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ARTICLE

Important tools for Amazon Parrot reintroduction programs

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ABSTRACT: Anti-predator behavior and personality have important consequences for the survival of captive-bred animals after reintroduction in the wild. The personality of an animal can be defined as low within-individual variation in behavior relative to between-individual variation in behavior. Mortality caused by predation is the main reason for reintroduction failure, and training captive-born animals to avoid predators can solve this problem. However, how anti-predator training affects the personality of animals is uncertain. The objective of this study was to test the behavioral responses of captive Amazon Parrots (*Amazona aestiva*, Psittacidae) submitted to an anti-predator conditioning protocol, and to evaluate if anti-predator training affects parrot personalities. Twenty-six parrots were trained against predators using taxidermized models; their personalities were evaluated by calculating boldness scores before and after anti-predator training sessions. Parrots increased the expression of anti-predator behaviors when tested with all models; control groups behaved in a more relaxed way. The anti-predator responses persisted for 60 days after the end of the training sessions. Boldness scores increased in 50% of cases after anti-predator training sessions, and in only 33% of cases did parrots become shyer after anti-predator training. The tendency of parrots to exhibit bold behaviors in the personality tests, even after the training sessions, may be explained by their early experiences, low behavioral plasticity or high cognitive ability. Training naive parrots was an effective tool to enhance behavioral responses against predators before reintroduction. The study of personality is of great importance in reintroduction and translocation programs to determine the position of each individual in the shy-bold continuum and to help select individuals more suited for reintroduction.

KEY-WORDS: anti-predator training, captivity, conservation, personality, Psittacidae.

INTRODUCTION

Predation is one of the most important factors that affect species distribution and abundance (McLean *et al.* 1999, Begon *et al.* 2006). Anti-predatory behavior has important consequences for the survival and population dynamics of prey, and in the stability of predator-prey interactions (Stankowich & Blumstein 2005). Anti-predator behavior has been studied mostly in fish, birds and mammals (Griffin *et al.* 2000), and the most commonly performed strategies by animals include hiding, escaping, freezing or fighting (Sanz & Grajal 1998, Eilam 2005, Rosier & Langkilde 2011, Yorzinski & Platt 2012, Miles *et al.* 2013). Captive-born animals or animals reared in predator-free territories may lose their anti-predator skills (Curio 1988), and because it is energetically costly to maintain these behaviors, they tend to disappear over time (Ryer & Olla 1998).

Mortality caused by predation has been critical in some reintroduction/translocation attempts (Beck *et al.* 1991, Short *et al.* 1992, Miller *et al.* 1994). Death of captive-born animals soon after reintroduction can be minimized by releasing the animals in predator-free areas, by building fences to avoid the entrance of predators, by eliminating the local predators by translocations or hunting, or by training naïve animals to recognize and to avoid predators (Griffin *et al.* 2000). The use of anti-predator conditioning has increased in the last decade (Miller *et al.* 1994, Maloney & McLean 1995, McLean 1996, Richards 1998, McLean *et al.* 1999, Azevedo & Young 2006, Specht 2007, Miles *et al.* 2013).

Many species, from humans to arthropods, differ individually in how they respond to environmental stimuli such as novelty, risk, and sociability (Lendvai *et al.* 2011). These differences are determined by behavioral and physiological traits, and can be described by their personalities (Groothuis & Carere 2005). The personality of an animal can be defined as the low within-individual variation in behavior relative to between-individual variation in behavior (Carter & Feeney 2012), *i.e.*

individual behaviors can consistently differ across situations or contexts, and these differences tend to be stable over time (Sih *et al.* 2004, Bell & Stamps 2004, Dingemanse & Réale 2005, McDougall *et al.* 2006, Stamps & Groothuis 2010, Wolf & Weissing 2012).

