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Adreno-immunological response to healthcare facility noise during rehabilitation of Tropical Screech Owls

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ABSTRACT

Wild animals, particularly those affected by urban expansion frequently face challenges such as vehicle collisions, and encounters with domestic dogs. Rehabilitation in care facilities usually conducted in *ex-situ* conditions poses considerable challenges. In rescue centers, these animals are exposed to a new environment characterized by human noise and the presence of multiple species in a confined space. This can potentially trigger stress responses and immunosuppression, further compromising their health. This study aimed to assess the endocrine and immunological parameters of owls (*Megascops choliba*) under human care for a continuous 49-day period. Twelve adult owls were exposed to audio recordings from a rescue center between days 15 and 35. Fecal samples were collected from each owl every seven days, as well as blood samples from eight of these birds. Fecal samples were analyzed for glucocorticoid metabolites (GCM), while blood samples were used to measure plasma corticosterone levels, bacterial killing ability (BKA), and heterophil-to-lymphocyte ratio (H/L). Our findings revealed that GCM concentration increased during noise exposure regardless of sex, while plasma corticosterone levels remained unchanged. BKA decreased during noise exposure compared to before treatment, but H/L did not differ between time points. These results show that exposure to rescue center noise induces alterations in allostatic mediators in owls, requiring further research to address other physiological changes in individuals that may affect their recovery and maintenance under human care.

1. Introduction

The decline of animal populations in their natural habitats cannot be attributed solely to wildlife trafficking (Simkin et al., 2022; Zain, 2020). This decline is further compounded by a range of anthropogenic activities that include habitat loss and urban sprawl. To facilitate the recovery and reintroduction of wild animals into their natural habitats, Brazilian government entities have established reception and care centers for injured animals (Giovanini, 2002). However, in these *ex-situ* conditions, wild species are subjected to

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management and care practices that can induce psychological stress during their recovery processes (Fischer and Romero, 2018). The perception of these stimuli as aversive triggers energy-dependent endocrine and immunological allostatic responses (Ellis et al., 2006; McEwen and Wingfield, 2010, 2003). When the energy demand of these responses exceeds the energy supply, it can lead to allostatic overload (Landys et al., 2006; Morgan and Tromborg, 2007).

Persistent allostatic overload may lead to pathophysiological conditions (McEwen and Wingfield, 2010), compromising the well-being of individuals and, in the worst-case scenario, potentially resulting in death (Broom, 2011; Broom and Molento, 2004; Morgan and Tromborg, 2007). One widely employed method for assessing animal welfare involves analyzing adrenal responses, often in response to stressors (Millspaugh and Washburn, 2004; Möstl and Palme, 2002; Tarlow and Blumstein, 2007), which includes measuring the levels of glucocorticoids (GCs) such as cortisol, corticosterone, and/or their metabolites (Möstl and Palme, 2002; Stocker et al., 2016). Glucocorticoids play a crucial role in regulating essential functions, including metabolism, growth, and resource allocation management (Breuner et al., 2013; Phuc Le et al., 2005). Furthermore, certain aspects of these processes are co-opted as part of a stress response (MacDougall-Shackleton et al., 2019). The production of these GCs is associated with the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Romero et al., 1998).

Given that stress can elicit a multisystemic response in individuals (MacDougall-Shackleton et al., 2019), and that changes in glucocorticoids following stressors are not always consistent or present (Bonier et al., 2009), researchers seek the integration of other physiological parameters, such as evaluating the immune response of animals. However, modulation of the immune system by stress is complex and can be immunosuppressive (Ilmonen et al., 2003; Keller et al., 1983; Kushima et al., 2003) or immunostimulatory (Dhabhar, 1998; Viswanathan et al., 2005). Previous studies in birds have shown that stress situations can alter the heterophil-to-lymphocyte ratio (H/L) (Campo et al., 2005; Davis et al., 2008; Davis and Maney, 2018; Gross and Siegel, 1983; Ilmonen et al., 2003), as well as affect the ability to kill bacteria (BKA) (Matson et al., 2006; Tieleman et al., 2005). However, it is important to note that each species exhibits significant variation in immune responses, and our understanding of this type of assessment in nocturnal birds of prey remains scarce.

