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POTENTIAL FOR REINTRODUCTION OF SAFFRON FINCHES (*SICALIS FLAVEOLA* LINNAEUS, 1766) REGARDING PREDATOR RECOGNITION

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Orientadora: Profª. Drª. Angélica da Silva Vasconcellos

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“O potencial para reintrodução de canários-da-terra (*Sicalis flaveola* Linnaeus, 1766) com relação ao reconhecimento de predador”

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Experiência não é o que acontece com um homem; é o que um homem faz com o que lhe acontece (Huxley).

RESUMO

O tráfico de animais silvestres é um dos principais fatores que contribui para a extinção de espécies e para a redução da diversidade biológica. No Brasil, os animais traficados são apreendidos por órgãos ambientais e prioritariamente reintroduzidos na natureza. No entanto, para a maioria das espécies não existem dados sobre o sucesso dessas reintroduções. A manutenção de animais silvestres em cativeiro pode afetar a habilidade do animal para sobreviver na natureza, por exemplo, reduzindo sua capacidade de reconhecer um predador, o que pode resultar em reintroduções mal sucedidas. Além disso, tem sido demonstrado que o temperamento de animais silvestres tem influência sobre a sua capacidade de sobreviver na natureza. Canários da terra (*Sicalis flaveola*) é a ave brasileira mais traficadas e apreendida. O objetivo deste estudo foi investigar se os canários da terra apreendidos reconhecem seus predadores e se o temperamento tem influência sobre a sua capacidade de diferenciar modelos de predador e não predador. Vinte e oito canários apreendidos foram submetidos a testes de temperamento e reconhecimento de predador. Para os testes de temperamento, foram utilizados quatro objetos novos; os comportamentos das aves foram registrados e os animais foram classificados de acordo com suas respostas aos objetos apresentados. Para os testes de reconhecimento de predador, dois predadores (um gavião vivo e um gavião taxidermizado) e dois modelos não predador (um tatu taxidermizado e um modelo de Lego) foram apresentados para os pássaros. As respostas comportamentais foram registradas em três tratamentos experimentais sequenciais (Linha de base, Modelos e Pós modelos) para avaliar influências temporais. Os modelos de predador e não predador tiveram influência sobre o comportamento dos canários. A frequência do comportamento "Alerta" aumentou e o uso do poleiro mais próximo aos estímulos diminuiu durante o tratamento Modelos, em comparação com as frequências observadas durante a Linha de base. As aves também passaram menos tempo “de Costas” para os estímulos e “Observando” o ambiente durante o tratamento Modelos. No entanto, as respostas dos animais para o gavião vivo, para o gavião taxidermizado e para o tatu não foram significativamente diferentes, o que indica uma deficiência no reconhecimento de uma ameaça de predação. Além disso, não houve diferença entre as frequências de outros comportamentos, como "Comer", "Cantar", "Bater asas" ou "Ajeitar as penas", em qualquer um dos tratamentos de teste, o que pode expor os animais ao perigo na presença de um predador. Não foi encontrada correlação significativa entre os traços de temperamento dos canários e suas respostas aos predadores: uma possível consequência das práticas de manejo em cativeiro. Nossos resultados sugerem que canários cativos podem ficar vulneráveis a predadores na natureza; o que indica a necessidade de treinamento anti-predação antes da reintrodução.

Palavras-chave: Comportamento anti-predação, reintrodução, *Sicalis flaveola*, sobrevivência, temperamento.

ABSTRACT

Wildlife trafficking is a major factor contributing to species extinction and to the reduction of biological diversity. In Brazil, trafficked animals are apprehended by Brazilian environmental agencies and reintroduced in the wild. However, for most species there are no data on how successful these reintroductions are. The maintenance of wild animals in captivity may affect the animal’s ability to survive in the wild, for example, reducing its ability to recognize a predator, which may result in unsuccessful reintroductions. In addition, the temperament of wild animals has been shown to have an influence on their ability to survive in the wild. Saffron finches are among the three most trafficked and apprehended Brazilian birds. The aim of this study was to investigate whether apprehended saffron finches recognize their predators and whether temperament traits have influence on their ability to differentiate predators from non-predator models. Twenty-eight apprehended saffron finches were submitted to Temperament and Predator-recognition tests. For the Temperament tests, four novel objects were used; birds’ behaviors were recorded and animals were ranked according to their responses to the objects presented. For the Predator-recognition tests, two predator (a live and a taxidermized hawk) and two non-predator models (an armadillo and a Lego model) were presented to the birds and their behavioral responses were recorded in three sequential experimental treatments (Baseline, Models, and Post-models) to control for temporal influences. Both predator and non-predator models had influence on the behavior of saffron finches, which increased the frequency of “Alert” behavior and used less often the perch closest to the stimuli during the Models treatment, compared to the frequencies observed during the Baseline. The birds also spent less time with their back to the stimuli and observing the environment during the Models treatment. However, some animals' responses to the live hawk, taxidermized hawk and to the armadillo were not significantly different, which indicates a deficiency in recognizing predator threat. Furthermore, there was no difference between the frequencies of other behaviors in any of the test treatments, such as “Eating”, “Singing”, “Wing-Flapping” or “Preening”, whose performance can expose the animals to danger in the presence of a predator. We found no relationship between temperament traits and responses to predators: a possible consequence of husbandry practices in captivity. Our results suggest captive saffron finches could be vulnerable to predators in the wild; thus, indicating the need for antipredator training before reintroduction.

Keywords: Antipredator behavior, Reintroduction, *Sicalis flaveola,* Survival, Temperament.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1 Introduction

Wildlife trafficking is considered the third largest illegal activity in the world and it is estimated that about 38 million specimens are captured from Brazilian biomes annually (Destro et al., 2012). Passerines are the most caged birds in the world and at least 2 million of them are involved in the global market annually (Renctas, 2001).

