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# The Effects of Interactive Object Provisioning on Corticosterone, Stress-Related Behaviors, and Cognition in Juvenile and Adult Zebra Finches (Taeniopygia guttata)

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# THE EFFECTS OF INTERACTIVE OBJECT PROVISIONING ON CORTICOSTERONE, STRESS-RELATED BEHAVIORS, AND COGNITION IN JUVENILE AND ADULT ZEBRA FINCHES (*TAENIOPYGIA GUTTATA*)

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## ABSTRACT

Identification and reduction of unnecessary stress in lab animals is important for welfare, and for scientific validity. Providing animals with infrastructure and objects that encourage exercise and species-typical manipulative behaviors can help reduce stress by meeting physical and psychological needs. The effects of such "environmental enrichment" (EE) have been heavily studied in both zoo animals—where it has been shown to reduce maladaptive behaviors—and in lab animals such as rodents, where it clearly reduces stress and anxiety, dampens reactions to stressful events, and increases cognition and the volume of related brain regions. While there is some evidence that EE is similarly effective in birds, no rigorous studies have been completed using the zebra finch (ZF) (*Taeniopygia guttata*), a common avian lab model. Although there are basic EE guidelines established by local IACUCs, it is unclear if the accepted minimal provisions reduce stress-related behaviors or provide a buffer for stressful events, and whether more advanced EE could reduce anxiety and improve cognition as enrichment does in rodents. Thus, I tested whether EE compared to basic housing reduced anxiety and improved cognition in juvenile and adult ZFs of both sexes by measuring baseline plasma levels of corticosterone (CORT), and changes in CORT induced by restraint; monitoring bodyweight; quantifying abnormal repetitive behaviors (ARBs); and assaying behavior in a Novel Object Test; a Hyponeophagia Test; and a spatial maze. In this research I found that in juveniles, EE prevents the development of ARBs, and in both age groups, EE birds weighed less than non-enriched birds. In adults, EE allows females to reach male levels of spatial cognition, with this difference not appearing in juveniles. In adults, the Novel Object test showed that enrichment was associated with activity or exploration in adults, but both female treatment groups moved sooner. In juveniles, it revealed that females of both treatments moved sooner than males. Hyponeophagia did not differ, baseline CORT levels did not change over time, and there were no differences in reactive CORT across any group. Given these results, I suggest implementation of EE for ZF starting at a young age for the greatest benefit.

## DEDICATION

This research is dedicated to Tiny and Scrunchie the zebra finches.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ARBs | abnormal repetitive behaviors |
| CORT | corticosterone |
| EE | environmental enrichment |
| ZFs | zebra finches |

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## INTRODUCTION

Identification and reduction of possible stress in laboratory animals is vital for both the animals' well-being and the quality of research being performed (National Research Council (NRC), 2008). An important part of laboratory animal care is providing animals with environmental enrichment (EE) via caging design and objects that enhance sensory and physical stimulation and allow for expression of species-typical behavior (NRC, 2011, pp. 52-53). Inadequate EE or impoverished housing can cause the development of stereotyped and self-harming behaviors, impair social interactions, and cause physiological disruptions such as immune system suppression and digestive system dysfunction (NRC, 2008, pp. 39-42). This, in turn, can affect the validity and repeatability of research within and between labs and generally undermine the extrapolation of results to non-stressed animal populations (Garner, 2005).

The National Research Council (NRC) publishes what is commonly known as the Guide, which provides recommendations for general laboratory animal care including EE; but detailed suggestions are lacking, particularly for non-standard animal models. Instead, local IACUCs (Institutional Animal Care and Use Committee) individually establish specific EE protocols for such animals, which has resulted in a lack of standardization across institutions. Furthermore, such protocols are rarely based on empirical evidence (Nager & Law 2010; Yamahachi et al. 2017) and are often adopted as mandates rather than recommendations (Nager & Law 2010) without clear rationale or consideration that sometimes, they may do more harm than good (Toth et al., 2011). Thus, it is important to formally test whether specific EE protocols contribute to animals' optimal well-being, as indicated by common measures of stress and anxiety, such as

high levels of abnormal repetitive behaviors, fearfulness, abnormal feeding behavior, cognitive deficits, or health complications.

Rodent studies have established that EE can positively affect animal behavior and welfare (reviewed in Simpson & Kelly, 2011). In general, rats with access to EE are easier to handle, less impulsive, and more relaxed; with such anxiety-reducing outcomes of EE being most obvious in novel situations that typically cause animals stress. However, summarizing the collective effects of EE on rats is complicated due to the great variety of variables across enrichment studies. The species, age, sex, and even social position of animals can impact EE success. Protocols can vary in efficacy through the duration of the enrichment, or the frequency they are changed. Even in well-known animal models, what would be considered standard conditions for implementing EE, and whether the effects of EE should be compared between impoverished or only standard housing is inconsistent (reviewed in Simpson & Kelly, 2011), making summarizing the effects of enrichment compared to controls difficult. Despite the variation in specifics, what many of these studies have in common is they make use of physiological and behavioral measures which are known to be indicators of stress and anxiety, and use them as markers of animal welfare.

These measures are useful for cross-species comparison due to the highly conserved nature of the stress-response system among vertebrates (Romero & Gormally, 2019). Common ways to quantify the function of this system are through measurements of glucocorticoids (Rensel & Schlinger, 2020), broadly classed as stress hormones, and the cognitive, behavioral, and physical impacts that correlate with them. A well-defined way that glucocorticoids affect animals cognitively is through spatial learning and other hippocampus-dependent tasks, due to the high levels of glucocorticoid receptors in this brain region (Kim et al., 2015). EE allows for

increased survival and proliferation of neurons in the dentate gyrus (Kempermann et al. 2002), which enhances performance on spatial memory tests compared to controls (Sisti et al., 2007). Spatial memory performance can therefore act as an indirect way of observing the effectiveness of enrichment across species, in conjunction with other measures of stress and anxiety.

Despite the corollaries in assessing the efficacy of EE in rodents and birds and the fact that birds are used only 1% less often than rats (Home Office, 2020), there has been little research into the effects of EE in laboratory birds. For example, using Google Scholar, the query "bird environmental enrichment" yields 57.1% fewer results than "rat environmental enrichment" as of the time of this search (6/12/22). Historically, non-mammalian vertebrates have been considered to be less sentient, less intelligent, and less capable of suffering than mammals (Hawkins et al., 2001), and so their welfare needs have been overlooked. Despite modern understanding of bird intelligence (Kverková et al., 2022) and experiential evidence that birds have advanced neural processing power and (Olkowicz et al., 2016) complex cognition (Emery, 2017; Ten Cate et al., 2017), studying how EE might benefit avian welfare is still not a research priority, even in zoological institutions (Woods et al., 2022).

EE research in this broad range of species, particularly those used as scientific-model species, is essential for welfare protection and for the sake of experimental validity and comparison of results across labs. The bulk of studies related to stress-reduction in birds comes from agricultural and biomedical research on chickens (*Gallus gallus domesticus*); with a focus on dietary supplementation (Gouda et al., 2020; Kucuk et al., 2003; Nelson et al., 2018) and environmental enrichment (Jones et al. 2020; Krause, et al., 2006; Liu et al., 2020; Ross et al., 2020). Outside of agricultural species, there have been a number of studies devoted to Psittaciformes including Amazon parrots (genus *Amazona*) and budgerigars (*Melopsittacus*

*undulatus*) that investigate how environmental variables relate to stress and welfare in these species (Cussen, & Mench, 2015; Ikkatai & Watanabe, 2015; Medina-García et al., 2017; Owen & Lane, 2006; Williams et al., 2017). Concern for these species has been prioritized due to their importance in studies of cognition, aging, and vocal communication (Hickman et al., 2017). Passerines are used as neurobiological model species as often or more often than Psittaciformes, but only a handful of studies focus on EE in these species. The most popular passerine species for laboratory studies is the zebra finch (*Taeniopygia guttata,* [ZF]) (Bateson & Feenders, 2010). This songbird is often chosen for research related to neurogenesis, speech learning, sexual dimorphism, memory, and aging (Hickman et al., 2017). Despite their great value to the field of neurobiology, there is little consistency among institutions in regards to their care, including enrichment efforts, husbandry practices, and experimental procedures (Schmidt, 2010). The available research suggests water baths (Jacobs et al., 1995; Krause & Ruploh, 2016), dust baths, larger cages, and additional perches (Jacobs et al., 1995) might reduce plasma CORT levels, increase locomotor activity, vocalization, and singing.

However, husbandry standards, even simple standards like minimum cage size, have not been established for ZFs, and EE is considered optional for ZFs (Olson et al., 2014). Although like rats and mice, birds are not included in the Animal Welfare Act, there is less effort in establishing consistent care and welfare guidelines for birds since they aren't as frequently used in the biomedical industry as with rodents (Bryda, 2013), they were historically assumed to be less intelligent than and capable of suffering than mammals (Hawkins et al., 2001), and there is no economic incentive to enhance productivity as in chicken research (Jones et al., 2020).

Given that we now understand the importance of appropriately stimulating environments and consistency of care in both welfare and repeatability of research (Garner, 2005), it is vital to

lay out more concrete guidelines for all model animals. Design of these enrichment programs must consider the sensory and physiological needs and limits of species, which may vary by age and sex. They must also account for the practical needs and safety of the animals, their caretakers, and the need to minimize experimental interference.

In ZF, olfactory enrichments—as are sometimes used in zoos for other species such as felids and primates (Clark & King, 2008)—are not likely to be as effective as visual and auditory enrichment due to the relatively limited scope of this sense (Krause et al., 2018). Such enrichment may even be ill-advised, as ZF and other birds' have chemical-sensitive respiratory systems (Brown et al., 1997). Although it has been shown to have some positive effects (Robbins & Margulis, 2016; UKEssays, 2018), auditory enrichment may also be problematic with model animals due to practical matters, such as exposing animal care staff to even higher volumes of sound beyond that of the birds' vocalizations, and the difficulty of isolating the enrichment to only a treatment group while keeping the animals in the same living space.

A type of enrichment that may have different effects depending on the sex and age of the subjects would be access to nesting material. In adult males, collection and placement of material in the nest is associated with activation of the dopaminergic reward system (Hall et al., 2014), meaning that nest-building may be more "enriching" to adult males than females. Since zebra finches do not build nests until reaching sexual maturity (Hauber et al., 2021), juveniles may also not experience interaction with nesting materials as rewarding in the same way or to the same degree as in adult males.

A common "shortcut" to designing enrichment programs to account for these issues is to model the enrichments on the needs of species' wild counterparts (Young, 2003, pp. 8-9). However, this assumes that species had no evolutionary pressure to be more tolerant, or even

better off in captivity. The needs of animals in captivity, especially ones that have been kept by humans for over 150 years (Mello, 2014) may differ from wild members of their species. It is well-established that living in captivity can lead to morphological, and functional changes in the brains and bodies of many species, including birds (Katajamaa & Jensen, 2020), mammals (Kruska, 1988), and fish (Pasquet, 2019). In captive ZFs, genetic diversity is reduced relative to native ZF (Forstmeier et al., 2007), which may allow cognitive and behavioral change along with the genetic changes. As female ZF have a preference for males with traits negatively associated with a high stress response (Roberts et al., 2007), part of this genetic change could have led to birds which have differing enrichment needs from their distant progenitors, as long as they are relevant to ZFs sensory abilities.

Considering what is known about the effects of EE on stress responses, body condition, cognition, and stress-related behavioral responses, I provided ZF of different ages and sexes with both artificial and semi-natural objects so that I may observe if there changes and differences in these measures in these groups compared to those with only basic environments. Overall, I am to determine whether environmental enrichment will alter indicators of stress and anxiety, such as baseline and reactive glucocorticoid levels, neophobia, and spatial cognition. I also aim to learn whether there will be differences in measures due to enrichment between adults and juveniles, and males and females. I anticipate that enrichment should reduce the proportion of time spent engaging in abnormal repetitive behaviors, the latency to eat in a new environment, and baseline and reactive glucocorticoid levels. I would also expect it to increase interactivity measures and the speed to interact with a novel object, use of spatial cue use during a spatial cognition test, and to have differing effects depending on the age of introduction of EE and the sex of the birds.

## GENERAL METHODS

### Subjects and Treatment Groups

Zebra finches (*Taeniopygia guttata*) were bred in flight cages (148.6 x 71.1 x 188.2 cm) at the University of Mississippi. Birds were maintained on a 14/10 h light/dark cycle with water, food (Kaytee Forti-Diet and Sunthing Special Vita Finch Formula), and cuttlebone available *ad libitum*, and bi-weekly supplementation of hard-boiled eggs and multigrain seed bread (IACUC protocol #19-018). Adults were transfered to single-sex flight cages upon sexual maturation, at which time they were banded with a single leg band. Males (n=16) and females (n=16) between 1-4 yo (ages balanced across treatments) were placed in single-sex enriched and non-enriched housing (60.3 x 40.6 x 40.6 cm, n=8 birds per cage) for a 10-14 days acclimation period prior to the introduction of interactive objects to EE birds (Figure 1). Before transfer to experimental housing, birds were also banded with two leg bands on one leg, to provide unique color combinations for each bird and aid identification from a distance.

Juveniles (males n=15, females n=12) were transferred from the breeding flight cage (148.6 x 71.1 x 188.2 cm) to cages with the same EE or non-enriched housing setups as adults upon independence at 31.2 ± 1.6 days. As nestlings, they received their single leg band to allow identification of nestmates. After sexing and prior to transfer into experimental cages, juveniles also received their two-band color identifiers. Since in ZFs, sexually dimorphic plumage develops between 40-60 days posthatching (Leader & Nottebohm, 2006), I used the beginning of chest stripe development at approximately day 25 post-hatching to identify males and distribute sexes across treatments. Since some males developed stripes later than others, there were ultimately 13 females and 15 males total. Although I considered using genetic sexing, mixed sex housing seemed preferable to submiting newly fledged juveniles to a blood draw or feather

removal to gather DNA. The birds were transferred to the cages in four "batches" where individuals of similar ages were distributed into each treatment cage, balanced by sex, nestmates, and batch. Cages Enriched 1 and NonEnriched 1 contained the first two batches (the first brood) , and Cages Enriched 2 and NonEnriched 2 contained the next two (the second brood). Nestmates were identified in order to control for having been raised by the same parents, and genetic relatedness (Table 1). Cage itself was also accounted for, since juveniles had two cages of each treatment group. Cage represented the place that the birds were housed, which varied slightly by sex ratio and positioning in the room. Cage was not accounted for in statistics for adults, since each cage already represented a distinct sex X treatment group. All subjects were provided interactive objects on the same day, but due to asynchronous hatching, acclimation in medium cages prior to treatment varied (10-18 days, 13.2 ± 3.1 SD) (see Table 2).

