ARIELA CASTELLI CELESTE

BEHAVIORAL RESPONSES OF VINACEOUS-BREASTED AMAZON PARROTS TO ANTI-PREDATOR TRAINING

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Manejo e Conservação de Ecossistemas Naturais e Agrários, para obtenção do título de *Magister Scientiae*.

Orientador: Leonardo Esteves Lopes

Coorientador: Cristiano Schetini de Azevedo

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Leonardo Esteves Lopes
Orientador

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Aos papagaios-de-peito-roxo, meus queridos roxinhos, seres incríveis e cativantes que despertaram meu interesse pelo comportamento animal, fizeram minha cabeça ferver em busca dos métodos de reabilitação mais adequados e me ensinaram tanto ao longo desse período. Nosso relacionamento foi intenso e não acaba por aqui! Acreditar que o trabalho que desenvolvemos juntos pode contribuir com a conservação não apenas dessa, mais de diversas outras espécies me inspira e renova minhas energias para continuar nessa luta!

RESUMO

CELESTE, Ariela Castelli, M.Sc., Universidade Federal de Viçosa, março de 2023. **Respostas comportamentais de papagaios-de-peito-roxo ao treinamento anti-predador.** Orientador: Leonardo Esteves Lopes. Coorientador: Cristiano Schetini de Azevedo.

Translocações conservacionistas possuem baixas taxa de sucesso, principalmente devido à falta de habilidades dos animais introduzidos para sobreviver no novo ambiente, como a ingenuidade diante de um predador em potencial. O treinamento pré-soltura, baseado no condicionamento clássico, pode permitir que esses animais identifiquem ameaças. O presente estudo avaliou as respostas comportamentais de uma espécie tropical ameaçada de extinção, o papagaio-de-peito-roxo (*Amazona vinacea*), ao treinamento anti-predador. Investigou-se a hipótese de que, em papagaios cativos, o treinamento anti-predador pode estimular comportamentos aversivos em relação a predadores, e investigou-se ainda se os animais se habituaram ao treinamento. Onze papagaios foram submetidos ao treinamento anti-predador, onde foram utilizados quatro diferentes modelos de predadores (onça-pintada, ave de rapina, cachorro e humano) e uma cadeira como controle. Durante o treinamento, os modelos de predadores foram associados a um estímulo aversivo, representado por uma pessoa disfarçada perseguindo os animais. Todo o treinamento foi gravado em vídeo e os comportamentos dos papagaios foram coletados por meio da análise das filmagens. Modelos Lineares Generalizados Mistos (GLMMs) foram ajustados para avaliar os comportamentos dos animais durante o treinamento e para avaliar diferenças nos comportamentos de acordo com o modelo de predador. Também foi realizada uma Análise de Séries Temporais (AST) por meio de uma Autocorrelação Temporal para identificar sinais de habituação. Os papagaios fugiram durante os treinos com todos os predadores, mas, em menor frequência, também andaram na presença do cachorro, do humano e da ave de rapina. Os comportamentos alimentando e preening foram os mais observados nos treinos com a cadeira. Os papagaios apresentaram mudanças comportamentais ao longo do treinamento, com diminuição da frequência do comportamento andando e aumento do comportamento alerta, o que é um indicativo que as aves não se habituaram ao treinamento. Os gráficos obtidos pela AST também corroboram esse resultado. Nossos resultados mostraram que o treinamento cumpriu seu objetivo em cativeiro, sendo necessário monitorar as aves após a soltura para identificar se o mesmo contribuirá para a sobrevivência dos animais na natureza.

Palavras-chave: *Amazona vinacea*. Translocação. Psittacidae. Condicionamento Clássico.

Conservação.

ABSTRACT

CELESTE, Ariela Castelli, M.Sc., Universidade Federal de Viçosa, March, 2023. **Behavioral responses of Vinaceous-breasted Amazon parrots to anti-predator training**. Adviser: Leonardo Esteves Lopes. Co-adviser: Cristiano Schetini de Azevedo.

Conservation translocations have a low success rate mainly due the lack of skills of introduced animals to survive in the new environment, such as naivety facing a potential predator. Pre-release training based on classical conditioning may allow those animals to identify threats. Here we evaluated the behavioral responses to anti-predator training by an endangered tropical species, the Vinaceous-breasted Amazon parrot (*Amazona vinacea*). We investigated the hypothesis that anti-predator training of captive parrots can stimulate aversive behavior towards predators, also investigating whether animals habituated to the training. Eleven parrots were submitted to anti-predator training using four different predator models (jaguar, bird of prey, dog, and human) and a chair as control. Training with predator models were associated with an aversive stimulus, represented by a disguised person chasing the animals. All training was video recorded and parrots' behaviors were collected through footage analysis. Generalized Linear Mixed Models (GLMMs) were adjusted to evaluate the animals' behaviors during training and to evaluate changes in behavior according to the predator model. We also carried out a Time Series Analysis (TSA) through a Temporal Autocorrelation to identify signs of habituation. Parrots escaped more in training with all predators but also walked in the presence of the dog, the human, and the bird of prey. The behaviors eating and preening were most observed in training with the chair. The parrots showed behavioral changes over training, with a decrease in the frequency of the behavior walking and an increase in the behavior alert, which is an indication that parrots did not habituate to training. The graphics obtained by TSA also corroborated this finding. Our results showed that training accomplished its purpose in captivity and it is now necessary to monitor the birds after release to identify if training will contribute to the animals survival in the wild.