In Brazil having birds as pets is common; however, these birds rarely come from legalized breeders; mostly they are captured in the wild (Sick, 1997) or illegally bred and sold. The extensive removal of animals from their natural habitats for trafficking or illegal breeding creates enormous challenges for governmental wildlife protection agencies and contributes to the reduction of biological richness and to species extinction (Licarião et al., 2013, Primack and Rodrigues, 2001).

Birds are the most confiscated animals by Brazilian environmental agencies, corresponding to 82% of the rescues from animal trafficking and illegal captive breeding (Pagano, 2009). According to the Normative Instruction no. 23 from the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), the options for destination of rescued wild animals are: immediate return to nature, maintenance in captivity, inclusion in reintroduction programs or remittance to research or educational institutions (Brasil, 2008). This Normative Instruction also determines that, before being reintroduced, every animal must undergo behavioral analysis; their skills for feeding, social interactions, reproduction, and predator responses should be observed; if the animal's performance is not appropriate in any of these categories, the individual must undergo appropriate training (Brasil, 2008). However, no guidelines are given regarding testing or training of these animals.

Behavioral analyzes are essential to identify deficiencies in behaviors that might constitute causes of unsuccessful reintroductions, for instance lack in predator recognition abilities and stress responses (Teixeira et al., 2007). Displacement behaviors can be considered as a behavioral expression, which is odd, unexpected and/or not adequate to the context. The causes for the development of these behaviors may vary, but their performance can be considered as an evidence of frustration or stress (Kruijit, 1964, Kuhne et al., 2013), since stressful situations have been pointed as a trigger for their performance (El Lethey et al., 2000, Vestergaard et al., 1997). Eating, scratching, beak cleaning, preening and many other behaviors associated with maintenance may be classified as comfort behaviors (Kruijit, 1964, Kuhne et al., 2013, Vestergaard et al., 1997), also included in the category of displacement behaviors. When animals are under stress, they can display comfort behaviors that help them either to terminate the frustration-evoking situation or to remove the stimulus (Kuhne et al., 2013, Vestergaard et al., 1997).

Another factor that compromises the success of reintroduction projects is the predation of released individuals, who do not recognize predators (Teixeira et al., 2007, van Heezik et al., 1999). Some captivity-reared birds show less appropriate responses to predators than their wild counterparts (Robertson and Dowell, 1990), and for this reason, conservation biologists have started including anti-predator training among the pre-release procedures (Griffin et al., 2000, van Heezik et al., 1999). This is an attempt to increase the birds' chances of survival, being proved economically viable (Azevedo and Young, 2006).

Sensing of environmental cues may be different for every individual; it is part of animal personality, which will affect an individual's ability to respond to environmental change (Minderman et al., 2009). Personalities have ecological and evolutionary relevance and its variation can be maintained within or among populations when they confer selective advantages to individuals experiencing different environmental conditions (Dingemanse and Rèale, 2005, Sih et al., 2004). Personality, temperament and individuality could be considered as synonyms, being defined as an individual's behavioral pattern that is consistent over time and across situations (Gosling, 1998, Réale et al., 2007). A behavioral pattern covers numerous aspects, such as aggressiveness - being an individual's agonistic reaction; sociability - the individual's reaction to the presence or absence of conspecifics; exploration-avoidance of novelty and shyness-boldness (Réale et al., 2007). The shyness-boldness aspect is defined as the tendency of an animal approaching an unfamiliar object or situation and, thereby, taking risks. It has been measured in wild captive animals using indexes such as the Boldness Score (Bremner-Harrison et al., 2004, Azevedo and Young, 2006).

Behavior variability is due partly to genetic variation and partly to variations in the phenotype structure, physiology, and behavior that arise during development, as a result of the individual's interaction with the environment. This variability may also arise through epigenetic mechanisms, that are molecular factors that modify gene expression, but do not change the DNA sequence, per se (Ledón-Rettig et al., 2012, West-Eberhard, 2005). When a behavior is adaptive or more sensitive to environmental change it may facilitate the fixation of genetic variants involved in this particularly behavior (Ledón-Rettig et al., 2012). Behavioral changes can lead to a rapid fitness declines whether by heritable epigenetic effects or relaxed natural selection that can be detected within one generation (Christie et al., 2012).

In birds, some anti-predator behaviors have been shown as correlated to personality traits, such as activity level and exploration (Jones and Godin, 2010). Bremner-Harrison et al. (2004) found that bold swift foxes (*Vulpes velox*) showed higher mortality rates than shy individuals upon reintroduction, indicating the personality of captive animals can be decisive for their survival after release into the wild. Although several studies have shown that temperament is linked to antipredator, foraging and exploratory behaviors, many of these links remain unexplored in reintroduction settings (McDougall et al., 2006).

Pagano (2009) demonstrated that 79% of the birds confiscated by IBAMA and received by the Wild Animal Triage Center (CETAS) were passerines, indicating a preference of illegal bird collectors for this group. Although not endangered, between 2001 and 2009, saffron finches, *Sicalis flaveola,* were the most confiscated animals by Brazilian environmental agencies (Souza et al., 2014). The great number of individuals maintained in Brazilian environmental institutions favored this study, whose results can be also meaningful to less abundant species (Destro, 2012, Ferreira and Glock, 2004).

The saffron finch (*Sicalis flaveola brasiliensis* Linnaeus, 1766, Thraupidae, Aves) measures between 11 and 15 cm and presents sexual dimorphism (BirdLife International, 2014, Figure 1a). The male is bright yellow with an orange crown, which distinguishes it from most other yellow finches; females are usually olive-brown with heavy dark streaks (Sick, 1997). They have a wide distribution throughout Brazil, from the Northeast to the Southeast, usually found in open grassland areas with scattered trees, as they are ground feeders (Sick, 1997, Figure 1b). Males are extremely territorial and remain constantly with the female and nestlings (Marcondes-Machado, 1982).