Personality traits, such as the shy-bold continuum (Wilson *et al.* 1994, Kurvers *et al.* 2010), can be used to describe and measure behavioral variation in humans and other species (Wilson *et al.* 1994, Watters & Powell 2012). A bold animal is one inclined to take risks, especially in novel situations, and a shy animal is one not inclined to take risks (Toms *et al.* 2010). Personality traits can be partly heritable (10–50%, van Oers *et al.* 2004, 2005, Taylor *et al.* 2012), and the topic of how personality is maintained in animal populations across time (evolutionary patterns) and had been subject of recent under study (Wolf & Weissing 2012).

The assessment of animal personality traits has some practical applications, especially for captive animals, since it can help keepers in the selection of the most suitable animals for exhibition, reproduction and handling, for instance (Carlstead 1999). Behavioral assessment can also be a tool for the selection of the best animals to reintroduce into the wild in conservation programs (Azevedo & Young 2006), since the reintroduction of captive-bred animals is an alternative approach to species conservation (Foose 1986, Cade 1988). For instance, bold individuals should not be reintroduced since they may suffer a high-risk of death due to their propensity to take risks (Bremner-Harrison *et al.* 2004). Alternatively, shy individuals should not be reintroduced since they may show reduction in foraging and growth rates (Biro & Stamps 2008). The ideal scenario would be the reproduction of individuals occupying intermediate positions in the shy-bold continuum, *i.e.* neither too bold or too shy; this would enable the correct response when individuals are exposed to a threat, such as a predator, or when searching for food or partners (Azevedo & Young 2006).

The Turquoise-fronted Parrot [*Amazona aestiva* (Linnaeus, 1758); hereafter Amazon parrot] is one of the most common Brazilian parrots (Béjcek & Stastný 2002), occurring in all biomes, except the Pampas (Schunk *et al.* 2011). Although *A. aestiva* are not considered threatened by extinction (IUCN 2015 – "Least Concern"; MMA 2014 – not threatened), chicks are frequentely captured in the wild and traded illegally (Beissinger & Bucher 1992, Seixas & Mourão 2002, Schunk *et al.* 2011), mainly due to their capacity to imitate the human voice (Ribeiro & Silva 2007). Many specimens are rescued annually by governmental agencies and are sent to rehabilitation centres for future reintroduction (Beissinger & Bucher 1992, Seixas & Mourão 2002). Consequently, testing an anti-predator conditioning protocol for this species is important because this could increase the chances of establishment of viable parrot populations after reintroductions.

Despite the importance of reintroduction as a tool for species conservation (Foose 1986, Cade 1988), without behavioral interventions in captivity, such as anti-predator conditioning, individuals can show high mortality rates after release in the wild, especially due to predation (Macias *et al.* 2003, White-Jr. *et al.* 2005, Valle *et al.* 2010, Veloso-Júnior *et al.* 2010, Alonso *et al.* 2011). Psittacines, as an example, were predated by snakes, hawks and ocelots in reintroduction programs with no pre-release anti-predator conditioning (Macias *et al.* 2003, Valle *et al.* 2010, Veloso-Júnior *et al.* 2010). Anti-predator conditioning for an Amazon parrot species is only known for *Amazona vittata* (Boddaert, 1783), reintroduced in Puerto Rico by the Puerto Rican Parrot Recovery Program, which reports a strong positive response of the parrots to the predator-aversion training (White-Jr. *et al.* 2005).

Few studies have evaluated if anti-predator training can change personality traits. Among these, the results are ambiguous, with some studies showing that personality was altered after anti-predator training sessions (Azevedo & Young 2006, Specht 2007), with bolder animals becoming less bold, and other showing that personality was not altered after anti-predator training sessions (Smith & Blumstein 2012).

The aim of this study was to test behavioral responses to an anti-predator conditioning program for captive Amazon Parrots, using *A. aestiva* as a model. We hypothesized that the predator-aversion behaviors would be enhanced after consecutive training sessions, helping the naive parrots (those living for longer periods in captivity that had no previous experiences with predators) to recognize and avoid predators. This study also intended to evaluate the personality of captive-bred *A. aestiva* individuals and tested the hypothesis that individuals become shyer after being trained against predators, due to increased fearfulness caused by predator visualization.

