.1). The count data fit a zero-inflated negative binomial model. The criteria for normality of residuals were met based on a Quantile-Quantile plot that included a Kolmogorov-Smirnov Test and dispersion test, along with a within group deviation from uniformity and a Levene Test for homogeneity of variance using the DHARMa package (Hartig, 2022) in R 4.0.5. An analysis of variance (ANOVA) table was created to attain an analysis of deviance. To understand the output of a model with a negative binomial regression, type II Wald chi-square tests were performed. Plots were created using the ggplot2 package (Wickham, 2016).

Results:

Correctly oriented mounting events performed by focal male ducks were not affected by rearing treatment ($\chi^2 = 1.26$, df = 1, $P = 0.261$; Fig. 1.2). However, correctly oriented mounting events were affected by drake age ($\chi^2 = 76.1$, df = 2, $P < 0.001$; Fig. 1.2). Furthermore, correctly oriented mounting events were not affected by an interaction of treatment and age ($\chi^2 = 5.77$, df = 2, $P = 0.056$). Between 26 and 32 woa, mounting events increased for both the same sex and mixed sex treatments (estimated means [95% CI]: 0.522 [0.374, 0.730] mounts/drake/12 hr and 1.61 [1.22, 2.12] mounts/drake/12 hr for same sex, respectively, and 0.893 [0.666, 1.20] mounts/drake/12 hr and 1.73 [1.32, 2.27] mounts/drake/12 hr for mixed sex, respectively). A decrease in mounting events was observed between 32 and 45 woa for both treatment groups (estimated means [95% CI]: 1.00 [0.741, 1.35] mounts/drake/12 hr for same sex and 1.10 [0.824, 1.48] mounts/drake/12 hr for mixed sex). The widest range in variation of individual mounting rates was observed at 32 woa, with 0 - 10 events for individual males with the lowest and highest rates, respectively, within a 12-hour period (Fig. 1.3).

Discussion:

We were interested in evaluating whether providing drakes with physical access to females during rearing would increase correctly oriented mounting rates. There was no difference in occurrences of correctly oriented mounts between ducks from mixed sex and same sex rearing treatments. Early exposure to information provided by cues in the social environment during a critical period of development can lead to a process known as imprinting. Two types of imprinting have been identified: filial and sexual (Bateson, 1966; Bolhuis, 1991; Bolhuis & Bateson, 1990). Sexual imprinting occurs when a bird learns about the social environment and connects the learned information to sexual behavior which is then stabilized (Oetting *et al.*, 1995). For commercial Pekin ducks, the use of imprinting hens during early development is encouraged to "sexually imprint" drakes reared in same sex groups (Cherry & Morris, 2008). However, whether sexual imprinting happens during this phase, which exact cues are crucial for sexual imprinting to occur, and the long-term effects of sexual imprinting in this system are unknown. Our findings suggest that if sexual imprinting affected the development of drake sexual behavior during rearing, it might have been facilitated through cues that include visual, auditory, and/or olfactory, either in conjunction with or instead of haptic. Visual and auditory cues are known to be important factors involved in behavioral development in Pekin ducks (Klopfer & Gottlieb, 1962). It could also be that rearing male ducklings with nonphysical access to female ducklings is adequate and provides cues (auditory, visual, olfactory) required for the development of reproductive behaviors. Characteristics of sexual behaviors may also have been established early on, then continually developed and refined into adulthood. Early research on sexual behavior proposed that copulatory behavior, specifically, is innate (Beach, 1939). However, it is likely that the performance of sexual behaviors is a combination of both innate and learned behaviors. Rodríguez-Manzo and Conseco-Alba (2014) advocate that organizing behaviors into either “innate” or “learned” categories may be inaccurate, and most behaviors are an amalgamation of both (Barnard, 2004; Bolhuis & Giraldeau, 2005; Rodríguez-Manzo & Conseco-Alba, 2013). If learning continued to occur into adulthood for males in this study, ducks from both treatment groups may have refined their mating behaviors after female and male groups were mixed in the breeder barn. Drakes may have gained valuable experience, as the heavily female-skewed sex ratios would have facilitated access to many females.

We observed individual variation in correctly oriented mounting behavior. While one drake properly mounted hens ten times in a single day, other male ducks in the flock made no correctly oriented mounts within the same time frame. Based on personal observations, some drakes did not attempt mounts, while other drakes were prevented from mounting by other ducks. Notable features of a commercial setting that are likely to impact individual duck behavior include that sex ratio is female-skewed and group sizes are very large. Early research observed individual variation in domestic drake mounting behavior by inducing sexual behaviors with testosterone injections in some Rouen ducklings and immature ducks (Balthazart & Stevens, 1975, 1976; Deviche, 1979). Differences in Pekin drake sexual activity have recently been explored in Ouyang *et al.* (2021). This study revealed that metabolic differences might affect gene expression and signaling pathways important for sexual behaviors and other reproductive traits. The group of drakes classified as having higher libidos showed greater testes and epididymis weights but lower body weights (Ouyang *et al.*, 2021). Additionally, variation in mounts could also be attributed to hen receptivity. In Pekin ducks, mounting and fertilization may sometimes be prohibited when the female is not receptive. Brennan *et al.* (2010) studied Muscovy ducks and identified that female genital morphology might work to prohibit forced copulations. Though we could not observe copulation directly, we did observe instances where female ducks that did not assume the receptive posture (reviewed in Brennan, 2019) hindered mounting attempts made by focal drakes. Furthermore, we observed groups of other males and females forcibly prohibiting males from properly mounting, and males continuously spending time mounting other males and females mounting females (personal observation). Research on wild mallards found that mates will often prevent forced copulations from other males (Davis, 2002); we were unable to identify if pairs developed in the commercial setting. Pekin ducks do form pairs under natural conditions (Miller, 1977). Thus, social dynamics and potential partner preferences could have affected which individual drakes were correctly oriented mounting females.

As predicted, the frequency of observed mounting behaviors changed with duck ages in both treatment groups. The number of properly oriented mounts increased between week 26 and 32, then decreased to week 45. A similar pattern was previously reported for White Leghorn chickens (Leonard *et al.*, 1993). The period of increased sexual activity coincided with peak egg production. Seasonal qualities are also likely to contribute to changes in mounting frequencies. Light and changes in temperature are known to help facilitate reproductive activity in Pekin ducks, which are domesticated from the seasonal breeding Mallard (Cherry & Morris, 2008). Though ducks were constantly exposed to supplemental lighting, as is standard practice on breeding duck farms (Cherry & Morris, 2008; Chen *et al.*, 2021), the sides of the barns allowed for exposure to natural light. When exposed to natural light conditions, Pekin ducks show cycles of reproductive activity similar to Mallards (Assenmacher *et al.*, 1975; Jallageas *et al.*, 1978). Thus, we were not able to separate the effects of seasonality and corresponding environmental changes (in temperature, photoperiod, humidity, and other environmental factors) and duck age, as this was outside the scope of our research objectives, and these factors were confounded.

Concluding remarks:

We hypothesized that access to females during early rearing would enhance normal development of male duck mating behavior. Specifically, we predicted that male ducks reared with physical access to females would have increased instances of correctly oriented mounts, which we used as a metric for determining potential reproductive success. However, we found no evidence to support that using imprinting hens affects correctly oriented mounting behavior in this strain of commercial Pekin ducks. Thus, rearing ducks in same sex groups, with a mesh fence in between groups, still allows for the development of correctly oriented mounting behavior. More investigation is needed to reveal which cues are important for the development of sexual behaviors in commercial Pekin ducks. We observed interesting individual differences in occurrences of correctly oriented mounts between drakes. This observed variation in the distribution of mounts by individuals may have impacted the study. More work should be done to examine sources of individual variation in reproductive behaviors that could contribute to differential fitness between drakes. Additionally, our study revealed that instances of correctly oriented mounts differed as ducks aged, though the effects of seasonality should be explored in the commercial environment.

Author Contributions:

MM, BL, LB contributed to the conception of work, study design, interpretation of findings and manuscript revision. Data collection and statistical analyses were carried out by LB.

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References:

Assenmacher, I., Astier, H., Daniel, J. Y., & Jallageas, M. (1975). Experimental studies on the annual cycles of thyroid and adrenocortical functions in relation to the reproductive cycle of drakes. *Journal de Physiologie, 70*(5), 507-552.

Balthazart, J., & Stevens, M. (1975). Effects of testosterone propionate on the social behaviour of groups of male domestic ducklings *Anas platyrhynchos* L. *Animal Behavior, 23*(4), 926–931. https://doi.org/10.1016/0003-3472(75)90116-5

Balthazart, J., & Stevens, M. (1976). Social Behaviour of Testosterone-Injected Male Ducklings in the Presence of Oestrogen-Treated Females. *Behavior, 57*(3), 288–306. https://doi.org/10.1163/156853976x00578

Barnard, C. J. (2004). *Animal Behavior: Mechanisms Development, Function and Evolution.* (p. 726). Pearson Education Ltd, Essex, UK.

Bateson, P. P. G. (1996). The characteristics and context of imprinting. *Biological Reviews, 41*(2), 177–220. https://doi.org/10.1111/j.1469-185x.1966.tb01489.x

Beach, F. A., Jr. (1939). The neural basis of innate behavior. III. Comparison of learning ability and instinctive behavior in the rat. *Journal of Comparative Psychology, 28*(2), 225–262. https://doi.org/10.1037/h0054839

Bolhuis, J. J. (1991). Mechanisms of avian imprinting: a review. *Biological Reviews*, *66*(4), 303–345. https://doi.org/10.1111/j.1469-185x.1991.tb01145.x

Bolhuis, J. J., & Bateson, P. (1990). The importance of being first: a primacy effect in filial imprinting. *Animal Behavior, 40*(3), 472–483. https://doi.org/10.1016/s0003-3472(05)80527-5

Bolhuis, J. J., & Giraldeau, L.-A. (2005). *The Behavior of Animals: Mechanisms, Function, and Evolution.* (p. 515). Blackwell Publishing, Malden, MA, USA.

Brennan, P. L. R. (2019). Bird with penises: Copulation mechanics and behavior. *Encyclopedia of Animal Behavior* (Second Edition, Vol. 4). Elsevier.

Brennan, P.L.R., Clark, C., & Prum, R. (2010). Explosive Eversion and Functional Morphology of Waterfowl Penis Supports Sexual Conflict in Genitalia. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1686), 1309–1314. https://doi.org/10.1098/rspb.2009.2139

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug,H. J., Maechler M., & Bolker, B. M. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal, 9*(2), 378-400. https://doi.org/10.32614/rj-2017-066

Chen, X., Shafer, D., Sifri, M., Lilburn, M., Karcher, D., Cherry, P., Wakenell, P., Fraley, S., Turk, M., & Fraley, G. S. (2021). Centennial review: history and husbandry recommendations for raising Pekin ducks in research or commercial production. *Poultry Science, 100*(8), 101241. https://doi.org/10.1016/j.psj.2021.101241

Cherry, P., & Morris, T. R. (2008). *Domestic Duck Production: Science and Practice.* CABI, Wallingford, Oxfordshire, UK; Cambridge, MA.

Davis, E. S. (2002). Male reproductive tactics in the mal- lard, *Anas platyrhynchos*: social and hormonal mechanisms. *Behavioral Ecology and Sociobiology, 52*(3), 224–231. https://doi.org/10.1007/s00265-002-0513-z

Deviche, P. (1979). Behavioral effects of castration and testosterone propionate replacement combined with ACTH in the male domestic duck (*Anas platyrhynchos* L.). *Journal of Experimental Zoology*, *207*(3), 471-480. https://doi.org/10.1002/jez.1402070315

Duncan, I. J. H, Hocking, P. M., & Seawright, E. (1990). Sexual behaviour and fertility in broiler breeder domestic fowl. *Applied Animal Behaviour Science*, *26*(3), 201-213. https://doi.org/10.1016/0168-1591(90)90137-3

Hartig, F. (2022). DHARMa: Residual Diagnostics for Hierarchical (multi-level/mixed) Regression Models. R Package Version 0.2.2. Available online: https://cran.r-project.org/package=DHARMa. (Accessed on 9 February 2022).

Jallageas, M., Tamisier, A., & Assenmacher, I. (1978). A comparative study of the annual cycles in sexual and thyroid function in male Peking ducks (*Anas platyrhynchos*) and teal (*Anas crecca). General and Comparative Endocrinol*ogy, *36*(2), 201-210. https://doi.org/10.1016/0016-6480(78)90023-0

Klopfer, P. H., & Gottlieb, G. (1962). Imprinting and behavioral polymorphism: Auditory and visual imprinting in domestic ducks (*Anas platyrhynchos*) and the involvement of the critical period. *Journal of Comparative and Physiological Psychology, 55*(1), 126–130. https://doi.org/10.1037/h0045463

Kruijt, J. P. (1971). Early experience and the development of social behavior in Junglefowl. *Psychiatria, Neurologia, Neurochirurgia, 74*(1), 7-20.

Laviola, G., & Terranova. M. L. (1998). The developmental psychobiology of behavioural plasticity in mice: the role of social experiences in the family unit. *Neuroscience & Biobehavioral Reviews, 23*(2), 197-213. https://doi.org/10.1016/s0149-7634(98)00021-9

Leonard, M. L., Zanette, L., & Fairfull, R. W. (1993). Early exposure to females affects interactions between male White Leghorn chickens. *Applied Animal Behaviour Science, 36(1),* 29-38. https://doi.org/10.1016/0168-1591(93)90096-8

MacFarlane, G. R., Blomberg, S. P., Kaplan, G., & Rogers, L. J. (2007). Same-sex sexual behavior in birds: Expression is related to social mating system and state of development at hatching. *Behavioral Ecology*, *18*(1), 21–33. https://doi.org/10.1093/beheco/arl065

Miller, D. B. (1997). Social Displays of Mallard Ducks (*Anas platyrhynchos*): Effects of Domestication. *Journal of Comparative and Physiological and Psychology, 91*(2), 221–232. https://doi.org/10.1037/h0077320

Moretz, J. A., Martins, E. P., & Robison, B, D. (2007). The effects of early and adult social environment on zebrafish (*Danio rerio*) behavior. *Environmental Biology of Fishes. 80*(1), 91-101. https://doi.org/10.1007/s10641-006-9122-4

Naguib, M., Florcke, C., & van Oers, K. (2011). Effects of social conditions during early development on stress response and personality traits in great tits (*Parus major*). *Developmental Psychobiology, 53*(6), 592-600. https://doi.org/10.1002/dev.20533

Oetting, S, Prove, E., & Bischof, H. J. (1995). Sexual Imprinting as a 2-Stage Process - Mechanisms of Information-Storage and Stabilization. *Animal Behaviour*, *50(2)*, 393–403. https://doi.org/http://dx.doi.org/10.1006/anbe.1995.0254

Ouyang, Q., Bao, D., Lu, Y., Hu, J., Hu, B., Lan, C., Hu, S., He, H., Liu, H., Li, L., & Wang, J. A. (2021). A comparative study of libido in drakes: from phenotypes to molecules. *Poultry Science, 10*(12), 101503. https://doi.org/10.1016/j.psj.2021.101503

Rodríguez, R., Rebar, D., & Fowler-Finn, K. D. (2013). The evolution and evolutionary consequences of social plasticity in mate preferences. *Animal Behavior, 85*(5), 1041–1047. https://doi.org/10.1016/j.anbehav.2013.01.006

Rodríguez-Manzo, G., & Canseco-Alba, A. (2014). A Role for Learning and Memory in the Expression of an Innate Behavior: The Case of Copulatory Behavior. The Case of Copulatory Behavior. In *Identification of Neural Markers Accompanying Memory*. (pp. 135–147). Elsevier. https://doi.org/10.1016/B978-0-12-408139-0.00009-2

Team, R. C. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

Tables and Figures:

Figure 1.1 A marked focal Pekin drake performs a correctly oriented mount on top of a hen. The orientation is head to head and tail to tail. High quality zoom capabilities allow for identification of both the drake and hen, with the drake having a blockier head and larger body size compared to the hen being mounted.

