

# Implications of Fecal Corticosterone on Welfare of Orange-Winged Amazons (*Amazona amazonica*) During Social Pairing

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## INTRODUCTION

- Orange-winged Amazon parrots are social birds, but are often housed singly<sup>2</sup>
- Birds living in a social environment have been reported to have fewer stereotypies and improved welfare<sup>4</sup>
- There is no established protocol to determine pairing compatibility in parrots
- Corticosterone is the primary stress hormone in birds<sup>1</sup> and can be used to measure the level of stress a bird experiences
- Corticosterone in birds can be measured via metabolites found in feces<sup>6</sup>
- High concentrations of fecal corticosterone equate to high levels of stress<sup>3</sup>

## OBJECTIVES

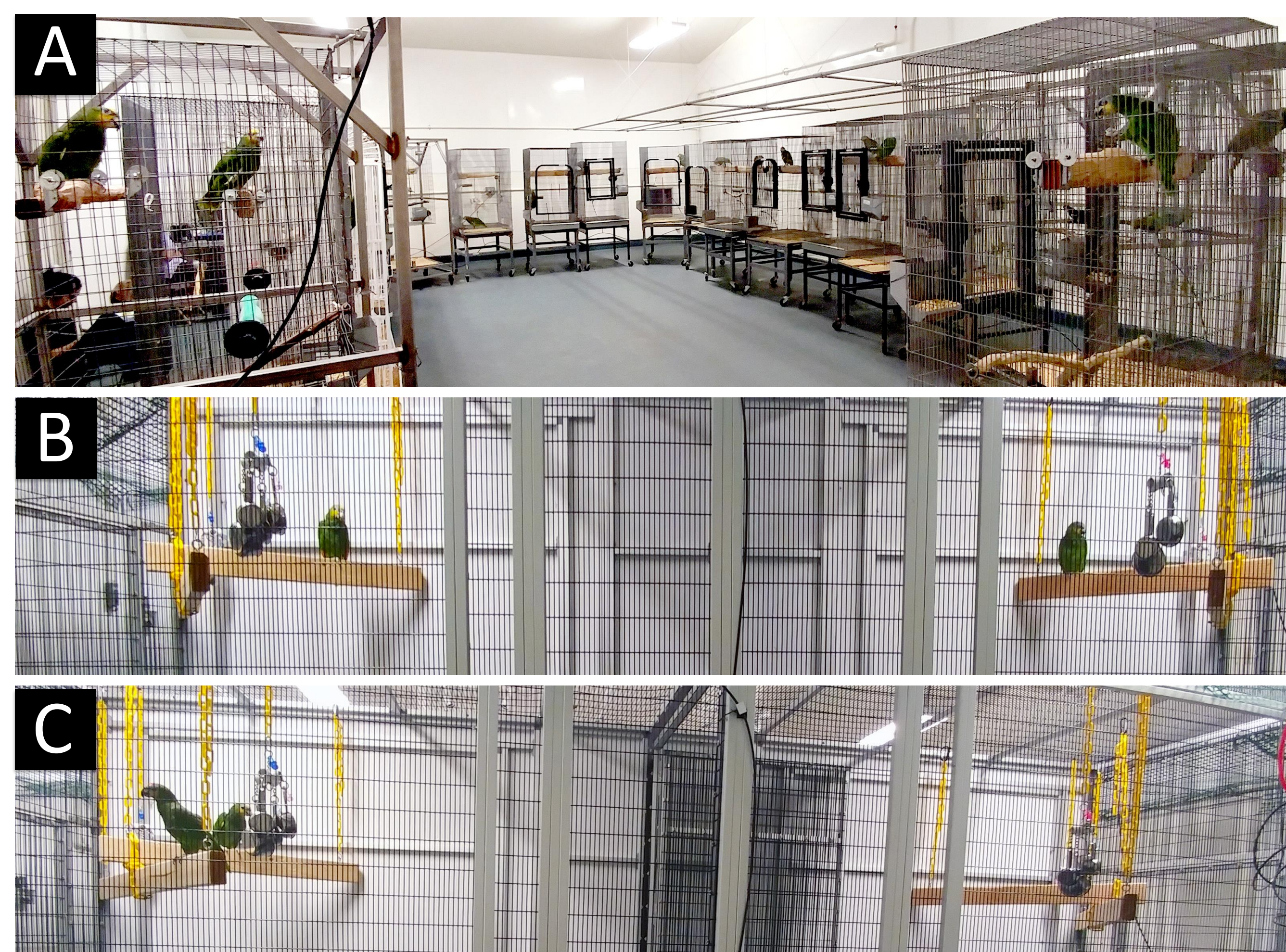
- To measure fecal corticosterone concentrations to monitor changes in the amount of stress parrots experience overtime
- Interpret fecal corticosterone concentrations to determine the most stressful aspects of the social pairing process