Keywords: *Amazona vinacea*. Translocation. Psittacidae. Classical Conditioning. Conservation.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE SIGLAS E ABREVIATURAS

CETAS	Centro de Triagem de Animais Silvestres
CEUA	Comitê de Ética no Uso de Animais
CRAS	Centro de Reabilitação de Animais Silvestres
IBAMA	Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis
IEF	Instituto Estadual de Florestas
UFV	Universidade Federal de Viçosa

SUMÁRIO

Behavioral responses of Vinaceous-breasted Amazon parrots to anti-predator training

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Abstract

Conservation translocations have a low success rate mainly due the lack of skills of introduced animals to survive in the new environment, such as naivety facing a potential predator. Pre-release training based on classical conditioning may allow those animals to identify threats. Here we evaluated the behavioral responses to anti-predator training by an endangered tropical species, the Vinaceous-breasted Amazon parrot (*Amazona vinacea*). We investigated the hypothesis that anti-predator training of captive parrots can stimulate aversive behavior towards predators, also investigating whether animals habituated to the training. Eleven parrots were submitted to anti-predator training using four different predator models (jaguar, bird of prey, dog, and human) and a chair as control. Training with predator models were associated with an aversive stimulus, represented by a disguised person chasing the animals. All training was video recorded and parrots' behaviors were collected through footage analysis. Generalized Linear Mixed Models (GLMMs) were adjusted to evaluate the animals' behaviors during training and to evaluate changes in behavior according to the predator model. We also carried out a Time Series Analysis (TSA) through a Temporal

Autocorrelation to identify signs of habituation. Parrots escaped more in training with all predators but also walked in the presence of the dog, the human, and the bird of prey. The behaviors eating and preening were most observed in training with the chair. The parrots showed behavioral changes over training, with a decrease in the frequency of the behavior walking and an increase in the behavior alert, which is an indication that parrots did not habituate to training. The graphics obtained by TSA also corroborated this finding. Our results showed that training accomplished its purpose in captivity and it is now necessary to monitor the birds after release to identify if training will contribute to the animals survival in the wild.

Keywords: *Amazona vinacea*, Translocation, Psittacidae, Classical Conditioning, Conservation

Introduction

The translocation of living beings, which consists in their human-mediated movement from one area with release into another, can have different purposes, such as commercial, recreational, aesthetic, biological control, or management (IUCN/SSC, 2013; Seddon et al., 2012). The latter purpose aims to restore, enhance, or sustain local biodiversity and has become a frequent strategy in the face of the growing number of endangered species. Conservation translocations, especially those carried out with animals, have aroused popular interest and gained media attention, being considered effective conservation events (Evans et al., 2023; Resende et al., 2020; Seddon et al., 2007). Even so, post-release evaluations show that conservation translocations have a low success rate (Morris et al., 2021), mainly due to the lack of skills of introduced individuals to survive in the new environment (Berger-Tal et al., 2020; Shier, 2016).

Predation of translocated individuals has been identified as one of the key issues in conservation projects, being responsible for the failure of many of them (Fischer and Lindenmayer, 2000; Moseby et al., 2011). Candidate animals for translocation usually have two different origins: they may come from nature (wild animals) or be raised in captivity. In both cases, the ability to recognize predators is crucial for the successful establishment of individuals in the wild (Griffin et al., 2000; Moseby et al., 2011). After translocation, wild animals may have trouble responding to predators not found in their original area, especially if the translocated group is composed of young individuals (Greggor et al., 2019). Even worse, animals that have spent long periods in captivity may no more be able to recognize predators or to elicit appropriate responses to situations of danger/threat (Berger, 1998; Blumstein and Daniel, 2005; Greggor et al., 2019; Jolly et al., 2018; Martin, 2014; Shier, 2016).

As the success of any conservation program depends on the survival of the individuals, the lack of an appropriate response when facing a potential predator is a risk factor to consider (Bell, 2016). Therefore, the learning capacity of animals can be used in favor of conservation, with training prior to translocation allowing those animals to identify potential predators and respond accordingly (Griffin et al., 2000). Such training is based on the classical conditioning and consists of the association of a conditioning stimulus (CS), represented by a model of a given predator or even predator cues (as body odor or feces), with an aversive unconditioned stimulus (US), such as a pursuit or spray of water (Edwards et al., 2021; Griffin et al., 2000). Training protocols usually take into account the specificities of the focal species, once as the choice of the CS and the US, or even the frequency with which the sessions are carried out, can generate habituation (Griffin et al., 2000; Moseby et al., 2014; Tetzlaff et al., 2019; Zhu et al., 2022). In order for training to achieve its purpose and influence the post-release survival of translocated animals, it needs to encourages appropriate and lasting behavior towards predators (such as flight, grouping, vigilance, warning signals) (Shier, 2016). Although training effectiveness has been hard to access after release (Greggor et al., 2019; Zhu et al., 2022), studies have obtained good results in captive trained animals such as Turquoise-fronted Amazon parrots (*Amazona aestiva*) (Azevedo et al., 2017), El Hierro giant lizard (*Gallotia simonyi*) (Burunat-Pérez et al., 2018), and eastern barred bandicoots (*Perameles gunnii*) (Taylor et al., 2022).

Many conservation efforts are addressed to parrots, macaws, parakeets and relatives (Psittacidae), once the family is among the most endangered group of birds, with 405 existing species worldwide, of which 109 are classified as threatened (HBW and BirdLife International, 2022). The main threats affecting the conservation status of the group are related to human activities, being habitat loss in first place, followed by hunting, and trapping (Olah et al., 2016). Trapped animals are frequently seized in actions to combat wildlife trafficking, and translocation programs are among their main destination (Vilela and Lopes, 2018). Despite that, the success of these translocation programs are uncertain, as predation is an important factor of reduced post-release survival (White et al., 2012). In this way, the pressure exerted by predation on translocated animals can cause major impacts on populations, especially of endangered species (IUCN/SSC, 2013; Scott and Carpenter, 1987).