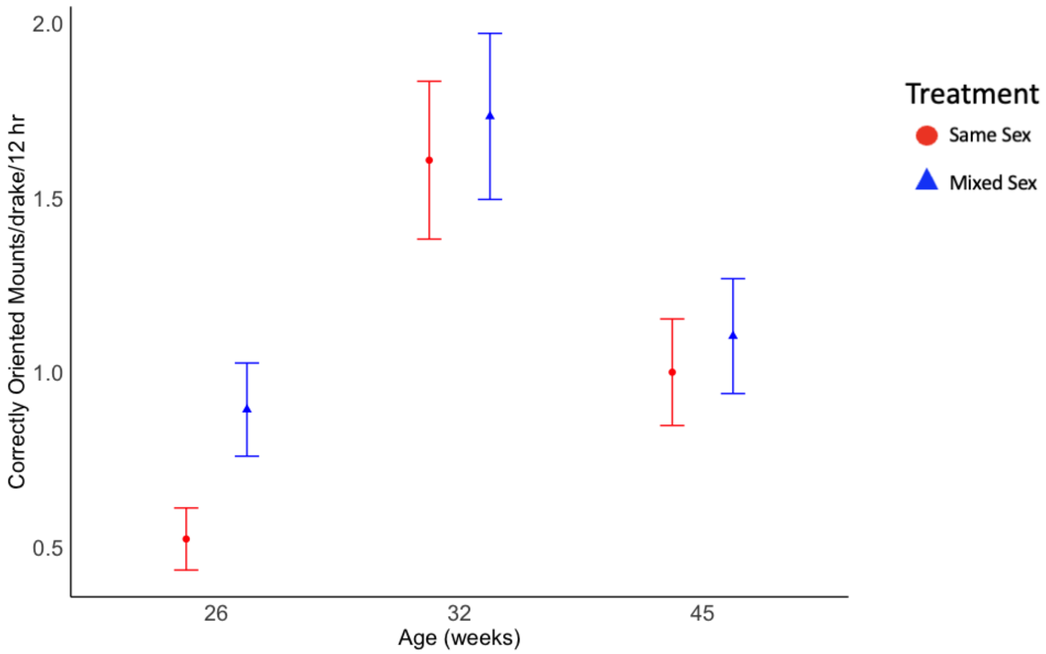

Figure 1.2 Average number of correctly oriented mounts for Pekin drakes over three 12 hour observation periods at 26, 32, and 45 weeks of age for same sex rearing groups and mixed sex rearing groups. Mount rate values are presented as estimated marginal means and standard errors.

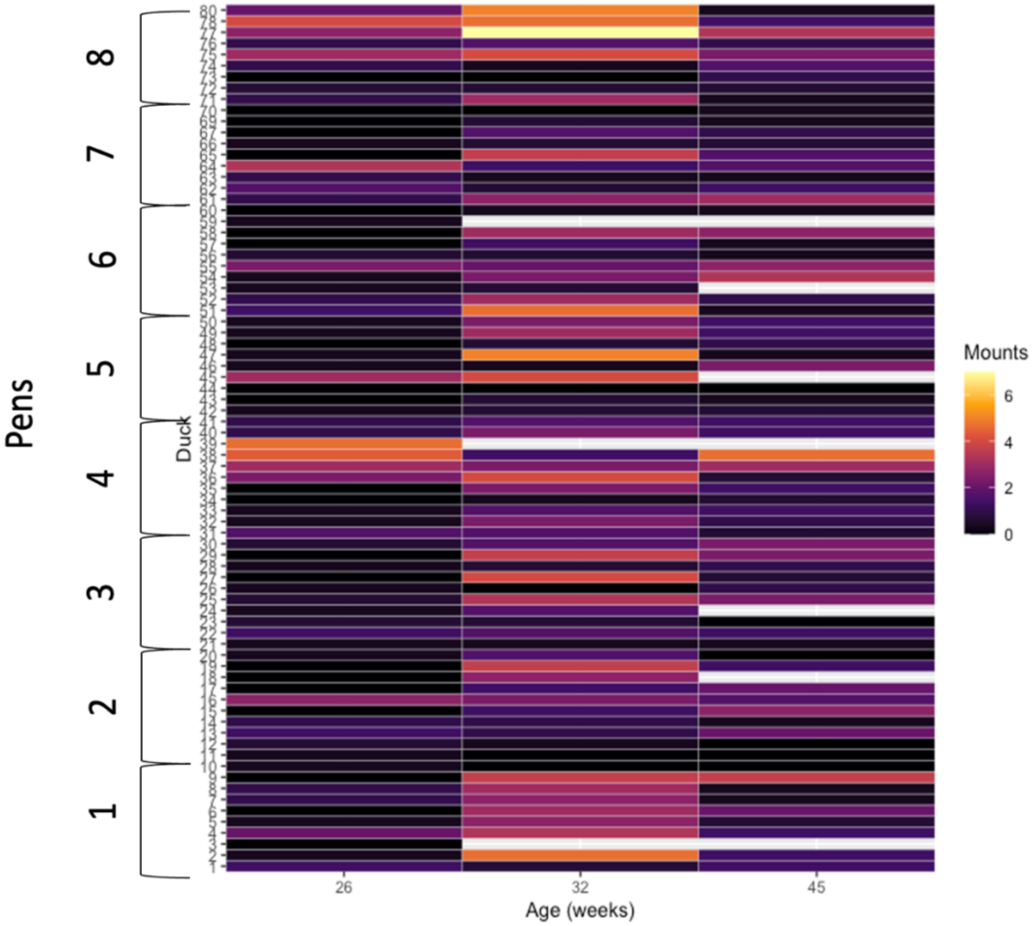

Figure 1.3 Average number of correctly oriented mounts for individual Pekin drakes over three 12 hour observation periods at 16, 32, and 45 weeks of age.

A. Mounting Behavior		
Model	AIC	△AIC
Full model (no dropped terms)	**2043.1**	**0**
No interaction	2044.9	1.8
No treatment	2044.1	1

Table 1.1 Akaike Information Criterion (AIC) values, and Delta AIC (△AIC) listed to display sensitivity to the loss of smoothed terms. Labels identify the dropped term in the model. The bolded label identifies the best fit model. Full model included treatment, age, and an interaction of both.

CHAPTER 2:

Does physical access to Pekin hens during rearing impact drake testosterone levels and pen level fertility?

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Abstract:

Pekin ducks (*Anas platyrhynchos*) housed in commercial breeder settings are often reared in same sex groups to facilitate separate diet provisioning. Some female ducklings may be raised with the male ducklings as a strategy for increasing flock reproductive success. However, the relationship between this management practice and the reproductive success of the ducks has not been confirmed. We assessed the effects of early social experiences (physical or nonphysical access to same age females) on circulating testosterone levels of drakes and egg fertility. Ducks were housed across 16 pens in a single barn located on a duck farm. Pekin ducklings (N=1440) were placed into same sex groups. At 12 days of age, three female ducklings were placed into four of the previously all-male pens. At approximately 20 weeks of age, adjacent male and female pens were combined creating eight mixed-sex pens of 180 ducks each. Circulating testosterone concentrations were analyzed from blood plasma samples collected from focal drakes at five time points. A baseline sample was taken before ducks reached reproductive maturity (15 weeks of age), and additional samples taken when the ducks were 22, 28, 34 and 45 weeks old. Pen-level fertility was determined based on candling of eggs collected at 33-34 and 45-46 weeks of age. Testosterone data were analyzed using a Linear Mixed Model in R 4.0.5, with duck in pen as a random effect. A Linear Mixed Model was also used to analyze fertility data in R, with pen as a random effect. Circulating testosterone levels were not impacted by rearing treatment ($P = 0.307$) or the interaction of treatment and age ($P = 0.083$) but did vary by age ($P < 0.001$). Egg fertility was not impacted by rearing treatment ($P = 0.233$) but did vary with flock age ($P < 0.001$). The results indicate that housing male ducklings and female ducklings in adjacent pens, without physical contact, is sufficient for promoting normal testosterone levels in males and good fertility in this strain of ducks.

Introduction:

In commercial settings for breeder Pekin ducks, females and males are often reared separately to account for differences in early diet provisioning. Some farms rear male ducklings with physical access to female ducklings, also referred to as ‘imprinting’ hens (often 7-10% females in the group; Cherry & Morris, 2008). This management strategy is maintained to encourage duck reproductive success in adulthood. However, the proximate physiological mechanisms that link the early rearing environment with adult duck reproduction are not well understood. Variation in adult drake Pekin duck reproductive hormone levels, primarily testosterone and fertility, may differ between birds that were reared in same sex or mixed sex groups.

A relationship between testosterone levels and the performance of drake sexual behaviors has been well documented in ducks and other seasonally breeding birds (Balthazart, 1976; Balthazart & Hendrick, 1976; Balthazart & Hendrick, 1979; Balthazart, 1983; Harding, 1983; Ball & Balthazart, 2004). This association between testosterone and drake reproductive behaviors has been described by the Challenge Hypothesis (Wingfield *et al.*, 1987; Wingfield *et al.*, 1990). The model describes circulating testosterone and other androgens as varying across three levels: Level A, where homeostatic activity is maintained, Level B, where a seasonal breeding baseline regulated mainly by long-term cues like photoperiod is maintained for secondary sex characters and reproductive activity, and Level C, which is facultative and triggered by stimulation associated with female presence or a male challenger (Wingfield *et al.*, 1990; Goymann *et al.*, 2007). Thus, testosterone may be acting organizationally in the early environment and may also have continuous activational effects. Moreover, early physical exposure to the opposite sex has been suggested to facilitate later display of sexual behaviors and male chickens exposed to the opposite sex engage in courtship behaviors with females differently than males reared in same sex groups (Leonard *et al.*, 1993). Changes in interactions with the opposite sex often affect circulating testosterone levels (Pinxten *et al.*, 2003). Higher testosterone levels are suggested to be closely related to fertility and reproductive success in ducks (Balthazart, 1978; Donham, 1979; Burns *et al.*, 1980; Penfold *et al.*, 2000).

The current study evaluated the assumed relationship between the practice of raising male ducklings with ‘imprinting’ hens and flock fertility and explored a possible underlying proximate mechanism. We predicted that (1) drakes would have higher circulating testosterone levels when raised with imprinting hens than drakes reared in same sex groups, and (2) flock fertility would be higher among flocks that utilized imprinting ducklings during rearing than in flocks reared in same sex groups. If testosterone concentrations facilitate increased fertility, we predicted a positive relationship between drake testosterone levels and flock fertility would be observed across all ducks.

Methods:

Ethical Statement

All procedures were approved by the University of California, Davis Animal Care and Use Committee (Protocol #20583).

Animals and Housing

The study was conducted at a breeder duck farm in central California. Day old ducklings (N=1,440) were placed, by sex, into 16 pens located within a single developer barn. In the barn, ducklings had access to light through windows, in addition to supplemental light. Ducklings were raised on wood shavings, which were topped off in the entire barn when needed. The birds had access to nipple drinkers and round feeders. All management protocols, including brooding protocol, diet composition, and temperature settings, followed company standard operating procedures. The pens, which spanned the width of the barn, were organized into eight groups of two: a pen with 30 male ducklings adjacent to a pen with 150 female ducklings. A walkway separated each set of adjacent male duckling and female duckling pens from the next. At 12 days of age, three female ducklings were added to every other male pen from the adjacent pen. Thus, male ducklings in four pens were raised with direct physical contact with female ducklings, and male ducklings in the other four pens had only visual, auditory, and olfactory exposure to female ducklings through the mesh fence. Six sets of adjacent female and male pens were mixed and moved into the breeder barn at 20 weeks of age (woa), with adjacent female and male pens being combined. The female and male groups from the remaining two sets of pens (one per treatment) were combined and moved to the breeder house at 22 woa due to farm staffing constraints. In total, the breeder house contained four pens of mixed sex ducks representing each of the two rearing treatments. To account for effects of location, pens containing each treatment were alternated within the barn, as was the case in the developer barn.

Blood Sampling & Hormone Analysis

Blood samples were collected from the same individually marked drakes between 0900 and 1100 hours at each of the five time points. A baseline sample was collected from 10 drakes per pen (N=80 drakes total across 8 pens) at 15 woa, before reproductive maturity. Additional samples were taken shortly after reproductive maturity was reached (22 woa), when collection of fertile eggs for incubation and hatching commenced at the farm (28 woa), near peak production

(34 woa), and after egg production had plateaued (45 woa). As part of standard management procedures, some ducks were removed from the flock due to injury or mortality, which impacted the number of focal birds available at each time point. A total of 78 focal ducks (39 from each treatment) were sampled at 22 and 28 woa. At 34 woa, 38 and 37 ducks were sampled from the same sex and mixed sex rearing treatment groups, respectively. The final blood collection included 71 ducks after an additional two focal ducks were removed from each treatment group.

At each time point, we drew 2 mL blood from the metatarsal vein using 21-G needles and 3cc syringes. Blood was immediately stored in EDTA vacutainers on ice in a cooler for transportation to University of California, Davis, where the samples were spun for 15 minutes at 1500 RPM. Plasma was siphoned off into duplicate cryovials and stored at -20°C until analyzed by competitive ELISA (Cayman Chemical, Ann Arbor, USA). The ELISA kit had been previously used for serum testosterone levels in the same genetic strain of Pekin duck (Van Wyk and Fraley, 2021). Following Love and Williams (2008) and Steenweg (2020), a modified protocol was utilized for validation. The protocol from the kit was followed, except plates were incubated at 26°C while shaking at 500 rpm. Samples were run in duplicate on a 96-well plate following a serial dilution protocol that replicated the standard curve dilutions of the buffer stock solution (1:8 for plasma samples taken before reproductive maturity and 1:64 for breeding age plasma samples). Each plate included a standard curve and duplicates of a control pool sample of male Pekin duck plasma. Absorbance was read at 405 nm, and the readings were analyzed using a Cayman-provided excel worksheet. The inter-assay coefficient of variation across all plates calculated based on pooled plasma was 3.36%. The intra-assay coefficient was 8.18%.

Egg Production Data

Egg production records were obtained from the Maple Leaf Farms, Inc. Hatchery (San Joaquin Valley, California). Eggs were considered fertile when containing an embryo as determined by candling. To determine impacts of age on fertility and facilitate comparisons with plasma testosterone levels, fertility data were averaged by pen from the weeks of 33-34 woa (January 2019) and 45-46 woa (April 2019).

Statistical Analysis

To assess the effect of the treatment (same or mixed sex rearing) on drake testosterone levels and pen-level fertility, Linear Mixed Models (LMM) were used (LMER in lme4 package by Bates *et al.*, 2015) in R 4.0.5 (R Core Team, 2020). Testosterone levels and fertility percentages were log-transformed for normality, which was assessed based on evaluation Q-Q and residual vs. fitted values plots and homoscedasticity of the independent variables. Thus, for the first model, log-transformed testosterone levels were the response variable. For the second model, log-transformed averaged fertility percentages at 33-34 woa and 45-46 woa were the response variable. For both models, treatment and age were both considered fixed effects (explanatory variables). For the model analyzing testosterone levels, an interaction between treatment and age was also included as a fixed effect. Individual focal drake nested in pen (model 1) or pen (model 2) were considered as random effects. Backward stepwise selection using ANOVA allowed for model comparison. A *P*-value of < 0.05 was considered statistically significant. Testosterone levels and fertility percentages were back-transformed for reporting means and standard errors and for use in figures. We relied on nonparametric statistics by utilizing a Spearman’s rank correlation coefficient (cor.test() function in R) to determine the relationship between testosterone levels and fertility, averaged at the pen level. We produced all plots using the ggplot2 package (Wickham, 2016).