To test whether the juveniles were appropriately balanced across sex, treatment, age of transfer, time to acclimate, and nestbox, I ran ANOVAs. Assumptions were met for all tests. For determining whether age of transfer was balanced across sex and treatment: treatment $F(1,22)=1.773$, $p=0.0469$; sex $F(1,22)=0.004$, $p=0.947$; treatment x sex $F(1,22)=1.296$, $p=0.267$. For acclimation time analysis: $F(1, 22)=0.123$, $p=0.729$; sex $F(1,22)=1.110$, $p=0.304$; treatment x sex $F(1,22)=0.242$, $p=0.628$. For nestbox analysis: treatment $F(1,22)=0.048$, $p=0.828$, sex $F(1,22)=1.151$, $p=0.295$, treatment x sex $F(1,2)=1.793$, $p=0.194$. These tests indicate that all cages were appropriately balanced for these variables, and so they do not need to be considered as covariates in further analysis.

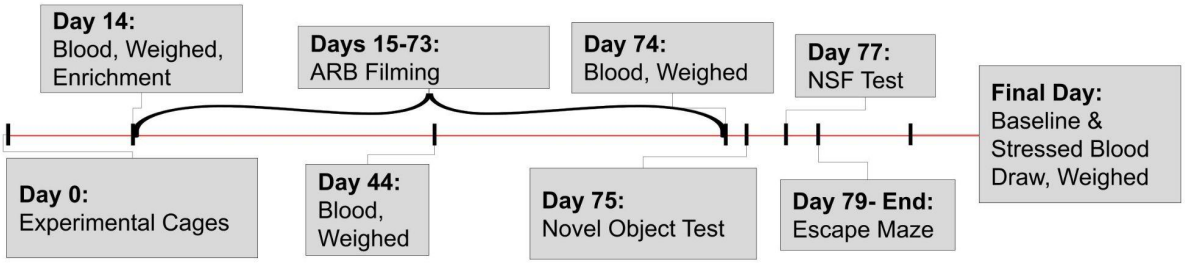


Figure 1. Timeline for adult and juvenile experiments. Birds were acclimated to experimental cages, enrichment objects were added on day 14, birds were weighed following blood draws. Behavioral tests are described in appropriate chapters. ARB = Abnormal Repetitive Behaviors. NSF=Novelty-Suppressed Feeding.

Table 1. Nestmates Distribution to Batches and Treatment to Each Experimental Cage

| Cages | Nest 2 | Nest 4 | Nest 9 | Nest 16 | Nest 18 | Nest 22 | Nest 25 | Nest 26 | Total |
|---|---|---|---|---|---|---|---|---|---|
| Enriched 1 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 0 | 7 |
| Non-Enriched 1 | 0 | 3 | 0 | 1 | 1 | 1 | 0 | 1 | 7 |
| Enriched 2 | 0 | 1 | 2 | 0 | 0 | 0 | 3 | 0 | 5 |
| Non-Enriched 2 | 1 | 1 | 1 | 0 | 0 | 0 | 4 | 0 | 7 |

Notes: Nestmates were distributed as equally as possible between pairs of cages of each treatment group.These were done in two sequential, but overlapping runs, with birds of batch 1 having similar ages to those of batch 2, and those of batch 3 with batch 4. Pairs of batches represented broods, with the subdivisions due to asynchronous hatching. Although we did not ascertain genetic parentage, birds hatched from the same nest likely came from the same parents, as offspring from extra-pair matings represent a minority of all offspring (Forstmeier et al., 2011), and couples tended to lay eggs in the same nest soon after the prior brood was independent.

Table 2. Distribution of Sexes, and Ranges of Days of Transfer and Acclimation per Cage

| Cage | # of Males | # of Females | Age Range of Day of Transfer | Days of Acclimation Range |
|---|---|---|---|---|
| Enriched 1 | 3 | 4 | 30-34 | 11-18 |
| Non-Enriched 1 | 3 | 4 | 30-38 | 10-18 |
| Enriched 2 | 3 | 3 | 30-31 | 11-15 |
| Non-Enriched 2 | 4 | 3 | 30-31 | 11-15 |

Notes: Sex, age, and times of acclimation were as equal as possible across cages.

**Interactive Objects**

Novel object provisioning was chosen for EE since the ZFs in this laboratory already have access to foraging opportunities (searching through seed on the floor lining) and social engagement (through social housing); both of which are common suggestions for enrichment in captive animals. The chosen enrichment items were olympic rings (JW Pet Activitoys Olympic Rings, 30 x 5 cm, with bell removed for safety reasons), a wooden swinging perch suspended with twine (17 cm wide), a stainless steel pipe bell (USQY Bird Toy Bell, 9 x 1.8 cm), natural perches (You & Me Bird Manzanita Wood Multi-Branch Bird Perch), and paper pet bedding (Healthy Pet Natural Paper Small Pet Bedding) (Figure 2). I chose not to provide food enrichment to avoid confounding effects of nutritional changes. In a pilot study, I filmed a cage of seven birds presented with a wicker rattan ball, the stainless steel bell, olympic rings, a swinging perch, a toilet paper roll, and nesting material. Birds interacted with or sat in close range with all objects except for the ball and toilet paper roll, and so the other objects were chosen.

ZFs were exposed to their distinct housing conditions 14 days for adults, and 10-18 days for juveniles (13.2 ± 3.1 SD, with variation due to asynchronous hatching) prior to introduction of enrichment to the treatment group. Two months was chosen as the duration of exposure since it is an intermediate of the two timespans tested by Fairhurst et al. (2011), in which they found that Clark's nutcrackers exposed to short term enrichment (10 days) experienced more stress than those with long-term exposure (92 days). In zebra finches, one month of restoration of bath water was enough to restore CORT levels to the prior state (Krause & Ruploh, 2006), demonstrating that this timeframe is sufficient to see baseline changes in response to enrichment.

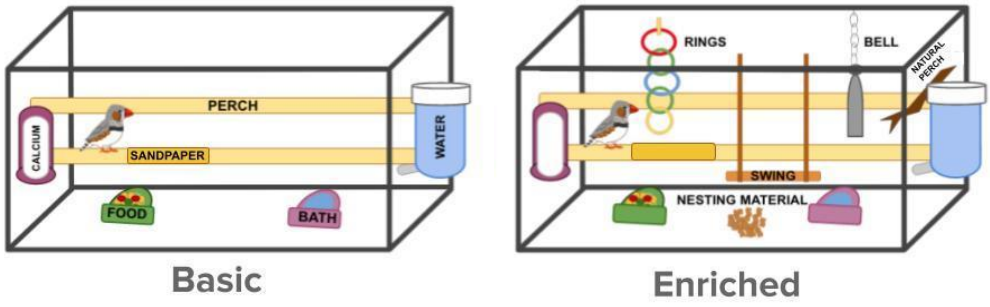


Figure 2. Comparison of non-enriched vs enriched cage set-ups. The basic cage contained a cuttlebone in a holder, basic wooden perches, food and bath bowls, and a water bottle. The "enriched" cage contains a natural perch, nesting material, olympic rings, a swing, and a bell. The latter three objects were completely novel. Each cage housed up to 8 birds each.

**Statistical Analysis**

All statistics were performed using JASP (Version 0.16.1) or SPSS Statistics (IBM, Version 27) depending on ease of running particular tests in each program. Alpha was set at $p \leq 0.05$. Appropriate effect sizes, eta squared ($\eta^2$), Cohen's D (d), Spearman's Rho ($\rho$), Pearson's R (r), etc. are provided. Data samples and residual errors were checked for distribution normality using Shapiro-Wilk tests, and Q-Q and residual plots; respectively. Data was transformed to improve normality if needed. All proportion data was angular transformed (arcsin (square root)). Sphericity was tested with Mauchly's sphericity test and violations corrected for using Greenhouse-Geisser or Huynh-Feldt (if epsilon was ~0.75 or greater) adjusted degrees of freedom. Homogeneity of group variance was tested with Levene's. ANOVA and other general linear models are robust to violations of normality and, compared to non-parametric tests, have greater power with continuous variables and are necessary to identify factor interactions. Thus, I typically report the parametric analyses, but back these up with the appropriate nonparametric

tests when assumptions can not be met. Sequential Bonferroni or Holms (repeated) were used for family wise alpha correction for post-hoc tests.

# CHAPTER 1: ABNORMAL REPETITIVE BEHAVIORS

## Introduction

Abnormal repetitive behaviors (ARBs) are defined as any repetitive and situation-inappropriate behavior that does not vary in either motor pattern or goal (Garner, 2005). Stereotypies are said to occur when these behaviors are inflexible in their motor sequence, while compulsive behaviors are said to occur when the motor sequence may differ, but the goal motivating it is the same. A well-known ARB observed in birds is feather plucking, although this is the most common among parrots. Other possible ARBs that can develop in birds, include spot picking (repeated pecking of spots on oneself, or something in the environment), or route tracing (pacing behavior, either on the floor, or hopping from perch to perch). Yamahachi et al. (2017) notes that in ZF in their laboratory, they have not observed self-feather plucking, or spot picking. However, they do have anecdotal observations of ZF exhibiting route tracing. The behaviors I counted as ARBs are based upon these possible behaviors and my own observations as further described in the methods.

I anticipate that birds with enrichment will spend a lower proportion of their time engaging in ARBs. I also expect that juveniles will differ more between treatment groups than adults, as young animals tend to be more behaviorally plastic than older animals, and males may spend a lower proportion of their time engaging in ARBs with enrichment than without, compared to females, since males tend to have greater stress-induced mortality than females (Jimeno et al., 2018

**Methods**

Abnormal repetitive behaviors (ARBs) counted were; repetitive movement from place-to-place, spot picking, self-plucking, and other-plucking. I defined repetitive movement from place-to-place as a bird moving between the two or more perchable surfaces at least 3 times. I defined spot picking as pecking at the same location on a surface 3 or more times unrelated to typical consummatory or nesting building behaviors. I differentiated self-plucking from normal grooming when it resulted in a visible bald spot. I counted instances of other-plucking unless the plucker was acting in the context of being attacked. Individual birds were identified via a sequence of two colored bands. Only behaviors in which the identity of the birds can be confidently established were included in the analysis.

All cages were video recorded simultaneously (camera, Sony HDR-XR100 or Canon VIXIA HF M52). Filming took place over a 56 day period with daily recording times and durations randomized by practical activities occurring in the aviary, such as feeding, cleaning, and other experimental procedures

For adults the average filming time began at 11:00 +/- 2 hrs. Focal observations were performed for the first 5 mins of each of three hours for each day with the following constraints: observations took place prior to or an hour after daily husbandry, sessions were excluded if all of birds' bands could not be identified, and days on which blood draws or other disruptive activities occurred. This sampling resulted in 36 days of footage with 33 days having the full 15 minutes of focal sampling and the remaining 3 having 10 minutes of sampling for a total of 8 hrs and 25 min of observations. The frequency of ARBs were relatively low, with some birds in each group performing no ARBs. Thus, all ARBs were summed and the proportion of time spent engaging in ARBs for daily observation times was calculated. To further condense the 36 days of focal

observations, I averaged across 12 day “timeframes” to equally represent early, middle, and late periods across the full 56 days ARBs were recorded .

For juveniles, cages were filmed simultaneously but in two separate cohorts (batch 1 and 2 and batch 3 and 4) given that birds were added to the experiment as they reached the appropriate age. For all juveniles, the majority of filming took place between 13:48 +/- 2 hrs 9 min. Sampling strategies for focal observations were the same for adults over the same 56 days duration of filming. With the constraints of excess human activity, inability to identify any birds, or behavioral or blood tests occuring within the cages, even if not all individuals in the cages were being sampled, there were 18 excluded days for batches 1 and 2, and 17 for batches 3 and 4. Five of these days had only 10 minutes of focal sampling. Across the overall 48 days retained, the daily proportion of ARBs/time observed were averaged across 16 day timeframes (early, middle, and late) to provide a total of 11 hrs and 55 min of observation for batches 1 and 2 and 11 hrs and 40 min for batches 3 and 4.

Subjects

For adults, there were 7 enriched and 7 non-enriched females, and 8 enriched and 8 non-enriched males used for analysis. This includes one enriched male who had died part-way through the two-month observation period, but was included in the analysis due to having been present for most of this period. For juveniles, there were 7 enriched and 7 non-enriched females, and 6 enriched and 7 non-enriched males used for analysis. This includes 2 enriched and 2 non-enriched males who were moved from the experiment due to fighting.

**Results**

Adults

Despite condensing data and performing an angular transformation for proportions, ARBs were still rare enough that data was skewed towards responses of zero for each time frame. However, one bird was hyper-responsive and excluded as an outlier (< interquartile range x 1.5 and < 3 S.D from mean). Even after excluding this outlier, inflated zero responses along with relatively high performance of ARBs by some individuals resulted in a skewed-distribution (Shapiro-Wilk p values all <0.001), and large and uneven variation within and between groups (Mauchly's W, p<0.001; Levene's for timeframe 1, p=0.001 and 3, p=0.006).

To examine interaction effects, I ran a repeated measures ANOVA (timeframe x sex x treatment). ARBs increased over timeframes ($F(1.2, 2.9)=4.44$, $p=0.04$, $\eta 2=0.07$) with timeframe 1 having significantly lower ARBs than timeframe 2 ($t(23)=-0.025$, $p=0.02$) and timeframe 3 ($t(23)=-0.52$, $p=0.005$) without increases between timeframe 2 and 3 ($t(23)=-0.26$, $p=0.26$). No other main effects or interactions were significant. These ANOVA results were supported by a Friedman test, verifying timeframe differences with marginal significance ($\chi^2$ (2) = 5.73, p=0.057), and a lack of differences between sexes or treatments for each timeframe and overall (Mann-Whitney U, ps range 0.28-0.81).

Juveniles

After angular transformation, data met the assumptions for a normal distribution (p=0.43, Shapiro-Wilk test) and sphericity (p=0.82, Mauchly's sphericity test). Although there was a violation of the assumption of equality of variance in timeframe 2 (Levene's test p=0.004), the data did meet the assumption of sphericity, and I proceeded analysis with a repeated

measures ANOVA. It revealed that enriched birds spent a lesser proportion of time engaging in ARBs than non-enriched birds regardless of sex ($F(1)=4.905$, 0.037, $\eta 2=0.089$).