Among the many parrot species that are currently threatened, the Vinaceous-breasted Amazon parrot (*Amazona vinacea*) is declining mainly due to habitat loss and the illegal capture of nestlings (Cockle and Bodrati, 2020; Forshaw, 2010; Juniper and Parr, 1998). Consequently, this species is considered Endangered at global level (IUCN, 2021). The historical range of the species, which is endemic to the Atlantic Forest (Vale et al., 2018), comprises the southeastern Brazil (from southeastern Bahia to Rio Grande do Sul), southeastern Paraguay and northeastern Argentina (Cockle and Bodrati, 2020; Forshaw, 2010; Juniper and Parr, 1998), covering more than 400,000 km^2 (Zulian et al., 2021). Its population is estimated in 8,500 individuals in the wild, located mainly (~90%) in Brazil (Zulian et al., 2020).

Despite being a target species of conservation projects (only in Brazil there are three ongoing projects with the species), behavioral studies with Vinaceous-breasted Amazon parrots are scarce. These studies focused on the analysis of reintroduction preparatory training (flight training and aversion to human training) (Pedroso, 2013), behavioral analysis of environmental enrichment (Martins, 2020), and personality analysis associated to environmental enrichment, flight ability, and response to humans (Ramos et al., 2021). Thus, this study intended to evaluate the behavioral responses to anti-predator training by Vinaceous-breasted Amazon parrots participating in a conservation project that aimed to rehabilitate, release, and monitor parrots victim of wildlife trafficking. We investigated the hypothesis that anti-predator training of captive parrots can stimulate aversive behavior towards predators. It was expected that: 1) training would stimulate aversive behaviors; 2) parrots would be capable of distinguishing between predator models and a harmless stimulus, showing distinct behavioral responses when faced with them; and, finally, 3) that parrots would not habituate to training sessions.

Materials and methods

Animals and study area

This study was carried out from June to September 2021 on the facilities of the Wild Animals Rehabilitation Center (CRAS – acronym in Portuguese), a structure maintained by the Instituto Estadual de Florestas (IEF) and by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), which are, respectively, the Minas Gerais state and the Brazilian environmental agencies. This study is part of a larger project that selected

22 adult Vinaceous-breasted Amazon parrots from seven different institutions, to be released in the wild. These animals were illegally trapped from the wild when juveniles and raised by humans, except for five parrots that were born in captivity and raised by their parents. There is no further information regarding previous experience of the animals. We selected 11 individuals to take part in the activities proposed here (n = 5 males and 6 females, 3 of which were captive-born). All the animals went through health exams, where it was investigated the presence of hemoparasites, *Chlamydia psittaci*, *Mycoplasma gallisepticum*, circovirus, avian bornavirus, and herpesvirus. Complete blood count, serum biochemistry, coproparasitological examination, and bacteria culture tests were also carried out. The birds were microchipped, received a numbered metal ring and, for individual identification, were marked in the chest with different colors with a non-toxic Acrilex™ paint.

The group was housed in an aviary (11m x 4m x 4m, 16m³/animal) located in an open area, with one of its smaller sides, made of wire mesh, facing the native forest vegetation. The other small side was made of brickwork and the two larger sides of the aviary, which were also made of wire mesh, were closed with canvas, avoiding visual contact of the animals with the external environment. The top of the aviary was covered by roof tiles in the part made of brickwork, and by wire mesh in the rest of it (Fig. 1). The canvas had small holes, allowing the researcher to observe the parrots. During anti-predator training, four cameras were positioned covering every angle of the aviary. The camera models used were a 1) Canon PowerShot SX70 HS; 2) GoPro Hero 4; 3) Cellphone Samsung Galaxy Note 10; 4) Nikon P510 (Fig. 1).

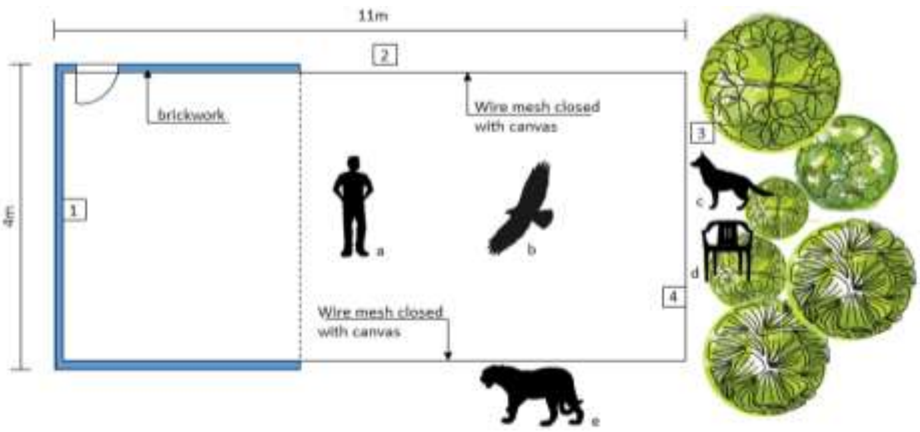

Figure 1. Aviary characterization, cameras positioning and place of presentation of predator models during anti-predator training. For details on the test arena and camera models, see the Materials and methods section of the text. During anti-predator training (a) the human was the only model presented inside the aviary, (b) the bird of prey was presented from above and the (c) dog, (d) the chair (control), and (e) the jaguar were presented on the sides of the aviary.

The parrots were fed twice a day with fruits, seeds, and a mixture of vegetables, extruded parrot food, and corn grit. Food was offered in different schedules to avoid habituation, and water was provided *ad libitum*. Feeding was provided through a hatch in the brick wall, so the birds did not have visual contact with the keeper. During the study, the parrots had contact with humans only at the aviary cleaning routine. The aviary was equipped with fixed and mobile perches of different diameters and textures and was frequently set with fresh branches and leaves. Whenever it was necessary to enter the aviary, the researchers used clothes that disguised the human silhouette.