Results:

Hormone Data

As shown in Figure 2.1, testosterone levels were affected by age ($\chi^2 = 4179$, df = 4, $P < 0.001$), but not treatment ($\chi^2 = 1.04$, df = 1, $P = 0.307$) or the interaction between treatment and age ($\chi^2 = 8.25$, df = 4, $P = 0.083$). As *P-value* for age was the same across top models, the results presented for treatment and interaction are from the second top model but not the most parsimonious model, which only had age (Table 2.1A). A distinct increase was observed between model-estimated marginal mean concentrations at 15 woa (before sexual maturity; estimated means ± SE: 0.186 ± 0.014 ng/mL and 0.166 ± 0.013 for the same and mixed sex rearing groups) and 22 woa (estimated means: 3.270 ± 0.256 ng/mL same sex rearing and 3.277 ± 0.256 ng/mL mixed sex rearing treatment). A numerical increase in mean testosterone concentrations was also observed between 22 and 28 woa for both treatment groups. Between 28, 34, and 45 woa, levels slightly increased then decreased for the same sex treatment (estimated means: 7.207 ± 0.564 ng/mL, 7.746 ± 0.613 ng/mL, and 7.267 ± 0.590 ng/mL, respectively) and slightly decreased for the mixed sex treatment (estimated means: 9.615 ± 0.752 ng/mL, 8.243 ± 0.661 ng/mL, and 7.921 ± 0.651 ng/mL, respectively). Pronounced individual variation was observed in circulating testosterone levels (ex. 1.31 ng/ml - 16.83 ng/ml at 34 woa; Figure 2.1), except at 15 woa.

Fertility Data

The percentage of fertile eggs was similar in the two treatments ($\chi^2 = 1.42$, df = 1, $P = 0.233$) but varied with age ($\chi^2 = 23.5$, df = 1, $P < 0.001$). The most parsimonious model only included age, so the values for treatment are reported from the second top model (Table 2.1B). Figure 2.2 describes fertility percentages across two time points determined for both treatment groups (estimated means ± SE: 71.6 ± 2.62 % for average fertility from 33-34 woa for the same sex treatment and 75.7 ± 2.77 % for the mixed sex treatment; 82.0 ± 3.00 % for average fertility from 45-46 woa for the same sex treatment and 86.7 ± 3.17 % for the mixed sex treatment). Spearman's rank correlation test was used to evaluate the relationship between fertility data collected from pens and drake testosterone levels averaged by pen and found no correlation between the two variables ($P = 0.223$).

Discussion:

We aimed to determine whether direct physical access to female ducklings of the same age during rearing influenced male Pekin duck circulating testosterone levels and flock fertility in adulthood. Both variables were affected by age, but not rearing treatment. Other sensory cues from female ducklings experienced in the early rearing environment may have been sufficient to promote development of the drake's physiological responses to hens later in life. Birds are known to rely on visual and auditory cues during social interactions (Ball & Balthazart, 2004), and all ducks, regardless of treatment, could see, hear, and smell ducks in adjacent pens. Additionally, the stimulation male ducks experienced after they were mixed with hens may have been sufficient to incite testosterone production, particularly under commercial housing conditions, where the sex ratio is heavily female skewed, increasing the likelihood of interactions among hens and drakes. Hen social cues have been shown to influence endocrine physiology in

Mallard drakes; testosterone levels are higher in drake groups that experience incitement displays by hens than groups that do not (Klint *et al.*, 1989).

We observed individual variation in circulating testosterone levels across multiple ages. Across free-living bird species, individual variation in circulating testosterone is affected by differences in both individual quality and environment (Kempenears *et al.*, 2008). In Mallards, drake testosterone levels under conditions with equal sex ratios did not differ between paired and unpaired drakes in the spring (during breeding) and fall, but paired drakes experienced a temporary decrease in testosterone levels after initial pair formation in the winter. When populations were male-skewed, paired drakes had higher testosterone levels than unpaired drakes during the breeding season (Davis, 2002). In our study, with female-skewed sex ratios, it is unknown whether focal drake ducks paired up, making it difficult to evaluate whether pair formation could have contributed to the pronounced individual variation in testosterone levels. Social experiences prior to sampling may also have influenced testosterone levels. In sheathbills, testosterone levels were found higher among males that had just performed mutual pair displays than other males, and testosterone was highest in the male that had been observed copulating within 10 minutes of sampling (Burger & Millar, 1980). Finally, although blood was quickly collected from focal individuals in each pen during our study, ducks in other pens may have been disturbed, which may have impacted their circulating testosterone levels. However, this was not likely to have a major influence, as the first pen sampled did not have ducks with the lowest average testosterone levels (data not reported). Thus, individual variation in Pekin drake testosterone levels observed in this study may be explained by a myriad of factors. Other studies have also noted individual variation in testosterone levels of sexually mature Pekin drakes. While direct comparison of data is not possible due to differences in study design and data collection protocols, reported average testosterone levels seem to be within a similar range to those reported in our study where ducks always had supplemental lighting (e.g., approximately 9 ng/ml in the winter with augmented light and approximately 18 ng/ml for ducks in the summer; Figure 4B in Porter *et al.*, 2018).

Differences in focal drake testosterone levels identified between ages followed a pattern that is predictably related to biologically relevant time points in the life cycle. Plasma samples taken before reproductive maturity at 15 woa were used as a baseline for comparison and were low, as expected. Samples taken at 22 woa, after all male duckling groups were mixed with female duckling groups, showed an increase in group testosterone levels. Highest average testosterone levels were observed at 28 woa, shortly after puberty (normally at around 26 woa). At 34 woa, after duck fertility peaked (which usually occurs by 32 woa), high testosterone levels were also reported (Cherry & Morris, 2008; Potter *et al.*, 2018), with a slight increase for the same sex treatment. When drakes were 45 woa, around when reproductive activity in commercial Pekin ducks plateaus (Cherry & Morris, 2008), testosterone levels decreased. During the current study, in addition to supplemental light, ducks were exposed to changes in natural day length at each sampling time point. Natural day length ranged from approximately 9 h 39 min for the shortest day length in December 2018, at 28 woa, to 13 h 15 min for the longest day length in April 2019, at 45 woa (NOAA/ESRL Global Monitoring Laboratory). Testosterone levels in ducks have previously been associated with diel and annual patterns. An increase in day length stimulates activation of the hypothalamic-pituitary-gonad (HPG) axis in Mallards, the wild ancestor of Pekin ducks and known seasonal breeders (Cherry & Morris, 2005; Porter *et al.*, 2018). The length of the natural photoperiod and light intensity have been shown to impact circulating testosterone levels in commercial Pekin drakes. Porter *et al.* (2018) supplemented lighting during the winter and measured fluctuations in Pekin drake testosterone levels. Ducks exposed to augmented lighting in winter and lighting in summer had higher testosterone levels than ducks exposed to regular winter lighting. When domestic Rouen ducks were placed under entirely natural conditions, drake testosterone levels fluctuated with seasonal variations influenced by both environmental and social factors, as predicted by the Challenge Hypothesis. The ducks exhibited peak testosterone levels in October, after the refractory period, in December, when drakes increase social displays, and in March-April, when the breeding season began (Balthazart & Hendrick, 1979). An earlier laboratory study (Garnier, 1971) revealed instances where Pekin drakes displayed slight peaks in testosterone levels in September-October and December-January, with the most pronounced elevation in March and April (Balthazart & Hendrick, 1979; Wingfield & Farmer, 1997). Similar findings were reported by Assenmacher *et al.* (1975), who also noted a pronounced peak in plasma testosterone levels starting in March in Pekin drakes housed under natural lighting conditions outdoors. Thus, entrained endogenous rhythms and natural environmental cues likely factor into Pekin duck breeding activity, in addition to the supplemental cues provided in the commercial setting. Investigating breeder housing light supplementation across all seasons in different commercial locations will reveal important information on how testosterone levels change in relation to multiple environmental and endogenous factors.

Early access to physical contact with female ducklings did not impact flock fertility. Furthermore, there was no correlation between flock level fertility percentages and drake testosterone levels. Although drake testosterone levels can influence drake reproductive success, post-copulatory female choice in Pekin hens may greatly dictate which drakes fertilize eggs and the overall fertility levels (Denk *et al.*, 2005). In captive Mallards, offspring were sired less often when forced copulations with drakes that were not paired occurred (Burns *et al.*, 1980; reviewed in Brennan & Prum, 2012). To our knowledge, the impacts of hen choice on flock fertility have not been examined in commercial settings. Such studies should be explored, as the female-skewed sex ratios in this setting are particularly unique, and findings may yield novel basic and applied information related to flock reproductive success.

Flock level fertility percentages varied between the two biologically relevant ages studied. As previously discussed, both age and seasonality are known to impact drake reproduction; thus, these factors may help explain the observed variation in fertility. Research on Muscovy ducks identified that drake and hen age could affect reproductive characteristics (Yakubu, 2013; Abd El-Hack *et al.*, 2019). Furthermore, female duck age is known to influence fertility more than male duck age (Brommer & Rattiste, 2008; Abd El-Hack *et al.*, 2019). Egg composition has been correlated with hen age. For example, yolk sac weight increased between Pekin duck hens that were 26 and 31 woa and hens that were 36 woa (Applegate *et al.*, 1998; Abd El-Hack *et al.*, 2019). Since season was confounded with age, we cannot speculate as to whether differences in seasonality affected flock fertility, which was averaged at 33-34 woa (January 2019) when day length was approximately 10 hours and 45-46 woa (April 2019) when day length was approximately 13 hours (day length information from NOAA/ESRL Global Monitoring Laboratory). Fertility in poultry can be affected by a multitude of seasonal factors, including but not limited to variation in humidity, rainfall, temperature, and wind speed (Buhr, 1995; Farghly *et al.*, 2018; Abd El-Hack *et al.*, 2019).

Based on the information gained from this study, we conclude that rearing male ducklings and female ducklings in adjacent pens is sufficient for flock reproductive success. Utilizing imprinting hens did not yield increased testosterone levels or flock fertility. Future research should consider other factors experienced during early rearing that may influence reproductive success in commercial ducks, including the influence of auditory and visual cues, possible interactions between duck age, light schedules and/or seasonality, and the impacts of hen choice. Revealing the implications of the observed individual variation in testosterone levels is another interesting direction for future research. The source of the variation is not clear; neither are the consequences for individual reproductive success of these birds.

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References:

Abd El-Hack, M. E., Hurtado, C. B., Toro, D. M., Alagawany, M., Abdelfattah, E. M., & Elnesr, S. S. (2019). Fertility and hatchability in duck eggs. *World's Poultry Science Journal, 75*(4), 599-608. https://doi.org/10.1017/s0043933919000060

Applegate, T. J., Harper, D., & Lilburn, M. S. (1998). Effect of hen production age on egg composition and embryo development in commercial Pekin ducks. *Poultry Science, 77*(11), 1608-1612. https://doi.org/10.1093/ps/77.11.1608

Assenmacher, I., Astier, H., Daniel, J. Y., & Jallageas, M. (1975). Experimental studies on the annual cycles of thyroid and adrenocortical functions in relation to the reproductive cycle of drakes. *Journal de Physiologie, 70*(5), 507-552.

Ball, G. F., & Balthazart, J. (2004). Hormonal regulation of brain circuits mediating male sexual behavior in birds. *Physiology & Behavior, 83*(2), 329–346. https://doi.org/10.1016/j.physbeh.2004.08.020

Balthazart, J. (1976). Daily variations of behavioural activities and of plasma testosterone levels in the domestic duck *Anas platyrhynchos*. *Journal of Zoology, 180*(2), 155–173. https://doi.org/10.1111/j.1469-7998.1976.tb04670.x

Balthazart, J. (1978). Behavioural and physiological effects of testosterone propionate and cyproterone acetate in immature male domestic ducks, *Anas platyrhynchos*. *Zeitschrift für Tierpsychologie, 47*(4), 410–421. https://doi.org/10.1111/j.1439-0310.1978.tb01845.x

Balthazart, J. (1983). Hormonal correlates of behavior. (pp. 221-365). In *Avian Biology*. National Academic Press, Washington, DC.

Balthazart, J., & Hendrick, J. (1976). Annual variation in reproductive behavior, testosterone, and plasma FSH levels in the Rouen duck, *Anas platyrhynchos*. *General and Comparative Endocrinology, 28*(2), 171-183. https://doi.org/10.1016/0016-6480(76)90169-6

Balthazart, J., & Hendrick, J. (1979). Relationships between the Daily Variations of Social Behavior and of Plasma FSH, LH and Testosterone Levels in the Domestic Duck *Anas platyrhynchos* L. *Behavioural Processes, 4*(2), 107–128. https://doi.org/10.1016/0376-6357(79)90027-5

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *arXiv preprint arXiv:1406.5823*.

Brennan, P. L. R., & Prum, R. O. (2012). The limits of sexual conflict in the narrow sense: New insights from waterfowl biology. *Philosophical Transactions of the Royal Society B: Biological Sciences, 367*(1600), 2324–2338. https://doi.org/10.1098/rstb.2011.0284

Brommer, J. E. & Rattiste, K. (2008). “Hidden” reproductive conflict between mates in a wild bird population. *Evolution; International Journal of Organic Evolution, 62*(9), 2326-2333. https://doi.org/10.1111/j.1558-5646.2008.00451.x

Buhr, R. J. (1995). Incubation relative humidity effects on allantoic fluid volume and hatchability. *Poultry Science, 74*(5), 874-884. https://doi.org/10.3382/ps.0740874

Burger, A. E., & Millar, R. P. (1980). Seasonal changes of sexual and territorial behaviour and plasma testosterone levels in male lesser sheathbills (*Chionis minor*). *Zeitschrift für Tierpsychologie, 52*(4), 397–406. https://doi.org/10.1111/j.1439-0310.1980.tb00726.x

Burns, J. T., Cheng, K. M., & McKinney, F. (1980). Forced Copulation in Captive Mallards. I. Fertilization of Eggs. *The Auk, 97*(4), 875-879.

Cherry, P., & Morris, T. R. (2005). The maintenance requirement of domestic drakes. *British Poultry Science, 46*(6), 725–727. https://doi.org/10.1080/00071660500392563

Cherry, P., & Morris, T. R. (2008). *Domestic Duck Production: Science and Practice.* CABI, Wallingford, Oxfordshire, UK; Cambridge, MA.

Davis, E. S. (2002). Female choice and the benefits of mate guarding by male mallards. *Animal Behaviour, 64*(4), 619–628. https://doi.org/10.1006/anbe.2002.3079

Denk, A. G., Holzmann, A., Peters, A., Vermeirssen, E. L. M., & Kempenaers, B. (2005). Paternity in mallards: Effects of sperm quality and female sperm selection for inbreeding avoidance. *Behavioral Ecology, 16*(5), 825–833. https://doi.org/10.1093/beheco/ari065

Donham, R. S. (1979). Annual Cycle of Plasma Luteinizing Hormone and Sex Hormones in Male and Female Mallards (*Anas platyrhynchos*). *Biology of Reproduction, 21*(5), 1273-1285. https://doi.org/10.1095/biolreprod21.5.1273

Farghly, M. F. A., Abd El-Hack, M. E., Alagawany, M., Saadeldin, I. M., & Swelum, A. A. (2018). Wet feed and cold water as heat stress modulators in growing Muscovy ducklings. *Poultry Science, 97*(5), 1588-1594. https://doi.org/10.3382/ps/pey006

Garnier, D. H. (1971). Variations de la testosterone du plasma peripherique chez le canard Pekin au tours du cycle annuel. *Comptes Rendus de l'Académie des Sciences (Paris), 272,* 1665-1668.