Figure 1 – (a) Male and female of saffron finches; (b) Saffron finches' world distribution (Ridgely et al., 2005)

This study aimed to evaluate whether captive saffron finches recognize natural predators and whether their predator-recognition responses are influenced by their temperament. We hypothesized that, due to the short time the study individuals spent in captivity, they will respond appropriately in the presence of predators, performing different behavioral responses to predator and non-predator stimuli, and the personality of individuals will affect their responses to predator and non-predator models.

2. Methods

2.1 Study place, animals, housing, and maintenance

This study was conducted in accordance with the Animal Ethics Committee of the Pontifical Catholic University of Minas Gerais, permit no. 0041/2013, between September and November 2013 in Belo Horizonte, Minas Gerais, Brazil. Our sample comprised 28 saffron finches (*S. flaveola*) obtained from IBAMA/CETAS of Belo Horizonte, kept in captivity and managed according to CETAS' instructions. Although, IBAMA/CETAS did not have precise data on the origins of the animals, confiscated birds were either captured in the wild or the first captive offspring from wild parents. The saffron finches were pair-housed in 120x30x40cm cages, containing six perches, set in three different horizontal positions, 20 cm distant from each other. The animals were fed daily with grain mixture for birds containing birdseed, millet, barley, fruits and vegetables. Food and water were offered *ad libitum*. After the experiments, the animals were returned to CETAS as per legal requirements.

2.2 Experimental procedures

Two sets of experiments, divided into five experimental treatments were run to test our hypothesis: Temperament tests and predator-recognition tests. Temperament tests occurred before and after the predator-recognition tests. Predator-recognition tests consisted in: a Baseline phase, a Models phase and a Post-models phase (Table 1).

Table 1 - Experimental treatments applied to saffron finches undergoing Predator-recognition and Temperament tests in Minas Gerais, Brazil

Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Temperament test I	Predator-recognition test - Baseline	Predator-recognition test - Models	Predator-recognition test - Post models	Temperament test II
32h	32h	32h	32h	32h

2.2.1 Temperament tests

Temperament tests were run twice to check for the consistency of the finches' personality over time. We presented the animals with four novel objects of the same size (approximately 20cm) and color, thus the novelty remained basically on the objects shape: a ball, a box, a toy and a plastic jar were used (Figure 2). As birds are more sensitive to some colors than others, we defined that all objects used in the test should have the same color, red, due to the animals' highest spectral sensitivity and hue discrimination at these longer wavelengths, avoiding any response towards different colorations (Kaczorowski et al., 2014).

Figure 2 - Novel objects used in the Temperament tests with the saffron finches.

The objects remained covered until the beginning of each test, which were videotaped. Each object was displayed individually, and only once for each pair per trial (Table 2). The objects were placed outside the cage, 100 cm distant from it. Inside the cage, perches were placed in three different horizontal positions. Based on these positions, we calculated the use of cage by the birds, regarding their distance from the objects: D1=<120cm, D2= 120cm, D3= 140 cm, D4= 160cm and D5= >160 cm. The tests were run between 07:00-17:00h and lasted 30 minutes per pair; the order of pairs for testing was defined following the Latin Square design. Each of the two test phases lasted 32 hours, totaling 64 hours of observation.

Table 2 – Example schedule of presentation of the novel objects to the saffron finches during the Temperament tests

	Pair 1	Pair 2	Pair 3	Pair 4
Afternoon 1	Ball	Box	Toy	Jar
Afternoon 2	Box	Toy	Jar	Ball
Morning 3	Toy	Jar	Ball	Box
Morning 4	Jar	Ball	Box	Toy

2.2.2 Predator-recognition tests

Throughout these tests, we evaluated the behavior of finches upon presentation of predator and non-predator models (see Azevedo et al., 2012). The tests were grouped into three sequential treatments: Baseline, Models and Post-models, to control for temporal effects. To avoid any carryover effects between the treatments, four-day intervals separated them. The order of stimuli presentation during the Models treatment is in Table 3; during the Baseline and the Post-models treatments, no stimulus was presented. The behaviors of the animals were videotaped during all treatments for posterior analysis. A live roadside hawk (*Rupornis magnirostris* – Live Hawk, Figure 3a) and a taxidermized yellow-headed caracara (*Milvago chimachima* – Taxidermized Hawk, Figure 3b) were used as predator stimuli. Hawks were chosen to represent the predator because they occur in the same biome as the study species and feed on small birds and other small vertebrates (Sick, 1997). The roadside hawk, obtained from IBAMA, was kept in a 100x100x60 cm cage, fed 40g mice every other day and had water *ad libitum*. To represent non-predator stimuli, a taxidermized armadillo (*Dasypus novemcinctus* – Armadillo, Figure 3c) and a cube (20x20x20 cm) made of green, red, blue, yellow, white and black LEGO® building blocks (Lego, Figure 3d) were used. The armadillo was chosen since it occurs in the area of saffron finches' geographic distribution (Naiff et al., 1986) and its diet consists mainly of Hymenoptera; therefore, it should not represent predatory threat to finches.