## MATERIALS AND METHODS

- Orange-winged Amazon parrots (OWA, n=20): 10 females, 10 males, ages 1-15 years old
- OWAs lived in individual cages in a common room (Fig. 1A) when not paired
- Each pairing included 10 birds (5 female, 5 male) in a specially-designed pairing cage (Fig. 1B, 1C)
- Each bird participated in 10 rounds of heterosexual pairing
- Each pairing lasted three days (Day 0, 1, 2)
- Fecal samples were collected on each pairing day (Table 1), between 7:20 – 8:00 AM
- Samples were stored at -20 °C for 24-72 hours, then at -80 °C for 1-5 weeks
- Fecal samples from round 1 and round 5 of pairing were sent to the St. Louis Zoo Endocrinology Lab for fecal corticosterone analysis via radioimmunoassay
- All OWAs had *ad libitum* access to food and water
- All procedures were approved by UC Davis IACUC (approval #19865)

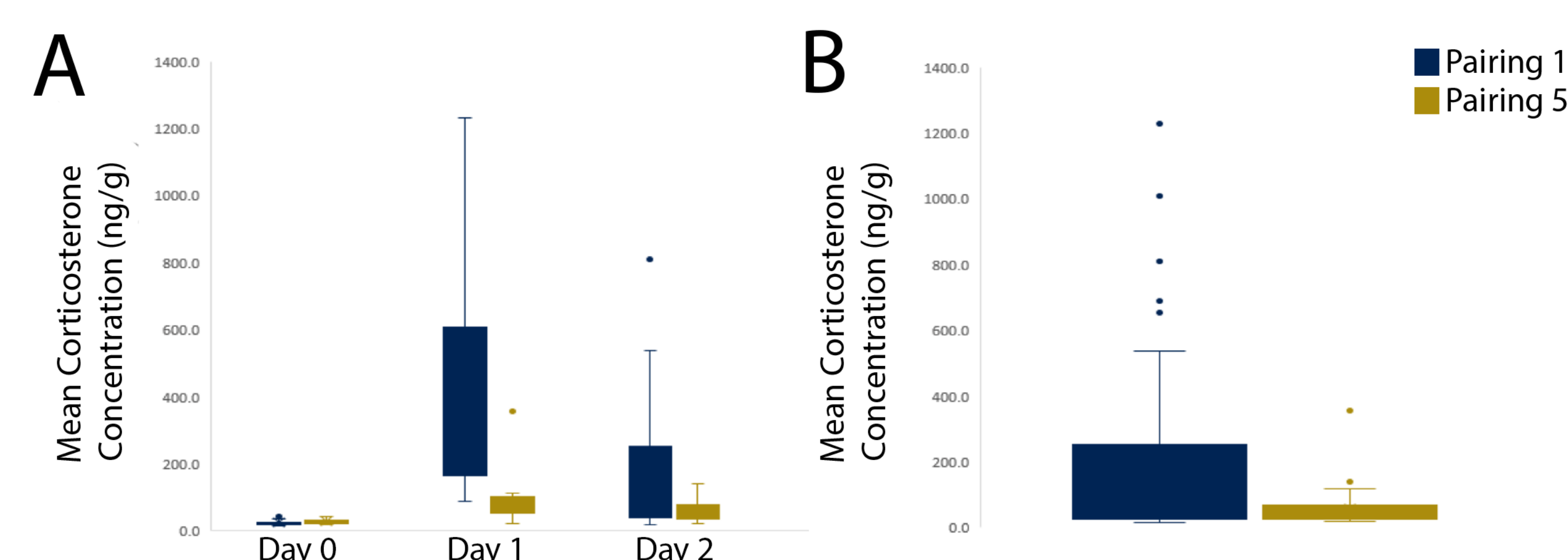
**Table 1:** Summary of fecal sample collection protocol