Goymann, W., Landys, M. M., & Wingfield, J. C. (2007). Distinguishing seasonal androgen responses from male – male androgen responsiveness — Revisiting the Challenge Hypothesis. *Hormones and behavior, 51*(4), 463–476. https://doi.org/10.1016/j.yhbeh.2007.01.007

Harding, C. (1983). Hormonal influences on avian aggressive behavior. (pp. 435-476). In *Hormones and Aggressive Behavior.* B.B. Svare, ed. New York: Plenum.

Kempenaers, B., Peters, A., & Foerster, K. (2008). Sources of individual variation in plasma testosterone levels. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1497), 1711–1723. https://doi.org/10.1098/rstb.2007.0001

Klint, T., Edsman, L., Holmberg, K., & Silverin, B. (1989). Hormonal correlates of male attractiveness during mate selection in the mallard duck (*Anas platyrhynchos*). *Hormones and behavior, 23*(1), 83-91. https://doi.org/10.1016/0018-506x(89)90076-7

Leonard, M. L., Zanette, L., & Fairfull, R. W. (1993). Early exposure to females affects interactions between male White Leghorn chickens. *Applied Animal Behaviour Science, 36*(1), 29-38. https://doi.org/10.1016/0168-1591(93)90096-8

Love, O. P., & Williams, T. D. (2008). Plasticity in the adrenocortical response of a free-living vertebrate: The role of pre- and post-natal developmental stress. *Hormones and behavior, 54*(4), 496–505. https://doi.org/10.1016/j.yhbeh.2008.01.006

NOAA/ESRL Global Monitoring Laboratory. Accessed April 2022. https://gml.noaa.gov/grad/solcalc/

Penfold, L. M., Wildt, D. E., Herzog, T. L., Lynch, W., Ware, L. B., Derrickson, S. E., & Monfort, S. (2000). Seasonal patterns of LH, testosterone and semen quality in the Northern pintail duck (*Anas acuta*). *Reproduction, Fertility and Development, 12*(4), 229-235. https://doi.org/10.1071/rd00093

Pinxten, R., De Ridder, E., & Eens, M. (2003). Female presence affects male behavior and testosterone levels in the European starling (*Sturnus vulgaris*). *Hormones and behavior, 44*(2), 103–109. https://doi.org/10.1016/s0018-506x(03)00120-x

Porter, L., Porter, A., Potter, H., Alenciks, E., Fraley, S. M., & Fraley, G. S. (2018). Low light intensity in Pekin duck breeder barns has a greater impact on the fertility of drakes than hens. *Poultry Science, 97*(), 262–4271. https://doi.org/10.3382/ps/pey289

Potter, H., Alenciks, E., Frazier, K., Porter, A., & Fraley, G. S. (2018). Immunolesion of melanopsin neurons causes gonadal regression in Pekin drakes (*Anas platyrhynchos domesticus*). *General and Comparative Endocrinology, 256*, 16-22. https://doi.org/10.1016/j.ygcen.2017.08.006

Steenweg, R. J. (2020). Carryover Effects of Winter and Pre-breeding Conditions on Reproduction in Northern Common Eiders *Somateria mollissima borealis* Nesting in Arctic Canada. Dissertation, Dalhousie University Halifax, Nova Scotia.

Team, R. C. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Van Wyk, B., & Fraley, G. (2021). Ontogeny of opn4, opn5, gnrh and gnih mrna expression in the posthatch male and female pekin duck (*Anas platyrhynchos domesticus*) suggests opn4 may have additional functions beyond reproduction. *Animals, 11*(4), 1121. https://doi.org/10.3390/ani11041121

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

Wingfield, J. C., Ball, G. F., Dufty, A. M., Jr, Hegner, R. E., & Ramenofsky, M. (1987). Testosterone and aggression in birds. *American Scientist, 75*(6), 602—608.

Wingfield, J. C., & Farner, D. S. (1978). The Endocrinology of a Natural Breeding Population of the White-Crowned Sparrow (*Zonotrichia leucophrys pugetensis*). *Physiological Zoology, 51*(2), 188–205. https://doi.org/10.1086/physzool.51.2.30157866

Wingfield, J. C., Hegner, R. E., Dufty, A. M., & Ball, G. F. (1990). The ‘Challenge Hypothesis’: Theoretical Implications for Patterns of Testosterone Secretion, Mating Systems, and Breeding Strategies. *The American Naturalist, 136*(6), 829–846. https://doi.org/10.1086/285134

Yakubu, A. (2013). Characterization of the local Muscovy duck in Nigeria and its potential for egg and meat production. *World's Poultry Science Journal, 69*(4), 931-938. https://doi.org/10.1017/s0043933913000937

Tables and Figures:

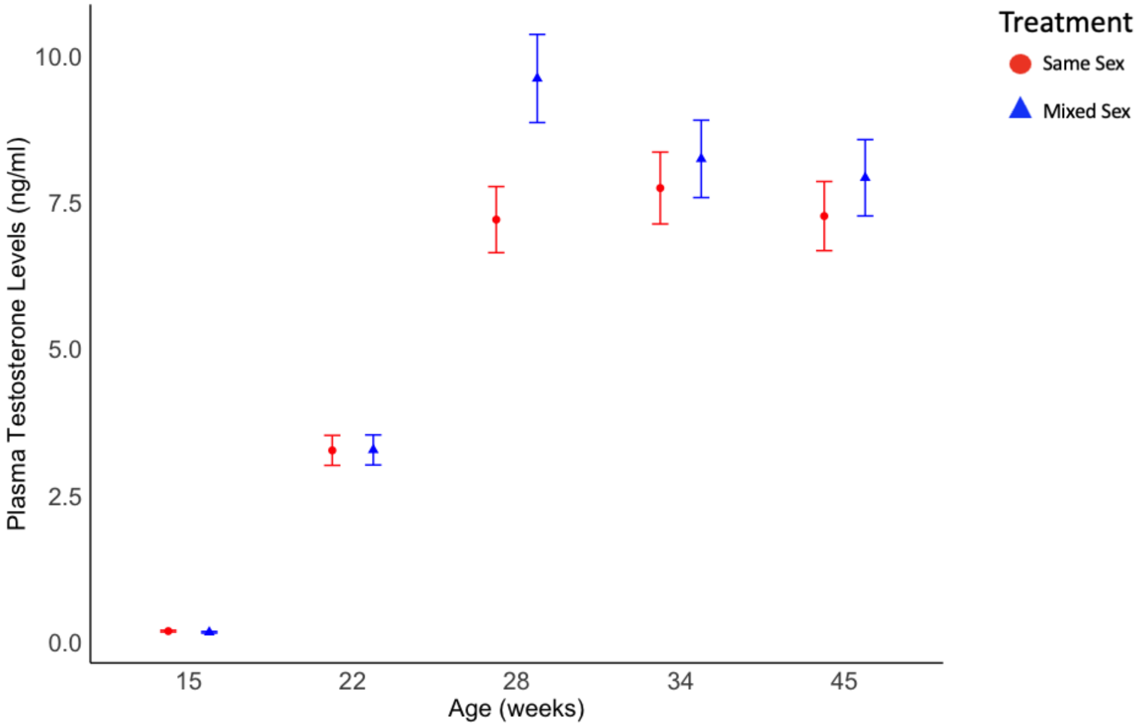

Figure 2.1 Circulating testosterone levels in drake Pekin ducks from same sex rearing groups and mixed sex rearing groups from 15 to 45 weeks of age. Testosterone concentration values are back-transformed from a logarithmic transformation and presented as estimated marginal means and standard errors.

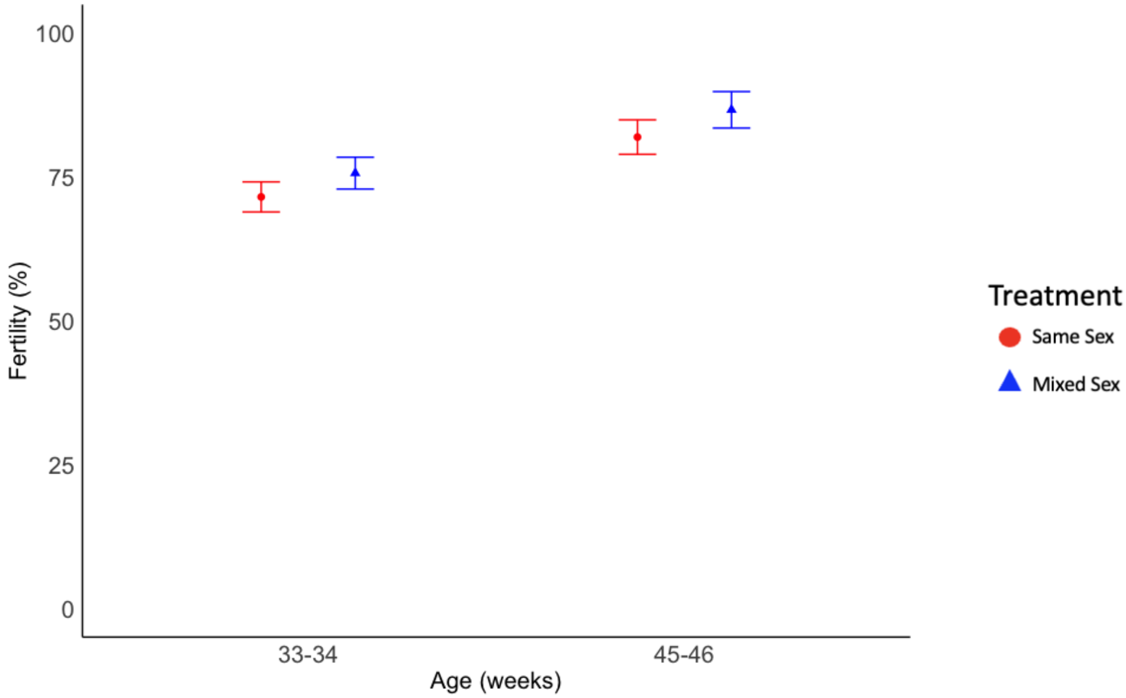

Figure 2.2 Average fertility percentages calculated based on pen-level data collected when ducks were 33 and 34, and 45 and 46 week of age. Average flock fertility differed by ages ($P < 0.001$) but not treatment. Fertility values are back-transformed from a logarithmic transformation and presented as estimated marginal means and standard errors. Fertility was determined based on candling of eggs.

A. Testosterone		
Model	AIC	△AIC
Full model (no dropped terms)	517.4	0.4
No interaction	517.8	0.8
No treatment	**517**	**0**
B. Fertility		
Model	AIC	△AIC
Full model (no dropped terms)	-29.2	1.9
No interaction	-30.8	0.3
No treatment	**-31.1**	**0**

Table 2.1 Akaike Information Criterion (AIC) values, and Delta AIC (△AIC) listed to display sensitivity to the loss of smoothed terms. Labels identify the dropped term in each model version (A is testosterone (ng/ml) as the response variable and B is fertility (% of fertile eggs) as the response variable). The bolded label identifies the best fit model. To determine estimate marginal means between treatment groups we used the full testosterone model, and for the fertility model, the version with no interaction was used because treatment was still not significant and *P* value for age remained at $P < 0.001$. Full models include treatment, age, and an interaction of both.

CHAPTER 3:

Evaluating variation in acute stress response in captive Wood Ducks (*Aix sponsa*)

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Abstract:

Acute stress response has often been linked to individual morphological state and associated with the allocation of finite energy resources in wild living bird populations. However, for many species, it is not clear whether this holds true under captive settings, where energy resources are abundant and readily available. We evaluated how individual variation in condition, measured as body mass, influences adult acute stress response for 28 captive Wood Ducks (*Aix sponsa*) fed *ad-libitum*, in and out of the breeding season. We predicted that a more attenuated stress response would be inversely correlated with mass as a measure of body condition. Additionally, we assessed whether early measurements of body condition would relate to current mass under conditions where food is abundant. In 2021, we collected blood from adult ducks within 3 minutes of capture and at 10, 30, and 60 minutes to analyze for plasma corticosterone (CORT) both in and out of breeding season and recorded body morphometric data once for adults. Total and maximum CORT secreted were analyzed in Generalized Linear Mixed Models (GLMM). No relationships between age, body mass, and total or maximum CORT were found. There was a significant difference in response in and out of the breeding season for total CORT ($P < 0.001$) and maximum CORT ($P < 0.001$), with a higher response during the breeding season. Repeatability in individual responses between seasons for total ($P < 0.001$) and maximum CORT ($P < 0.001$) were found. Baseline CORT levels were consistently low, with 53.3% and 78.6% of the samples below the detection limit during and outside the breeding season, respectively. A regression revealed a correlation between adult body mass and early maximum growth rates ($P = 0.002$), as well as early tarsus growth rates ($P = 0.03$). Our results highlight possible seasonal variation in captive Wood Duck acute stress response, though more research across multiple seasons is warranted. This study also reveals a relationship between

early and adult morphological traits and suggests the need for further investigation of underlying factors affecting phenotypic traits that may be important for coping with changes across life stages.

Introduction:

Energetically demanding life history stages (i.e., reproduction, migration) occur throughout an animal's life (Hennin *et al.,* 2016; Landys *et al.*, 2007). Animals may face energetic trade-offs during these stages, with variation in individual behavioral, morphological, and physiological state influencing success of response and potential long term fitness consequences (McNamara & Houston, 1996). For instance, individuals in better condition often manage stressful situations with greater success than those in poorer condition (Hennin *et al.*, 2016; Kisdi *et al.*, 1998; McNamara & Houston, 1996; Rowe *et al.*, 1994; Stearns, 1992). Individuals can encounter a variety of factors (e.g., maternal effects, resource availability, social factors, weather conditions) starting early on and continuing throughout life that interact with genetics and physiology to help develop differences in quality of state (Afton & Paulus 1992; Butler & McGraw, 2010; Hayward & Wingfield, 2004; Hepp *et al.*, 1987; Mousseau & Fox, 1998; Muller *et al.*, 2009; Sedinger *et al.*, 2018; Stamps & Groothuis, 2010).

The silver spoon hypothesis has been used to explain how early experiences can have lasting consequences on phenotypic quality. According to this hypothesis, an individual who experienced good conditions early on would be of increased quality, and an individual who experienced poor early conditions would be of reduced quality later in life (Minias *et al.*, 2015; Monaghan, 2008; Song *et al.*, 2019; van de Pol *et al.*, 2006). One aspect of individual quality that has been studied with the silver spoon hypothesis is body condition. Early body condition is often related to adult condition across species (Monaghan, 2008). Metrics used to assess body condition can involve body quality measurements (such as mass and bone size) and early growth rates, which can be measured over time to help understand the impact of early experiences. Positive relationships between increased early growth rates, survival, and body size have been demonstrated in wild living bird species (e.g., Cooch *et al.*, 1991; Krementz *et al*., 1989; Perrins, 1966; Perrins *et al.*, 1973). Wood Ducks (*Aix sponsa*) in captivity have exhibited numerically large differences in early growth rates (Hinton *et al.*, in progress). These differences were observed despite birds experiencing potentially less challenging captive conditions, with *ad-libitum* feeding and safety from predation. This variation in growth rates poses the question of whether any lasting consequences would be detected similar to what has been observed in the wild.