METHODS

Animals

We randomly selected thirty adult *Amazona aestiva* individuals spontaneously returned to the Brazilian Environmental Agency (*Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis - IBAMA*) at a 1:1 sex ratio (parrots were sexed through DNA analysis). All parrots had lived for at least 5 years in captivity. The study was conducted in the IBAMA facilities, at Belo Horizonte, Minas Gerais state, southeastern Brazil. Parrots were held in two enclosures measuring 7.10 m length × 1.8 m width

× 2.45 m height (15 parrots in each enclosure), placed 2 m apart, away from human interferences and surrounded by natural habitat. Two parrots died during the study due to injuries caused by fights inside the aviaries, thus data from only 28 individuals were included in the study. All experiments were approved by IBAMA's Animal Ethics Committee.

The birds were fed twice daily, in the morning (around 08:00 h) with Psittacidae feed (Evicanto Papagaios©) and in the afternoon (around 14:00 h) with fruits and seeds. Water was provided *ad libitum*. Birds were marked with colored rings on their legs to facilitate individual recognition.

Anti-predator training ethogram

An ethogram for the Turquoise-fronted parrots (Table 1) was compiled based on 20 h of *ad libitum* sampling during 20 days of preliminary observations (Altmann 1974) and previous studies (Prestes 2000, Andrade & Azevedo 2011). Behaviors were classified into aversion (anti-predator behaviors) and relaxing behavior (those not displayed in predatory situations) categories, based on the preliminary observations and in the studies of Andrade & Azevedo (2011).

Anti-predator training protocol

Anti-predator training was done using two taxidermized models of potential predators – an Ocelot [*Leopardus pardalis* (Linnaeus, 1758)] and a Harris's Hawk [*Parabuteo unicinctus* (Temminck, 1824)] – and an adult human; a chair was used as a control model. The presentation of models was followed by an aversive stimulus (chasing by an unfamiliar human). All training sessions were done in groups of three parrots each (except two groups with two parrots; the parrots remained in the same group during the entire experiment): two groups were trained against the three predator types (mixed group); two groups were trained against the ocelot model (ocelot group); two groups were trained against the hawk model (hawk group); two groups were trained against the human (human group), totaling six groups (two groups per predator type); and two groups received no training (control) (Table 2).

Table 1. Behavioural ethogram of *Amazona aestiva* individuals kept at IBAMA/BH with behavior description used during anti-predator training sessions and classification used to calculate boldness scores.

Behavior	Acronym	Description	Category	Classification
Self or Allopreening	PREE	Parrot preens own feathers or feathers of other individuals.	Relax	Boldness
Nodding head	ND	Parrot nods its head.	Anti-predator	-
Aggression	AGR	Parrot pecks conspecifics aggressively.	Relax	Boldness
Yawning	YA	Parrot yawns.	Relax	-
Walking on perch	WP	Parrot walks on the perch.	Relax	Boldness
Walking on the floor	WF	Parrot walks on the floor.	Relax	Shyness
Walking on wire	WW	Parrot walks on the enclosure's wiremesh.	Relax	Shyness
Inactive	IN	Parrot remains inactive or sleeping.	Relax	Boldness
Inactive on wire	IW	Parrot remains inactive on the wiremesh.	Relax	Shyness
Alert	AL	Parrot adopts an alert posture (head up, looking fixedly towards something).	Anti-predator	Shyness
Hiding behind the shrub	HID	Parrot hides behind the shrub, avoiding the predator models.	Anti-predator	Shyness
Sleeping	SLEE	Parrot sleeps.	Relax	-
Pacing	PAC	Parrots walks from one side to another on the perch, on the wiremesh or on the floor, using the same route for no apparent reason.	Abnormal	Shyness
Flying	FLY	Parrot flies away from the predator models.	Anti-predator	Shyness
Vocalizing	VOC	Parrot emits social vocalizations.	Relax	Shyness
Pecking on feather/leaf	PF	Parrot pecks on free feathers or tree leaves on the ground.	Relax	Boldness
Head scratching	HS	Parrot scratches its head with its feet.	Relax	Boldness
Wing/leg stretching	WS	Parrot stretches its wings or legs.	Relax	Boldness
Pecking on plastic markings or on perch		Parrot pecks plastic markings of the perches or the perches inside the enclosure.	-	Boldness
Pecking on the platform		Parrot pecks the wooden platform of the novel objects.	-	Boldness
Playing with object		Parrot plays with the novel object.	-	Boldness
Cleaning the beak		Parrot scratches its beak on the perch to clean it.	-	Boldness
Not visible	NV	Parrot is not visible.	-	-