In recent years, studies have explored the impact of anthropogenic noise pollution on wildlife, specifically examining its effects on animal behavior and reproductive success (Kight and Swaddle, 2011; Zollinger et al., 2019). For instance, studies have shown that forest management activities and the intensity of harvesting can noticeably affect the wild populations of northern spotted owls (*Strix occidentalis*), primarily influencing male owls by increasing their glucocorticoid metabolite levels (Wasser and Hunt, 2005). When wild animals, including owls, arrive at a rescue center, they encounter *ex-situ* conditions characterized by a complex soundscape, which includes not only human-generated noises but also the presence of various species within a confined space. Additionally, owls are particularly sensitive to auditory stimuli due to their sensitivity across a wide range of frequencies, especially at high frequencies compared to most other birds (Brittan-Powell et al., 2005; Kraemer et al., 2017). Notably, one of the most frequently received raptor species in Brazilian rescue centers is the Tropical Screech Owl (*Megascops choliba*) (Lima et al., 2021). Natural populations of this species can be found in various environments, ranging from forest peripheries to urban settings in Central and South America (BirdLife, 2021). This owl species, distinguished by a wingspan of up to 50 cm and weighing as much as 120 g (Enríquez et al., 2017), exhibits seasonal reproduction, with a significant chick hatching during the spring season in South American countries (Barbosa-Moyano et al., 2024; Enríquez et al., 2017; Motta-junior, 2002). Research focusing on this species, addressing issues related to its immune and endocrine systems, will significantly contribute to the development of conservation methodologies applicable to other nocturnal raptor species. The present study hypothesizes that the endocrine and immunological parameters of tropical screech owls respond to simulated noise within the healthcare facility of a rescue center, suggesting that the animals perceive this stimulus as a stressor, potentially leading to an increase in HPA axis activity and subsequent immunosuppression.

2. Materials and methods

2.1. Ethical permissions

This study complies with the Brazilian norms related to research involving wild species and is registered in the National System of Management of Genetic Resource, Management, and Associated Traditional Knowledge - SisGen No. A15F992. Permits for the collection of biological material were granted by the Biodiversity Information and Authorization System - SISBIO, process No. 70120–1. This research was previously approved by the Ethics Committee for the Use of Animals (CEUA) of the Faculty of Veterinary Medicine and Animal Science, University of São Paulo (FMVZ USP) No. 8414141021. The activities conducted at the Centro de Manejo e Conservação de Animais Silvestres (CeMaCAS) were also approved and registered in process No. 6027.2020/0005242 0.

2.2. Animals and experimental design

During the non-breeding season, between May and June 2021, twelve adult *M. choliba* – six males and six females – were continuously evaluated over a 49-day period. The technical staff at the rescue center (23° 24' 47"S 46° 47' 29"W) received these animals due to poor nutritional status or injuries that affected their ability to fly. However, these animals were in their final phase of rehabilitation (pre-release) when they were selected one month before the start of the test. It was confirmed that they were all in excellent overall health, exhibiting characteristics such as optimal body condition, pristine plumage, and even the ability to fly. Their health was confirmed through veterinary clinical evaluation, complemented by coprological and serological laboratory tests. They remained in the rescue center solely for this study, no longer under clinic care or direct handling by rescue center staff. Each individual was housed in a galvanized wire cage measuring 0.7 m × 0.6 m × 0.7 m. The cages containing the owls were placed in an isolated

enclosure at the rescue center facilities, allowing the individuals to be exposed to natural weather conditions. This isolation extends to the absence of vocalizations from both people and other animals, including opossums and felines. The animals received daily water *ad libitum* and a diet based on mice and quail offered as dead prey.

Between the fifteenth and thirty-fifth day, the animals were exposed to audio recordings simulating typical sounds encountered during routine hospital management in a rescue center. These included staff voices, noises associated with cage transfers, and vocalizations of different species, such as parrots and opossum grunts. To minimize predictability, five different audio tracks were randomly played between 7 a.m. and 5 p.m., ensuring each track was played at least once every hour. Each audio recording lasted 15 min and did not exceed 62 dB, representing the average sound level typical in the rescue center where this study was conducted. This level was determined by collecting data over three days with five measurements taken daily. We categorized the duration of audio stimulus exposure into three distinct periods: pre-stimulus (0–14 days before), stimulus (15–35 days during acoustic treatment), and post-stimulus (36–49 days after), as illustrated in Fig. 1.

Twelve evaluated owls were categorized into a non-manipulated group (n = 4, for fecal samples) and a manipulated group (n = 8, for blood and fecal samples). The selection of individuals was random, and group distribution remained consistent throughout the study. Every seven days, fecal samples were collected from all owls at four-hour intervals (09:00, 13:00, 17:00, and 21:00). On the same day, the owls from the manipulated group had blood samples obtained between 8:00 a.m. and 9:00 a.m., ensuring that the manipulation time for each individual did not exceed 1 min. A volume of up to 200 μL of blood was collected via jugular vein puncture using a 1 mL syringe containing heparin. Additionally, a small drop of blood was immediately placed on glass microscope slides to create a blood smear (see leukocyte profile methodology). Subsequently, blood samples were centrifuged for 5 min at 3500*g*, and the resulting plasma was separated and stored in 2 mL conical tubes. All samples were shipped at −4 °C and stored at −80 °C.