Day of Collection	Collection Parameters	Number of Samples Collected
Day 0	Sample collected from individual cages from the common room	n=10 for both Pairing 1 and Pairing 5
Day 1	Sample collected after 24 hours in the pairing cage with the divider between the two birds. After collection, the door dividing the birds is removed	n=10 for both Pairing 1 and Pairing 5
Day 2	Sample collected 24 hours after door between the two birds is removed	n=9 for Pairing 1 and n=10 for Pairing 5. Inadequate fecal sample collected from parrot 14 during Pairing 1. Feces from day 2 Pairing 5 from parrot 14 was subsequently thrown out



**Figure 1:** A) Day 0, common room. Fecal samples collected from OWAs housed in their individual cage B) Day 1, pairing cage. Fecal samples collected from OWAs housed in the pairing cage, separated by door C) Day 2, pairing cage. Fecal samples collected from OWAs cohabitating in the pairing cage

## RESULTS

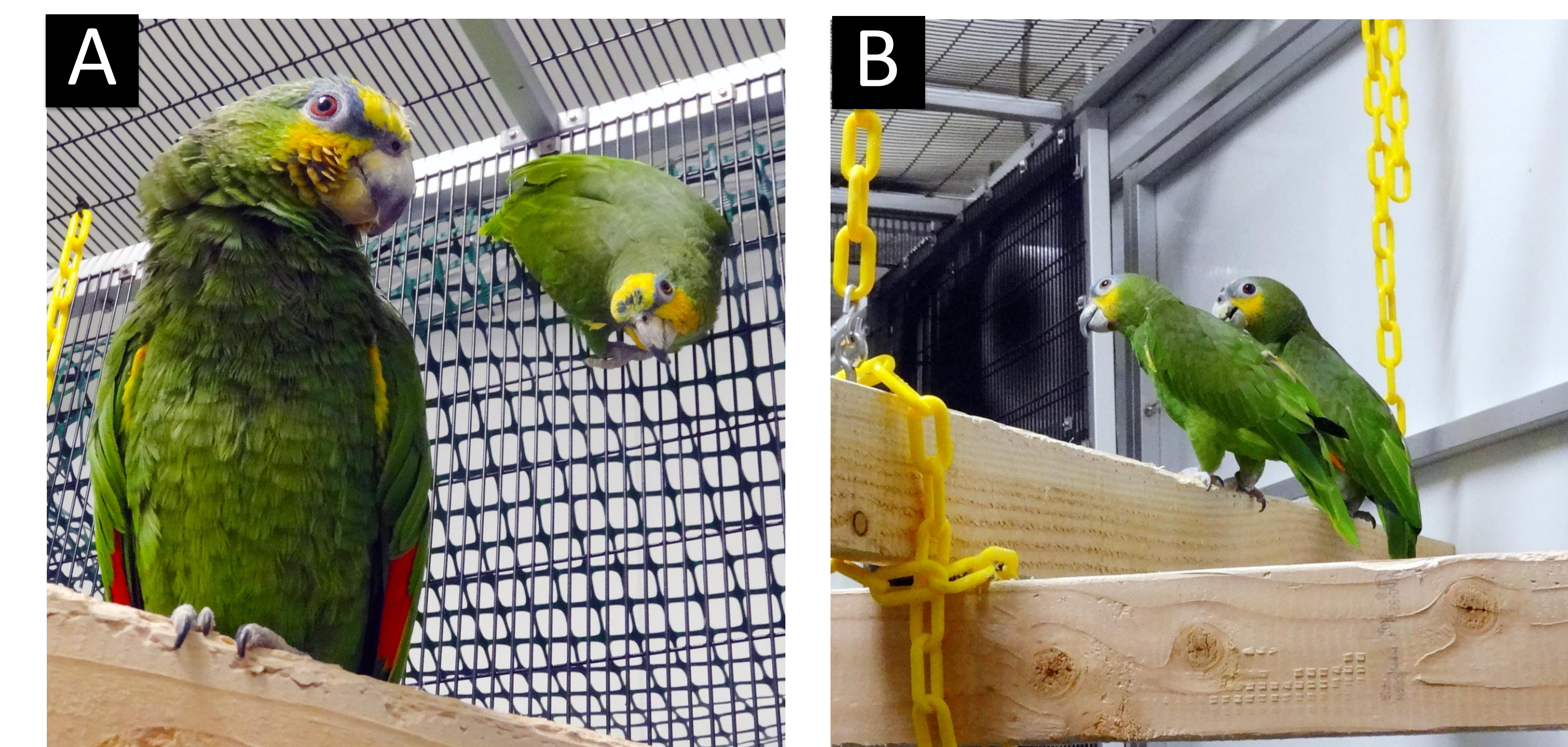


**Figure 2:** A) Mean  $\pm$  SEM fecal corticosterone concentrations from Day 0, 1, and 2 (n=20, 20, and 19) from Pairing 1 compared to Day 0, 1, and 2 (n=20, 20, and 19) from Pairing 5. Fecal samples from Pairing 5 were collected 35 days after Pairing 1. Day 1 from Pairing 1 had the highest mean fecal corticosterone levels ( $398.1 \pm 76.83$  ng/g) B) Overall mean  $\pm$  SEM of fecal corticosterone concentrations (n=59) of Day 0, 1, and 2 from Pairing 1 compared to those from Pairing 5

- 118 samples from Pairing 1 and Pairing 5 were analyzed for fecal corticosterone