Ethogram

An ethogram was designed based on previous studies with the species (Prestes, 2000; Queiroz et al., 2014; Ramos et al., 2021) and on behavioral observations conducted from March to May 2021 at CRAS and at the Wild Animal Screening Center (CETAS – acronym in Portuguese), another facility administered by the Brazilian environmental agencies (IEF and IBAMA). *Ad libitum* observation (Altmann, 1974; Bateson and Martin, 2021) were carried out from 8:00 h to 17:00 h, totaling 7 hours of records. During this period, 35 behaviors were recorded and classified into 10 categories (see Appendix A - supplementary material, table 1). Such material was used to elaborate the behavioral assessment sheets used to collect anti-predator training data.

Anti-predator training

Anti-predator training was designed using classical conditioning techniques and following the methods proposed by Griffin et al. (2001), where the figure of a potential predator is associated with an aversive stimulus. The following predator models were used: a life-size photograph of a jaguar (*Panthera onca*) printed and pasted on a wood board; a 3D model of a bird of prey in flight, measuring 70cm wingspan and colored according to the plumage of a Harris's Hawk (*Parabuteo unicinctus*); a large adult dog (*Canis lupus*) of the German Shepherd breed; and a person wearing everyday clothes (pants and t-shirt) (Fig 2). A white plastic chair was used as control to assess whether animals responded differently when faced with a predator and a non-dangerous object. The aversive stimulus was represented by a person who entered the aviary carrying a net and wearing clothes that disguised the human silhouette, then chasing the parrots (Fig 2e). The clothes used during the aversive stimulus were different than those used when the researches needed to enter the aviary.

Figure 2. Predator models used during anti-predator training with the Vinaceous-breasted Amazon parrot (*Amazona vinacea*): (a) a printer model of a jaguar, (b) a 3D printed model of a bird of prey, (c) a real dog and (d) a person. A disguised person carrying a net.

All predator models were presented to the parrots from outside the aviary except for the person. Tie rods were attached to the jaguar model, allowing it to pass between the canvas and the aviary wire mesh when pulled, making it visible to the birds, where it was held static for a short time period (see below). The bird of prey was attached to a rod and presented above the aviary, as soaring; the dog was attached to a long leash (5m) and presented on the smaller side of the aviary, facing the vegetation, where it remained in silence, standing still or making short walks in front of the wire mesh; the chair was presented in the same side of the aviary as the dog, and was positioned with the aid of a rod. The person entered the aviary through the entrance door, positioning himself in the center of it, standing still for a short time period (Fig 1).

All training sessions were video recorded and data collection was carried out through the footage analysis, using the focal animal method with instantaneous recordings every 15 seconds (Altmann, 1974; Bateson and Martin, 2021). However, it was not possible to collect vocalization behaviors due the poor audio quality of the footages. Training protocol consisted in presenting the predator model to the birds for 30 seconds. The model was then removed, and an aversive stimulus (disguised person chasing parrots) was carried out for another 30 seconds. After the chase ended, the predator model was displayed again, for another 30 seconds. The control training performed with the chair followed the same procedures described, except that the disguised person did not entered the aviary, neither chased the parrots.

All training procedures were previously tested without the presence of parrots and five field assistants helped the senior author to conduct the experiment. The positioning of the cameras and subsequent analysis of the video recordings were performed by the same researcher, who also monitored all the training sessions through small holes in the canvas and registered the positioning of each individual at the beginning and at the end of the procedures. Such records helped in the parrot's identification when performing the analysis of the footage (see below). The five field assistants took turns to perform the aversive stimulus (chasing) and the predator's presentation.

Training sessions were carried out each time with a different predator and conducted twice a week between 8:00 h and 10:00 h, period of the day when the birds are active and when there was no natural light interference on the video records. Three repetitions were carried out with each one of the four predator models as well as with the chair randomly, totaling 15 sessions.

All 11 parrots were trained at the same time, once the Vinaceous-breasted Amazon parrot is a social species and the aviary size was appropriate for the number of individuals, so that there was no risk of accidental collision, thus avoiding unnecessary handling.

Statistical analysis

Analyses were performed using R Studio Software (v.4.2.1; R Core Team, 2022). To evaluate behavioral responses of Vinaceous-breasted Amazon parrots to anti-predator training, we used generalized linear mixed-effects models (GLMMs), associating the behaviors expressed by the parrots with the different predator models exhibited during training. We included in the models the predators as independent variable, the behaviors as dependent variable and the number of training repetition as random variable. Tukey *Post hoc* tests were carried out in order to identify significant differences between predators, by using the emmeans R package (v1.7.5; Lenth, 2022).

We also evaluated changes over time in the behaviors expressed during training to identify if the procedure stimulated aversive behaviors in the parrots and also to indicate if the animals habituated to training or not. For that, GLMMs were adjusted with the number of training repetitions as independent variable, the behaviors as dependent variable and the predator types as random variable. All models were run with the package ‘lme 4’ (Bates et al., 2015). For all models, data-distributions were tested through residual analysis. To identify if changes in behavior in a training affected subsequent training, we conducted a Time Series Analysis through a Temporal Autocorrelation (already available in the R base package) with the significant results obtained by GLMMs analysis, correlating the behaviors expressed in front of predators (time series) by days of training (units of time) (Crawley, 2007). Alpha level adopted was of 5%.

Ethics approval

This study is part of a larger project entitled “Voar Project – Rehabilitation, release, and monitoring of the Vinaceous-breasted Amazon parrot (*Amazona vinacea*) in the state of Minas Gerais/ Brazil”, carried out by Waita – Instituto de Pesquisa e Conservação. This project has been approved by the Ethics Committee for the Use of Animals at the Universidade Federal de Viçosa (CEUA – UFV, protocol n. 22/2021), by IEF (Authorization n. 28097356) and IBAMA (Process n. 02015.000745/2021-78).