**Interpretation**

Adults

The increase in ARBs over time is not unexpected, as the adult birds had previously been housed in larger aviaries, and were accustomed to having more space to move. The smaller space of the experimental cages may have been considered sufficiently stressful for the interactive objects to not be effective at preventing the development or worsening of ARBs. Alternatively, what I counted as ARBs may not have been manifestations of stress, and instead were products of a stronger drive for exercise as birds gained weight (see Bodymass Measurements section below) from the reduced space for activities in the smaller cages. This is consistent with my observations of bodyweight fluctuations discussed later in this thesis.

Juveniles

As ARBs take time to develop, introduction of enrichment at a young age may have prevented these behaviors from forming and causing permanent developmental changes in the brain (Garner, 2005). Environments in which animals are unable to exhibit natural behaviors, or are unable to at least partially predict or control stimuli can lead to stress (Watters, 2009). Chronic stress can lead to the development of ARBs, immunoinsufficiency, disrupted feeding and grooming behavior, and abnormal reactions to stimuli (Garner, 2005). By providing EE, the juveniles may have had a greater sense of control over their environment (Coleman & Novak, 2017), preventing the development of ARBs.

The differences in behavior between enriched and non-enriched birds may be greater if the non-enriched cages were even less stimulating, since smaller and less complex cages are associated with more anxiety indicators (An et al., 2021; Kitchen & Martin, 1996).

Examples of ways I had observed non-enriched birds "making their own fun" were by tearing off the cardboard lining the cage bottoms, pulling off their perch covers, and grabbing nesting material that had fallen from the enriched cages to carry around and build nests with. Birds regularly preened each other and mated in both the single and mixed sex cages. As ZF are a highly social species (Elie et al., 2015), the presence of other ZF may have been sufficient to meet most of their stimulation needs, making the enriched cages less of a reprieve from impoverished conditions, and more of an enhancement of acceptable conditions.

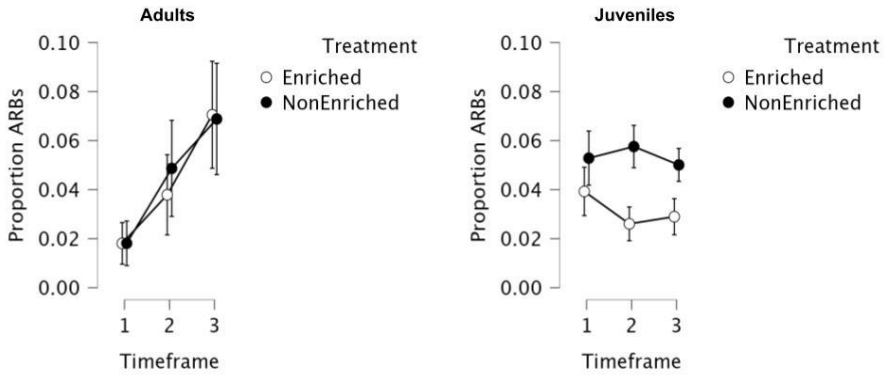


Figure 3. Adult and juvenile abnormal repetitive behavior results. The left graph shows the average proportion of time adults engaged in ARBs in both treatment groups with the sexes combined. This proportion increased over time. The right graph shows the deviation in the proportion of time engaging in ARBs in juveniles, in which enriched birds spent less time doing these behaviors, regardless of sex.. The values on the y axis are arcsine transformed. Error bars represent +/- 1 SE.

# CHAPTER 2: NEOPHOBIA MEASUREMENTS

## PART 1: Novel Object Test

### Introduction

Neophobia is the fear of new objects and environments. Chronic stress can have varying impacts upon neophobia, depending on species and individuals' behavioral tendencies (Bruijn & Romero, 2019). Considering this, it is important to test each species in more than one way to understand how their stress status affects their behavior, which can be used to test whether enrichment interventions are beneficial. There are varying measures of neophobia when presented with novelty. In the case of birds, this can include latency to approach an unknown object, latency to peck it, and how many times these actions are performed (Kulke et al., 2021). To evaluate the overall stress state of an animal, it is important to evaluate a variety of measures, since CORT levels themselves do not necessarily predict the response to the degree of neophobia an animal may exhibit (Bruijn & Romero, 2019).

### Methods

Subjects

For adults, there were 7 enriched and 6 non-enriched females, and 7 enriched and 8 non-enriched males used for analysis. An enriched male had died prior to this test. For juveniles, there were 7 enriched and 7 non-enriched females, and 4 enriched and 5 non-enriched males used. This did not include the 2 enriched and 2 non-enriched males who were removed prior

Materials

The arenas for the neophobia test consisted of experimental cages (30.5 x 16.5 x 15.2 cm) with a small perch placed 2 cm from one end of the cage. There were two pieces of red tape on the top to mark the midpoint of the longest dimension of the cage. Four of these cages were set side-by-side within the aviary, and visually isolated from each other with a barrier. The experimental birds not currently being tested were prevented from seeing the arenas with a black cloth over their cages. The tests took place within the aviary to isolate the effect of a novel object, and not their response to a less common environment. Preliminary tests of non-experimental birds showed that the birds tended to freeze for extended periods of time when the test was conducted in a less familiar environment.

For adults, one bird from each cage was tested at a time, with the individuals chosen being predetermined by a randomly ordered list. For juveniles, birds from each eligible cage were distributed as evenly as possible, with the individuals chosen also predetermined by a randomly ordered list. The birds were placed in the dark into the cages, and after I left the room and turned the lights on, were allowed to acclimate for 3 min. After this, I turned off the lights, placed the object in each cage, and gently pushed the birds to the side opposite of the object. This object was a white clothespin wrapped in red tape, with black drawn-on eyes and two small cuts of artificial blue feather projecting from the top (Figure 4). Following placement, I initiated filming (with a Sony HDR-XR100 or Canon VIXIA HF M52), left the room, and turned on the lights for a trial length of 5 min. After the trial, I turned off the lights to enter the aviary, and transferred all birds into temporary group cages until each batch of birds were completed.

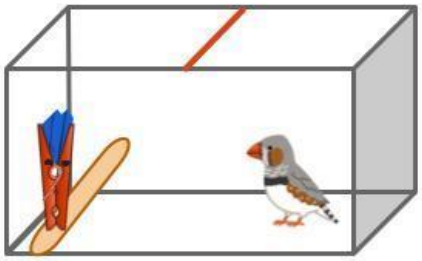

Figure 4. Novel Object Test. In the Novel Object Test, the object is placed on the side of the perch, and upon turning on the lights, the birds are filmed for 5 minutes to monitor activity and interactions with the object.

Statistical Analysis

In this test, I measured the latency to cross the midline towards the object, latency to perch, latency to peck the object, number of times crossing the midline (towards the object, and in total), number of times perching, and number of times pecking the object. These variables were analyzed with Kruskal-Wallis tests when they violated the assumption of normality.

**Results**

Adults

Latency to cross the midline and latency to perch is highly correlated (Pearson's $r=-0.40$, $p=0.035$), as was the number of times crossed and the number of times perched (Pearson's $r=0.994$, $p<.0001$). Therefore, only latency to cross and the number of times perched were used for further analysis. Enriched birds perched more times ($H(1)=7.95$, $p=0.005$) than non-enriched birds regardless of sex. Females of either treatment had a lower latency to cross the line than males of either treatment ($H(1)=4.50$, $p=0.034$). Since birds touched the object too few times to be analyzed statistically, it implies that this test was more of a measure of hyperactivity or exploration than a measure of neophobia.

Juveniles

All these variables except for the latency to touch the object and the number of times touching it were highly correlated with each other (p value between <.001 and .007), since birds who tended to rapidly initiate movement towards the midline also tended to perch whenever they crossed towards the side with the perch, and rapidly repeat this behavior. Thus; only Times Perched, and Latency to Cross the Midline were included for further analysis. Touching the object happened to infrequently to be analyzed, so latency to touch and the number of times touching the object were also not included. There was a significant effect of sex with females having a lesser latency to cross the line towards the object, ($H(1)=7.527$, $p=.006$) and a higher number of times perched ($H(1)=5.565$, $p=.018$), regardless of treatment.

**Interpretation**

Adults

Since they moved back and forth more times than non-enriched birds, enriched birds were either hyperactive or more exploratory than non-enriched birds, assuming that non-enriched birds represented the "baseline" behavior, or non-enriched birds had these behaviors or motivations depressed lower than the "baseline" of what would be expected of random birds chosen from a non-experimental aviary. This could mean that the smaller experimental cages, or the reduced stimulation compared to normal aviaries led to more stress, despite assumptions that the experimental cages were simply a "scaled-down" version of their normal living conditions. Although it occurred too infrequently to analyze statistically, it is of note that the only birds that touched the novel object were enriched males. Females approaching the midline sooner may

have been a carry-over from this effect present in the juvenile females, as described below.

Juveniles

Juvenile females may have perched more times, and had a lower latency to perch than males if the females had a different coping style (see Discussion section) than males in the face of stress. This may have occurred if coping style can change over time with sex hormones.

The near-total absence of interacting with or paying attention to the object, again, implies that this test was more of a measure of hyperactivity and exploratory behavior than a measure of neophobia. It is possible that, for juveniles, there is no effect of treatment since all birds did not have as much as a frame-of-reference of what larger, more stimulating housing conditions could be like since they were all transferred to experimental cages shortly after fledging.

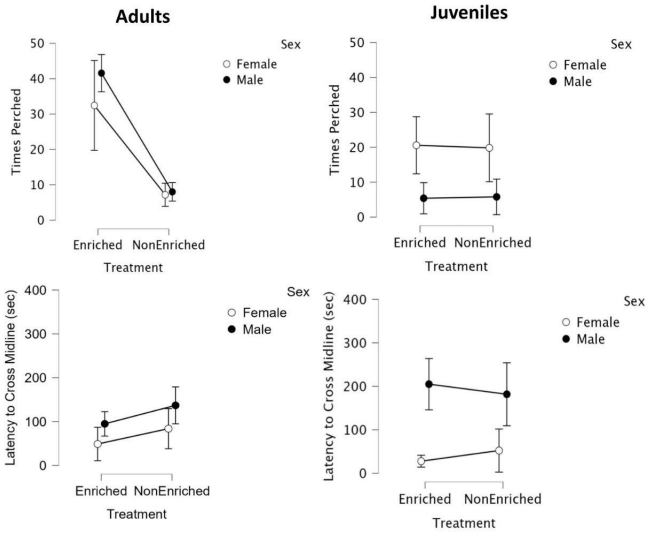


Figure 5. Novel Object Test results. In adults, as seen in the top left graph, enriched birds perched more times regardless of sex. The graph below shows how females of both treatments had a lesser latency to cross the midline than males. The top right graph demonstrates how, regardless of treatment, juvenile females perched more times over the course of 5 minutes than males, and the bottom right graph shows how juvenile females also had a lesser latency to cross than males, regardless of treatment. Error bars represent +/- 1 SE.

## PART 2: Novelty-Suppressed Feeding Test

### Introduction

The Novelty-Suppressed Feeding Test, or Hyponeophagia Test (Samuels & Hen, 2011), has long been used as an indicator of an anxious or depressed state in laboratory animals. Hyponeophagia is the increased latency to eat observed in anxious or depressed animals compared to controls following a fast. Unlike the Novel Object Test, this is not influenced as much by birds' tendency to explore. In the Hyponeophagia Test, feeding motivation is expected to be similar among animals, so fearfulness or depressed behavior can be isolated as a function of affective state and used as an indicator of welfare.

### Methods

Subjects

For adults, there were 7 enriched and 7 non-enriched females, and 7 enriched and 8 non-enriched males used for analysis. This did not include the one enriched male who had died prior to the test. For juveniles, there were 7 enriched and 7 non-enriched females, and 4 enriched and 5 non-enriched males used for analysis. This did not include the 2 enriched and 2 non-enriched males removed due to fighting.

Materials

The experimental cage (76.8 x 36.2 x 40.6 cm) was placed in a less familiar environment outside of the birds' aviary room. The cage had a divider in the middle which had a piece of string attached to the side. The first two groups of birds (enriched females and non-enriched males) had the food dishes removed from their cages and their cage bottoms changed out (to remove residual seed) the night prior, after the birds had stopped eating for the day. They were tested two hours after the lights turned on (7:30 AM), with the shortest time fasted being 2 hours

after waking, and the longest being 4 hours due to limitations in the number of birds that can be tested at a time. The food and old cage liners were removed from the other two cages two hours prior to their testing period during the day. For each test, two birds (pre-determined from a randomized list within each cage) were placed in two separate experimental arenas in the dark. If there was an uneven number of birds to be tested, then a random non-experimental bird was chosen as a "filler" to ensure no birds were unpaired. A piece of cardboard separated the arenas from each other visually. The lights were turned on and they were allowed to acclimate for 3 min. I then turned off the lights, turned on the camera, turned on the lights as I left, and simultaneously opened the barrier to both experimental cages with strings passed under a door with a one-way window, revealing a cup of food on the other end of each cage (Figure 6). The birds were given up to 10 minutes to eat the food. The trial ended either when both birds ate or when 10 minutes had passed. They were then placed in temporary cages in another room while other trials were ongoing. After all trials were completed, all birds were returned to their home aviary.

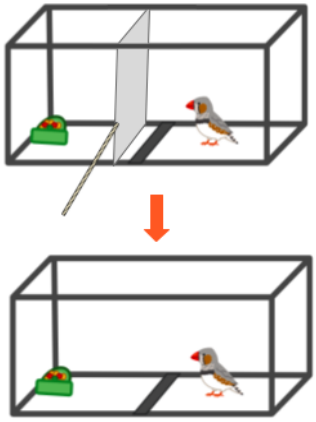

Figure 6. Hyponeophagia test. Following acclimation, the divider between the bird and the food is removed using a string. The trials end when either both birds have eaten, or 10 minutes has passed. Error bars represent +/- 1 SE.

Statistical Analysis

Since the data was non-normal, Kruskal-Wallis tests were used for analysis.

**Results**

Adults

There was no correlation between time fasted and the latency to eat (Pearson's r=0.226, p=.239), so time fasted was not used for further analysis. Analysis with a Kruskal-Wallis test revealed that there was no significant effect of sex (H(1)=0.9744, p=0.324) or treatment (H(1)=2.406, p=0.121) on latency to eat.

Juveniles

There was no correlation between the amount of time fasted prior to the trial, and the latency to eat (Pearson's r=0.1038, p=0.637), so this variable was not used in analysis. Data violated the normality assumption and the equality of variances assumption, so a Kruskal-Wallis Test was used. Enriched birds had a significantly lower latency to eat than non-enriched birds (H(1)=6.54, p=0.011), regardless of sex.