Some behaviors were observed only during anti-predator training sessions and others only during personality tests.

Table 2. Identification number and sex of parrots submitted to anti-predator training sessions and to the personality tests before and after the application of the anti-predator training sessions, and the predator stimuli used.

Anti-predator training sections				
Hawk	**Ocelot**	**Human**	**Mixed (Hawk/Ocelot/Human)**	**Control**
7♀	13♀	19♀	1♂	24♀
8♂	14♀	20♀	2♀	25♀
9♂	15♂	21♂	3♂	26♂
10♀	16♀	22♀	4♀	27♀
11♂	17♀	23♂	5♂	28♀
12♂	18♂	29♂†	6♂	30♂†

† Parrots that died during anti-predator training sessions.

Training sessions were run in an enclosure similar to the maintenance aviary (50 m from the maintenance aviaries), but with its laterals covered by an opaque black plastic to prevent birds from seeing outside surroundings. The roof of the aviary was not covered by a black plastic. A circular opening of 0.3 m in diameter in the black plastic in the front of the enclosure allowed the researcher to video record all training sessions using a webcam COMPAQ. The enclosure's door was also covered with the black plastic and during the experiment was opened to show the models to the parrots inside. A bush at the end of the enclosure provided the parrots with shelter.

Each parrot group received three training sessions, except the mixed group, who received nine training sessions (three with each predator model); control groups, although not trained with predator models, also received three sessions with a chair. Training sessions were run in four consecutive days of February 2012, always between 08:00–09:00 h and 16:00–17:00 h, since these birds are diurnal and inactive in the hottest periods of day (Collar 1997, Pitter & Christiansen 1997, Gilardi & Munn 1998).

Parrots were captured in the maintenance enclosure and then transferred to the test enclosure each day, and a 15-min period was adopted for birds' acclimation and relaxing. All captures were done quietly, with minimal interference to avoid stressing the parrots and no influences of this procedure were detected during data analysis. When in the test enclosure, parrots in the maintenance enclosures could not see or hear the test groups. Each trial lasted 18 min, which consisted of 2 min filming before the presentation of the predator (phase 1), 1-min of conditioning (phase 2), and 15 min of filming after the end of conditioning (phase 3), thus, more than one group received anti-predator training in the same day, but not simultaneously.

The 1-min of conditioning was adapted from Griffin *et al.* (2001): the stimulus (predator) was shown to the animals for 15 s before a human dressing a costume (see below) and carrying a net entered the enclosure and began a 30 s simulated capture procedure (aversive experience; birds were never caught). After the capture simulation, the predator appeared again to the parrots for more 15 s (Fig. 1).