Results

Behaviors facing predators

Six of the behaviors described in the ethogram were frequently performed by the parrots during anti-predator training (resting, walking, feeding, preening, alert, and escaping). Another six behaviors (flying relaxed, foraging, pecking, cleaning the beak, yawning, and hiding) were also recorded, but at a very low frequency (less than 10 records) and therefore were excluded from the analysis.

The type of predator modified ($p<0.05$) the behaviors walking, feeding, preening, and escaping (Table 1). The *post-hoc* tests showed that the parrots walked more in the presence of the dog, the human, and the bird of prey, than in the presence of the chair and the jaguar (Fig. 3a). The parrots fed more in the presence of the chair, and the frequency of this behavior was lower in front of the human in relation to other predators (dog, jaguar, and bird of prey) (Fig. 3b). The behavior preening also showed higher frequency in the training sessions with the chair when compared to the training sessions with the predators (Fig. 3c). Parrots escaped more in the training sessions with all the predators than with the chair (Fig. 3d).

Table 1. GLMM results for behavioral responses in front of predators. The different predator models were used as independent variable, the behaviors as dependent variable and the training repetition as random variable.Significant results highlighted in bold.

Dependent variable	DF	AIC	Distribution	Deviance	x^2	p-value
Inactive	4	659.62	Poisson	647.62	3.5568	0.4693000
Walking	**4**	**278.88**	**Poisson**	**266.88**	**14.596**	**0.0056180**
Eating	**4**	**221.12**	**Poisson**	**209.12**	**14.762**	**0.0052200**
Preening	**4**	**183.21**	**Poisson**	**171.21**	**15.616**	**0.0035810**
Alert	4	110.49	Poisson	98.493	4.8117	0.3446000
Escaping	**4**	**376.04**	**Poisson**	**364.04**	**46.627**	**1.823e-09**

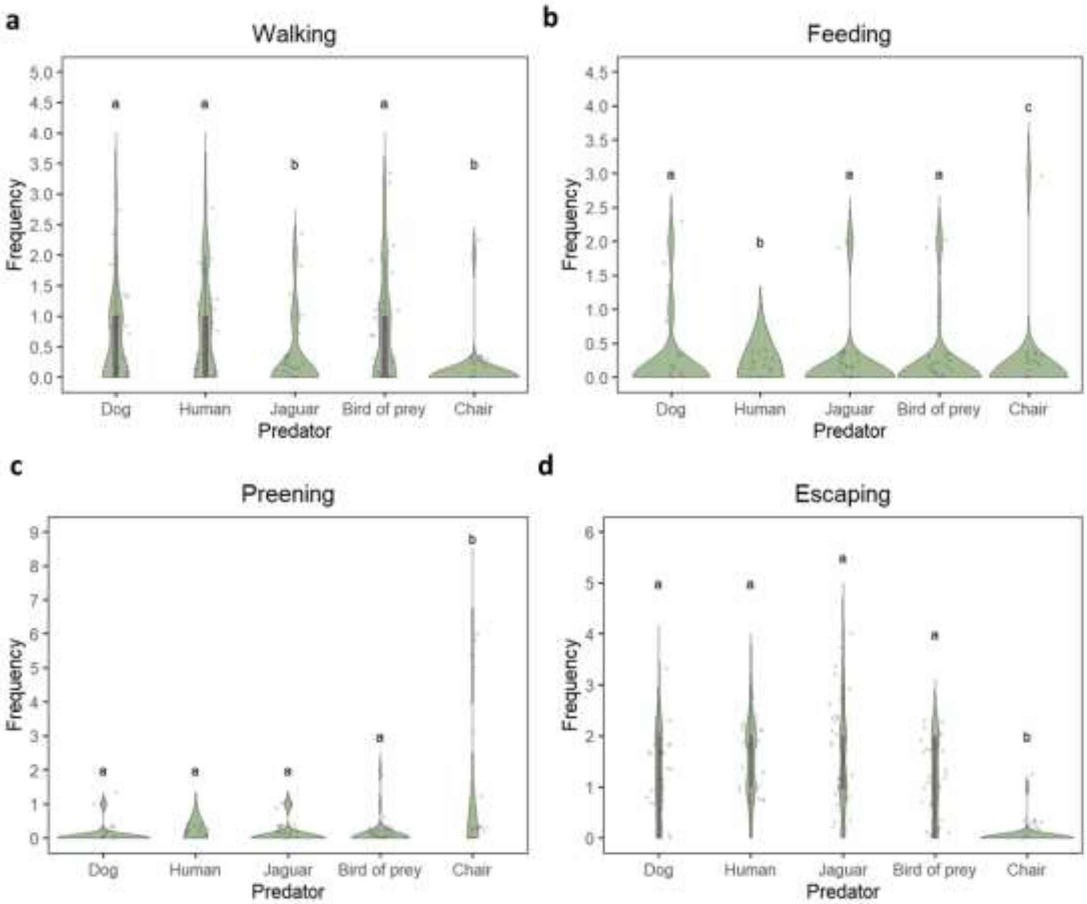

Figure 3. Violin plots of the behaviors performed by Vinaceous-breasted Amazon parrots when facing four different predators (dog, human, jaguar, bird of prey) and a control model (chair) during anti-predator training. Different letters represent significant statistical differences.

Behavioral responses over time

GLMM analyzes identified behavioral changes over the course of training for the behaviors walking and alert (Table 2). Throughout the training period the parrots showed a decrease in frequency of the behavior walking (Fig. 4a) and an increase in the behavior alert (Fig. 4b).

Table 2. GLMM results for behavioral changes of the Vinaceous-breasted Amazon parrot (*Amazona vinacea*) over the course of anti-predator training. The number of training repetition was used as independent variable, the behaviors as dependent variable and the predators as random variable.Significant results highlighted in bold.