Table 3 – Example schedule of presentation of predator and non-predator models to the saffron finches during the Models treatment of the Predator-recognition tests

Day period	Pair 1	Pair 2	Pair 3	Pair 4
Afternoon 1	Live Hawk	Hawk model	Armadillo model	Lego
Afternoon 2	Hawk model	Armadillo model	Lego	Live Hawk
Morning 3	Armadillo model	Lego	Live Hawk	Hawk model
Morning 4	Lego	Live Hawk	Hawk model	Armadillo model

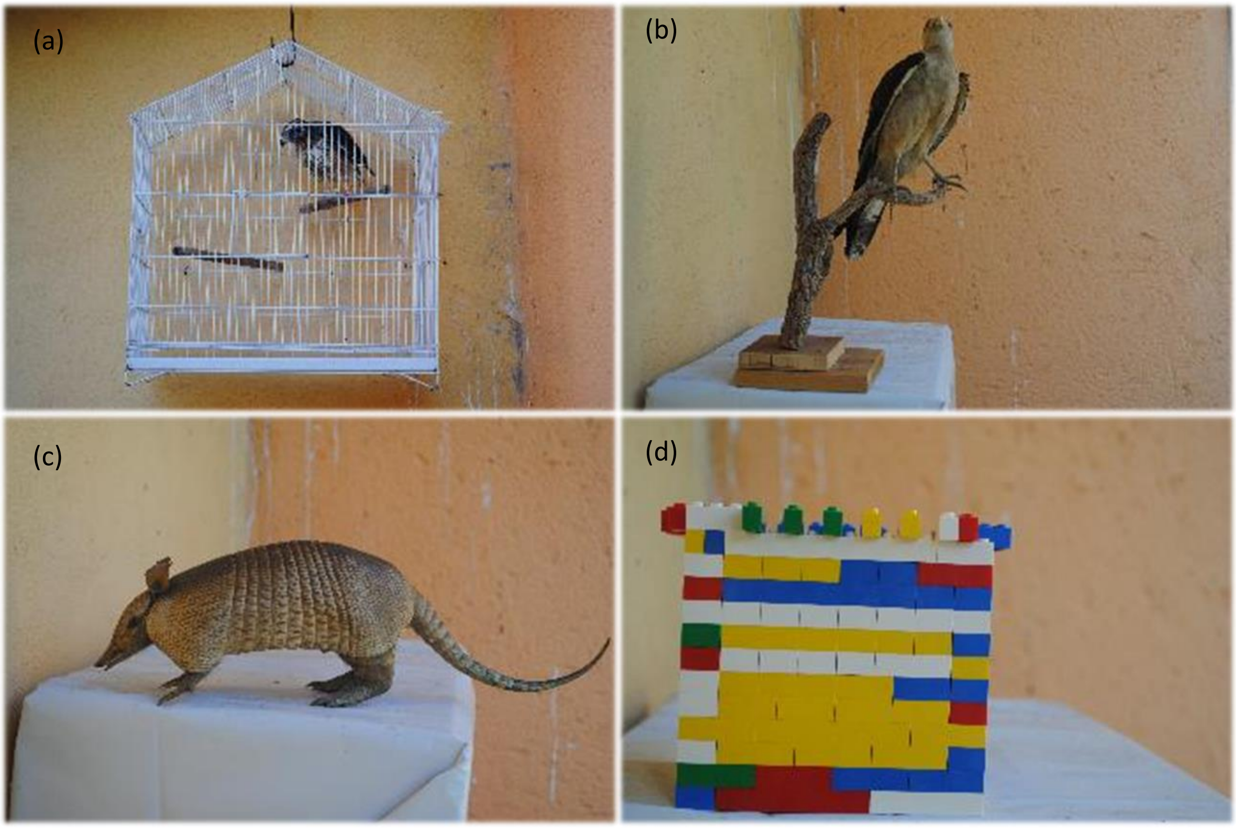

Figure 3 - Predator and non-predator stimuli used in the models treatment of the Predator-recognition tests. (a) Live roadside hawk (*Rupornis magnirostris* – Live Hawk); (b) Taxidermized yellow-headed caracara (*Milvago chimachima* – Taxidermized Hawk); (c) Taxidermized armadillo (*Dasypus novemcinctus* – Armadillo); (d) 20x20x20 cm cube made of LEGO® building blocks (Lego).

Tests were run only once a day, between 07:00-17:00h and lasted 30 minutes per pair. Each stimulus was presented only once to each pair. Each of the three test treatments lasted 32 hours, totaling 96 hours of observation.

2.3 Data collection and statistical analysis

All tests were videotaped, and were coded using focal animal sampling and instantaneous recording of behavior, with one minute interval (Martin and Bateson, 2007) through the Solomon Coder software (version beta 14:03:10, 2006-2011 © András Péter). Based on *ad libitum* pilot observations, an ethogram was constructed (Martin and Bateson 2007; see Table 4). We applied the method used by Bremner-Harrison et al. (2004, the Boldness Score), to evaluate the animals' responses to the stimuli. Behaviors were classified as "Fear", "Shy" or "Bold". The Boldness Score is calculated multiplying the frequency of fear behaviors by 0, the shy behaviors by 1 and the boldness behaviors by 2. Subsequently, the values for all categories are summed up; a higher score represents a bolder personality. This score was used to evaluate behaviors performed during the temperament tests and the predator-recognition tests.

Table 4 – Ethogram of behaviors recorded from preliminary observations of saffron finches, without interventions

Categories	Behavior	Description
Fear	Grabbing cage away the object	Grabbing the cage grid from the object opposite direction.
	Flying away	Flying to the object opposite direction.
Shy	Head-Flicking	Turning the head quickly in any direction.
	Being Alert	Observing the environment.
Bold	Wing-Flapping	Opening and flapping the wings.
	Back-facing	Standing on the perch, with the back turned to the object.
	Eating	Manipulating or ingesting food.
	Drinking	Dipping the beak into the watering cup or ingesting water.
	Beak-cleaning	Scratching the beak repeatedly on the perch.
	Preening	Manipulating feathers using the beak.
	Singing	Vocalizing.
	Grabbing cage towards the object	Grabbing the cage grid facing the object.
	Flying toward	Flying toward the object.