**Interpretation**

Counterintuitively, only 21.7% of juveniles ate within 10 minutes, while 45.2% of adults did. It is possible that since the juveniles had reached adult size and were no longer rapidly growing at the time of testing, the fear of the testing environment was stronger than the need to eat. Since adult birds had previously been in the testing room more times than juveniles due to this being where the birds are moved to during aviary cleanings, adult birds may have been partially habituated to this environment. To better understand if the enrichment was altering birds' feeding behavior, it would be beneficial to compare the latency for individual birds to eat following the same period of fasting in their home cage versus the testing arena. This would illuminate whether the reluctance to feed is related to the stress of the new environment, or insufficient feeding motivation.

Despite research showing that short-term fasting (between 2-6 hours) is enough to increase plasma CORT, reduce testosterone levels, and alter courtship behavior and singing (Fokidis et al., 2013), this duration may not have been sufficient to produce feeding motivation great enough for any treatment or sex differences to appear in the form of hyponeophagia. This means that the treatment, if effective, is not effective enough to override neophobia if conflicting motivations are not strong enough to desire initiation of a behavior in the first place.

As only the enriched juveniles ate within the time allotted, enrichment may have reduced the birds' perception of my activities as a stressor, or made them better able to cope in the face of a novel situation.

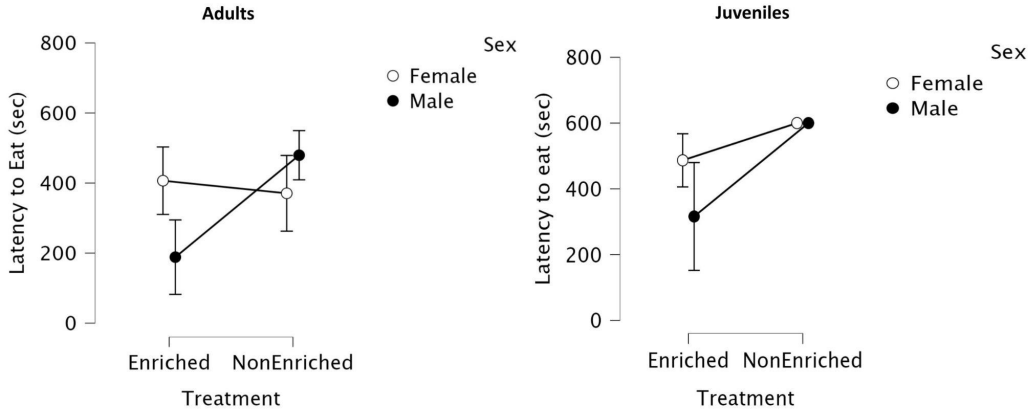


Figure 7. Hyponeophagia test results. As presented in the left graph, adult birds of both sexes and treatments ate within the time allotted. However, there were no significant differences. As seen in the right graph, in the juvenile Hyponeophagia Test only enriched birds ate the food provided within 10 minutes. Note that 600 seconds was the maximum time in testing, which was assigned to the birds which did not eat. Error bars represent +/- 1 SE.

# CHAPTER 3: ESCAPE MAZE

## Introduction

The Escape Maze (Figure 8) was developed in the Day Lab (Williams, 2014) to measure spatial learning and memory in small passerines. The Escape maze is a Morris water maze analog that uses mild heat to motivate escape from a clear cylinder. Unlike other adaptations (Mayer et al., 2013), food is not used as a reward. Thus, birds do not have to be pre-trained to search for food, deprived of food prior to testing, and the assumption that all birds have the same motivation to feed does not have to be applied. Additionally, no local cues can be used to locate the escape and spatial cue use can be easily identified using probe trials.

One can indirectly assess brain function and compare the effects of treatments on the long-term stress status of animals by evaluating spatial cognition. The hippocampus, the region of the brain vital for spatial cognition, is well-established to be negatively affected by chronic stress (Kim et al., 2015).

## Methods

### Subjects

For adults, 7 enriched and 7 non-enriched females, and 7 enriched and 6 non-enriched males were tested. This excluded three individuals whose identity I could not ascertain due to them having lost their leg bands. For juveniles, there were 7 enriched and 7 non-enriched females, and 4 enriched and 6 non-enriched males tested. This excluded 2 enriched and 1 non-enriched male removed due to bullying, a normal result of birds forming pecking orders in

group cages. Adults had previously been tested in a similar version of the maze that did not result in use of spatial visual cues by the majority of test subjects. This result was likely due to inadvertently having the maze, camera, and resting perches uncentered in the testing aviary. Birds appeared to use room geometry or auditory cues rather than 3 visual cues on the walls of the aviary. While, this is an interesting result in itself, to keep the focus on sex and treatment effects it will not be further reported here.

Materials

The testing arena is bordered by a clear plastic cylinder (30 cm in diameter), with a clear lid to prevent non-target escaping. The arena is held in place by a clear plexiglass tube (3 mm diameter) glued to a ceramic tile (30.5 cm x 30.5 cm) floor sitting on a hot plate, the tile absorbs and releases the heat to regulate the floor temperature ($\bar{X}$=50.1±1.8 °C). This temperature is sufficient to motivate escape in most birds without seeming to cause excessive stress (Dearman et al., 2019). This lack of excessive stress or pain is evident when, upon exiting the maze, some birds voluntarily stand on the hottest part of the floor to wait to be placed back in their cages, despite having access to perches. The side of the cylinder has a 5.5 cm diameter hole, cut 2.3 cm above the floor, through which the birds can escape. Birds are unable to locate this hole visually, initially tapping along the walls to learn its location (Hirbar, 2015). The Escape Maze is placed in the center of an aviary flight cage (148.6 x 71.1 x 188.2 cm) with black cloth blocking the birds' view of the testing room outside of the cage. Several visual cues of different colors and shapes made from poster board or craft foam (four cues of size range 19.0-20.3 cm L, 14- 22.9 cm W are attached to the black cloth so that approaching or avoiding any single cue will not lead to locating the escape hole. The quadrants of the maze are designated by artificial cardinal points NE, NW, SW, and SE, and behavior is tracked using Ethovision software (Noldus Information

Technology, Wageningen, The Netherlands) receiving input from a camera centered over the arena. The software quantified latency to escape (s), average velocity (cm/s), and total distance traveled across and within the quadrants (cm). Juvenile acquisition and probe trials were identical to that of adult trials.

Acquisition Trials

I tested the birds sequentially, with birds randomly selected for each grouping of up to 8 birds, under the constraint that each treatment group and sex was balanced. Group sizes were chosen to keep inter-test waiting periods for birds to no more than 30 minutes. Birds were transferred on a cart in individual carrying cages (30.5 x 16.5 x 15.2 cm) covered by a black cloth so as to minimize stress. I held them in a Bander's Grip (Lincoln, 1929), and tucked them in my lab coat pocket as I opened the lid of the maze, and placed the bird in either the N, S, W, or E cardinal direction of the arena (with the release location being pseudo-random, under the constraint that all release sites are used for each bird per day). They were placed facing towards or away from the center of the maze in a pre-randomized order.

Trials were monitored remotely and behavioral tracking starting as soon as the lights were turned on in the maze. Each trial lasted no longer than two minutes. If the bird did not escape by 120s, I manually guided them out through the escape hole and recorded their latency to escape as 120s. After escape, the bird rested in the aviary outside the maze for one minute on the inner walls of the aviary or one of two perches, which were 25 cm from the top of the aviary, projected out 17.9 cm, and were placed midway down the longest end of the aviary walls.

After this rest period, the lights were turned off again, and the bird was captured and returned to their holding cage (30.5 x 16.5 x 15.2 cm). All birds had four trials in the maze per day until the performance in one of the treatment groups leveled off in. This was the endpoint of

data collection for statistical comparisons between groups. Birds were given 2-3 more days of training to make sure the majority of birds had an opportunity to learn the escape location, so that comparisons of the strategy used to orient to the escape could be tested in "probe trails".

Probe Trials

On the last day of testing, I ran four acquisition trials to reinforce escape strategies prior to performing a fifth trial as a probe trial. On the fifth trial, the cues were turned 180$^{o}$ from their normal direction and the clear cylinder was swapped out for an identical one except that it had no exit.

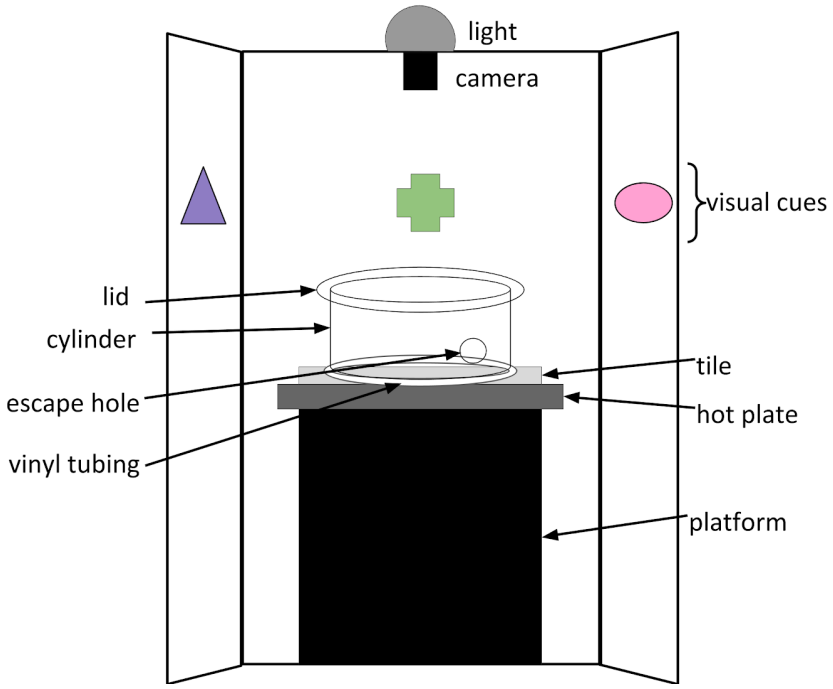


Figure 8. Escape Maze side view. Birds are placed in a clear cylinder, topped by a clear lid, with an escape hole cut on the side. Vinyl tubing encircles the cylinder to hold it in place. It is positioned on a ceramic tile heated by an underlying hot plate. This arena is centered in a flight aviary with visual cues of different shapes and colors attached to the walls, which allow the birds to learn the position of the escape hole relative to these cues. For the second version of this maze, there was a yellow star directly across from the green plus sign (not pictured), the hole was facing the NE direction instead of the SE, and the cues are bigger. Image used with permission from Chyna-Rae Dearman.

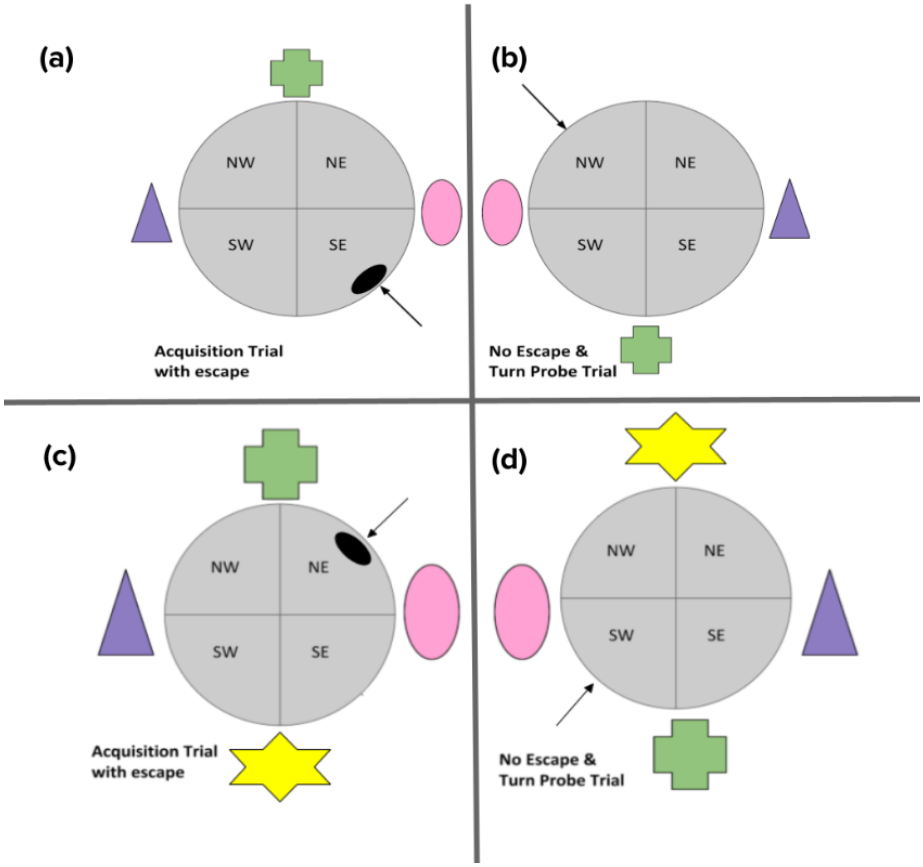


Figure 9. Escape Maze top view. (a) Represents a top view of the maze during the learning period, where there is an escape hole present. (b) Shows how the visual cues are rotated 180º during the probe trial, in which there is no escape hole. The expected goal location is represented by an arrow in both schematics. The bottom images are the top view of the maze during the second acquisition (c) and probe trial periods (d). Images modified with permission from Chyna-Rae Dearman.

Statistical Analysis

The dependent variables were latency to escape, distance traveled, and velocity. These variables were averaged across the four-daily trials. Learning in the escape maze is identified by a reduction in latency to escape and total distance traveled demonstrating efficient search patterns. Velocity measurements can reflect the same overall efficiency in escape path as latency and distance, but can also indicate differences in motivation or hyperactivity that might have an impact on how fast or how far birds travel to escape. To isolate the impact of velocity on latency

and distance the association between these variables was scatter plotted and evaluated. In juveniles and adult experiments, correlations of velocity with distance and latency were due only to the ceiling effect of birds assigned the maximum 120 seconds due to lack of escape which had over all velocities similar to other birds but high distance and latency because they did not escape. For birds that found the escape hole and exited prior to the maximum 120 seconds, velocity did not correlate with distance or latency. Thus, velocity was not examined as a covariate for distance or latency analyses. Each dependent measure was analysed using a three-way repeated measures ANOVAs (Days X Treatment X Sex). Dependent measures were log transformed to improve normality. For the probe trial, three-way repeated measures ANOVAs (Treatment X Sex X Quadrant) were used to compare the proportion of total distance covered and total time spent in the goal quadrant to the proportion of time and distance in the other quadrants of the maze. The visually cued quadrant was initial compared to each of the other three quadrants. If none of the other three was preferred over the goal quadrant, I report the comparison between the goal quadrant and an average of the other three quadrants. All of this proportion data was arcsine transformed.