Body condition has been associated with stress mediation ability throughout life (Monaghan, 2008). Like body condition, an individual's ability to cope with stress in their environment can be considered an indicator of individual quality (Angelier *et al.*, 2010). Considering the silver spoon effect, the ability for birds to respond to an acute stressor imposed upon them may be correlated with quality during early development. Measuring glucocorticoid hormone response to a stressful stimulus is a common approach to quantifying the stress response (Dallman *et al.*, 1993; Dupont *et al.*, 2019; Landys *et al.*, 2006). Corticosterone (CORT) is the primary avian glucocorticoid released from the adrenal gland as part of the hypothalamic pituitary-adrenal (HPA) axis and involved in stress and energetic regulation (Sapolsky, 1987; Sapolsky *et al.*, 2000; Wingfield, 1994, 1997; Wingfield & Ramenofsky, 1997). Normally, baseline CORT concentrations regulate energetic demands and daily activity in birds, but monitoring CORT response to an imposed acute stressor can reveal information about individual quality and ability to handle stress. A greater ability to mediate energetic demands often includes lower baseline CORT (Crespi *et al.*, 2013; Landys *et al.*, 2006; Love *et al.*, 2005; Romero, 2002). Moreover, a better-quality response to stress may involve less magnified stress-induced CORT secretion, and, when the stressor recedes, quickly dampening CORT levels through negative feedback (Breuner *et al.*, 2008; de Kloet *et al.*, 2005; Romero, 2004; Schmidt *et al.*, 2012).

In several species of birds, handling-induced CORT responses are lower in individuals with more fat than in individuals with less fat (Breuner & Hahn, 2003; Cherel *et al.*, 1988; Kitaysky *et al.*, 1999; Love *et al.*, 2005; Perfito *et al.*, 2002; Schwabl, 1995; Smith *et al.*, 1994; Wingfield, 1994; Wingfield *et al.*, 1994). In wild Wood Duck hens, a negative correlation was found between adult body mass and stress response. Wood Ducks are seasonal breeders, and this correlation was determined to occur late in the breeding season. Thus, birds were likely regulating stress response while in reproductive condition (DuRant *et al.*, 2013). During the breeding season, most birds experience increased plasma concentrations of baseline and stress-induced CORT (DuRant *et al.*, 2013; Romero, 2002). An exploration of connections between ability to handle stress and adult body condition may reveal connections with state experienced in early life.

In this study, questions about the relationship between early development, current state, and adult ability to respond to stress were addressed with a captive colony of Wood Ducks, a precocial bird species. The common garden setting has allowed for long term collection of a plethora of life history data. Additionally, in this environment, individuals often have access to food *ad libitum* and no predation events, which can alter energetic issues experienced during life stages that are normally considered stressful for wild bird species (Heath & Dufty, 1998; Kitaysky *et al.*, 1999). Studying precocial birds in captivity allowed us the opportunity to evaluate connections between early and current state when many selective pressures were removed. We were able to explore the relationship between current body condition and stress response in Wood Duck hens and drakes during and outside of the breeding season.

Our objective was to evaluate whether early body condition predicts current body condition, and whether current body condition, and, therefore, early body condition, metrics relate to acute stress response. We compared individual ability to cope with imposed acute stress through CORT responses quantified during a capture period. We hypothesized that early life body condition and adult body condition would share a positive relationship, so birds that were in better body condition as juveniles, which was determined by early growth rates, would be in better body condition as adults, which was determined by body mass. In a study on wild Wood Duck hens, body mass alone was correlated with lipid reserves prior to egg-laying and determined to be a good indicator of individual quality. Body mass was also determined to be independent of both structural size and age (Hepp *et al.*, 1987). We hypothesized that if energetic requirements mitigate stress response, then there would be an inverse relationship between body condition and magnitude of stress response so adults that had higher body mass would have a more dampened acute stress response than adults with a lower mass.

Methods:

Study site and animals

This study was conducted on a captive colony of Wood Ducks (total n = 28) housed at the University of California, Davis in Davis, CA, USA. Birds were kept in three mixed-sex, outdoor 3m x 6m enclosures with shade (male: n = 1, female: n = 8 in pen A; male: n = 3, female: n = 14 in pen B, male: n = 1, female: n = 1 in pen C). Enclosures included concrete flooring with water pools, logs for perching, and artificial grass patches. One circular food tray was provided per pen and was refilled as needed to maintain access to food. Nest boxes with pine shavings as nest material were provided during the breeding season. All birds were hatched and reared in captivity as part of a previous study in either 2015, 2016, or 2017 cohorts. All procedures were in accordance with U.S. state and federal laws and were approved by the University of California, Davis Institutional Animal Care and Use Committee protocol number 20423.

Blood collection

To assess individual variation in sensitivity of the HPA axis to acute handling stress, captive ducks were subjected to a standardized capture stress protocol and bled in a series (Wingfield, 1994; Wingfield *et al.*, 1994; Wingfield *et al.*, 1995). The CORT series was taken twice: once during the breeding season in June-July 2021 and once outside of the breeding season in November 2021. Within each season, blood was collected from two ducks per day until all 28 ducks were sampled. To minimize daily disturbance, we captured ducks from a single pen on any given day and tested birds from different pens on subsequent days. The baseline sample was obtained between 0800 h and 1000 h, always within three minutes of handlers entering the pen. If a baseline was not successfully obtained within this time limit, handlers returned the bird to the pen and recaptured them at a later date. Additional samples were taken at 10, 30, and 60 minutes. Approximately 200 μL blood was collected into two to three heparinized capillary tubes from the brachial vein at each sampling point. Whole blood samples were kept on ice until plasma was separated by centrifugation and stored at –20°C at UC Davis for further analysis. The birds were contained in a dark box (one per individual) in between sampling. Birds were fitted with a hood during blood collection. Birds were individually marked with two plastic tags on their rectrices (tail feathers) before being returned to their enclosure to prevent them from being recaptured within the same sampling season.

Corticosterone assays

Corticosterone assays were performed using a CORT assay kit based on competitive binding (enzyme-linked immunoassay [ELISA]: Enzo Life Sciences, Farmingdale, NY). Concentrations of CORT in non-extracted plasma were determined using a four-parameter logistic fit (outlined in Love & Williams, 2008). All samples were run in triplicate at a volume of 100 μl with 1:40 dilution with steroid displacement reagent provided by the kit. Each plate included a pooled sample control and a standard curve generated by serially diluting 200,000 pg/ml kit-provided CORT standard. According to the kit and methods outlined in Love and Williams (2008), plates were incubated at 26°C while shaking at 500 rpm for two hours, washed, then incubated again at 26°C without shaking for one hour. Plates were read at 405 nm wavelength (see Hennin *et al.*, 2015 & 2016). Intra and inter-assay coefficients of variation were 9.5% and 13.1%, respectively. The detection limit of the assay was determined to be 0.59 ng/ml.

Metrics of body condition

In March 2021, body mass and tarsus measurements were recorded for each duck. Body condition values were based on body mass (outlined in Labocha & Hayes, 2012; Schamber *et al.*, 2009). Body morphometric data for juvenile body condition was obtained for 19 focal birds from flock records. Measurements included early growth rate data that had been calculated for both tarsus length and body mass for these ducks using the slope of the tangent at each point of measurement from a Logistic 3P growth curve for each individual (Hinton *et al.*, in progress).

Statistical analysis

Corticosterone response

We used a simple regression to assess whether time of sampling predicted baseline CORT (nlme package; Pinheiro *et al.*, 2022). To evaluate the total increase in CORT across the 60 minute capture period, we subtracted our baseline time point values from all time points (0, 10, 30, and 60), and calculated the area underneath the resulting curve (Breuner *et al.*, 1999; Romero & Wingfield, 2015). Baseline CORT, maximum CORT (determined to be a single secretion at the 10, 30, or 60 minute collection period depending on the bird), and total CORT as determined by the area under the CORT response curve were used to evaluate individual CORT response. Current body condition values, taken as adult mass, were compared to both maximum and total CORT values. We were interested in determining if current body condition predicts acute stress response. We used R 4.2.0 (R Core Team, 2022) to run Generalized linear mixed effects models (GLMM; glmmTMB package; Brooks *et al.*, 2017) with normal distribution and a log link function were used to determine the effects of adult body mass, cohort, series taken during or out of the breeding season, and sex on total CORT, and separately on maximum CORT. Body mass was log-transformed to fit normality of model residuals. A *P*-value of < 0.05 was considered statistically significant. Day of blood draw and individual duck identity were included as crossed random effects (Table 3.1). We used backward model selection with ANOVA to improve model fit. Multiple models had similar Akaike Information Criterion (AIC) values to the best fit model determined by Delta AIC (△AIC). In order to evaluate a key predictor variable of interest, body mass, the models selected for both total and maximum CORT as response variables contained body mass, cohort, and season as predictor variables, and the △AIC between those models and the best fit models were numerically small (< 2). Models met criteria for normality of residuals based on a Quantile-Quantile plot with a Kolmogorov-Smirnov Test and dispersion test, and a within group deviation from uniformity and a Levene Test for homogeneity of variance using the DHARMa package (Hartig, 2022). Linear regressions (nlme package; Pinheiro *et al.*, 2022) with continuous variables were used to assess relationships between total and max CORT, and total and max CORT between breeding season and out of breeding season. Plots were created using the ggplot2 package (Wickham, 2016).

Morphometric data

The residuals of a regression of body mass on tarsus length have often been used in past research to account for body size (Peig & Green, 2009), but we did not find a significant relationship with a regression (nlme package; Pinheiro *et al.*, 2022) in R 4.2.0 (R Core Team, 2022), and this method has not been validated for Wood Ducks. Additionally, linear regressions with continuous variables were used to determine whether body mass growth rates and tarsus length growth rates predicted adult body mass as a metric of body condition. The maximum rates of body mass and tarsus length growth for each individual up to 50 days of age (juvenile body condition data) were taken for comparison. For all analyses, a P-value of < 0.05 was considered statistically significant. Plots were produced using the ggplot2 package (Wickham, 2016).

Results:

The percentage of baseline samples with CORT levels that fell below the limit of detection (0.59 ng/ml) was 53.3% during the breeding season and 78.6% outside the breeding season. Time of sampling under three minutes did not affect baseline CORT levels (Adjusted $R^2 = 0.01$, $P = 0.19$). All individuals had increased CORT levels to above the limit of detection at the 10 minute collection period, with all levels remaining detectable at 30 and 60 minute collection periods, as well. Total CORT levels were 1256.0 ± 299.7 ng/ml/hr; maximum CORT levels were 32.80 ± 8.0 ng/ml across all birds sampled (model estimated means and standard error measurements).

The relationship between tarsus and body mass was not significant (Adjusted $R^2 = 0.002$, $P = 0.31$). Thus, we used body mass alone as a metric of body condition (following Hepp *et al.*, 1998; Labocha & Hayes, 2012; Schamber *et al.*, 2009) rather than using the residuals (e.g., Peig and Green, 2009). Body mass did not have an effect total CORT secreted over the hour ($\chi^2 = 1.37$, df = 1, $P = 0.24$; Fig. 3.1A) or maximum CORT ($\chi^2 = 1.48$, df = 1, $P = 0.22$; Fig. 3.1B). However, there was a difference in total CORT measured during and out of the breeding season ($\chi^2 = 10.9$, df = 1, $P < 0.001$; Fig. 3.2A) and maximum CORT measured during and out of the breeding season ($\chi^2 = 15.9$, df = 1, $P < 0.001$; Fig 3.2B). Cohort did not have a significant effect on total CORT ($\chi^2 = 5.39$, df = 2, $P = 0.07$) or maximum CORT ($\chi^2 = 3.97$, df = 2, $P = 0.14$). The best fit model for total CORT included cohort, season, and intercept, and the best fit model for maximum CORT only included season and the intercept. However, we chose to use the models with body mass included as a predictor of interest because body mass was a key factor in our predictions and the difference in AIC values was numerically small between the chosen models and the best fit models (0.6 for total CORT and 1.4 for max CORT). We found that maximum CORT levels correlated with total CORT secreted (Adjusted $R^2 = 0.93$, $P < 0.001$; Fig. 3.3).

Early body mass growth rates predicted adult body mass (Adjusted $R^2 = 0.40$, $P = 0.002$; Fig. 3.4). Tarsus length growth rated also predicted adult body mass, though the prediction was not as strong (Adjusted $R^2 = 0.20$, $P = 0.03$; Fig. 3.5). Total and maximum CORT responses were not predicted by residuals of a regression of body mass on early growth rates (Adjusted $R^2 = -0.06$, $P = 0.84$ for breeding season total CORT; Adjusted $R^2 = -0.02$, $P = 0.46$ for out of breeding season total CORT; Adjusted $R^2 = -0.05$, $P = 0.76$ for breeding season maximum CORT; Adjusted $R^2 = -0.02$, $P = 0.44$ for out of breeding season maximum CORT).

Individual variation persisted for CORT responses (Fig. 3.6 & 3.7). The responses during the breeding season predicted the responses out of breeding season for both total (Adjusted $R^2 = 0.65$, $P < 0.001$; Fig. 3.8) and maximum CORT secretions (Adjusted $R^2 = 0.71$, $P < 0.001$; Fig. 3.9). The predictive value decreased for both total (Adjusted $R^2 = 0.19$, $P = 0.01$) and maximum CORT (Adjusted $R^2 = 0.22$, $P = 0.008$) when one outlier duck was removed.

Discussion:

Differences in individual state (e.g., body condition) can affect how wild birds respond to energetically expensive life stages. Wild precocial birds often experience intense selection during early life that influences the development of differences in individual state. In captivity, birds encounter the same life stages but do not experience the same constraints (i.e., resource availability, predation avoidance). Despite the lack of constraints, captive Wood Ducks have still shown variation in early body condition as indicated by growth rates (Hinton *et al.,* unpublished). We are interested in understanding the intersection of physiology facilitating stress response and body condition, and relationships with early body condition, during an important life stage, breeding, and how these factors compare when birds are out of the breeding season. We did not find that body mass predicted CORT response, but we did find that CORT response varied in and out of the breeding season. Adult body condition, using body mass as a metric, related to early body condition, using maximum body mass and tarsus length growth rates as metrics. Though not part of our original objectives, we discovered novel information on individual variation in stress response. Individual CORT responses showed consistency across the breeding season and out of the breeding season.

We did not find a relationship between body mass and total or maximum CORT response. With *ad-libitum* feeding, birds may be able to consistently maintain good body condition while coping with stressors. Adequate variation in body condition may be needed for the relationship between CORT response and body condition to exist (Wingfield *et al.*, 1994). Residuals of body mass on tarsus have been negatively correlated with CORT concentration at baseline (Hope *et al.*, 2020) and 30 minutes (as a single measure of stress-induced response) in wild breeding Wood Duck hens (DuRant *et al*., 2013; Hope *et al.*, 2020). The relationships between both baseline and stress-induced CORT levels and body condition in wild Wood Ducks still need to be investigated outside of the breeding season, as it can be difficult to capture non-nesting birds.

We found differences in both total and maximum CORT secretions from samples taken during the breeding season (June-July) and outside of the breeding season after molt (November). The higher levels found during the breeding season show similarities with wild Wood Duck research. DuRant *et al.* (2013) found that stress-induced CORT levels increased for wild Wood Duck hens over the incubation period (from March to May).

There are differences between captive and wild conditions that affect some of the costs associated with incubation. For example, the captive ducks in our study lay eggs during the breeding season, but the eggs are removed regularly to prohibit prolonged incubation. Additionally, as there are three nest boxes per pen, each Wood Duck hen does not have their own box to tend, which could save energy otherwise spent incubating (DuRant *et al.*, 2013). Wood Duck clutch sizes are large, often consisting of 10-13 eggs (Semel & Sherman, 1992), so removing the option to maintain a clutch may remove energetic demands associated with breeding. Conversely, increased energetic demands that wild birds experience during the breeding season, when food availability may not be sufficient to maintain body weight, create a stressful situation that can be measured by increased CORT secretion (Romero, 2002). Thus, it is interesting that we still found a difference in total and maximum plasma CORT secreted in and out of the breeding season for captive ducks.