2.3. Extraction and quantification of the glucocorticoid metabolites (GCM)

Fecal glucocorticoid metabolites were measured using enzyme immunoassay (EIA) methods that utilized polyclonal corticosterone antibodies (CJM006; 1:4000) and HRP-conjugated corticosterone (1:40,000) from Coralie Munro in Davis, CA, USA. The EIA method had been previously validated for the species under study, following the feces sample treatment described by Barbosa-Moyano et al. (2023). Briefly, 0.050 g of lyophilized fecal sample was placed in a 16 × 120 mm glass tube, and 1.5 mL of 80 % methanol was added. The mixture was shaken three times for 5 min each at 2200 rpm, then centrifuged for 10 min at 1500 g. One mL of the supernatant was transferred to a new glass tube and dried using airflow and a 60 °C water bath. Finally, 0.3 mL of buffer solution (NaH2PO4, Na2HPO4, NaCl, BSA) was added and the suspension was vortexed for 5 min. All samples were analyzed in duplicate, and the plates were read at a wavelength of 405 nm using an ELX 808TM spectrophotometer from Bio Tek Instruments Inc, USA.

2.4. Plasma corticosterone (CORT)

Total corticosterone levels were assessed by the EIA DetectX-K014 (Arbor Assays™, Eisenhower Place, Ann Arbor, MI, USA) with the analytical validation parameters previously determined. To perform specificity tests, 30 μL of plasma from two males (K #95849 and B #94875) and two females (A #83763 and D #97263) were combined in a conical polypropylene tube, resulting in a pooled volume of 120 μL. Subsequently, 120 μL of a dissociation reagent provided by the EIA kit was added and mixed by vortexing. The sample was then subjected to successive 2-fold dilutions in EIA buffer solution, resulting in nine separate vials, each with a different concentration. A calibration curve was prepared using the corticosterone standard provided in the EIA kit, following the same conditions. Each sample was analyzed in duplicate, adhering to the manufacturer's instructions. The ELISA plates were read at a wavelength of 450 nm using a spectrophotometer (ELx 808™ Bio Tek Instruments Inc, USA). Accuracy was assessed through the recovery test (%REC), wherein a known amount of corticosterone standard was added in equal aliquots to a previously quantified owl plasma sample. The precision test involved analyzing intra-assay and inter-assay coefficients of variation (CV) for the same owl plasma sample

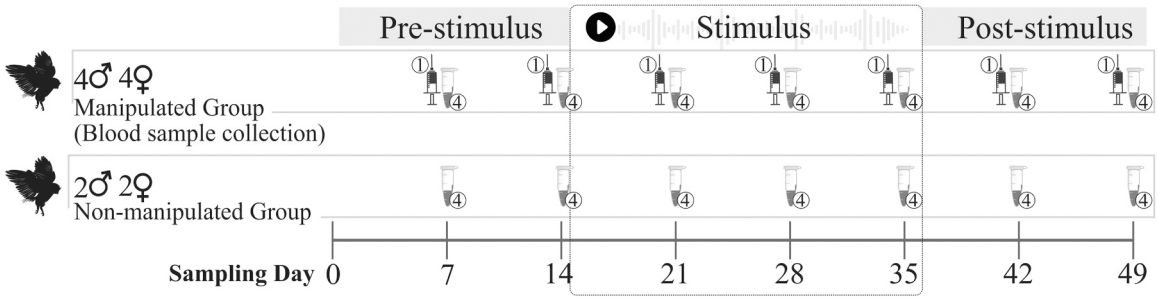

Fig. 1. Experimental Design Overview. The study's timeline included two pre-stimulus sampling days (7 and 14) and two post-stimulus sampling days (42 and 49). Between days 15 and 35, during auditory stimulation, three additional sampling days occurred (21, 28, and 35). The study involved two distinct groups: a non-manipulated group (for only fecal sample collection) consisting of four animals and a manipulated group (for blood and fecal sample collection) with eight individuals. The composition of both groups remained consistent throughout the entire experiment. On each sampling day, researchers collected four pooled stool samples and one blood sample from each individual.

to evaluate the repeatability of the measured values. All plasma samples collected during different treatment periods (pre-, during, and post-auditory stimulus) were treated with a dissociating agent at a 1:1 ratio and subsequently diluted 34 times in EIA buffer (determined through the parallelism test). The total corticosterone concentration in each plasma sample was evaluated in duplicate using the EIA, and the results were reported in μg/dL units.