**Results & Interpretation**

Adult Acquisition

There was a significant effect of sex in the latency to exit ($F(1,99)=4.131$, $p=0.045$), with females having a lesser latency to exit than males regardless of treatment. There was also an interaction effect between sex and treatment ($F(1,99)=39.880$, $p=.001$), with enrichment seeming to hinder female latency to exit, and the absence of enrichment enhancing it. The opposite was true of males. For distance moved, there was only an effect of sex X treatment ($F(1,99)=26.997$, $p<.001$), with the same trend as in the latency to exit interaction. For velocity, there is also an

effect of sex (F(1,99)=7.001, p=.009) and an effect of sex X treatment (F(1,99)=18.626, p<.001). Males had a lower velocity overall, although the difference in velocity between the sexes is only present between the non-enriched groups. When velocity is used as a covariate, only sex X treatment effect remains for latency to exit (F(1,98), p<.001) and distance moved (F(1,98)=13.306, p<.001), with enrichment hindering female performance and enhancing that of males.

Juvenile Acquisition

For latency to exit, there was an effect of sex (F(1,20)=6.196, p=0.022), with females exiting sooner regardless of treatment. For distance moved (F(1,20)=5.137, p=0.035), females also moved less within the maze, which along with the brief latency, indicates a more direct exit. Surprisingly, there was no difference in velocity depending on sex or treatment (treatment F(1,20)=2.143, p=0.159, sex F(1,20)=0.573, p=0.458, treatment X sex (F(1,20)=0.590, p=0.452)), meaning that the reduced latency to exit observed in the males is solely due to a more circuitous path to exiting.

Adult Probe Trials

For the probe trials, one enriched male was exhibiting stress during the trial and was removed (enriched females n= 7 non-enriched females n=7, and enriched males n=6, non-enriched males n=6). In the pre-probe acquisition trials prior, birds spent more time and traveled more distance in the escape quadrant than each of the other three (all $p_{holm}$ <0.003) so we averaged the three non-escape quadrants for all other analyses. Birds spent a greater proportion of their time (F(1, 21)=14.88, p<0.0009, $\eta^2$=0.35) and traveled a greater distance ((F(1, 21)=37.82, p<0.0001, $\eta^2$=0.57) in the current goal quadrant than the average of the other quadrants with some suggestive interactive effects in parametric tests that may not be valid due

to violations of heterogeneity between groups (Levene's test p=0.01-0.05) and neither sex nor treatment differences held up in non-parametric tests. A bias to the goal quadrant at this stage could actually indicate low learning as high accuracy would limit time and distance in the goal quadrant when escaping. Thus, performance on the turned cue trials is important for interpretation of use of visual cues.

Unlike acquisition trials when escape was possible, when escape was not possible in the probe trials, there was a significant linear correlation between velocity and both distance and duration. If birds moved faster, they ended up with higher proportions of time spent and distance covered in the escape quadrant simply because they moved more in the 120 sec probe trial than other birds. Thus, I used velocity as a covariate in analyses of distance and duration. Note that initial model checks that included interaction terms with the covariate, velocity, and sex and treatment were not significant. However, for duration the velocity x sex interaction was marginally significant (p=0.06), thus; the possibility that any sex differences in duration are counfounded by sex differences in velocity should be considered. Removing the covariate interaction terms, I found that overall, birds spent a greater proportion of time ($F(1,20)=16.15$, $p<0.007$) and distance (($F(1,20)=11.57$, $p<0.002$) searching for the escape in the visually cued quadrants than other quadrants indicating they were using the visual cues for escape. For both duration and distance, there were a number of marginally significant effects suggesting differential performance of the sexes and effects of treatment on the sexes (Duration: sex p=0.08, quadrant x sex p=0.07; Distance: quadrant x sex p=0.06, sex p=0.06). Only for the proportion of distance spent searching in the visually cued quadrant did this conclusively show that enriched females were similar to males in there spatial cognition while non-enriched females did not have a preference for the visually cued escape quadrant ($F(1,20)=4.85$, $p=0.04$). While sex differences

in spatial ability in zebra finches would not be expected based on ecological factors, several studies have shown that both adult and elderly female zebra finches appear to have less robust spatial cognition than males. In this context, it would appear that EE allowed adult females to have spatial cognition that was on par with males.

Juveniles Probe Trials

With the normal cues, there was a significant effect of quadrant, with birds spending a greater proportion of their time in the goal quadrant ($t=5.317$, $p_{holm}<.001$), and traveled a greater distance in the current goal quadrant than the arcsine average of the other quadrants ($t=8.855$, $p_{holm}<.001$). The significance of quadrant does not change with velocity as a covariate.

For the turned cues, there was a significant effect of quadrant, with birds spending a greater proportion of their time in the current goal quadrant ($t=9.959$, $p_{holm}<.001$), and traveled a greater distance in the current goal quadrant than the arcsine average of the other quadrants ($t=4.192$, $p_{holm}<.001$). Again, including velocity does not change the significance of any results.

The absence of a sex X treatment effect as was seen in adults may indicate that the disparitiy in performance between enriched and non-enriched females is something that takes time to develop, so any "rescuing effect" that enrichment may have on female performance is not evident at the age that the birds were tested. Kosarussavadi et al. (2017) observed in their tests of spatial cognition among different ages and sexes of ZF that older females showed slower gains in accuracy in spatial learning than young females. They noted that this sex difference in spatial cognition or motivation is not observed in young birds, and that it may result from estrogen's putative effect upon hippocampal excitability, or known sex differences in the strategies individuals use to solve spatial tasks that are influenced by sex hormones. Since the sex differences do not arise until later in life, all of the juvenile ZF may have been using a spatial

strategy that is not optimal for learning the escape maze, with a better strategy only developing in males later.

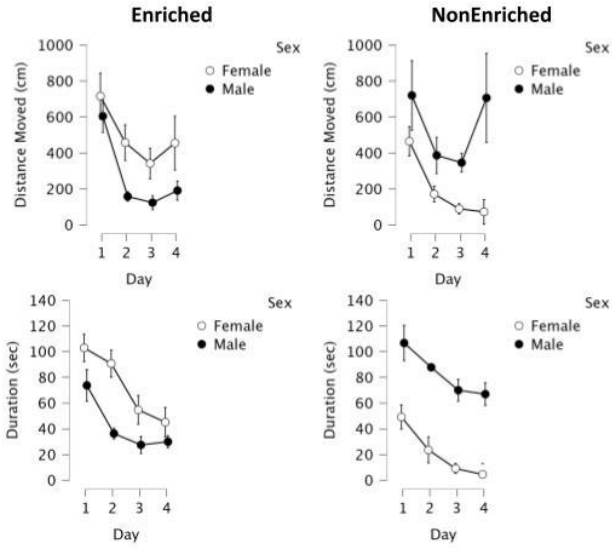


Figure 10. Adults second attempt acquisition results. The top graphs show the interaction effect between sex and enrichment, with the left graph demonstrating the increase in distance traveled by females in the enriched group, and the right graph showing the increased movement by males in the non-enriched group. This pattern is mirrored in the bottom graphs which showed the latencies to exit. During spatial memory acquisition, female performance seems to be hindered with enrichment. Error bars represent +/- 1 SE.

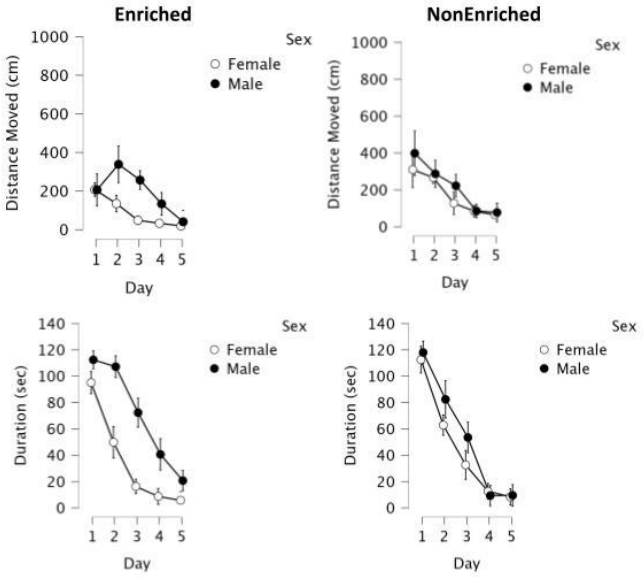


Figure 11. Juvenile acquisition results. The top graphs demonstrate how enriched females moved more directly than enriched males, while the non-enriched birds of both sexes had similar distances. In the bottom graphs, it shows how the enriched females also had a lesser latency to exit than males, while the non-enriched birds had similar latencies. Error bars represent +/- 1 SE.

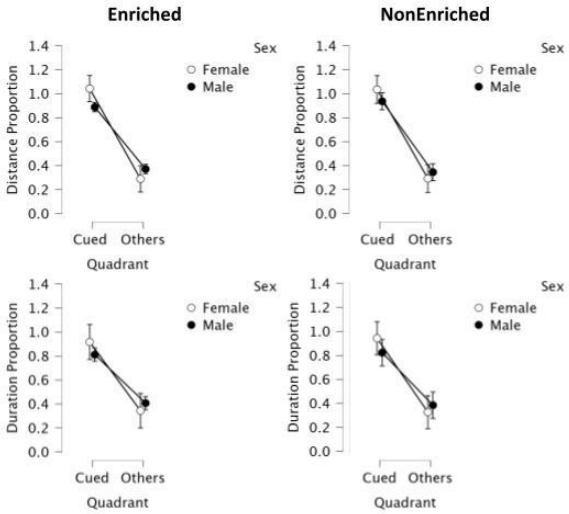


Figure 12. Adult second attempt probe trial normal cues. These trials were identical to acquisition trials, but served to provide a point of comparison against the turned cue trials. Birds traveled more distance and spent more time in the appropriate quadrant than the average of the others, demonstrating that they have learned the maze sufficiently to be tested with turned cues. Error bars represent +/- 1 SE.

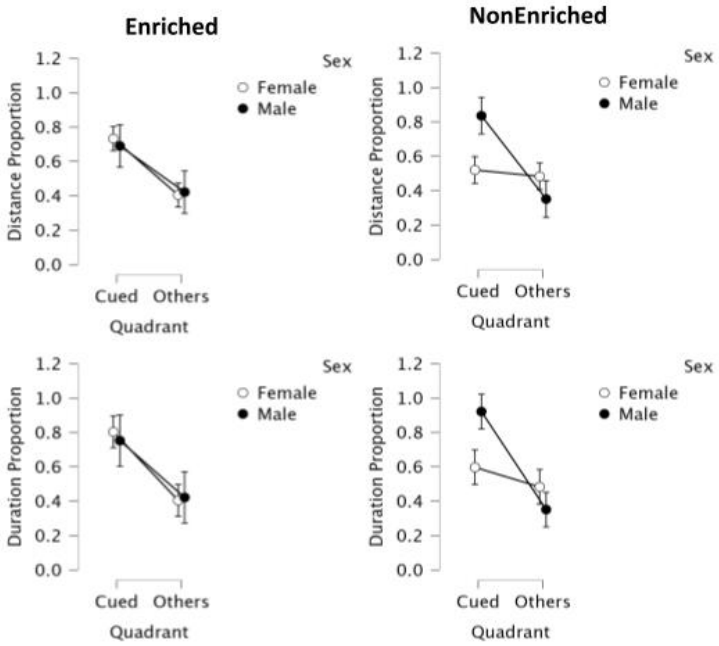


Figure 13. Adult second attempt probe trial turned cues. Among enriched birds, there is no sex difference in the distance traveled and the time spent in the new goal quadrant. However, among non-enriched birds, males identified the new goal quadrant more successfully while females did not. In adults, enrichment seems to allow females to reach male performance in spatial cognition and identify the correction goal quadrant. Error bars represent +/- 1 SE.

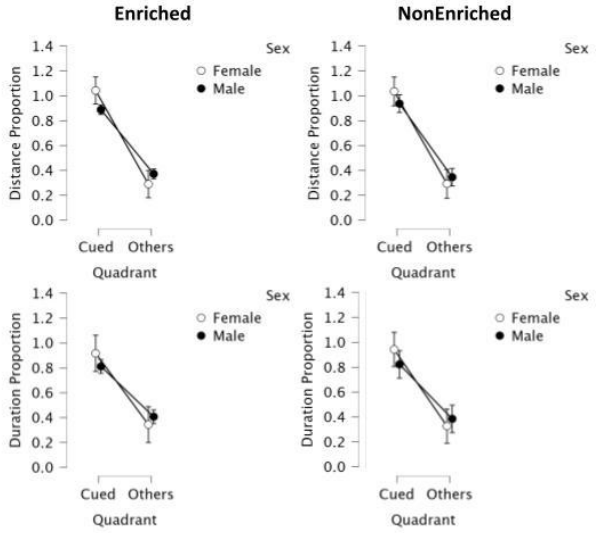


Figure 14. Juvenile probe trial normal cues. These trials were identical to acquisition trials, but served to provide a point of comparison against the turned cue trials. Birds traveled more distance and spent more time in the appropriate quadrant than the average of the others, demonstrating that they have learned the maze sufficiently to be tested with turned cues. Error bars represent +/- 1 SE.

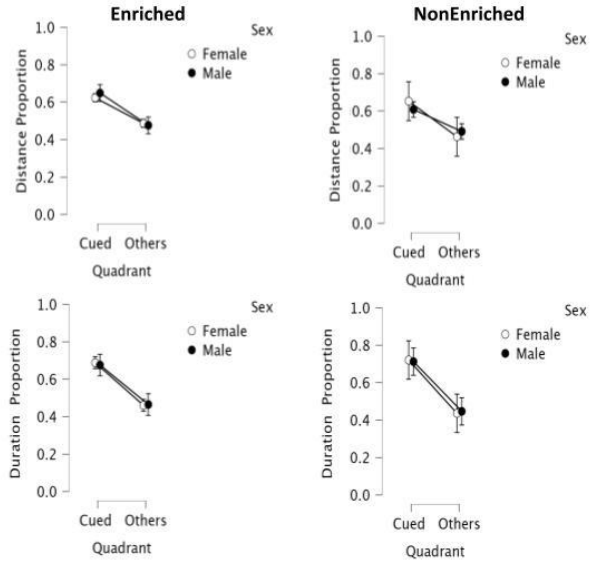


Figure 15. Juvenile probe trial turned cues. Birds traveled more distance in, and spent more time in the correct goal quadrant, regardless of sex or treatment. Error bars represent +/- 1 SE.