It is also interesting that baseline CORT levels were consistently low, with most levels falling below 0.59 ng/ml. Such low levels could highlight that in captivity, ducks likely have sufficient resources to maintain energy stores that manage demands related to daily activities at various life stages (DuRant *et al.*, 2013; Landys *et al.*, 2006). The captive female ducks could be expending less energy on incubation and do not have to cope with nest predation and lack of resources, so they are not exposed to the same factors that could affect the mobilization of energy stores as in the wild.

Differences in CORT response observed in and out of the breeding season in this study could also relate to habituation to handling via the capture-restraint protocol and other variables, such as fluctuations in hormonal secretion patterns associated with the environment. The effects of handling habituation, environmental fluctuations, and costs associated with breeding on CORT response are confounding. CORT responses across more seasons should be evaluated to parse apart which of these factors may contribute to the observed effect.

In wild birds, CORT response levels can vary with age of the individual; as age increases, birds often have a more dampened response to experimentally induced stress (Heidinger *et al.*, 2006; Wilcoxen *et al.*, 2011). While other studies have found that differences in CORT response vary by age (Heidinger *et al.*, 2006; Wilcoxen *et al.*, 2011), we did not find any significant difference between birds of different ages. This result may be due to high overall variation in CORT measures or low sample size.

Using a dataset containing growth rates for body mass and tarsus length, we confirmed our prediction that early body condition is related to adult body condition. We found that maximum growth rate values of both body mass and tarsus length were associated with adult body mass. Thus, early morphological patterns predicted that differences in body condition were maintained into adulthood. This result is similar to effects seen in research on lesser snow geese, where young that grew more slowly became smaller adults (Cooch *et al.*, 1991). Wood Duck ducklings showed individual variation in development through differences in growth rates, even with *ad-libitum* food intake providing an opportunity for expeditious development (Hinton *et al.*, unpublished). In a common garden setting, ducks still may experience differences in development relating to factors such as genetics, the social environment, or interactions between both.

We were able to begin to reveal previously unknown levels of individual variation in acute stress response. Our results also revealed repeatability of inter- and intra-individual variation in responses. Though our findings were surprising, Wood Duck research has shown pronounced individual variation for a variety of traits. For example, interesting variation in life history in Wood Duck breeding consists of some birds laying their eggs in other Wood Duck nests, some making nests and laying eggs in those nests, some performing a combination of both, and some birds skipping years altogether (Bellrose & Holm, 1994; Semel & Sherman, 1986; Eadie *et al.*, 1998; Semel & Sherman, 2001). Furthermore, there are early differences in life history, including factors such as temperature during early incubation, that have large effects on variation among individual ducklings in terms of stress response (DuRant *et al*., 2010). Moving forward, more life history data, including personality, can be utilized to look at other potential connections with variation in stress response. One recent study determined that juvenile CORT response at a 30 minute collection period did not relate to early activity, exploratory, and boldness behaviors and growth rates in captive Wood Ducks (Hope *et al.*, 2018). The relationship between adult activity, exploratory, and boldness behaviors and total CORT secretion in response to stress should be explored.

Our findings suggest that the captive Wood Ducks may all be in good enough body condition that they do not need to tightly regulate response to stress. Our results highlight possible differences in captive Wood Duck acute stress response in and out of the breeding season, though more research across multiple seasons is warranted. This study also reveals a relationship between early and adult morphological traits and suggests the need for further investigation of factors influencing variation in phenotypic traits that may be important for coping with changes across life stages. Understanding variation can begin to tell us about which traits are important in terms of survival and reproduction and what potential evolutionary consequences there may be from differential survival and reproductive success.

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References:

Angelier, F., Wingfield, J. C., Weimerskirch, H., & Chastel, O. (2010). Hormonal correlates of individual quality in a long-lived bird: A test of the "corticosterone-fitness hypothesis". *Biology Letters, 6*(6), 846–849. https://doi.org/10.1098/rsbl.2010.0376

Bellrose, F. C., & Holm, D. J. (1994). *Ecology and Management of the Wood Duck.* Stackpole Books, Mechanicsburg, PA, USA.

Breuner, C. W., & Hahn, T. P. (2003). Integrating stress physiology, environmental change, and behavior in free-living sparrows. *Hormones and Behavior, 43*(1), 115–123. https://doi.org/https://doi.org/10.1016/S0018-506X(02)00020-X

Breuner, C. W., Patterson, S. H., & Hahn, T. P. (2008). In search of relationships between the acute adrenocortical response and fitness. *General and Comparative Endocrinology*, *157*(3), 288–295. https://doi.org/10.1016/j.ygcen.2008.05.017

Breuner, C. W., Wingfield, J. C., & Romero, L. M. (1999). Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. *Journal of Experimental Zoology, 284*(3), 334–342. https://doi.org/10.1002/(sici)1097-010x(19990801)284:3<334::aid-jez11>3.0.co;2-%23

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug,H. J., Maechler M., & Bolker, B. M. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal, 9*(2), 378-400.

Butler, M. W., & McGraw, K. J. (2011). Past or present? Relative contributions of developmental and adult conditions to adult immune function and coloration in mallard ducks (Anas platyrhynchos). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 181*(4), 551–563. https://doi.org/10.1007/s00360-010-0529-z

Cherel, Y. V., Robin, J. P., Walch, O. D., Karmann, H. U., Netchitailo, P. I., & Le Maho, Y. V. (1988). Fasting in king penguin. I. Hormonal and metabolic changes during breeding. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 254*(2), R170-R177. https://doi.org/10.1152/ajpregu.1988.254.2.r170

Cooch, E., Lank, D., Dzubin, A., Rockwell, R., & Cooke, F. (1991). Body Size Variation in Lesser Snow Geese: Environmental Plasticity in Gosling Growth Rates. *Ecology, 72*(2), 503-512. https://doi.org/10.2307/2937191

Crespi, E. J., Williams, T. D., Jessop, T. S., & Delehanty, B. (2013). Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Functional Ecology*, *27*(1), 93-106. https://doi.org/10.1111/1365-2435.12009

Dallman, M. F., Strack, A. M., Akana, S. F., Bradbury, M. J., Hanson, E. S., Scribner, K. A., & Smith, M. (1993). Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Frontiers in neuroendocrinology*, *14*(4), 303-347. https://doi.org/10.1006/frne.1993.1010

de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience, 6,* 463-475. https://doi.org/10.1038/nrn1683

Dupont, S. M., Grace, J. K., Brischoux, F., & Angelier, F. (2019). Post-natal corticosterone exposure affects ornaments in adult male house sparrows (*Passer domesticus*). *General and Comparative Endocrino*logy, *276,* 45-51. https://doi.org/10.1016/j.ygcen.2019.02.021

DuRant, S. E., Hepp, G. R., Moore, I. T., Hopkins, B. C., & Hopkins, W. A. (2010). Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (Aix sponsa) ducklings. *The Journal of Experimental Biology*, *213*(1), 45–51. https://doi.org/10.1242/jeb.034488

DuRant, S. E., Hopkins, W. A., Hepp, G. R., & Walters, J. R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biological Reviews*, *88*(2), 499–509. https://doi.org/10.1111/brv.12015

Eadie, J., Sherman, P., & Semel, B. (1998). Conspecific brood parasitism, population dynamics, and the conservation of cavity-nesting birds. In Caro, T. ed. *Behavioral ecology and conservation biology*. Oxford Univ. Press, pp 306-340.

Hartig, F. (2022). DHARMa: Residual Diagnostics for Hierarchical (multi-level/mixed) Regression Models. R Package Version 0.2.2. Available online: https://cran.r-project.org/package=DHARMa. (Accessed on 9 February 2022).

Hayward, L. S., & Wingfield, J. C. (2004). Maternal CORT is transferred to avian yolk and may alter offspring growth and adult phenotype. *General and Comparative Endocrinology, 135*, 365–371. https://doi.org/10.1016/j.ygcen.2003.11.002

Heath, J. A., & Dufty, Jr, A. M. (1998). Body condition and the adrenal stress response in captive American kestrel juveniles. *Physiological Zoology, 71*(1), 67-73. https://doi.org/10.1086/515888

Heidinger, B. J. Nisbet, I. C. T. & Ketterson, E. D. (2006). Older parents are less responsive to a stressor in a long-lived seabird: a mechanism for increased reproductive performance with age? *Philosophical Transactions of the Royal Society B: Biological Sciences, 273*, 2227-2231. https://doi.org/10.1098/rspb.2006.3557

Hennin, H. L., Legagneaux, P., Bêty, J., Williams, T. D., Gilchrist, H. G., Baker, T. M. *et al.* (2015). Pre-breeding energetic management in a mixed-strategy breeder. *Oecologia, 177*(1), 235–243. https://doi.org/10.1007/s00442-014-3145-x

Hennin, H. L., Wells-Berlin, A. M., & Love, O. P. (2016). Baseline glucocorticoids are drivers of body mass gain in a diving seabird. *Ecology and Evolution, 6*(6), 1702–1711. https://doi.org/10.1002/ece3.1999

Hepp, G. R., Stangohr, D. J., Baker, L. A., & Kennamer, R. A. (1987). Factors affecting variation in the egg and duckling components of Wood Ducks. *The Auk, 104*(3), 435-443. https://doi.org/10.2307/4087542

Hope, S. F., DuRant, S. E., Angelier, F., Hallagan, J. J., Moore, I. T., Parenteau, C., … Hopkins, W. A. (2020). Prolactin is related to incubation constancy and egg temperature following a disturbance in a precocial bird. *General and Comparative Endocrinology*, *295*, 113489. https://doi.org/10.1016/j.ygcen.2020.113489

Hope, S. F., Kennamer, R. A., Moore, I. T., & Hopkins, W. A. (2018). Incubation temperature influences the behavioral traits of a young precocial bird. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(4–5), 191–202. https://doi.org/10.1002/jez.2176

Kisdi, E. (1998). Individual optimization: mechanisms shaping the optimal reaction norm. *Evolutionary Ecology*, *12*(2), 211-221. https://doi.org/10.1023/a:1006535829880

Kitaysky, A. S., Piatt, J. F., Wingfield, J. C., & Romano, M. (1999). The adrenocortical stress-response of black-legged kittiwake chicks in relation to dietary restrictions. *Journal of Comparative Physiology B*, *169*(4), 303-310. https://doi.org/10.1007/s003600050225

Krementz, D. G., Nichols, J. D., & Hines, J. E. (1989). Post-fledging survival of European Starlings. *Ecology, 70*(2), 646-655. https://doi.org/10.1002/jwmg.670

Labocha, M. K., & Hayes, J. P. (2012). Morphometric indices of body condition in birds: A review. *Journal of Ornithology*, *153*(1), 1–22. https://doi.org/10.1007/s10336-011-0706-1

Landys, M. M., Ramenofsky, M., & Wingfield, J. C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology, 148*(2), 132–149. https://doi.org/10.1016/j.ygcen.2006.02.013

Love, O.P., Chin, E.H., Wynne-Edwards, K.E., & Williams, T.D. (2005). Stress hormones: a link between maternal condition and sex-biased reproductive investment. *American Naturalist, 166*(6), 751–766. https://doi.org/10.1086/497440

Love, O. P., & Williams, T. D. (2008). Plasticity in the adrenocortical response of a free-living vertebrate: The role of pre- and post-natal developmental stress. *Hormones and Behavior, 54*(4), 496–505. https://doi.org/10.1016/j.yhbeh.2008.01.006

McNamara, J. M., & Houston, A. I. (1996). State-dependent life histories. *Nature, 380*(6571), 215-221. https://doi.org/10.1038/380215a0

Minias, P., Włodarczyk, R., Surmacki, A., & Iciek, T. (2015). Silver spoon effects on plumage quality in a passerine bird. *Royal Society Open Science*, *2*(6), 140459. https://doi.org/10.1098/rsos.140459

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1497), 1635–1645. http://doi.org/10.1098/rstb.2007.0011

Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology and Evolution, 13*(10), 403–407. https://doi.org/10.1016/S0169-5347(98)01472-4

Perfito, N., Schirato, G., Brown, M., & Wingfield, J. C. (2002). Response to acute stress in the harlequin duck (*Histrionicus histrionicus*) during the breeding season and moult: relationships to gender, condition, and life-history stage. *Canadian Journal of Zoology, 80*(8), 1334-1343. https://doi.org/10.1139/z02-118

Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos, 118,* 1883e1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x

Perrins, C. M. (1966). Survival of young Manx Shearwaters *Puffinus puffinus* in relation to their presumed date of hatching. *Ibis, 108*(1), 132-135. https://doi.org/10.1111/j.1474-919x.1966.tb07259.x

Perrins, C. M., Harris, M. P., & Britton, C. K. (1973). Survival of Manx Shearwaters *Puffinus puffinus. Ibis, 115*(4), 53 5-548. https://doi.org/10.1111/j.1474-919x.1973.tb01991.x

Pinheiro, J., Bates, D., & Team, R. C. (2022). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-158

Romero, L. M. (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and comparative endocrinology*, *128*(1), 1-24. https://doi.org/10.1016/s0016-6480(02)00064-3

Romero, L.M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology and Evolution*, *19,* 249-255. https://doi.org/10.1016/j.tree.2004.03.008

Romero, L. M., & Wingfield, J. C., (2015). *Tempests, poxes, predators, and people: stress in wild animals and how they cope.* Oxford University Press.

Rowe, L., Ludwig, D., & Schluter, D. (1994). Time, condition, and the seasonal decline of avian clutch size. *The American Naturalist*, *143*(4), 698-722. https://doi.org/10.1086/285627

Sapolsky, R. (1987). Stress, social status, and reproductive physiology in free-living baboons, in: Crews, D. (Ed.), *Psychobiology of Reproductive Behavior: An Evolutionary Perspective.* Prentice Hall, Englewood Cliffs, NJ, pp. 291–392.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21*(1), 55–89. https://doi.org/10.1210/er.21.1.55

Schmidt, K. L., Furlonger, A. A., Lapierre, J. M., MacDougall-Shackleton, E. A., & MacDougall-Shackleton, S. A. (2012). Regulation of the HPA axis is related to song complexity and measures of phenotypic quality in song sparrows. *Hormones and Behavior, 61*(4), 652–659. https://doi.org/10.1016/j.yhbeh.2012.02.027

Schwabl, H. (1995). Individual variation of the acute adrenocortical response to stress in the white-throated sparrow. *Zoology, 99*(2), 113-120.

Sedinger, B. S., Stewart, K. M., & Nicolai, C. A. (2018). On the importance of having a good mother: maternal investment affects duckling mortality risk in wood ducks. *Journal of Avian Biology*, *49*(10), 1–10. https://doi.org/10.1111/jav.01802

Semel, B., Sherman, P. W. (1986). Dynamics of nest parasitism in wood ducks. *The Auk, 103*(4), 813-816.

Semel, B., & Sherman, P. W. (1992). Use of clutch size to infer brood parasitism in wood ducks. *Journal of Wildlife Management, 56*(3), 495–499. https://doi.org/10.2307/3808864

Semel, B., & Sherman, P. W. (2001). Intraspecific parasitism and nest-site competition in wood ducks. *Animal Behaviour, 61*(4), 787–803. https://doi.org/10.1006/anbe.2000.1657

Smith, G. T., Wingfield, J. C., & Veit, R. R. (1994). Adrenocortical response to stress in the common diving petrel, *Pelecanoides urinatrix*. *Physiological Zoology, 67*(2), 526-537. https://doi.org/10.1086/physzool.67.2.30163862

Song, Z., Zou, Y., Hu, C., Ye, Y., Wang, C., Qing, B., ... & Ding, C. (2019). Silver spoon effects of hatching order in an asynchronous hatching bird. *Behavioral Ecology, 30*(2), 509-517. https://doi.org/10.1093/beheco/ary191

Stamps, J. A. & Groothuis, T. G. G. (2010). Developmental perspectives on personality: implications for ecological and evolutionary studies of individual differences. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*, 4029–4041. http://doi.org/10.1098/rstb.2010.0218

Stearns, S. C. (1992). *The evolution of life histories*. Oxford: Oxford University Press.