2.5. Heterophil to lymphocyte ratio (H/L)

Immediately after blood collection, a drop of blood was spread onto the glass microscope slides to create a blood smear. Once naturally dried, the sample was stained using the Rosenfeld method. The concentration of leukocytes per mm^3 of blood was determined using the methodology proposed by Ilmonen et al. (2003). This method involves estimating the total number of white blood cells (WBC) by counting the number of leukocytes in ~10,000 erythrocytes. The relative abundance of heterophil to lymphocyte, denoted as the H/L ratio, was obtained by multiplying their proportion by the WBC.

2.6. Bacterial killing ability (BKA)

Non-pathogenic *Escherichia coli* pellets (Microbiologics, 324311 - ATCC 8739) were diluted in 1 mL of sterile phosphate-buffered saline (PBS). Next, 100 μL of the *E. coli* + PBS solution was mixed with 5 mL of sterilized trypticase soybean (TSB) solution and incubated overnight at 37 °C (De Assis et al., 2013). An aliquot of 300 μL of this culture was transferred to a 96-well ELISA microplate to determine bacterial concentration by measuring optical density at 600 nm using a spectrophotometer. For a working concentration of 1×10^6 microorganisms/mL, dilutions were prepared with sterile Ringer's solution [1 L contains 6.5 g NaCl, 2.0 g C6H12O6, 100 μL NaH2PO4 (10 %), 400 μL KCL (25 %), 450 μL CaCl2 (25 %), and 0.2 g NaHCO3].

In polypropylene tubes, 10 μL of each owl plasma sample was diluted in sterile Ringer's solution (190 μL). To this preparation, 10 μL of *E. coli* dilution (5×10^4 microorganisms/mL) was added. The positive control of the assay was determined by adding 10 μL of *E. coli* working solution to 200 μL of sterile Ringer's solution, while the negative control consisted of 210 μL of sterile Ringer's solution. All plasma samples containing the *E. coli* dilution, as well as the positive and negative controls, were incubated at 37 °C for 1 hour. After incubation, 500 μL of TSB solution was added to each sample. Subsequently, 300 μL of each sample was shaken and transferred in duplicate to a 96-well culture plate that had been preheated to 37 °C, ensuring a controlled temperature throughout the 6-hour duration. After the designated time had passed, four readings were taken from the plate at 1-hour intervals. The antimicrobial activity of the owl plasma was calculated as follows: activity = 1 - (optical density of the sample / optical density of the positive control). This calculation represents the proportion of microorganisms killed in the sample relative to the positive control.

2.7. Data analysis

The specificity of the immunoassay for quantifying corticosterone plasma levels was confirmed using the parallelism test. Student's t-test was used to compare the angular coefficients of the equations for the corticosterone standard curve and the owl plasma pool curve. The %REC was calculated by dividing the percentage of obtained binding by the percentage of expected binding. CV values were expressed in percentage units and calculated by dividing the standard deviation by the mean value. Statistical analysis of parallelism was conducted using GraphPad Prism (version 5.0, Windows, USA).

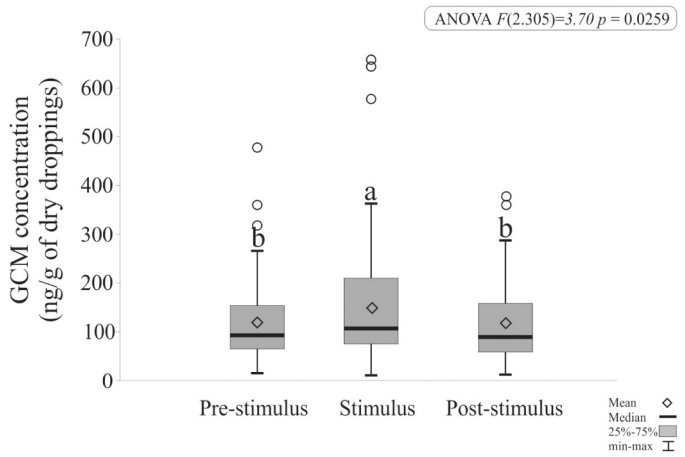

Fig. 2. Glucocorticoid metabolite concentrations (GCM) in owls (*Megascops choliba*) submitted to the acoustic treatment (pre-, during and post-stimulus moments). The two-way ANOVA showed that there is an effect of the time of collection and treatment on GCM levels, showing a significant interaction in the factors [F (2.305) = 3.70 p = 0.0259]. Outliers within the analyzed group are denoted by circles, while values sharing the same letter are statistically similar.