# CHAPTER 4: MEASURES OF STRESS PHYSIOLOGY

## PART 1: Body Mass Measurements

### Introduction

Body mass is correlated with baseline and reactive CORT in a variety of avian species and can indicate reactivity to current or prior environmental and social conditions in wild and captive birds (Schoech et al. 1997; reviewed in Wingfield et al., 1995). The effects that stress can have on body mass can vary depending on species and source of stress, but in animals, generally, stress is associated with weight loss (Harris, 2015).

Thus, I predicted that birds without EE, compared to those with EE, would have lower body mass as an indicator that environmental deprivation impacts metabolism, activity level, or food consumption. Body mass can be influenced by factors other than stress and high CORT can have counterintuitive effects on body mass, such as inducing hyperphagia and weight gain in migrating birds (Löhmus et al. 2003), therefore; changes in body mass should be interpreted in light of other physiological, behavioral, and cognitive measures.

### Methods

Birds were weighed to within 0.25 g using a Pesola spring scale (30g ±0.3%) following blood draws on day 14 (first enrichment day), 44, 74, and upon completion of the experiment (see Figure 1 for timeline).

Statistical Analysis

Change in mass was calculated by subtracting each individual's starting mass from the other measures providing three relative mass timepoints for analysis. Measures of mass were compared using a repeated measures ANOVA (Sex X Treatment X Timepoint) after confirming the data met all assumptions for this test.

**Results**

Adults

For adults, there were 7 enriched and 7 non-enriched females, and 8 enriched and 8 non-enriched males used for analysis. Unexpectedly, enriched birds had a lower body mass than non-enriched birds overall ($F(1,24)=5.04$, $p=0.034$, $\eta^2=0.08$; Figure 17). Planned contrasts showed significant differences only at timepoint 2 ($F(1, 24) = 8.50$, $p = 0.008$) and there was a trend towards the enriched birds losing mass over time, and non-enriched birds gaining mass ($F(2,48)=3.01$, $p=0.06$, $\eta^2=0.05$; Figure 17) though they had similar changes in mass by timepoint 3. There were no other significant main effects (sex $F(1,24)=1.05$, $p=0.32$, $\eta^2=0.02$; timepoint $F(2,48)=0.01$, $p=0.99$, $\eta^2<0.001$) or interactions (treatment x sex $F(1,24)=0.29$, $p=0.60$, $\eta^2=0.01$; timepoint x sex $F(2,48)=1.52$, $p=0.23$, $\eta^2=0.026$; or timepoint X treatment X sex $F(2,48)=0.1185$, $p=0.89$, $\eta^2=0.002$).

Juveniles

For juveniles, there were 7 enriched and 7 non-enriched females, and 6 enriched and 7 non-enriched males used for analysis. As seen in Figure 17, birds in both treatment groups initially gained mass and then lost mass ($F(2,36)=5.86$, $p=0.006$, $\eta^2= 0.33$) with a significant increase from timepoint 1 to timepoint 2 ($t(1)=2.75$, $p<0.02$) and decrease from timepoint 2 to 3 ($t(1)=3.14$, $p<0.01$). As for adults, it was the non-enriched birds that weighed more, although

treatment differences did not quite reach significance ($F(1,18)=2.70$, $p=0.12$, $\eta^2= 0.11$). There were no main effects of sex ($F(1,18)=0.50$, $p=0.49$, $\eta^2=0.02$) or interactions of sex with time ($F(2,36)=1.13$, $p=0.33$, $\eta^2=0.064$) or treatment $F(1,18)=0.25$, $p=0.62$, $\eta^2= 0.01$). Differences across time were not influenced by treatment ($F(2,36)=0.111$, $p=0.90$, $\eta^2=0.001$) nor was there a three way interaction of time, sex, and treatment ($F(2,36)=0.90$, $p=0.42$, $\eta^2=0.005$).

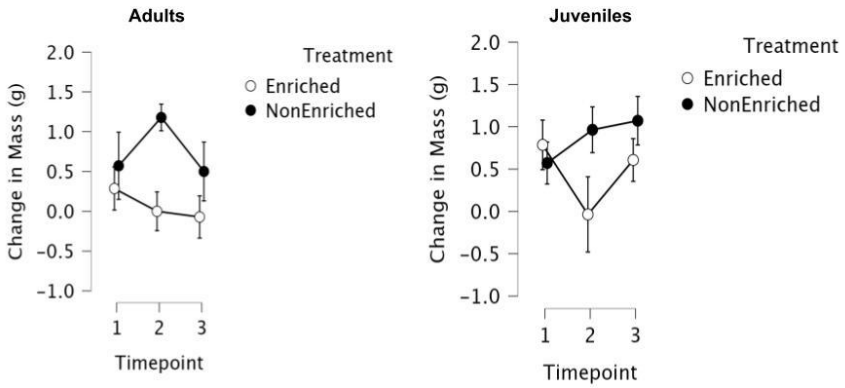


Figure 17. Change in body mass over time. In both adults and juveniles, non-enriched birds had higher body mass than enriched birds, although treatment was only significant in adults, and only for timepoint 2 with an interaction effect evident. In juveniles, only timepoints differed with significant changes from timepoint 1 to 2 and 4. Error bars represent +/- 1 SE.

**Interpretation**

Changes in body mass are fairly subtle. While in both adults and juveniles overall increases in body mass from the start of the experiment favor non-enriched birds, adult mass changes are similar at timepoint 3 and no juveniles have no significant treatment differences. It is possible that enriched birds found the interactive items stressful and avoided food early in the enrichment period as has been found for short-term enrichment in nutcrackers (Fairhurst et al. 2011) Although not as common a response to stress as avoiding food in response to stress (Yamahachi et al., 2017), non-enriched birds may have been eating more as a means to reduce stress in support of the "Comfort Food Hypothesis" (Tomiyama et al., 2011). Regardless, the

trend in body mass having nearly returned to baseline for both groups by the end of the experiment suggests that interactive housing does not have a long-term effect on body mass whether such short-term changes are a result of altered exercise or food consumption. In juveniles, the increase and and later decrease in mass is likely due to intial growth as birds reached sexual maturity, but also gained nutritional independence from parents (Elie et al. 2015).

**PART 2: Corticosterone Measurements**

**Introduction**

Corticosterone (CORT) is the primary glucocorticoid in birds (Palme et al., 2005). The normal function of CORT is to match metabolic response to demand; such that a "stress" response is characterized by increased peripheral blood flow, mobilizing glucose reserves, and reducing digestion. This response is normally adaptive, allowing animals to respond to threats or conserve energy as needed. However, chronic elevation of CORT and other glucocorticoids can suppress the immune system, impair digestion, limit cognitive abilities, increase abnormal behaviors such as self-harming, and negatively impact exploration, drinking, eating, grooming, and sleeping (NRC, 2008).

Due to the association of circulating CORT levels with an array of indices of health and well-being and its quantifiability, CORT measurement is regularly used as a proxy for "stress" levels, with elevated baseline CORT suggesting poor environmental conditions are influencing animal welfare. Obviously, circulating CORT levels and the animal's response to CORT are influenced by a number of other factors such as the number of receptors sensitive to CORT in significant areas of the brain and body, dysregulation of the HPA axis by chronic stress or other

factors, circulating binding globulins, enzymatic activity and other factors (Otovic, 2015). In songbirds, like the zebra finch, regulation of CORT at local circuits is also possible, however, with few exceptions, CORT levels in the periphery reflect the activity of CORT regulation at local neural circuits (Rensel & Schlinger, 2020).

Thus, we can generally rely on circulating CORT levels as an index of stress, but should keep caveats related to regulatory factors in mind. Additionally, CORT measurements should be interpreted in the context of other indicators of stress and anxiety levels, such as performance on test of neophobia and spatial cognition and comparisons of baseline and reactive stress CORT levels should be measured over short time periods and within subjects, as I have done in the experiments reported.

**Methods**

Subjects

For the pre- and post-stress test comparisons, the same individuals were used and sample sizes were the same. Adult and juvenile data was combined. There were 5 enriched females, 2 non-enriched females, 2 enriched males, and 3 non-enriched males. For the baseline comparison analysis in adults, there were 7 enriched females, 8 non-enriched females, 7 enriched males and 8 non-enriched males. With juveniles, there were 7 enriched females, 7 non-enriched females, 6 enriched males, and 6 non-enriched males. Age groups were combined for analysis.

Baseline Blood Samples

For all birds, blood was drawn following the cage acclimation period, at the midpoint of the experiment (day 30), the day prior to the start of the behavioral testing, and the day after the last behavioral test. Birds were transferred to the procedure room in individual carrying cages (30.5 x 16.5 x 15.2 cm) covered with a black cloth, which creates a restive state in birds. I

randomly selected one bird from each of the four treatment cages, and performed blood draws for each of these sets of birds until all birds had been sampled.

Once in the procedure room, birds rested in the carrying cages for at least 20 minutes prior to blood draw. After cleaning the skin with sterile saline, blood samples were taken by venipuncture of the ulnar vein with a 27-gauge needle and blood was collected in micro hematocrit capillary tubes and transferred to microcentrifuge tubes held on ice until centrifugation (10-20 min, 6000-10,000 r.p.m.), which varied in speed based upon my observation that low volume samples spun for a longer period of time had less hemolysis then when spun faster for a shorter period of time. I isolated the plasma fraction using a pipette and stored the plasma at −20°C until time for analysis. All baseline samples were completed in less than 4 minutes, with the great majority within 3 minutes, as recommended by Wingfield, Smith & Farner (1982). As circadian rhythm affects corticosterone secretion in several bird species (Krause & Ruploh, 2016), I took all blood samples at the same time of day. When blood draws were complete, I offered each bird oral electrolyte solution (Parent's Choice Electrolyte Solution, unflavored, or water solution with 3.2% sucrose, 0.255% NaCl), weighed them, and transferred them to group holding cages (76.8 x 36.2 x 40.6 cm, with half of this being allotted to two different groups) until all blood draws were complete.

Reactive Stress

After the fourth baseline sample, I conducted a reactive stress test. Instead of being placed in the holding cage, the bird was placed in a standard bird bag, a breathable cloth bag, for 20 minutes. This is a typical way to induce reactive stress in birds (Hodgson et al., 2007) without risking injury.

Hormone Assay

Plasma CORT concentrations were determined using a CORT Enzyme Immunoassay (EIA) kit (Cat No. ADI 900-097, Enzo Life Sciences) previously validated for use in ZF (Cooper et al., 2019; Crino et al., 2018; Jimeno et al., 2018b). The manufacturer's recommended procedures for small sample sizes were followed with the exception that sample concentrations were optimized prior to the start of the experimental assay (1:20 dilution factor). Samples were run in duplicate or triplicate and standards were run in triplicate. For triplicates, outliers from the other two samples were removed. For standards, this eliminated 14/120 or 10% of triplicates. Samples with concentrations below the detection limit of the kit were assigned the minimum value of detectability, which was 32 pg/mL. These samples were not included for determination of CVs. For sample duplicates with unacceptable CVs, results were not included in any analysis. Eight assays were performed and a pooled sample of zebra finch plasma was included for six assays with an inter-assay CV based on this sample of 13.8% calculated from final concentrations for this sample across these assays. The average CV for standards across plates was 8.9%, with a standard deviation of 5.7 across the 8 plates. The overall CV for samples calculated as the average CV across all plates was 17.8%, standard deviation of 15.79. While this is a little higher than desired, this average included some samples with CVs over 20% that were necessary to include so that each treatment x sex group had at least three individuals. For final publication, these will likely be eliminated.

Statistical Analyses

Given low subject numbers, we used non-parametric tests for all analyses.

**Results**

The expected expected effects of the reactive stress test were found for both adults (Wilcoxon signed-rank test Z=-267, p=0.005) and juveniles (Z=-2.13, p=0.03) with CORT levels higher after restraint than for the baseline 20 minutes prior. Neither treatment nor sex influence the initial baseline or reactive stress results (Mann-Whitney ps>0.07). For baseline samples, there were no significant changes in CORT levels over time depending on age (Friedman test $\chi^2$ (2) = 3.56 p=0.17), treatment ($\chi^2$ (2) = 3.56 p=0.17) or sex ($\chi^2$ (2) = 3.56 p=0.17).

**Interpretation**

The lack of treatment effects on CORT, paired with elevation of CORT after bag restraint in both adults and juveniles suggest that CORT levels do not reflect any distress or eustress caused by lack of environmental enrichment or having interactive objects present. This result could suggest that housing up to eight zebra finches in the cages we used does not cause undue stress as measured by CORT levels. However, given small sample sizes and the fact that circulating CORT levels may not accurately reflect responses to a variety of external factors (Otovic, 2015), and may not mirror responses to CORT at local neural circuits (Rensel & Schlinger, 2020) a lack of differences between groups should not be considered strong evidence for the basis of animal welfare guidelines. CORT measurements should be considered along with results of behavioral tests and observations to more fully understand the relationship between interventions such as enrichment and stress on welfare status.

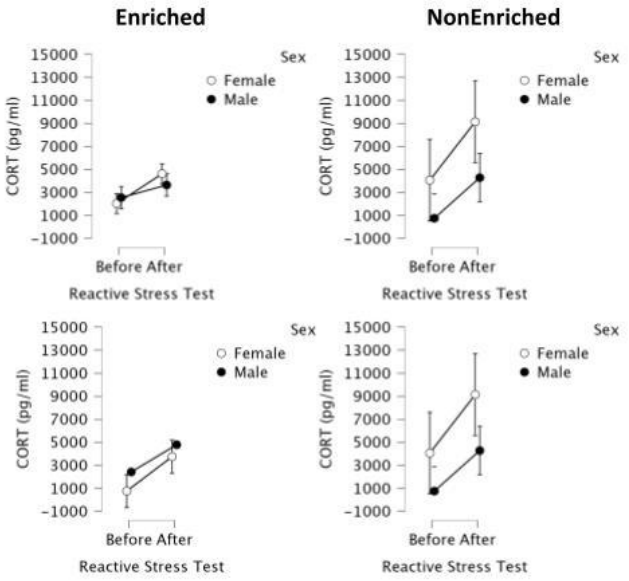


Figure 16. CORT levels before and after the reactive stress test. The top graphs contain results for adult birds and the bottom set for juveniles. The increase in CORT levels following the stress test is clear.

## DISCUSSION

Overall, this collection of physiological and behavioral measures offers insight not only into animal welfare, but also demonstrates the importance of pairing physiological measurements with behavioral tests to gain a rich understanding of the impacts of environmental enrichment.