Team, R. C. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

van de pol, M., Bruinzeel, L. W., Heg, D. I. K., Van der Jeugd, H. P., & Verhulst, S. (2006). A silver spoon for a golden future: long-term effects of natal origin on fitness prospects of oystercatchers (Haematopus ostralegus). *Journal of Animal Ecology, 75*(2), 616-626. https://doi.org/10.1111/j.1365-2656.2006.01079.x

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

Wilcoxen, T. E., Boughton, R. K., Bridge, E. S., Rensel, M. A., & Schoech, S. J. (2011). Age-related differences in baseline and stress-induced corticosterone in Florida scrub-jays. *General and Comparative Endocrinology*, *173*(3), 461–466. https://doi.org/10.1016/j.ygcen.2011.07.007

Wingfield., J. C. (1994). Modulation of the adrenocortical response to stress in birds. R.E. Davey, R.E. Peter, S.S. Tobe (Eds.), *Perspectives in comparative endocrinology,* National Research Council of Canada, Ottawa, pp. 520-528.

Wingfield, J. C. (1997). Corticosterone and behavioral responses to unpredictable events. In S. Harvey and. R. J. Etches (eds.), *Perspectives in avian endocrinology,* pp. 267-278.

Wingfield, J.C., Deviche, P., Sharbaugh, S., Astheimer, L.B., Holberton, R., Suydam, R., & Hunt, K. (1994). Seasonal changes of the adrenocortical responses to stress in Red- polls, *Acanthis flammea*, in Alaska. *Journal of Experimental Zoology, 270*, 372–380. https://doi.org/10.1002/jez.1402700406

Wingfield, J.C., O'Reilly, K.M., & Astheimer, L.B. (1995). Modulation of the adrenocortical responses to acute stress in arctic birds—a possible ecological basis. *American Zoologist, 35*, 285–294. https://doi.org/10.1093/icb/35.3.285

Wingfield, J. C., & Ramenofsky, M. (1997). Corticosterone and facultative dispersal in response to unpredictable events. *Ardea*, *85*(1), 155-166.

Wingfield, J. C., Suydam, R., & Hunt, K. (1994). The adrenocortical responses to stress in snow buntings (Plectrophenax nivalis) and Lapland longspurs (Calcarius lapponicus) at Barrow, Alaska. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *108*(3), 299-306.

Tables and Figures:

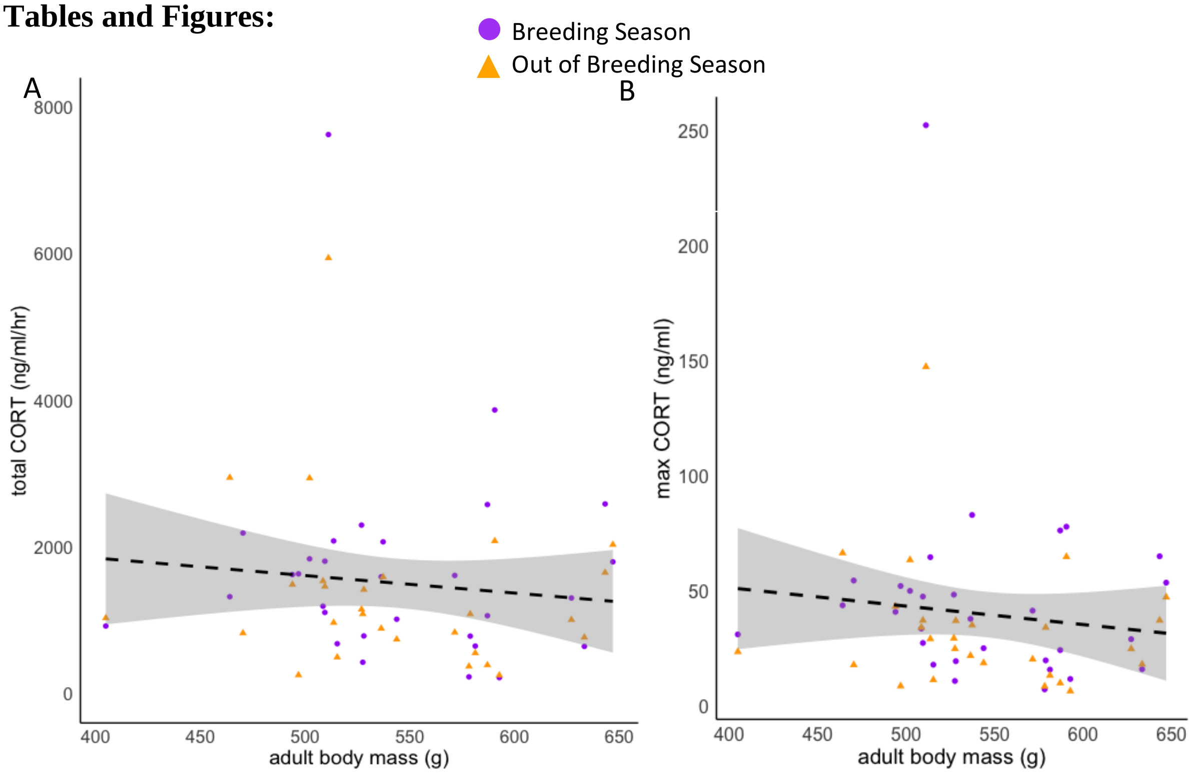

Figure 3.1 Raw values of total plasma CORT levels (A) and maximum plasma CORT levels (B) compared to adult body mass values for Wood Ducks in June/July 2021 (Breeding Season) and November 2021 (Out of Breeding Season). Shaded areas show 95% confidence around dashed trendlines for all samples. Body mass did not predict total or maximum CORT in GLMMs.

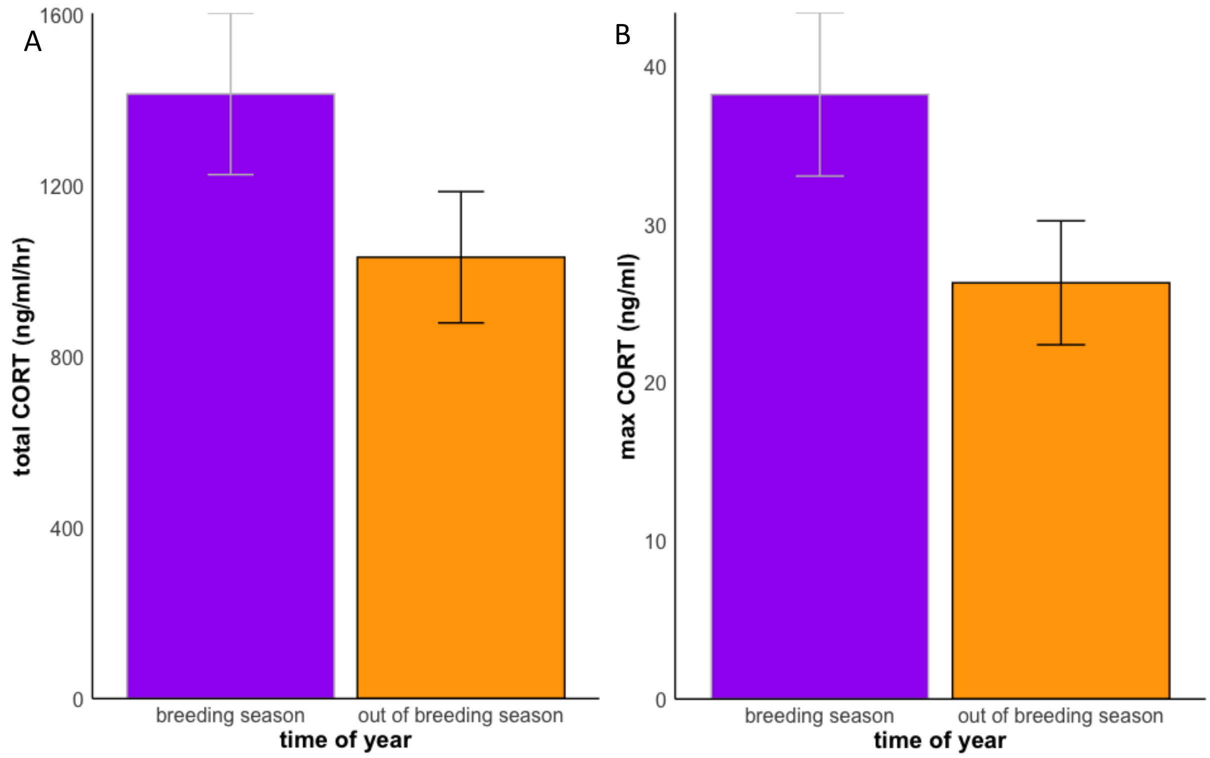

Figure 3.2 Total (A) and maximum (B) plasma CORT secretions determined from samples collected from 28 Wood Ducks in June/July 2021 (breeding season) and November 2021 (out of breeding season). Both total and maximum CORT differed between the breeding season and out of breeding season ($P < 0.001$). CORT concentration values are presented as model estimated means and SEM.

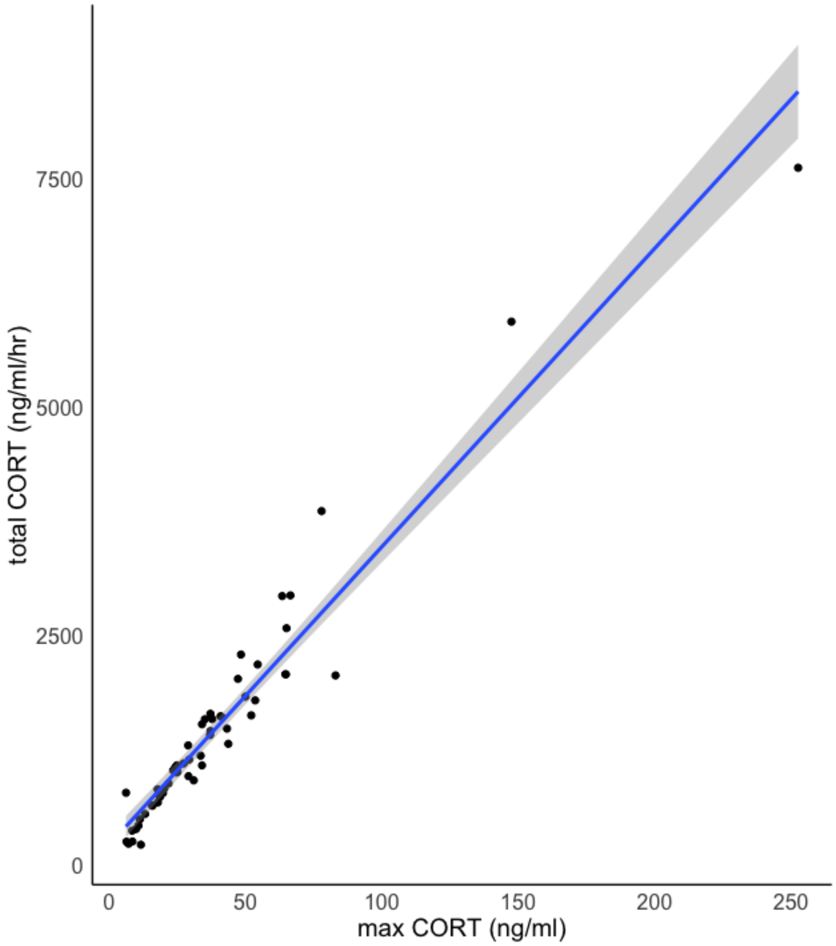

Figure 3.3 A positive relationship (Adjusted $R^2 = 0.93$, $P < 0.001$) between Maximum CORT levels compared to total CORT levels for Wood Ducks in June/July 2021 (Breeding Season) and November 2021 (Out of Breeding Season). The shaded area displays 95% confidence around the trendline for samples.

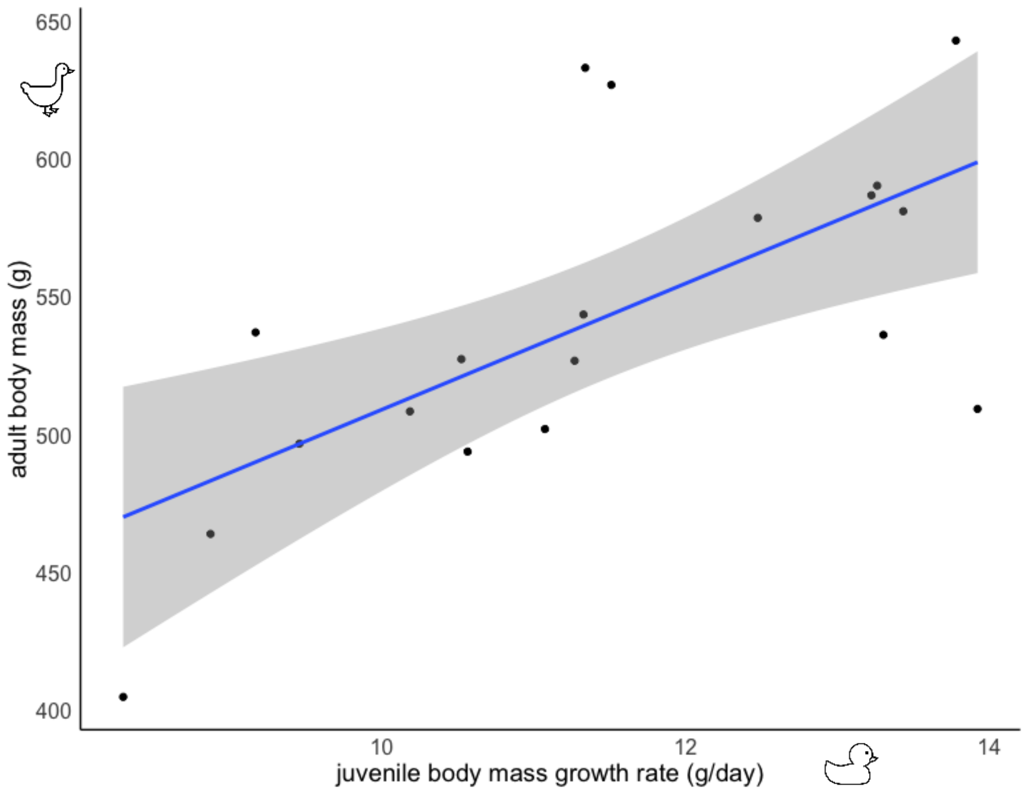

Figure 3.4 A positive relationship between adult body mass and early maximum body mass growth rates for 19 total birds from the 2016 and 2017 cohorts (Adjusted $R^2 = 0.40$, $P = 0.002$). The shaded area shows 95% confidence around the trendline for samples.