The concentrations of corticosterone and its metabolites, H/L ratio, and plasma bactericidal capacity were analyzed using descriptive statistics. This analysis considered different time sample points: before (0–14 days pre-stimulus), during (15–35 stimulus days), and after (36–49 post-stimulus) the acoustic treatment. Data normality and homogeneity of variances were assessed using the Shapiro-Wilk test and the Levene test, respectively. If necessary, data was subjected to transformation based on its distribution. A repeated measures ANOVA was employed to evaluate the impact of the auditory stimulus on GCM levels (ng/g) while considering factors such as sex and the group of animals (manipulated for blood sample collection and non-manipulated). Subsequently, a mixed ANOVA was performed to assess all dependent variables in relation to sex and treatment (pre-, during, and post-stimulus moments). Significant differences among groups were determined using Tukey post-hoc tests. The analyses were carried out using the Statistical Analysis System software (version 9.3 - SAS, Windows, USA) with a significance level set at $\alpha \leq 0.05$.

3. Results

3.1. Glucocorticoid metabolites

A total of 308 fecal samples were evaluated, with missing samples attributed to insufficient mass for extraction. The ANOVA analysis did not reveal any significant differences in GCM levels between the manipulated and non-manipulated animal groups [$F(1,305) = 2.43$, $p = 0.1499$]. We then considered the GCM values independently of manipulation for the next analysis. Two-way ANOVA demonstrated a significant effect of the treatment on GCM levels [$F(2,305) = 3,70$ $p = 0.0259$], while no sex effect was observed. The GCM concentrations in each group averaged 120.63 ng/g (pre-stimulus), 151.81 ng/g (during stimulus), and 120.23 ng/g (post-stimulus). There was a significant increase in GCM levels on the day of acoustic treatment, as illustrated in Fig. 2.

3.2. Plasma corticosterone level

In the analytical validation of the EIA, it was observed that the angular coefficient of the curve derived from the owl plasma pool (slope = −32.86) was not statistically different to that of the corticosterone standard curve (slope = −36.53, $F = 1.08$, $p = 0.321$), confirming the parallelism between the two curves (Fig. 3). This result indicates the immunogenic similarity between the antigens of the standard and the Tropical Screech Owl samples. By performing successive dilutions of the plasma pool, the optimal dilution (1:34) of the samples was determined, which corresponds to the closest percentage of binding to 50 %. The accuracy of the assay demonstrated a mean hormonal recovery of 95 ± 10 %. The intra- and inter-assay coefficients of variation were 3.5 % and 8.1 %, respectively.

A total of 55 plasma samples were analyzed, comprising 15 samples before, 24 during, and 16 after the stimulus phases, respectively. Plasma corticosterone exhibited mean values of 2.6 μg/dL for the pre-stimulus treatment, 2.4 μg/dL during the stimulus, and 2.3 μg/dL in the post-stimulus phase (Fig. 4). In this way, the plasma levels of this steroid showed no significant differences between treatments [$F(2,52) = 0.32$ $p = 0.7299$] or between sexes [$F(1,53) = 0.40$ $p = 0.5525$].

3.3. Heterophil to lymphocyte ratio (H/L)

A total of 55 smears were analyzed, and the H/L ratio displayed mean values of 0.64 in the samples collected during the pre-stimulus treatment, 0.53 during the stimulus, and 0.52 in the post-stimulus phase (Fig. 5). Thus, the H/L ratio showed no significant differences between treatments [$F(2,52) = 0.83$ $p = 0.44$] or between sexes [$F(1,53) = 0.05$ $p = 0.82$].

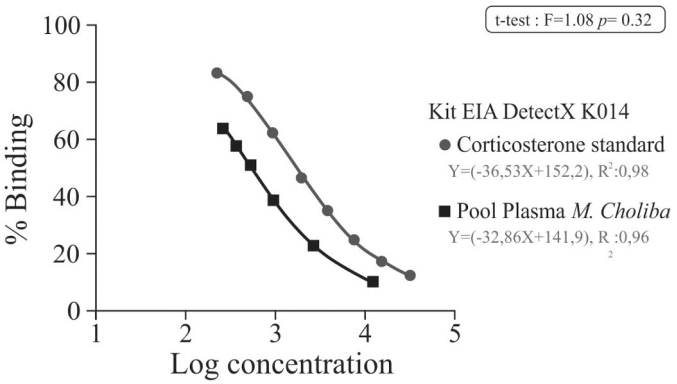

Fig. 3. Parallelism test between curves generated by diluting plasma samples from owls *Megascops coliba* and the corticosterone standard curve. The specificity of the Arbor Assay's DetectX K014 EIA was indirectly confirmed through the parallelism test. Standard curve of corticosterone diluted with a 2-fold dilution factor (circle) ($Y = 36.53x+152.5$, $R^2=0.98$); owl plasma pool (square) ($Y = -32.86x+141.9$, $R^2=0.96$).