Dependent variable	DF	AIC	Distribution	Deviance	x^2	p-value
Inactive	1	129.32	Poisson	123.33	0.5637	0.452800
Walking	**1**	**84.890**	**Poisson**	**78.890**	**4.1543**	**0.041530**
Eating	1	78.513	Poisson	72.513	0.1156	0.733900
Preening	1	62.400	Poisson	56.400	7e-04	0.979400
Alert	**1**	**46.194**	**Poisson**	**40.194**	**4.8488**	**0.002057**
Escaping	1	91.424	Poisson	85.424	0.8088	0.368500

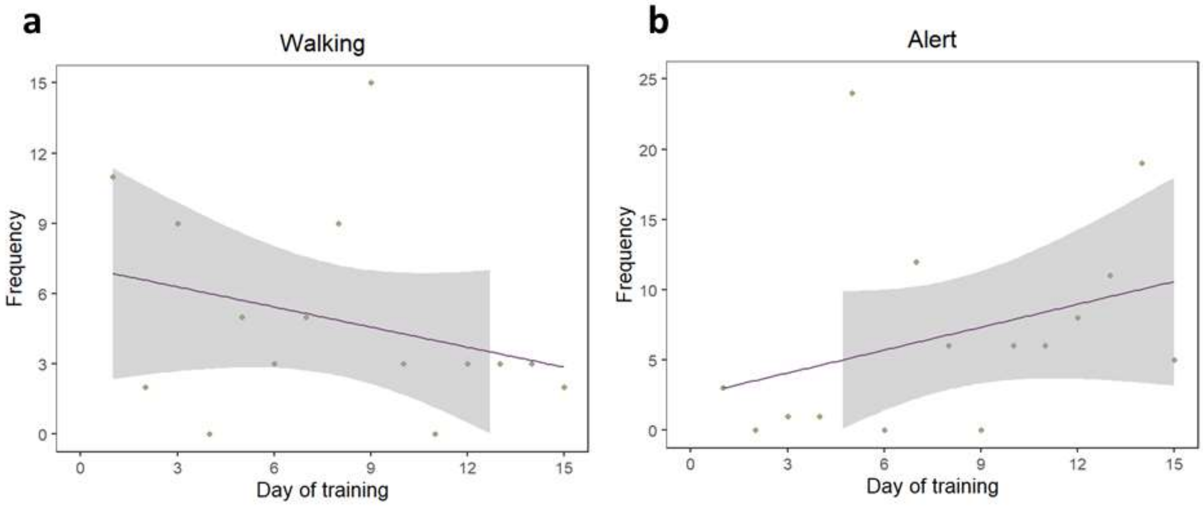

Figure 4. Plots displaying Vinaceous-breasted Amazon parrots behavioral changes facing anti-predator training over time, showing a decrease in the frequency of the behavior walking (a) and an increase in the frequency of the behavior alert (b).

Habituation to training

Once behavioral changes over time were identified for the behaviors walking and alert (Table 3), a temporal autocorrelation was performed. The graphical result of this analysis showed that the behaviors expressed by the parrots in a given training session did not influence the behaviors in the following sessions, once significant autocorrelation ($p<0.05$) is expressed only when vertical bars reach or cross the dotted lines (Fig. 5). This indicates that the parrots did not habituate to training (Fig. 5).

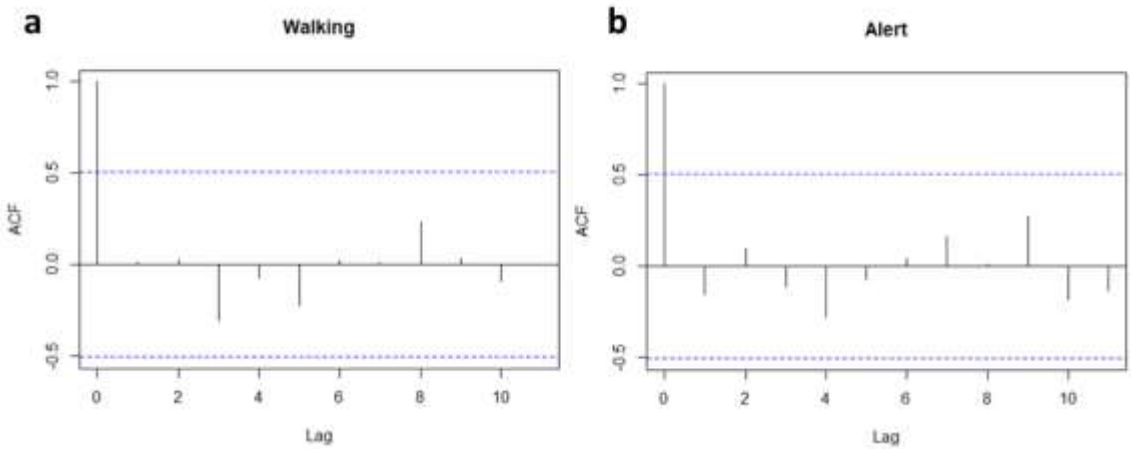

Figure 5. Time Series Analysis showing the relationship of the behaviors and subsequent training sessions. Bar above/below dotted lines express significant autocorrelation ($p<0.05$). Lag = Number of training session. ACF = Auto correlation factor.

Discussion

In this study we corroborated the hypothesis that training can stimulate aversive behaviors in Vinaceous-breasted Amazon parrots, which was demonstrated by an increase in the behavior alert and a reduction in the behavior walking during training period. We found that parrots were capable to distinguish between predator models and a harmless stimulus, by showing more relaxed behaviors (eating and preening) when facing harmless stimulus, and we also found no signs of habituation of the parrots to the training protocol. All our finds show that training had good results in captive Vinaceous-breasted Amazon parrots.