All data were tested for normality (Kolmogorov-Smirnov Test) and results of all statistical tests were considered significant at $P < 0.05$. Not all data met the requirements for parametric statistics and transformation was not effective to meet these requirements. Thus, we have used a mixture of parametric and nonparametric statistical tests. For statistical analysis, we used the programs BioEstat (version 5.3), Minitab Statistical Software (version 13.20, copyright© 2000 Minitab Inc.) and GraphPad InStat (version 3.00, GRAPHPAD Software Inc. 2000). We used a General Linear Model (GLM) test to determine whether sex, object, day period, test phase and the Boldness scores had any effects on the responses to predator and non-predator models. As ”Alert” was the only behavior whose performance increased in the presence of the predator, we used a Chi-square *One Sample* Test to access the inter-individual variability of this response.

The behavioral responses to the different models (Live Hawk, Taxidermized Hawk, Armadillo and Lego) were tested using ANOVA test for parametric data, followed by Tukey's *post hoc* test or Friedman's test for non-parametric data, followed by Dunn's *post hoc* test.

3. Results

3.1 Temperament tests

The mean Boldness score (Table 5) was 38.79 (±4.93), and the scores for each novel object were not different from each other ($p > 0.05$). Also, the sex of the birds did not have significant influence on the Boldness score ($p > 0.05$). The finches did not behave differently in the morning and afternoon periods ($p > 0.05$), and no difference was found between Boldness scores from the treatment I and treatment V ($p > 0.05$).

Table 5 – Mean Boldness scores of male (M) and female (F) saffron finches during the Temperament tests

Subject	Boldness score	Subject	Boldness score
M3	27.875	F7	39.625
F3	28.375	F14	40.000
F2	29.625	M14	40.250
F13	33.125	F9	40.375
M13	33.625	F8	40.625
M2	36.125	M12	41.375
M1	36.250	M11	42.750
F5	36.500	F1	42.750
F6	36.750	M7	42.875
M8	38.000	M4	43.125
F12	38.250	M10	45.250
M9	38.375	F10	45.375
M6	38.625	F4	45.500
M5	39.125	F11	45.625

*F1= Female 1, M1= Male 1 etc.

3.2 Predator recognition tests

3.2.1 Saffron finches' responses to different treatments during the Predator-recognition tests

Saffron finches modified their behaviors when exposed to both predator and non-predator models (see Table 6, Figure 4). "Alert" (Fr = 40.89, DF= 2, $p<0.01$, Dunn's post hoc: Baseline x Models $p<0.01$, Models x Post-models $p<0.01$) was expressed more often during the Models treatment, compared to the other two treatments. This response did not vary significantly between individuals in any of the treatments (Baseline χ^2=17.94; Models phase χ^2=25.61; Post-models phase χ^2=22.03; DF= 27 and $p> 0.05$ for all tests). During the Models treatment, "Head-Flicking" (Fr = 14.95, DF=2, Dunn's post hoc: Baseline x Models $p< 0.01$) was significantly less expressed compared to the Baseline, and "Back Facing" (Fr = 9.43, DF=2, $p<0.00$, Dunn's post hoc: Baseline x Models $p<0.05$, Models x Post-models $p<0.05$) was less expressed than during the other two phases. "Flying Away", "Flying Towards", "Beak-Cleaning", "Grabbing cage grid away", "Grabbing cage grid towards", "Wing-Flapping", "Eating", "Preening", "Singing", "Drinking" and "Non Visible" were performed equally often across treatments. The perch closest to the models (D1= <120cm) was used significantly less during the Models treatments, when compared to Baseline, having its usage decreased by 26% from the Baseline levels (Fr = 9.52, DF=2, $p<0.00$, Dunn's post hoc: Baseline x Models $p<0.05$). The usage of the other perches showed no differences across treatments ($p>0.05$).

Table 6 – Mean frequencies per treatment and significant differences (Dunn's *post hoc* test) of behaviors performed by the saffron finches during the Predator-recognition tests

Behavior/Distance	Baseline	Model	Post-models
Alert	7.63	11.95** $_{b, pm}$	8.37
Head-Flicking	2.99*	1.67** $_{b}$	2.71
Back Facing	9.62*	8.04** $_{b. pm}$	10.51
Flying Away	1.28	1.07	0.94
Flying Towards	1.27	0.90	0.88
Beak-Cleaning	0.39	0.52	0.64
Grabbing cage grid away	0.40	0.49	0.44
Grabbing cage grid towards	0.26	0.23	0.19
Wing-Flapping	0.34	0.26	0.29
Eating	1.52	1.44	1.54
Preening	0.45	0.57	0.47
Singing	0.66	0.83	1.01
Drinking	0.00	0.03	0.02
Non Visible	1.76	1.42	1.38
D1	2.31	1.71* $_{b}$	2.29
D2	6.87	5.68	6.54
D3	7.07	7.40	7.56
D4	5.75	6.28	5.86
D5	5.97	7.38	6.42

*Asterisks indicate significant differences between phases *p<0.05, **p < 0.01.