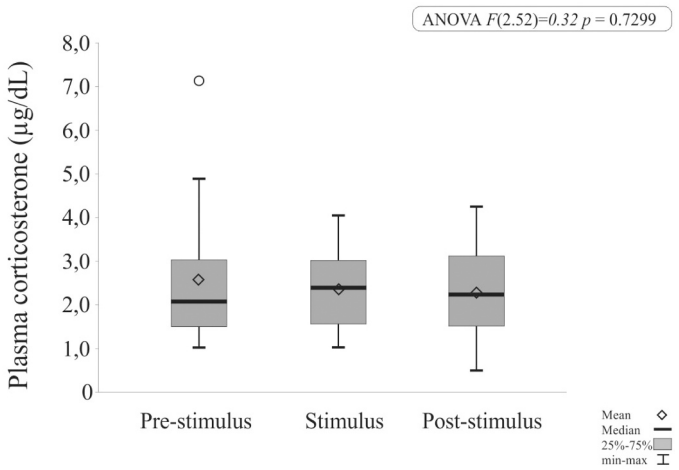

Fig. 4. Plasma levels of free corticosterone expressed in units of µg/dL in owls (*Megascops choliba*) submitted to the acoustic treatment (pre-, during and post-stimulus moments). ANOVA did not identify differences in the levels of this steroid hormone between treatments.

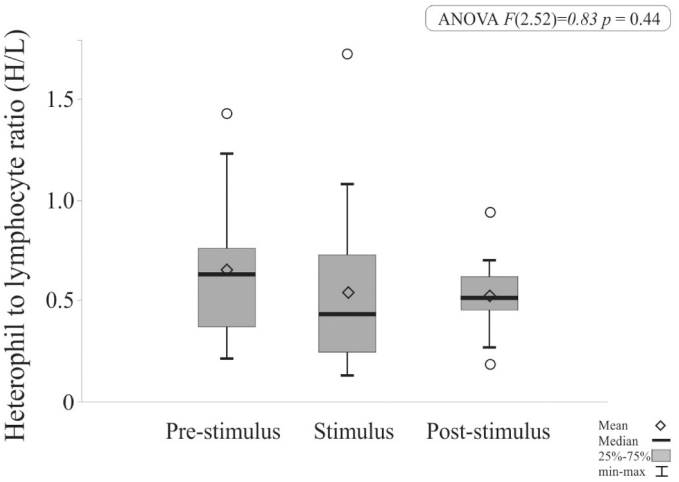

Fig. 5. The heterophil to lymphocyte ratio in owls *Megascops choliba* submitted to the acoustic treatment (pre-, during and post-stimulus moments). ANOVA did not identify differences in the H/L ratio between treatments.

3.4. Bacterial killing ability (BKA)

The values of BKA showed differences between treatments [F (2,49) = 6.51, p = 0.0137], but not between sexes [F (1,50) = 0.12, p = 0.7407]. Tukey's test reports higher BKA values for the pre-stimulus days (0.91 ± 0.09), while the values during stimulus (0.62 ± 0.28) and post-stimulus (0.66 ± 0.30) showed no differences (Fig. 6).

4. Discussion

We show that exposure of owls to audio simulations of a hospital environment within a rescue center results in elevated levels of glucocorticoid metabolites and significantly reduced plasma BKA. However, it is important to note that in response to the treatment applied in this study, no statistical differences were found in plasma corticosterone levels or the H/L. In addition to offering a non-invasive alternative for the evaluation of plasma glucocorticoid events (Goymann, 2012), the GCM analysis also offers a broader view of the stress response, as it represent accumulated levels over hours or days depending on the species (Goymann and Mostl, 2002; Palme, 2019). Therefore, the interpretation of the GCM concentration to explain a particular stressful event must be carried out considering, among others, the half-life of these steroids (Romero and Beattie, 2022). In a prior investigation, it was noted that *M. choliba* displayed an increase in GCM production hours after ACTH stimulation (Barbosa-Moyano et al., 2023). This observation confirms that the timing and duration of fecal sampling applied in this study can effectively reflect the adrenal response to the auditory stimuli used.