The behavior walking was observed in the initial trainings carried out with the dog, the human, and the bird of prey. This is a mild response, but demonstrates the perception of the risk present in the situation. During anti-predator training, tammar wallabies (*Notamacropus eugenii*) responded with fear responses, which were characterized by an increase in locomotion (Griffin et al., 2001). The same was observed in a study with crab spiders (*Xysticus koch*), that responded walking when exposed to ant's cues (*Formica clara*) (Mestre et al., 2014), and hellbender salamanders (*Cryptobranchus alleganiensus*), that increased locomotion when exposed to chemical cues of conspecifics associated with the presence of a predator (Crane and Mathis, 2011). Once the sampled group comprises animals victims of wildlife trafficking, which for some period of their lives maintained contact with humans and, possibly, with dogs, walking may be the result of their previous experience. Additionally, vultures were constantly flying over the parrots' aviary. The species does not represent a threat to the parrots, but their shape resembles of the bird of prey model used during this study. So, walking could be a sign of the restlessness observed in the initial training sessions with this predator. Nevertheless, parrots reduced this behavior and increased in alert over time, which is evidence that training accomplished its purpose. Anti-predator training with other species also had good results in captivity, with eastern barred bandicoots (*Perameles gunnii*) learning to avoid areas with cat (*Felis catus*) cues, El Hierro giant lizards (*Gallotia simonyi*) fleeing from models of kestrel and cat, and Turquoise-fronted Amazon parrots (*Amazona aestiva*) increasing awareness and reducing relaxing behaviors against predator models (Taylor et al., 2022; Burunat-Pérez et al., 2018; Azevedo et al., 2017).

Here we also evaluated the behavioral responses of Vinaceous-breasted Amazon parrots during anti-predator training in front of four different predator models (human, dog, jaguar, and bird of prey) and a control (chair). As expected, the parrots responded to all predators with aversive behaviors, with escaping being the most performed behavior. This result is consistent with that found for wild-caught Northern quolls (*Dasyurus hallucatus*) and their captive-born and predator-naïve chicks, where they recognized cues of predators and promptly escaped (Jolly et al., 2018). The predator size can interfere in the prey's perception of fear (Stankowich and Blumstein, 2005), with large predators inducing escaping as soon as they are noticed. This association is in line with our findings: escaping frequency was slightly higher in training with the jaguar, the human, and the dog, predator models larger than the bird of prey.

When exposed to a predator model, the parrots displayed aversive behaviors. Contrarily, when exposed to a control model (chair), the parrots displayed more relaxed behaviors (eating and preening). This result shows that the chair did not elicit fear in the birds, which can be explained once it is a model that does not represent a threat, in addition to the fact that the control training procedure did not pair the presented model with an aversive stimulus. Animals as diverse as tammar wallabies (*Macropus eugenii*), blue, great and willow tits (*Cyanistes caeruleus, Parus major,* and *Poecile montanus*), and greater rhea (*Rhea americana*) also didn’t show fear for harmless models (Griffin et al., 2001; Hogstad, 2017; Azevedo and Young, 2006). Thus, in our study, parrots learned to associate danger to only significant stimuli, i.e., predators.

Another point evaluated refers to the possibility that parrots habituated to training. Designing training procedures, from choosing the right aversive stimulus to how many sessions must be carried out and by which time interval, is a hard task that must consider ecological and biological specificities of the targeted species and particularities of the life history of the studied animals (Griffin et al., 2000). The number of training sessions can make individuals used to the procedure, failing to fulfill its role. We verified that the proposed protocol was effective: Vinaceous-breasted Amazon parrots underwent fifteen training sessions, three with each of the four predator models, and exhibited adequate anti-predator responses, showing no signs of habituation. However, when it comes to anti-predator training, “one size does not fit all” (Tetzlaff et al., 2019), and this is not a formula that can be generalized to other taxa. A survey carried out by Rowell et al. (2020) showed that the number of training sessions varies greatly between studies with different species (from 1 to 40 repetitions), as does the risk of habituation. Red-legged partridges (*Alectoris rufa*) submitted to more than 40 training sessions did not show habituation to training, contrary to houbara bustards (*Chlamydotis macqueenii*) and North American bullfrogs (*Rana catesbeiana*), that show habituations signs after three and 20 training sessions, respectively (Gaudioso et al., 2011; Heezik et al., 1999; Teixeira and Young, 2014).

Identifying adequate training protocols can be ideal for saving time and resources. More than that, once translocation projects have shown low success rates (Morris et al., 2021), the development of judicious techniques and protocols both for the pre- and post-release stages of these projects must be considered. By assessing the behaviors of a group of parrots we found that the treatment carried out was effective, but an individual evaluation of each parrot behavior can improve our approach to a more detailed level. Studies have explored animal’s particularities, such as temperament/personality, connecting it to post-release behavior and survival. Personality traits were correlated to survival after release in swift foxes (*Vulpes velox*), with shier foxes surviving longer than bolder ones, and European mink (*Mustela lutreola*), with bolder minks surviving longer than shier ones (Bremner-Harrison et al., 2004; Haage et al., 2017). Personality was also correlated with the avoidance of predators in gray partridge (*Perdix perdix*), with bolder birds maintaining greater distance from areas usually frequented by carnivores than shier birds (Harmange et al., 2021). In

Turquoise-fronted Amazon parrots, shy individuals showed greater ability to interact with conspecific and lesser propensity to learn human vocalization, adequate features to animals candidate to release (Lopes et al., 2017). In captive Vinaceous-breasted Amazon parrots personality was categorized along the dimensions vigilance and risk taking, with vigilant parrots characterized by alertness behaviors while risk taking parrots were more neophilic and had a tendency to vocalize more (Ramos et al., 2021). This behavioral difference suggests that vigilant parrots may be less prone to predation and capture by traffickers, but this assumption has yet to be tested after release (Ramos et al., 2021). Thus, individual behavioral assessments can be a tool that, in addition to anti-predator training, may improve translocation success and should be considered in conservation programs for parrots and other species.