**Subscribed letters indicate the treatment in which the behavior was significantly different: $_{b}$ = Baseline; $_{m}$ = Models; $_{pm}$ = Post-Models

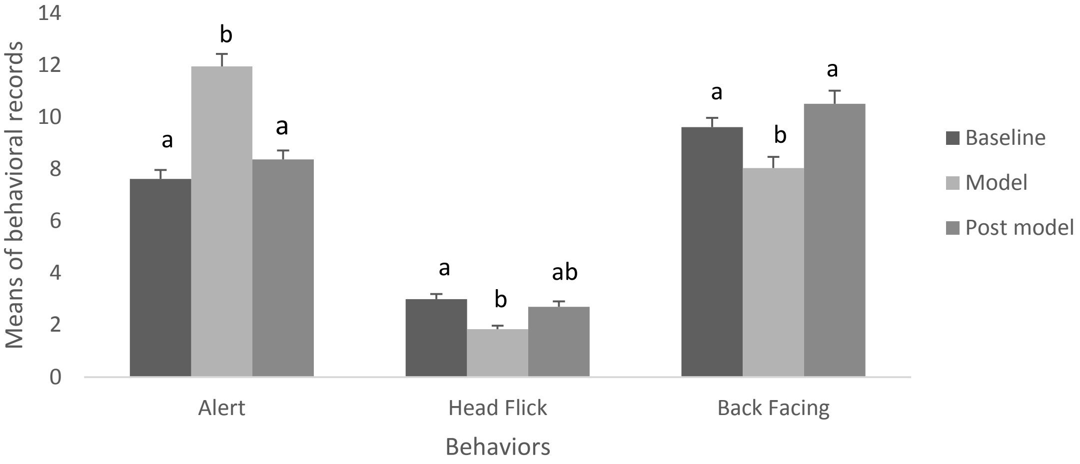

Figure 4 – Mean frequencies (+SE) of saffron finches' behaviors that differed across treatments during the Predator-recognition test. Different superscript letters represent statistically significant differences.

3.2.2 Predator versus non-predator models during the Models treatment of the Predator-recognition Test

Saffron finches responded differently towards some stimuli; the highest Boldness score was shown to the Lego and the lowest score, to the Live Hawk (DF= 3, F = 2.93, $p<0.01$, Tukey's post hoc: Live Hawk x Lego $p<0.05$). However, the Boldness score calculated for the other stimuli were not statistically different (Figure 5).

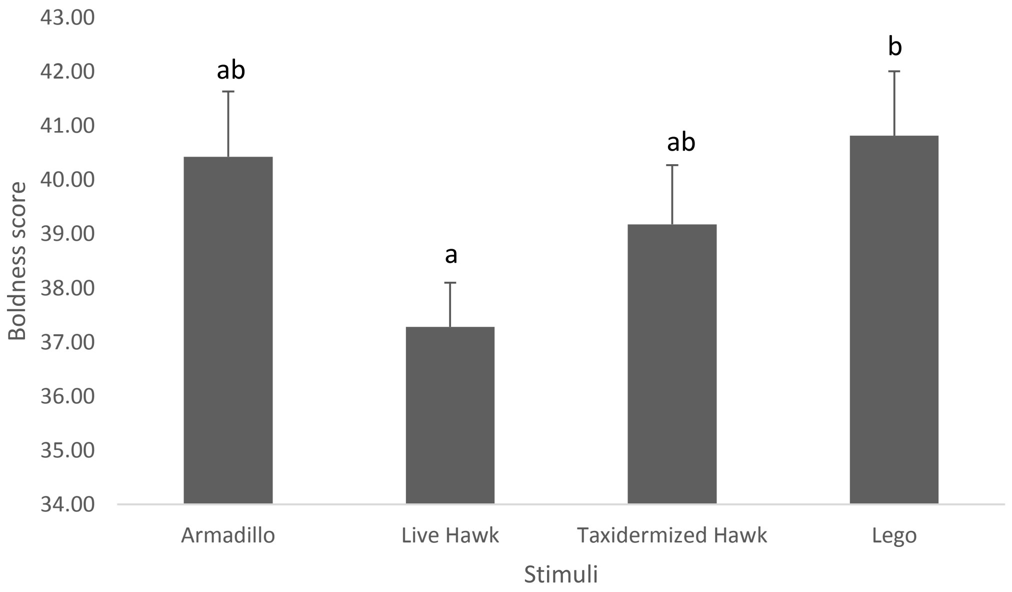

Figure 5 – Mean Boldness score (+SE), calculated for the saffron finches during the presentation of the four stimuli of the Models treatment in the Predator-recognition test. Different superscript letters represent statistically significant differences.

The behavior “Alert” (F = 18.52, DF= 3, $p<0.01$; Tukey’s post hoc: Armadillo x Live Hawk $p<0.01$; Armadillo x Lego $p<0.05$; Live Hawk x Taxidermized Hawk $p<0.05$; Live Hawk x Lego $p<0.05$; Taxidermized Hawk x Lego $p<0.05$) was expressed more often to the Live Hawk and the Taxidermized Hawk, followed by the Armadillo model and lastly to the Lego. The behaviors “Head-Flicking” (F = 3.16, DF= 3, Tukey’s post hoc: Armadillo x Live Hawk $p<0.05$), and “Beak-Cleaning” (Fr = 17.90, DF= 3, $p<0.01$; Dunn’s post hoc: Armadillo x Lego $p<0.05$) were recorded more often during the Armadillo presentation. “Back Facing” (F = 9.82, DF= 3, $p<0.01$; Tukey’s post hoc: Live Hawk x Lego $p<0.05$; Taxidermized Hawk x Lego $p<0.05$) and “Non Visible” (Fr = 16.14, DF= 3, Dunn’s post hoc test: Live Hawk x Lego $p<0.05$; Taxidermized Hawk x Lego $p<0.05$) were more recorded during the Lego presentation (Figure 6).