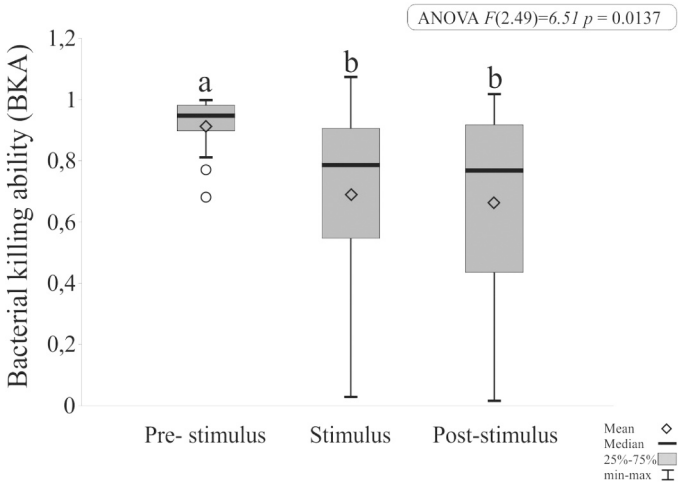

Fig. 6. Bacterial killing ability in plasma samples in owls *Megascops choliba* submitted to the acoustic treatment (pre-, during and post-stimulus moments). Different letters mark significant differences between treatments as determined by Tukey comparisons ($p < 0.05$).

The increase in glucocorticoid metabolite levels in owls suggests their sensitivity to auditory stimuli, with potential implications for their well-being, particularly in animals maintained under human care. This observation aligns with the concept of Reactive Homeostasis (Romero et al., 2009), wherein physiological mediators like glucocorticoids rise above normal levels to restore balance in response to stressors. Similar increases in GCM levels have been observed in other bird species under stress (Sinhorini et al., 2020; Stocker et al., 2016), showing allostatic overload in response to the presence of tourists in their natural environment (Arlettaz et al., 2015). On the other hand, research on Northern Spotted owls (*Strix occidentalis caurina*) has demonstrated that forest management practices can notably impact only males by increasing their GCM levels (Wasser and Hunt, 2005). Additionally, a study on free-living population of California Spotted owls (*Strix occidentalis occidentalis*) did not find a correlation of habitat condition and road proximity (factors potentially associated with chronic stress) with fecal corticosterone levels (Tempel and Gutiérrez, 2004). These researchers propose that the absence of confirmation regarding road-related stress may indicate that the owls of their study area were habituated to road traffic (Tempel and Gutiérrez, 2004). In this way, we propose that the owls of our study are not habituated to the noise of the rescue center, since the animals exhibited high levels of GCM during stimulus.

Despite observing an increase in GCM levels in owls, there was no variation in plasma corticosterone levels between treatments. The lack of correspondence between plasma corticosterone levels and its metabolites has been documented in other avian species (Ninnes et al., 2010). This discrepancy could be due to the inherent limitations of blood samples in offering insights into hormonal levels, as they provide merely a momentary snapshot of hormone levels within a constantly fluctuating production process (Goymann, 2012; Newman et al., 2017; Ninnes et al., 2010). In this way, probably a corticosterone response had occurred earlier, but the circulating concentration of this hormone had returned to the baseline level before the sampling point. In fact, chicks exposed to constant noise, for example, do not exhibit plasma corticosterone level modulation 7 days after the sound stimuli (McFarlane and Curtis, 1988). As both blood and fecal samples here were collected every 7 days - not necessarily immediately after the noise stimuli - our results contribute to illustrating that plasma corticosterone level is a more sensitive indicator of the immediate stress response, while GCM may play an important role in illustrating prolonged stress response. It is worth mentioning that the EIA DetectX K014 presented analytical parameters accepted for its validation in the detection and quantification of plasma corticosterone in the tropical screech owl (Brown et al., 2005), which are consistent with findings from other studies in avian species where the same kit was utilized (Mohammed et al., 2021; Ramos-Güivas et al., 2021; Wilsterman et al., 2020).