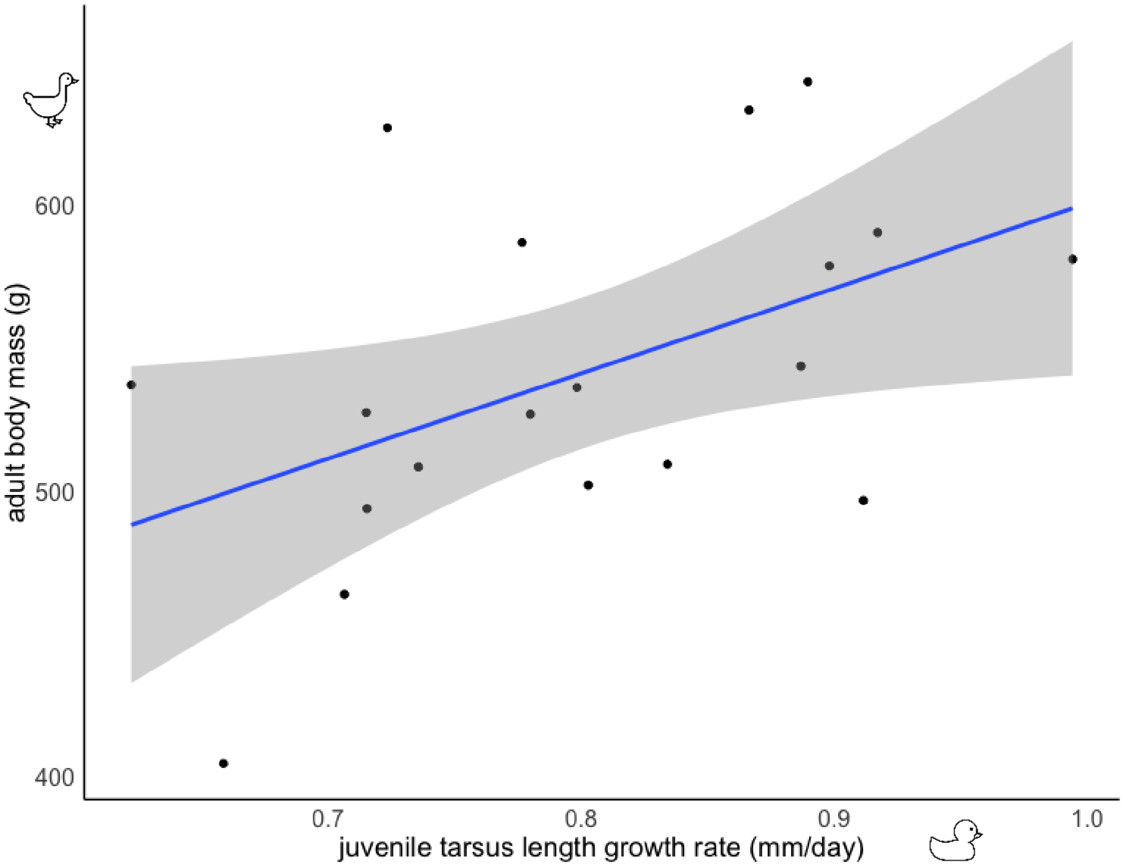

Figure 3.5 A positive relationship between adult body mass and early maximum tarsus length growth rates for 19 total birds from the 2016 and 2017 cohorts (Adjusted $R^2 = 0.20$, $P = 0.03$). The shaded area shows 95% confidence around the trendline for samples.

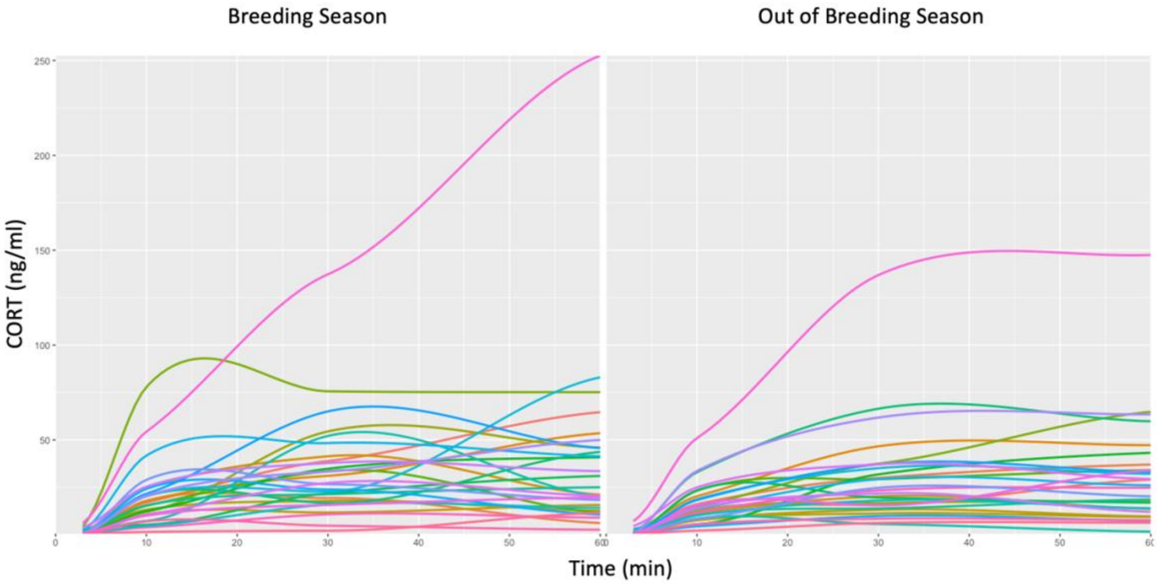

Figure 3.6 CORT level changes in response to capture and handling stress. Data collected in 28 Wood Ducks in June/July 2021 (during the breeding season) and November 2021 (out of the breeding season).

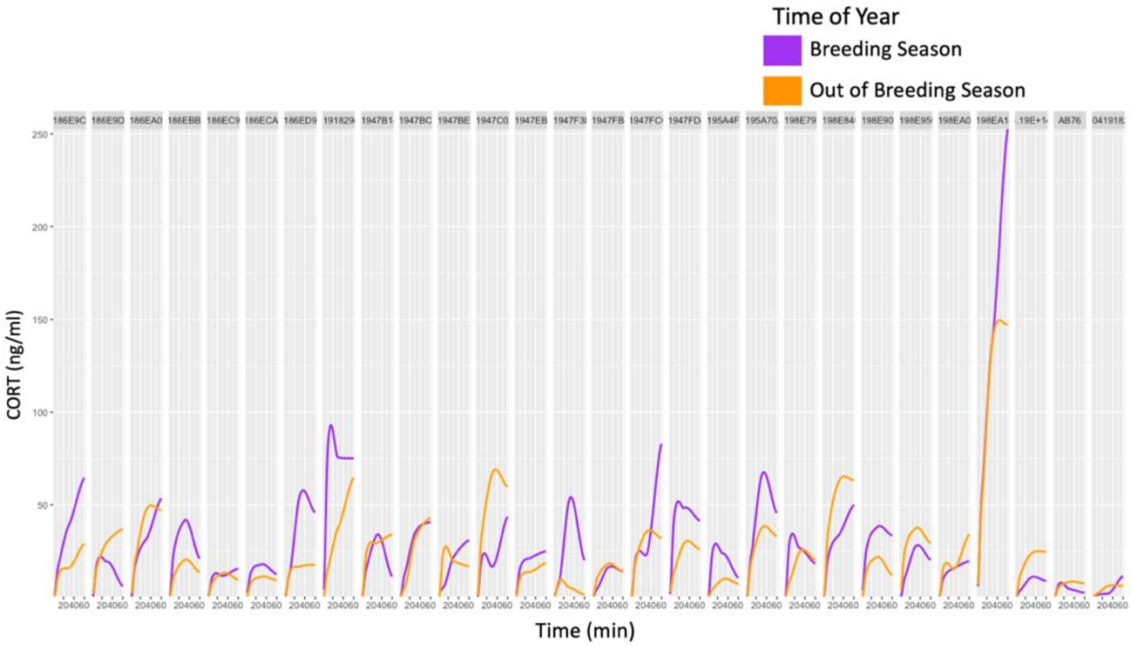

Figure 3.7 Individual changes in CORT levels in response to 60 min. of capture and handling stress. Data collected in 28 Wood Ducks in June/July 2021 (during the breeding season) and November 2021 (out of the breeding season).

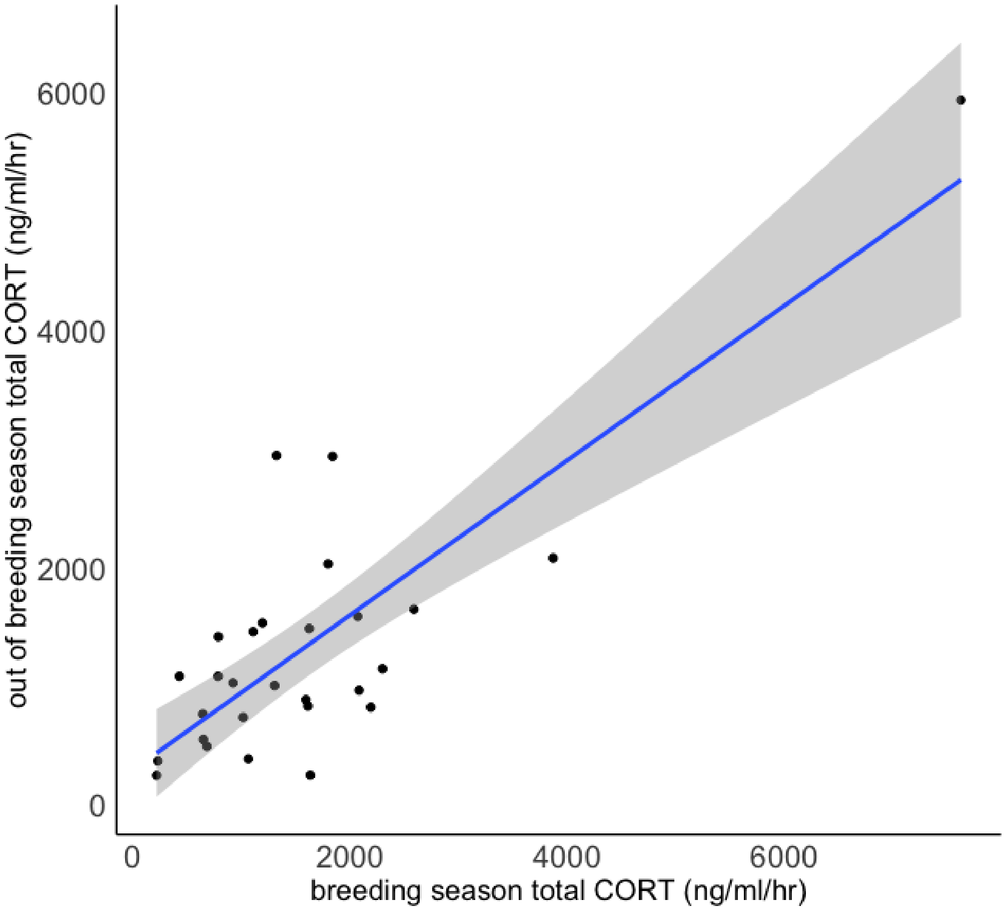

Figure 3.8 Values presented for 28 total birds from 2015, 2016, and 2017 cohorts show that there was a correlation between total CORT secreted during the breeding season and total CORT secreted out of breeding season (Adjusted $R^2 = 0.65$, $P < 0.001$). However, the predictive value decreased when one outlier duck was removed (Adjusted $R^2 = 0.19$, $P = 0.01$). The shaded area denotes 95% confidence around the trendline for all samples.

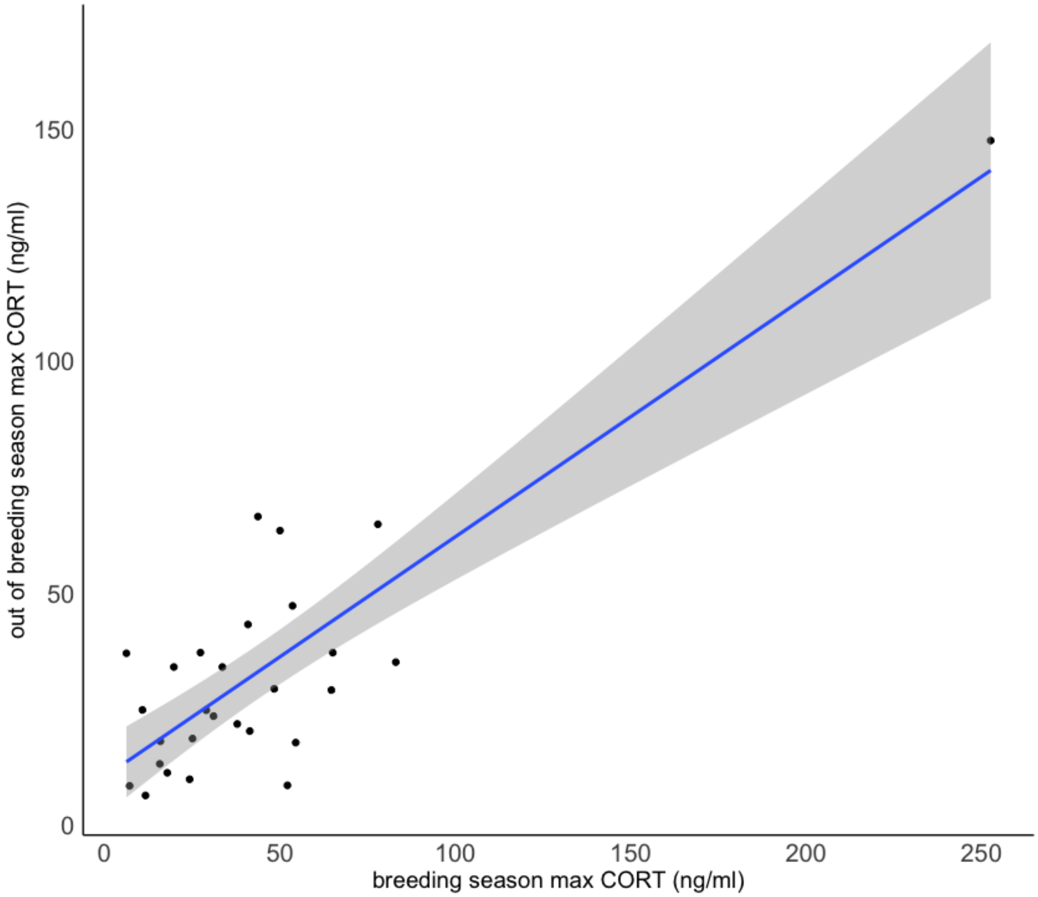

Figure 3.9 Values presented for 28 total birds from 2015, 2016, and 2017 cohorts show that there was a correlation between maximum CORT secreted during the breeding season and maximum CORT secreted out of the breeding season (Adjusted $R^2 = 0.71$, $P < 0.001$). However, the predictive value decreased when one outlier duck was removed (Adjusted $R^2 = 0.22$, $P = 0.008$). The shaded area denotes 95% confidence around the trendline for all samples.

A. Total CORT		
Model	AIC	△AIC
Full model (no dropped terms)	954.6	8.1
Season*Sex	952.6	6.1
Mass*Cohort	951	4.5
Mass*Sex	950.3	3.8
Sex*Cohort	947.8	1.3
Season*Cohort	949.3	2.8
Sex	947.1	0.6
Mass	**946.5**	**0**
Cohort	947	0.5
B. Max CORT		
Model	AIC	△AIC
Full model (no dropped terms)	534.1	9.1
Mass*Cohort	531.8	6.8
Season*Sex	530.7	5.7
Sex*Cohort	530	5
Season*Cohort	529.5	4.5
Mass*Sex	527.5	2.5
Sex	526.4	1.4
Mass	525.9	0.9
Cohort	**525**	**0**

Table 3.1 Akaike Information Criterion (AIC) values, and Delta AIC (△AIC) listed to display sensitivity to the loss of smoothed terms. Labels identify the dropped term in each model version (A is total CORT as the response variable and B is maximum CORT as the response variable). The bolded label identifies the best fit model. Though the best fit model for A included all dropped terms except Season and Cohort and the best fit model for B included all dropped terms except Season, the model selected for both contained body mass, cohort, and season because all were predictor variables of interest and the △AIC between those models and the best fit models were numerically small (< 2).