For ARBs, the lack of difference between the treatment groups in adults may be due to the reduced behavioral plasticity exhibited by older animals (Stamps & Krishnan, 2017). Since once they develop, ARBs are hard to extinguish (Garner et al., 2005), introduction of ARB-preventing interventions may have little effect upon the behavioral aspect of stress-indicators in birds. The importance of preventing ARBs from developing in order for

enrichment to have a behavioral effect is evident in the juveniles, in which enriched birds spent less time engaging in these behaviors than non-enriched birds. It is possible that the interactions with the items are also an expression of stress-related behavior, or the presence of the items interfered with the space necessary to carry out the ARB sequence, despite the birds experiencing no less stress with their presence.

The Novel Object Test, although not clearly serving its intended purpose as a neophobia measure, was among the more informative tests in this experiment, in which adults strongly demonstrated an effect of enrichment. Enriched birds were much more likely to cross the midline of the cage, and subsequently perch than non-enriched birds. This may be evidence of enrichment altering the coping style of ZF when exposed to novel stimuli in a stressful situation. Coping style, also known as stress response pattern, is broadly classified into two categories. The "proactive" style is evident in individuals who have an active fight-flight response, with fast exploration of the environment, and rigid behavior minimally influenced by external stimuli. Conversely, "reactive" individuals have a conservation-withdrawal ("freeze-hide") response pattern, in which they tend to be immobile in the face of a stressor, although they have greater behavioral flexibility (van Zeeland et al., 2013). It seems that the enriched birds took on a more proactive approach by rapidly moving back and forth in the cages, while non-enriched birds primarily stood still in the location they were when the lights were turned on, or tried to exit from the side of the cage they were placed in without moving much throughout the environment. This difference in responses may also be due to the greater opportunities for exercise in the enriched birds, perhaps allowing them to be more physically fit than the non-enriched birds and facilitating the proactive coping style. A way to redesign the novel object test in the future to better understand the birds' response to novelty-induced stress may be to allow a longer or

unlimited amount of time to interact with the object, reducing the basement effect.

Although I anticipated the Hyponeophagia Test to be informative about birds' degree of neophobia, it seems that the time fasted was insufficient for there to be a difference in behavior. Although a fasting period between 2 and 6 hours is enough to alter hormone levels and change courting behavior (Fokidis et al., 2013), this duration was not sufficient to increase feeding motivation enough for any treatment or sex differences to be evident for adult birds. In juveniles, enriched birds were the only group that ate, regardless of sex. Although adult birds were overall more likely to eat within the time allotted, possibly due to their greater familiarity with the testing room, only enriched juveniles ate. For juveniles, the enrichment may have served as a buffer to stress in the novel environment, allowing feeding motivation to overcome neophobia. To more effectively perform this test in the future, one should ideally increase the amount of time fasted, and if possible, design the experiment such that all groups can be tested in an evenly distributed way across multiple experimental arenas.

In the first acqusition attempt of the Escape Maze, there were no significant differences in the latency for birds to exit the maze based upon sex or treatment. The turned-cue probe trial revealed that the birds were not relying upon the provided cues, and likely were depending on auditory or non-target visual cues to know where to exit. In the second acquisition attempt of the Escape Maze, group differences were more evident, with enriched females, on average, traveling more distance to exit over the acquisition days than non-enriched females, but enriched males traveling less than non-enriched males. For the duration spent in the maze, enriched females, on average, spent more time than non-enriched females, while enriched males spent less time than non-enriched males. The relationship between distance and duration is obvious with the velocity

results, in which enriched females were slower, and enriched males faster. Overall, during acquisition, enriched females traveled slowly and less directly towards the exit, while enriched males moved quickly and more directly. The behavior of the enriched females may either represent a greater tolerance to stress, if they feel less pressure to exit despite knowing how, or poorer spatial abilities. In the turned-cue probe trial, non-enriched females did not differ in the proportion of distance they traveled in the cued quadrant vs the average of the other quadrants, while enriched males traveled a greater amount of distance in the cued quadrant. This difference still remains even after accounting for velocity.

From these results, it seems that non-enriched females either did not learn the maze, or were not motivated to exit the maze, while the enriched females and the males of both treatment groups did learn the maze or were motivated to exit. Since in many species males have better spatial cognition (Jones et al., 2003), enrichment may not have made as much of a difference in enhancement of spatial memory in the males, while females may have required such conditions to have spatial cognition performance similar to males. This effect may have occurred due to a lower subjective sense of stress in the female birds due to having more activities available to them, or through the increased physical activity that these objects may induce. Enrichment has been shown to reverse learned helplessness, including the effects of cognitive deficits (Ilin & Richter-Levin, 2009), and exercise itself (Taati et al., 2022) has been shown to enhance spatial learning and memory.

In juveniles the lack of difference in performance on the Escape Maze regardless of treatment or sex supports prior research indicating that deviations in spatial performance by sex only develops over time. Considering the rescuing effect that enrichment has on adult females, I

suggest a future study observing whether access to enrichment can prevent the deviation in spatial cognition that happens from sex hormones with age. This can be accompanied by long-term monitoring of stress-associated hormones and sex hormones to see whether enrichment affects these hormones, and after death, whether enrichment affected gross and microscopic hippocampal structures.

These experiments also demonstrate that measures of body mass and circulating CORT indicate that enrichment is unnecessary to mitigate stress in zebra finches. Behavioral tests revealed the importance of introducing stress-mitigation measures at a young age, and showed the differential effects of enrichment on the sexes.

Understanding of body mass effects might be improved by including measurements of caloric intake and expenditures given that adjustments back to an equilibrium mass are common in birds. Unlike mammals, in the face of unrestricted food, birds rarely become overweight due to a refined self-regulation system, in which birds limit their weight changes by adjusting activity and feeding (Mathot et al., 2020).

## CONCLUSIONS

Upon observing the ARB prevention effects and apparent spatial cognition effects that enrichment can have on ZF, I would recommend enrichment implementation for laboratory birds from a young age. Since better spatial cognition and lower prevalence of ARBs are associated with these indices of welfare, providing enrichment to juveniles may be optimal both ethically and from the perspective of the need for healthy, psychologically normal animals for research, even if only as a point of comparison. Although it may require that laboratory animal housing

needs slightly more cleaning, and depending on the type of enrichment chosen, some initial cost, it is a moral imperative for scientists to use such measures to strive for the three R's: Replacement, Refinement, and Reduction (Fenwick et al., 2009). By studying means to implement enrichment in animals such as ZF, fewer animals will be needed to acquire quality data (reduction), and the animals may experience less pain and distress in the face of daily stressors or experiments (refinement). Taking enrichment into account, especially for historically cognitively underestimated species, is both better for the animals, and for the work of the people that study them.

## LIMITATIONS

In this work, I acknowledge the caution one must employ in directly comparing the results of the adults and the juveniles due to the variability in the time of acclimation, as well as the juveniles being kept in mixed-sex cages as opposed to single-sex cages as in adults. Adults had also undergone two training and probe trial periods due to problems in the original set-up of the Escape Maze, which likely led them to no longer perceive the maze environment as novel, despite the goal quadrant and the available visual cues being different. It is possible that having a second, contradicting period of training led the birds to be confused and have poorer performance than they otherwise would have.

The parentage of the adult birds, and their exact age was not as precisely known as in juveniles. The adult birds also had previously been housed in larger aviaries, and consequently, had become used to having more space to move in, while the juveniles had only briefly experienced the larger cages once they left their nest. This may mean that the juveniles are less likely to perceive the experimental cages as a "downgrade" in terms of space and stimulation,

which could be a stressor.

For the ARBs, it is important to note the limitation of knowing the motivations of the birds' behaviors. It is possible that events I counted as ARBs were actually expressions of excitement. Considering this, the non-enriched birds may have appeared to be presenting more ARBs since their energy is not directed towards enrichment objects. The assumption in this paper is that birds who choose to perform repetitive behaviors with no clear stimulus are doing so out of stress. Although I had occasionally observed males performing a repetitive movement sequence prior to singing, the lack of sex effect in my ARB findings suggests that singing-related movement motivation is not a major contributer to data I collected.

# LIST OF REFERENCES

An, W., Zhang, Y., Zhou, A., & Hu, Y. (2021). Detrimental effects of restricted cage size on reproductive performance, exploration ability, and anxiety but not working memory of kunming mice. *Front. Behav. Neurosci.*, *15*. https://doi.org/10.3389/fnbeh.2021.651782

Bateson, M., & Feenders, G. (2010). The use of passerine bird species in laboratory research: Implications of basic biology for husbandry and welfare. *ILAR Journal*, *51*(4), 394–408. https://doi.org/10.1093/ilar.51.4.394

Brown, R. E., Brain, J. D., & Wang, N. (1997). The avian respiratory system: A unique model for studies of respiratory toxicosis and for monitoring air quality. *Environ. Health Perspect., 105*(2), 188–200. https://doi.org/10.1289/ehp.97105188

Bruijn, R., & Romero, L. M. (2019). Prior restraint stress inhibits habituation to novel objects in the European starlings (*Sturnus vulgaris*). *J. Exp. Zool. A. Ecol. Integr. Physiol., 333*(2), 88–95. https://doi.org/10.1002/jez.2327

Bryda E. C. (2013). The Mighty Mouse: the impact of rodents on advances in biomedical research. *Mo Med, 110*(3), 207–211.

Clark, F., & King, A. J. (2008). A critical review of zoo-based olfactory enrichment. *Chemical signals in vertebrates 11*, 391-398.

Coleman, K., & Novak, M. A. (2017). Environmental Enrichment in the 21st Century. *ILAR journal*, *58*(2), 295–307. https://doi.org/10.1093/ilar/ilx008

Cooper, L. N., Mishra, I., & Ashley, N. T. (2019). Short-term sleep loss alters cytokine gene expression in brain and peripheral tissues and increases plasma corticosterone of zebra finch (*Taeniopygia guttata*). *Physiol. Biochem. Zool., 92*(1), 80-91.

Crino, O. L., Jensen, S. M., Buchanan, K. L., & Griffith, S. C. (2018). Evidence for condition mediated trade-offs between the HPA-and HPG-axes in the wild zebra finch. *Gen. Comp. Endocrinol., 259*, 189-198.

Cussen, V. A., & Mench, J. A. (2015). The relationship between personality dimensions and resiliency to environmental stress in orange-winged Amazon parrots (*Amazona amazonica*), as indicated by the development of abnormal behaviors. *PLoS ONE*, *10*(6), 1–11. https://doi.org/10.1371/journal.pone.0126170

Dearman, C., Boutwell, L., Jiwani, Z., McFatridge, E., Powell, J., Echoles, T. & Day, L. B. (2019, April). *No sex differences in spatial memory ability or response to aromatase inhibition after cerebellar lesion in zebra finches*. [Poster presentation]. University of Mississippi, Oxford, MS.

Elie, J. E., Soula, H. A., Trouvé, C., Mathevon, N., & Vignal, C. (2015). Housing conditions and sacrifice protocol affect neural activity and vocal behavior in a songbird species, the zebra finch (*Taeniopygia guttata*). *Comptes Rendus Biologies*, *338*(12), 825-837.

Emery, N. J. (2017). *Bird brain: An exploration of avian intelligence*. Ivy Press.

Fairhurst, G. D., Frey, M. D., Reichert, J. F., Szelest, I., Kelly, D. M., & Bortolotti, G. R. (2011). Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. *PLoS ONE*, *6*(3). https://doi.org/10.1371/journal.pone.0017663

Fenwick, N., Griffin, G., & Gauthier, C. (2009). The welfare of animals used in science: how the "Three Rs" ethic guides improvements. *Can. Vet. J. 50*(5), 523–530.

Forstmeier, W., Martin, K., Bolund, E., Schielzeth, H., & Kempenaers, B. (2011). Female extrapair mating behavior can evolve via indirect selection on males. *Proc. Natl. Acad. Sci. U.S.A.*, *108*(26), 10608–10613. https://doi.org/10.1073/pnas.1103195108

Forstmeier, W., Segelbacher, G., Mueller, J. C., & Kempenaers, B. (2007). Genetic variation and differentiation in captive and wild zebra finches (*Taeniopygia guttata*). *Mol. Ecol.*, *16*(19), 4039–4050. https://doi.org/10.1111/j.1365-294X.2007.03444.x

Fokidis, H. B., Prior, N. H., & Soma, K. K. (2013). Fasting increases aggression and differentially modulates local and systemic steroid levels in male zebra finches. *Endocrinology, 154*(11), 4328–4339. https://doi.org/10.1210/en.2013-1171

Garner, J. P. (2005). Stereotypies and other abnormal repetitive behaviors: Potential impact on validity, reliability, and replicability of scientific outcomes. *ILAR Journal*, *46*(2), 106–117. https://doi.org/10.1093/ilar.46.2.106

Gouda, A., Amer, S. A., Gabr, S., & Tolba, S. A. (2020). Effect of dietary supplemental ascorbic acid and folic acid on the growth performance, redox status, and immune status of broiler chickens under heat stress. *Trop. Anim. Health Prod.*, *52*(6), 2987–2996. https://doi.org/10.1007/s11250-020-02316-4

Hall, Z. J., Bertin, M., Bailey, I. E., Meddle, S. L., & Healy, S. D. (2014). Neural correlates of nesting behavior in zebra finches (*Taeniopygia guttata*). *Behav. Brain Res. 264*, 26–33. https://doi.org/10.1016/j.bbr.2014.01.043

Harris, R. B. S. (2015). Chronic and acute effects of stress on energy balance: Are there appropriate animal models?Am. J. Physiol. Regul. Integr. Comp. Physiol. *308*(4), R250–R265. https://doi.org/10.1152/ajpregu.00361.2014

Hawkins, P., Morton, D. B., Cameron, D., Cuthill, I., Francis, R., Freire, R., ... & Townsend, P. (2001). Laboratory birds: refinements in husbandry and procedures. *Lab. Anim., 35*(Suppl 1), 1-163.

Hickman, D. L., Johnson, J., Vemulapalli, T. H., Crisler, J. R., & Shepherd, R. (2017). Commonly used animal models. *Principles of Animal Research for Graduate and Undergraduate Students*, 117–175. https://doi.org/10.1016/B978-0-12-802151-4.00007-4

Hodgson, Z. G., Meddle, S. L., Roberts, M. L., Buchanan, K. L., Evans, M. R., Metzdorf, R., Gahr, M., & Healy, S. D. (2007). Spatial ability is impaired and hippocampal mineralocorticoid receptor mRNA expression reduced in zebra finches (*Taeniopygia guttata*) selected for acute high corticosterone response to stress. *Proc. R. Soc. B: Biol. Sci.*, *274*(1607), 239–245. https://doi.org/10.1098/rspb.2006.3704

Home Office. (2020). *Annual Statistics of Scientific Procedures on Living Animals, Great Britain 2019* (p. 6).