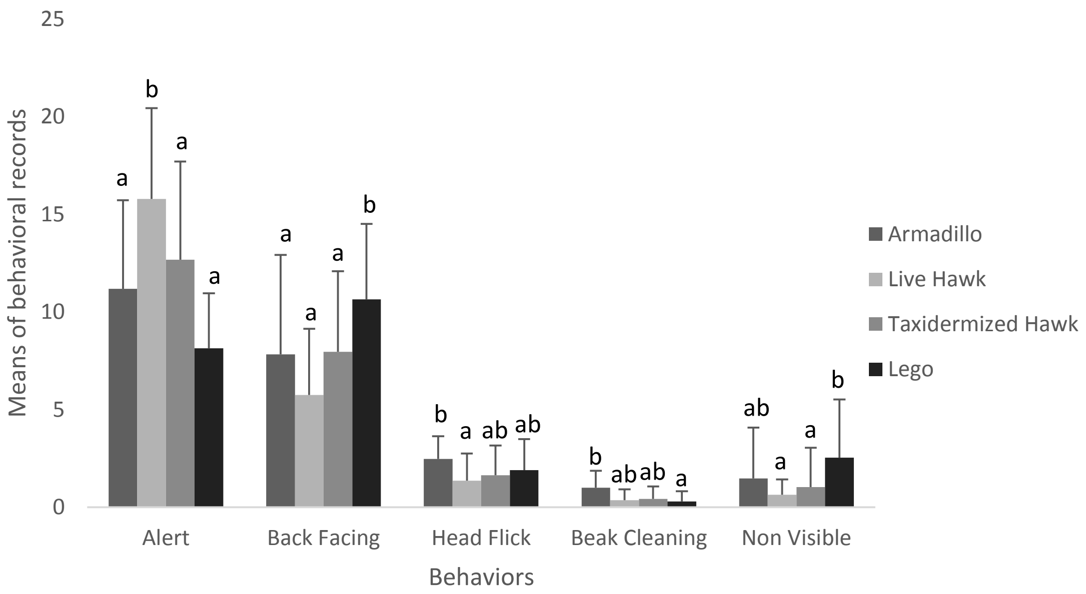

Figure 6 – Mean frequencies (+ SE) of behaviors performed by the saffron finches during the presentation of the four stimuli of Models treatment in the Predator-recognition test. Different superscript letters represent statistically significant differences.

The frequencies of the behaviors “Grabbing cage grid away”, “Grabbing cage grid towards”, “Flying Away”, “Wing-Flapping”, “Flying Towards”, ”Eating”, “Preening”, “Singing”, and “Drinking” showed no differences between stimuli ($p>0.05$). Saffron finches used more frequently the perches that were less than 120cm (D1, Fr = 13.38, DF= 3, $p<0.01$, Dunn’s post hoc: Live Hawk x Lego $p<0.05$) and were 120cm from the object (D2, F = 3.10, DF= 3, $p<0.05$, Tukey’s post hoc: Live Hawk x Lego $p<0.05$) when the Lego was exposed in comparison to the Live Hawk. The perch that was farthest from the stimulus was used more often when the Live Hawk was presented than during the presentation of any other stimuli (D5= >160cm, F = 5.03, DF= 3, $p<0.01$, Tukey’s post hoc: Armadillo x Live Hawk $p<0.05$; Live Hawk x Lego $p<0.05$; Live Hawk x Taxidermized Hawk $p<0.01$). We investigated the effects of temperament of the animals on their predator recognition ability by making a correlation between the Boldness scores calculated during the Temperament tests and during the Predator-recognition tests, but there was no significant correlation (Pearson’s r = 0.19).

4. Discussion

The results of our study suggested that confiscated saffron finches have retained general anti-predator responses, such as increasing alertness and keeping distance when faced with potential predators. However, they were not able to discriminate between predator and non-predator models neither decrease some inappropriate behaviors when in the presence of a predator. Similar results were found for captive greater rheas (*Rhea americana*) (Azevedo et al., 2012).

The saffron finches increased the performance of “Alert” behavior and the use of the farthest available perch when the Live Hawk was presented. Other studies report that captivity did not avoid the display of alert behaviors in greater rheas (Azevedo et al., 2012) or in Vancouver Island marmots (*Marmota vancouverensis*) (Blumstein et al, 2006).

Responses to predators are often costly because they must be traded off with other activities such as feeding, resting or looking for mates (Jones and Godin, 2010). When faced with a particular class of predator, animals must make a rapid decision and select the effective response in their repertoire; adequate responses imply the presence of a recognition process (Curio, 1993). Life in captivity presents the animals with fewer challenges to survival and, therefore, weaker selective pressures. The maintenance of antipredator behaviors in captivity can be explained by the ‘‘Ghost of Predators Past” hypothesis, which states that a species that has been subjected to past selection for antipredator behavior will retain this behavior if it is not too costly (Connel, 1980). Another explanation for the phenomenon is the ‘‘Functional Integration” hypothesis, which argues that behaviors used when dealing with predators may have multiple functions or otherwise be genetically linked to other favorable characteristics. Thus, such behaviors may be retained simply because there is selection on a locus maintained for other reasons (Coss et al., 2005).

As our study animals were wild born or captive-born from wild-born parents, their time in captivity, maximum of one generation, was not long enough to put them under evolutionary pressure for captivity conditions. Furthermore, we did not find any significant difference in individual responses in any test, discarding the possibility of different captivity time influencing on saffron finches' predator-recognition ability. Animals in these conditions can be considered as ontogenetically isolated, but can have the capacity to express at least some aspects of antipredator behavior, differently from those who have been isolated from predators for many generations, such as in evolutionary isolation (Griffin et al., 2000). Low-cost defense behaviors are likely to persist in captivity and the persistence of such behaviors will also depend on the number of generations under which the relaxed selection has taken place (Coss, 2005, McDougall et al., 2006).