The increase in the H/L ratio is one of the multisystem responses to stress that has been studied in avian species (Breuner et al., 2013; Davis et al., 2008). In our study, owls were expected to show an increase in the H/L ratio after exposure to acoustic treatment, as demonstrated in chicks exposed to constant noise (McFarlane and Curtis, 1988). Nevertheless, the absence of leukocyte profile modulation has also been recorded in other species of owls (*Athene cunicularia*), in which increased H/L was expected in individuals residing near urban areas (Cavalli et al., 2018). The H/L increase due to stress stimuli can be attributed, at least in part, to the plasma corticosterone level enhancement. Birds such as the common kestrel (*Falco tinnunculus*), for example, show leukocyte redistribution, increasing H/L, after exogenous corticosterone treatment (Muller et al., 2011). In this way, the absence of leukocyte modulation concomitant with the absence of plasma corticosterone modulation shown here agrees with previous studies. As it requires minimal materials, we encourage further research into the use of the leukocyte profile. Its applicability has previously been demonstrated, showing that exposure of wild animals to new environmental and social situations, increases the N/L ratio (Minias, 2019; Müller et al., 2011; Vleck et al., 2000). The implementation of this method is also useful in the evaluation of enrichments to be implemented to minimize stress in animals kept under human care (Mohammed et al., 2021).

Previous research on other bird species has documented a decrease of up to 40 % in bactericidal capacity following exposure to containment stress (Matson et al., 2006). This aligns with our study's findings, confirming the negative impact of acoustic treatment on

the plasma components responsible for killing the *E. coli* strain in vitro. Such a reduction in plasma innate immunity could compromise the birds' ability to resist infections. While this study does not delve into the specific identification of plasma components responsible for bacterial lysis, it is plausible that innate immunity involves natural antibodies, serving as non-specific recognition molecules capable of limiting early microbial infections (Names et al., 2021; Ochsenbein et al. 1999; Sarrigeorgiou et al., 2023). Moreover, the complement enzyme cascade, a component of plasma, can lyse specific cells either through the formation of a membrane-attack complex end product or via protein by-products (Esser, 1994; Nordahl et al., 2004; Ostrycharz and Hukowska-Szematowicz, 2022). Additionally, lysozyme, present in plasma, exhibits bactericidal potential by enzymatically digesting structural carbohydrates in bacterial cell walls (Ferraboschi et al., 2021; Selsted and Martinez, 1978). The in vitro bacterial lysis capacity, a method less demanding than specific antibody assays has proven to be an effective means of evaluating the non-cellular innate immunity of individuals (Matson et al., 2006; Tieleman et al., 2005). To enhance our understanding in this area, we recommend future studies with larger sample sizes, extended study periods, or the inclusion of additional bacterial strains in the analysis. For instance, examining bacteria linked to conditions such as bumblefoot, a prevalent disease among captive raptors (Poorbaghi et al., 2012), could provide valuable insights.

Although captive animals have shelter and abundant food, urban conditions can often have negative physiological outcomes (Busch and Hayward, 2009; Fischer and Romero, 2018; Landys et al., 2006; Mason, 2010; Morgan and Tromborg, 2007; Tarlow and Blumstein, 2007). Thus, the acoustic exposure that simulates the hospital environment of a rescue center generated endocrine and immunological alterations in the owls. Given that the prolonged increase in glucocorticoid levels and compromised innate immunity negatively affect the health of animals; it is advisable to implement protocols that consider separating individuals based on their predator-prey status. Additionally, simple measures such as minimizing sudden movements during cage cleaning and conducting technical personnel communication away from the animals' housing can be beneficial. Apart from the ongoing initiatives aimed at mitigating the decline of wild animals, there is a need for additional research to investigate and evaluate the influence of anthropogenic stimuli on birds that are under human care (Tarlow and Blumstein, 2007; Woods et al., 2022). This necessitates the incorporation of ethological, immunological, and endocrine methods to gain a deeper understanding of the adaptability processes of animals in captive conditions and enhance our comprehension thereof.

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CRediT authorship contribution statement

Ribeiro Gomes Fernando: Supervision, Writing – review & editing. **Aymam De Cobo Figueiredo:** Methodology, Writing – review & editing. **Stefanny Christie Monteiro Titon:** Methodology, Validation, Writing – review & editing. **Melissa Peixoto Prosperi:** Methodology. **Mayra Hespanhol Frediani:** Methodology, Writing – review & editing. **Heriberto Barbosa-Moyano:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Claudio Alvarenga De Oliveira:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Heriberto Barbosa-Moyano reports financial support was provided by State of Sao Paulo Research Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Declaration of AI-ASsisted Technologies

During the preparation of this work, the author(s) utilized Conversational AI assistance from ChatGPT, a language model developed by OpenAI, to review and enhance the English grammar of this article. Following the use of this tool/service, the author(s) thoroughly reviewed and edited the content as necessary and assumed full responsibility for the publication's content.

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