- A one-way ANOVA with data clustered by bird and the day of sample collection as the fixed effect ( $p < 0.001$ ) and post-hoc Tukey test of mean fecal corticosterone concentrations found that Day 1 of Pairing 1 was statistically different from the means of all other pairing days ( $p < 0.001$ )
- There was no statistical difference between Day 0 of Pairing 1 and 5 ( $p = 0.781$ )
- All other pairing samples were statistically different when compared to Day 0 from each pairing round ( $p < 0.001$ )
- A paired t test determined significant differences in mean fecal corticosterone concentrations between Pairing 1 and Pairing 5 ( $p < 0.001$ )
- Fecal corticosterone concentrations (mean  $\pm$  SEM) decreased from  $193.50 \pm 35.22$  ng/g in Pairing 1 to  $58.20 \pm 6.44$  ng/g in Pairing 5
- All statistical analysis was conducted in R<sup>®</sup>, Version 3.4.1<sup>5</sup>

## CONCLUSIONS

- The highest fecal corticosterone concentrations were measured on Day 1 in Pairing 1. This correlates to the first time OWAs were placed in the pairing cage, suggesting a novel environment is stressful for OWAs
- Fecal corticosterone decreased from Pairing 1 to Pairing 5, indicating that the parrots experienced less stress as the pairing process continued
- Repetition in the social pairing process may be an important factor accounting for the decrease in fecal corticosterone concentrations over time
- In the future, stress experienced by OWAs transitioning to social housing may be reduced by introducing parrots to a new environment over the course of several days



**Figure 3:** A and B) : Successful cohabitation in two different OWA pairs

## ACKNOWLEDGEMENTS

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