Hauber, M. E., Louder, M. I., & Griffith, S. C. (2021). Neurogenomic insights into the behavioral and vocal development of the zebra finch. *eLife, 10*, e61849. https://doi.org/10.7554/eLife.61849

Ilin, Y., & Richter-Levin, G. (2009). Enriched environment experience overcomes learning deficits and depressive-like behavior induced by juvenile stress. *PLoS ONE*, *4*(1), e4329. https://doi.org/10.1371/journal.pone.0004329

Ikkatai, Y., & Watanabe, S. (2015). Eye surface temperature detects stress response in budgerigars (*Melopsittacus undulatus*). *NeuroReport*, *26*(11), 642–646. https://doi.org/10.1097/WNR.0000000000000403

Jacobs, H., Smith, N., Smith, P., Smyth, L., Yew, P., Saibaba, P., & Hau, J. (1995). Zebra finch behaviour and effect of modest enrichment of standard cages. *Anim. Welf.*, *4*(1), 3-9.

Jimeno, B., Briga, M., Hau, M., & Verhulst, S. (2018). Male but not female zebra finches with high plasma corticosterone have lower survival. *Funct. Ecol.*, *32*(3), 713-721.

Jimeno, B., Hau, M., & Verhulst, S. (2018). Glucocorticoid–temperature association is shaped by foraging costs in individual zebra finches. *J. Exp. Biol., 221*(23), jeb187880.

Jones, C. M., Braithwaite, V. A., & Healy, S. D. (2003). The evolution of sex differences in spatial ability. *Behav. Neurosci.*, *117*(3), 403–411. https://doi.org/10.1037/0735-7044.117.3.403

Jones, P. J., Tahamtani, F. M., Pedersen, I. J., Niemi, J. K., & Riber, A. B. (2020). The productivity and financial impacts of eight types of environmental enrichment for broiler chickens. *Animals*, *10*(3), 1–14. https://doi.org/10.3390/ani10030378

Katajamaa, R., & Jensen, P. (2020). Selection for reduced fear in red junglefowl changes brain composition and affects fear memory: Brain and cognition in red junglefowl. R. Soc. Open Sci., 7(8). https://doi.org/10.1098/rsos.200628rsos200628

Kempermann, G., Gast, D., & Gage, F. H. (2002). Neuroplasticity in old age: Sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann. Neurol.*, *52*(2), 135–143. https://doi.org/10.1002/ana.10262

Kim, E. J., Pellman, B., & Kim, J. J. (2015). Stress effects on the hippocampus: A critical review. *Learn. Mem., 22*(9), 411–416. https://doi.org/10.1101/lm.037291.114

Kitchen, A. M., & Martin, A. A. (1996). The effects of cage size and complexity on the behaviour of captive common marmosets, *Callithrix jacchus jacchus*. *Laboratory Animals*, *30*(4), 317–326. https://doi.org/10.1258/002367796780739853

Kosarussavadi, S., Pennington, Z. T., Covell, J., Blaisdell, A. P., & Schlinger, B. A. (2017). Across sex and age: Learning and memory and patterns of avian hippocampal gene expression. *Behav. Neurosci.*, *131*(6), 483–491. https://doi.org/10.1037/bne0000222

Krause, E. T., Bischof, H. J., Engel, K., Golüke, S., Maraci, N., Mayer, U., Sauer, J., & Caspers, B. A. (2018). Olfaction in the zebra finch (*Taeniopygia guttata*): What is known and further perspectives. *Adv. Study Behav., 50*, 37–85. https://doi.org/10.1016/bs.asb.2017.11.001

Krause, T. E., Naguib, M., Trillmich, F., & Schrader, L. (2006). The effects of short term enrichment on learning in chickens from a laying strain (*Gallus gallus domesticus*). *Appl. Anim. Behav. Sci., 101*(3–4), 318–327. https://doi.org/10.1016/j.applanim.2006.02.005

Krause, E. T., & Ruploh, T. (2016). Captive domesticated zebra finches (*Taeniopygia guttata*) have increased plasma corticosterone concentrations in the absence of bathing water. *Appl. Anim. Behav. Sci.*, *182*, 80–85. https://doi.org/10.1016/j.applanim.2016.06.003

Kruska, D. (1988). Effects of domestication on brain structure and behavior in mammals. *Hum. Evol., 3*(6), 473–485. https://doi.org/10.1007/BF02436333

Kverková, K., Marhounová, L., Polonyiová, A., Kocourek, M., Zhang, Y., Olkowicz, S., ... & Němec, P. (2022). The evolution of brain neuron numbers in amniotes. *Proc. Natl. Acad. Sci. U.S.A., 119*(11), e2121624119.

Kucuk, O., Sahin, N., & Sahin, K. (2003). Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens. *Biol. Trace Elem. Res.*, *94*(3), 225–235. https://doi.org/10.1385/BTER:94:3:225

Kulke, K., Kemper, N., & Stracke, J. (2021). Boys (toms) don't try. Behaviour of turkeys in a novel object test – Influence of age and sex. *Appl. Anim. Behav. Sci.*, *244*, 105484. https://doi.org/10.1016/j.applanim.2021.105484

Leader, N., & Nottebohm, F. (2006). Delayed plumage maturation in socially isolated juvenile zebra finches, *Taeniopygia guttata*. *Anim. Behav., 72*, 113-121.

Lincoln, F. C., 1892-1960. (1929). Manual for bird banders. United States.

Liu, Z., Torrey, S., Newberry, R. C., & Widowski, T. (2020). Play behaviour reduced by environmental enrichment in fast-growing broiler chickens. Appl. Anim. Behav. Sci., *232*(March), 105098. https://doi.org/10.1016/j.applanim.2020.105098

Löhmus, M., Sandberg, R., Holberton, R. L., & R.Moore, F. (2003). Corticosterone levels in relation to migratory readiness in red-eyed vireos (*Vireo olivaceus)*. *Behav. Ecol. Sociobiol.*, *54*(3), 233–239. https://doi.org/10.1007/s00265-003-0618-z

Mathot, K. J., Kok, E. M. A., van den Hout, P., Dekinga, A., & Piersma, T. (2020). Red knots (*Calidris canutus islandica*) manage body mass with dieting and activity. *J. Exp. Biol.*, https://doi.org/10.1242/jeb.231993

Mayer, U., Watanabe, S., & Bischof, H. J. (2013). Spatial memory and the avian hippocampus: Research in zebra finches. *J. Physiol. Paris, 107*(1–2), 2–12. https://doi.org/10.1016/j.jphysparis.2012.05.002

Medina-García, A., Jawor, J. M., & Wright, T. F. (2017). Cognition, personality, and stress in budgerigars, *Melopsittacus undulatus*. *Behav. Ecol.*, *28*(6), 1504–1516. https://doi.org/10.1093/beheco/arx116

Mello C. V. (2014). The zebra finch, *Taeniopygia guttata*: an avian model for investigating the neurobiological basis of vocal learning. *Cold Spring Harb. Protoc.*, *2014*(12), 1237–1242. https://doi.org/10.1101/pdb.emo084574

Nager, R. G., & Law, G. (2010). The zebra finch. *The UFAW handbook on the care and management of laboratory and other research animals, (Ed. 8), 674-685.*

Nelson, J. R., McIntyre, D. R., Pavlidis, H. O., & Archer, G. S. (2018). Reducing stress susceptibility of broiler chickens by supplementing a yeast fermentation product in the feed or drinking water. *Animals*, *8*(10), 173. https://doi.org/10.3390/ani8100173

National Research Council (NRC). 2008. *Recognition and alleviation of distress in laboratory animals*. National Academies Press, Washington, D.C.

National Research Council (NRC). 2011. *Guide for the care and use of laboratory animals: eighth edition*. National Academies Press, Washington, D.C.

Olkowicz, S., Kocourek, M., Lučan, R. K., Porteš, M., Fitch, W. T., Herculano-Houzel, S., & Němec, P. (2016). Birds have primate-like numbers of neurons in the forebrain. *Proc. Natl. Acad. Sci. U.S.A., 113*(26), 7255-7260.

Olson, C. R., Wirthlin, M., Lovell, P. V., & Mello, C. V. (2014). Proper care, husbandry, and breeding guidelines for the zebra finch, *Taeniopygia guttata*. *Cold Spring Harb. Protoc., 2014*(12), 1243-1248. https://doi.org/10.1101/pdb.prot084780

Otovic, P. (2015). Limits to using HPA axis activity as an indication of animal welfare. *ALTEX*, 41–50. https://doi.org/10.14573/altex.1406161

Owen, D. J., & Lane, J. M. (2006). High levels of corticosterone in feather-plucking parrots (*Psittacus erithacus*). *Vet. Rec., 158*(23), 804–805. https://doi.org/10.1136/vr.158.23.804

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S., & Möstl, E. (2005). Stress hormones in mammals and birds: Comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Ann. N. Y. Acad. Sci., 1040*(1), 162-171. doi: 10.1196/annals.1327.021

Pasquet, A. (2019). Effects of domestication on fish behaviour. *Animal Domestication*. https://doi.org/10.5772/intechopen.78752

Rensel, M. A., & Schlinger, B. A. (2020). The stressed brain: Regional and stress‐related corticosterone and stress‐regulated gene expression in the adult zebra finch (*Taeniopygia guttata*). *J. Neuroendocrinol., 32*(5), e12852.

Robbins, L., & Margulis, S. W. (2016). Music for the birds: Effects of auditory enrichment on captive bird species. *Zoo Biol., 35*(1), 29-34.

Roberts, M., Buchanan, K., Bennett, A., & Evans, M. (2007). Mate choice in zebra finches: Does corticosterone play a role? *Anim. Behav., 74(4)*, 921–929. https://doi.org/10.1016/j.anbehav.2006.12.021

Romero, L. M., & Gormally, B. M. (2019). How truly conserved is the “well-conserved” vertebrate stress response?. *Integr. Comp. Biol., 59*(2), 273-281.

Ross, M., Rausch, Q., Vandenberg, B., & Mason, G. (2020). Hens with benefits: Can environmental enrichment make chickens more resilient to stress? *Physiol. Behav., 226*(July 2020), 113077. https://doi.org/10.1016/j.physbeh.2020.113077

Samuels, B. A., & Hen, R. (2011). Novelty-suppressed feeding in the mouse. In *Mood and anxiety related phenotypes in mice* (pp. 107-121). Humana Press.

Schmidt, M. F. (2010). An IACUC perspective on songbirds and their use in neurobiological research. *ILAR Journal*, *51*(4), 424–430. https://doi.org/10.1093/ilar.51.4.424

Schoech, S. J., Mumme, R. L., & Wingfield, J. C. (1997). Corticosterone, reproductive status, and body mass in a cooperative breeder, the Florida Scrub-Jay (*Sphelocoma coerulescens*). *Physiol. Zool.*, *70*(1), 68–73. https://doi.org/10.1086/639545

Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats-Behavioural and neurochemical aspects. *Behav. Brain Res.*, *222*(1), 246–264. https://doi.org/10.1016/j.bbr.2011.04.002

Sisti, H. M., Glass, A. L., & Shors, T. J. (2007). Neurogenesis and the spacing effect: learning over time enhances memory and the survival of new neurons. *Learn. Mem., 14*(5), 368–375. https://doi.org/10.1101/lm.488707

Stamps, J. A., & Krishnan, V. (2017). Age-dependent changes in behavioural plasticity: insights from Bayesian models of development. *Anim. Behav.*, *126*, 53–67. https://doi.org/10.1016/j.anbehav.2017.01.013

Taati, M., Barzegar, P. E. F., & Raisi, A. (2022). Exercise improves spatial learning and memory performance through the central GLP-1 receptors. *Behav. Neurol.*, *2022*, 1–6. https://doi.org/10.1155/2022/2900628

Ten Cate, C., Healy, S. D., & Healy, S. (Eds.). (2017). *Avian cognition*. Cambridge University Press.

Tomiyama, A. J., Dallman, M. F., & Epel, E. S. (2011). Comfort food is comforting to those most stressed: Evidence of the chronic stress response network in high stress women. *Psychoneuroendocrinology, 36*(10), 1513–1519. https://doi.org/10.1016/j.psyneuen.2011.04.005

Toth, L. A., Kregel, K., Leon, L., & Musch, T. I. (2011). Environmental enrichment of laboratory rodents: The answer depends on the question. *Comp. Med., 61*(4), 314–321.

UKEssays. (November 2018). Effect of sound enrichment in captive zebra finches (*Taeniopygia guttata*) Behaviour. Retrieved from https://www.ukessays.com/essays/biology/effect-of-sound-enrichment-in-captive-zebra-finches-taeniopygia-guttata-behaviour.php?vref=1

van Zeeland, Y. R., van der Aa, M. M., Vinke, C. M., Lumeij, J. T., & Schoemaker, N. J. (2013). Behavioural testing to determine differences between coping styles in Grey parrots (*Psittacus erithacus erithacus*) with and without feather damaging behaviour. *Appl. Anim. Behav. Sci., 148*(3–4), 218–231. https://doi.org/10.1016/j.applanim.2013.08.004

Watters J. V. (2009). Toward a predictive theory for environmental enrichment. *Zoo Biol.*, *28*(6), 609–622. https://doi.org/10.1002/zoo.20284

Williams, I., Hoppitt, W., & Grant, R. (2017). The effect of auditory enrichment, rearing method and social environment on the behavior of zoo-housed psittacines (Aves: Psittaciformes); implications for welfare. *Appl. Anim. Behav. Sci.*, *186*(November 2016), 85–92. https://doi.org/10.1016/j.applanim.2016.10.013

Williams, T., "The effects of adrenergic antagonists on spatial memory in the zebra finch (*Taeniopygia guttata*)" (2014). Honors Theses. 907. https://egrove.olemiss.edu/hon_thesis/907

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. *Am. Zool., 35*(3), 285-294.

Wingfield, J. C., Smith, J. P., & Farner, D. S. (1982). Endocrine responses of white-crowned sparrows to environmental stress. *The Condor, 84*(4), 399-409.

Woods, J. M., Eyer, A., & Miller, L. J. (2022). Bird welfare in zoos and aquariums: General insights across industries. *J. Zool. Bot. Gard., 3*(2), 198-222.

Yamahachi, H., Zai, A., Tachibana, R., Stepien, A., Rodrigues, D., Cavé-Lopez, S., Narula, G., Lee, J., Huang, Z., Hörster, H., Düring, D., & Hahnloser, R. (2017). Welfare of zebra finches used in research. *BioRxiv*, 154567. https://doi.org/10.1101/154567

Young, R. (2003). *Environmental enrichment for captive animals*. Iowa: Blackwell Publishing.

# VITA

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