On the other hand, while this short time in captivity can have preserved the animals' basic antipredatory responses (McDougall et al., 2006), relaxed predation pressures and epigenetic processes, which facilitate the rapid fixation of environmentally adaptive phenotypes and favor fitness in the wild (Ledón-Rettig et al., 2012), might have affected their specific predator-recognition abilities. For example, mothers exposed to predators produce offspring with tighter shoaling behavior, an antipredator response in fish (Giesing et al., 2011); therefore, fish mothers living in a predation-free environment may produce bolder offspring. If the wild population of a certain species contains the necessary genetic variation for rapid adaptation to captivity, in one or a few generations, we can see a rapid, epigenetically based fitness reduction. Therefore, unintentional selection or pressures in captivity can cause rapid fitness declines (Christie et al., 2012). The study animals in this research, living in captivity, may have lost their full capacity to respond to predators. Although the birds became more alert during the Models treatment of the Predator-recognition test, they showed no difference in their reactions towards the Live Hawk and the Armadillo for example. This result points to a deficiency in recognizing predatory threats, which can result in loss of energy and time when responding to innocuous stimuli, or in the risk of being killed in the case of a lack of appropriate reaction (Ferrari et al., 2007, Teixeira and Young, 2014). Rapid reduction in adequate responses in animals was also observed in other studies. Robins *(Petroica australis)* translocated to an island without predators had their predator recognition weakened within one generation (Jamieson and Ludwing, 2012). Wild primates that were constantly observed by humans suffered fewer leopard predatory attacks and consequently reduced their vigilance behavior (Isbell and Young, 1993), and released grey partridges (*Perdix perdix*) spend more time feeding than wild ones (Rantanen et al., 2010), probably a result of the constant availability of food in the rearing pens, which compromises individual alertness to predators.

Our animals also did not decrease their ‘bold’ behaviors when facing predators, which included singing, eating, preening, wing-flapping etc. The expression of such behaviors in the presence of a predator is inappropriate, since they may increase risk, or even attract the predator’s attention. Singing, particularly, may expose the birds to a greater risk of predation (Schmidt and Belinsky, 2013). Behaviors like wing-flapping, singing, beak-cleaning, preening and head-flicking have been reported as displacement activities in birds (Duncan and Wood-Gush, 1972, Vestergaard et al., 1997); that is, motor patterns not belonging to the primarily activated tendencies (Kruijt, 1964, Kuhne et al., 2013), which function as comfort behaviors and are performed when the bird faces stressful situations. However, had our study animals perceived any of the treatments as stressful, we would expect an increase in the frequencies of these behaviors, which was not the case. On the contrary, we observed no change in the performance of these behaviors, except for head-flicking, which even decreased during the Models treatment.

Several studies have shown a reduction in appropriate responses to predators in captivity. Captive-born greater rheas, as per the saffron finches, modified their behaviors when presented with predator and non-predator models, but were not able to discriminate between these stimuli (Azevedo et al., 2012). Seress and collaborators (2011) also did not succeed in evoking anti-predator responses by simulated cat attacks, because house sparrows (*Passer domesticus*) responded similarly to the predator model and to the control object. Rantanen et al. (2010) observed that captive grey partridges show poor vigilance behavior compared to wild grey partridges. These results point to the potential harmful effects of releasing reintroduction candidates back into the wild without training or behavioral testing. As our study animals are intended for release in the wild, inefficiency in anti-predator defenses may contribute to high losses in the reintroduced population. However, the retention of the basic responses by the animals suggests such losses might be decreased through the training of antipredator behavior (Azevedo et al., 2012). The Live Hawk stimulus resulted in the strongest anti-predator response from saffron finches, showing that a live animal could be used in training sessions to enhance the response to predation risk, since some species can improve their anti-predator responses with experience (Griffin et al., 2000).

We found no correlation between the animals' temperaments and their antipredator responses. This outcome differs from the findings of other studies, such as the work of Bremner-Harrison et al (2004), which found that the most exploratory individuals have a higher mortality rate, probably due to inadequate anti-predator responses. Explorative and risk-taker individuals of collared flycatchers (*Ficedula albicollis*) consistently sing at lower song posts than shy individuals in the presence of a human observer, representing the predator threat (Garamszegi et al., 2008). Cichlids (*Amatitlania nigrofasciata*) that are more exploratory in a novel environment are slower to react to a simulated fish predator attack (Jones and Godin, 2010). Both in captivity and in the wild, the association between temperament and fitness traits has been reported just as the existence of some links between temperament and morphological or physiological traits is common to many species, suggesting a complex relationship between temperament and individual fitness (McDougall et al., 2006). The reason for the lack of correlation between temperament and antipredator responses observed in our study animals is not clear, but might be related to a non-intentional directional selection to which our animals may have been subjected. A potential side-effect of captive breeding is a change in the evolutionary trajectory of wild animals, resulting from captivity conditions and from husbandry practices that would favor particular geno/phenotypes, disadvantageous in the wild (McDougall et al., 2006). As we worked mostly with wild-captured animals and their first captive progeny, a directional selection might have acted, homogenizing their anti-predator responses. Captured birds may not represent a random sample of the population, but a sample of more exploratory and less careful individuals: the study individuals might have been captured because they, or their parents, were bolder than their wild companions; therefore easier to capture. It is also possible that individuals that were more nervous have been more sensitive to capture/captivity stress and, as a consequence, more prone to develop stress-related diseases (Koolhaas et al., 1999), and had died soon in captivity, leaving bolder individuals to compose the illegal captive population.

5. Conclusions

Our results indicated that saffron finches, although retaining general antipredator responses, were not able to discriminate between predator and non-predator models, illuminating the potential detrimental effects of reintroducing such individuals without training or testing. However, the retention of basic predator recognition responses by the animals suggests such effects might be mitigated through the training on antipredator behavior. Our animals showed a general homogenization in their behaviors, a possible side effect of capture and husbandry practices. Likely due to this homogenization, we were not able to correlate personality traits and predator recognition responses.

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