Conclusion

Once predation has been a critical risk factor in translocation programs with captive animals, identifying how animals react in front of a threat and instigate adequate behaviors that may contribute to its survival can increase those initiatives success. Here we have part of our sample composed by parrots victims of wildlife traffic and part composed by their captive-born offspring. Once our sample size is reduced, which is a constant limitation when studying endangered species, it was not possible to explore and discuss the effects of these variables. Nevertheless, our results could bring interesting insights that may be useful in future research in the field of study. We explored Vinaceous-breasted Amazon parrots behaviors during anti-predator training and found that 1) the proposed protocol elicited aversive behaviors in the parrots; 2) the animals showed appropriate behavioral change through time; 3) a harmless stimulus didn’t elicited fear in the parrots; 4) and that the animals did not habituate to the experiment.

All these results correspond with the expected and demonstrate that anti-predator training can elicit proper behaviors in captive animals. Nevertheless, although the experience proportioned to the parrots in this experiment consists of an attempt to improve survival chances in the wild, it is hard to know if the same responses will be performed, or even effective, in a real predator-prey encounter (Griffin et al., 2000). Thus, monitoring the survival of the parrots after the release into the wild would bring important data about the efficacy of the anti-predator training for this species.

Declaration of Competing Interest

The authors report no declarations of interest.

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APPENDIX A - supplementary material, table 1

Supplementary table 1. Ethogram of the Vinaceous-breasted Amazon parrot (*Amazona vinacea*) obtained during seven hours of observations conducted from March to May 2021, using *ad libitum* observations and the literature (Prestes, 2000; Queiroz et al., 2014; Ramos et al., 2021).

Category	Behavior	Description
Resting	Sleeping	The bird is standing still, with eyes closed, in a neutral position or with the head turned back with the beak positioned between the feathers.
	Inactive	The bird is standing on a perch, on a branch, at the top of a trunk, in the grund or on the wire mesh, where it also fixes its beak. Their eyes are open. The beak is closed, and the wings are held close to the body.
Locomotion	Flying relaxed	The bird calmly flies, can follow social vocalization.
	Walking	The bird walks on a perch, on the ground or across the wire mesh, using its feet and beak.
Exploration	Foraging	The bird walks on the ground, with its head down, or among branches, looking for food.
	Pecking	The bird pecks at the masonry base of the aviary, at perches, leaves, and branches, as if “cutting/crushing”, and lets them fall to the ground, or holds them with one of its feet and continues pecking. Pecks items of environmental enrichment (coconut, corn) or aviary setting (trunk).
Feeding	Feeding	The bird feeds at the feeder, on the perch or on

		the ground of the aviary. During this action, it takes the beak to the food or picks up the food with the foot and takes it to the beak, ingesting it.
Maintenance	Scratching	The bird takes one foot to part of the body and uses the two front fingers to scratch it.
	Preening	The bird uses its beak to clean the feathers, pulling them quickly and smoothing them.
	Cleaning the beak	The bird quickly and repeatedly sweeps its beak on both sides of a branch or perch, performing a “horizontal figure 8” motion with its head, or just scraps the upper jaw on the lower.
	Shaking	The bird is at rest, then ruffle the feathers on the back of the neck and/or the body. Then, the bird shakes the head or the whole body. The feathers then return to their normal position.
	Ruffling the feathers	The bird is standing still and then ruffles its body plumage and spreads its tail. The plumage then returns to its normal position.
	Stretching	The bird slightly stretches its wings backward, or stretches one wing and one leg simultaneously backwards.
	Yawning	The bird is standing still, opens its beak slowly, remaining open for a few seconds, and then closes.
	Grooming feet	The bird grooms its feet to remove rests of food and dead skin.

Vocalization	Social vocalization	The bird emits typical vocalization of the species.
	Human vocalization	The bird performs imitation of human sounds, such as whistles and words.
Social affiliation	Allopreening	One bird cleans the feathers of other bird, pulling and smoothing them. The receiver can lean their body, and both can close their eyes during the action.
	Requesting allopreening	One bird approaches the other, touches it with its foot and lowers its head. Can receive allopreening or not.
Social agonistic	Moving away	After being approached by another bird, the – bird that was already there flies or walks away.
	Attacking conspecifics	The bird raises its head, stretches its neck, opens its beak, and moves towards another individual, vocalizing or not. The body and nape feathers may be ruffled, and the tail may be fanned out. There may or may not be physical contact.
	Threatening	The bird, standing still, looks at another individual and opens its beak. May or may not lift head, ruffle feathers on nape, body, and tail, and move head back and forth. The bird may also lift one of its legs.
	Fighting	Two birds attack each other and may even attach beak to beak.
	Attacking in flight	The bird flies towards another individual, hitting it or not with its beak and/or feet.

	Ignoring threat	The bird is indifferent to being threatened.
	Retreating	After being attacked or threatened by a conspecific, the bird moves away in the opposite direction, walking, with the body being lowered, or flying. There may be vocalization.
	Defending	The bird that is under attack or threat raises one of its feet towards the aggressor, positioning it in front of the body, which is lowered and slightly inclined.
Abnormal	Pacing	The bird walks to one side, turns, and then returns to the same point it was at before, repeatedly.
	Bobbing head	The bird quickly and repeatedly throws its head back in a half-turn motion.
	Regurgitating	The bird performs undulating neck motion repeatedly. It may regurgitate or not.
	Balancing	The bird swings its body from side to side, in place, taking one leg off the support and then the other.
Anti-predator	Alert	The bird stands with its neck stretched and staring.
	Escaping	The bird moves quickly (walking or flying), in the opposite direction to a stimulus, vocalizing or not.
	Alert vocalization	The bird repeatedly makes alert calls while stationary or moving.

	Hiding	The bird hides in the vegetation.
Not visible		The bird is out of the observer's field of view.