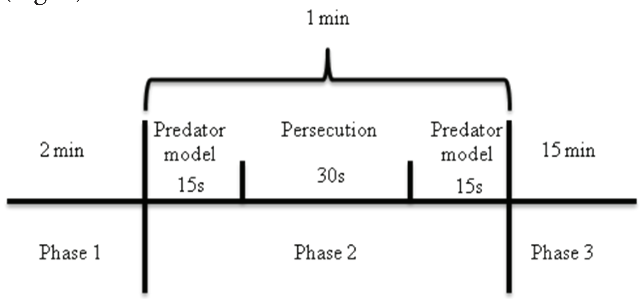

Figure 1. Protocol used in the anti-predator training of the Amazon parrots.

The ocelot, human and chair stimuli appeared to the parrots through the frontal enclosure's door; the hawk appeared to the parrots through the enclosure's roof. The costume used by the man had the objective to camouflage his silhouette, *i.e.* for the parrots, the chaser was not a human. Control groups received the same training protocol used for the other groups, but the human persecution never occurred. Data were collected using focal sampling with instantaneous recordings in 15 s intervals (Altmann 1974).

Memory tests

Memory tests were performed 30 and 60 days after the anti-predator training session. These tests consisted in showing the predator models to all groups of parrots (trained and controls) similar to that of the anti-predator training sessions, except that the chasing simulation did not occur.

Personality tests

Behaviors recorded in the ethogram representing boldness and shyness were identified (Table 1). Risk-taking behaviors, normally expressed during encounters with predators or in stressful situations, were considered shy behaviors (van Oers & Naguib 2013), and aggression, exploratory and maintenance behaviors, exhibited during calm, non-stressful events, were selected as bold (Smith & Blumstein 2013). A boldness score was calculated for each individual following Bremner-Harrison *et al.* (2004). Boldness scores were calculated per individual before and after anti-predator training sessions, during the novel object trials (personality tests; see below). The number of occurrences for shyness and boldness behaviors was counted to calculate the score. The number of shyness behaviors were multiplied by 1 and the number of boldness behaviors were multiplied by 2, and the higher the score, the bolder the individual was considered (Bremner-Harrison *et al.* 2004, Kurvers *et al.* 2010).

Personality tests consisted in presenting two unknown objects to the parrots: a traffic cone and a pot of potato chips connected to a bottle of milk; one object was shown to the birds before the anti-predator training sessions and the other object was shown to the birds after the anti-predator training sessions. The objects were presented to the birds on a platform at the centre of the enclosure. Four perches, with markings indicating the distances to the object (less than 1.3 m and more than 1.3 m), were connected to the platform.

The tests were conducted in the maintenance aviaries at 08:00 h and each group of parrots participated in only one test before and only one test after anti-predator training sessions. The behaviors and the distance of the birds to the objects (approach distance) immediately after its presentation were recorded for 60 min for each object, using the instantaneous focal-animal sampling method with 1-min intervals (Martin & Bateson 2007). The tests were filmed using the webcam of a COMPAQ notebook. Personality tests after anti-predator training sessions occurred before the memory tests, in the day following the end of the anti-predator training sessions.

Data analysis of anti-predator tests

Data normality was evaluated using the Anderson-Darling test. Since the data did not meet the requirements for normality, we used Friedman's non-parametrical ANOVA test, with Dunn's *post-hoc*, to evaluate differences between parrots' responses to the different predator models (control, ocelot, hawk, mixed, mixed hawk, mixed ocelot, mixed human, and human), between phases (before, during and after the appearance of the predator model). Wilcoxon's test was used to compare the parrots' behaviors between 30 and 60 days after training (memory tests). The results for the mixed group were evaluated pooled (data from the tests with all predator models together) and separated (with only the responses showed for each predator model; hawk, ocelot and human). For all statistical analyses, the confidence level was 95% ($\alpha = 0.05$) (Zar 1998).

Data analysis of personality tests

Using an Anderson-Darling test we determined that our data did not meet the requirements for parametric statistics, so the data were square-root transformed and parametrical statistical tests were used throughout.

A paired *t*-test was used to test whether boldness scores differed significantly between treatments (before and after anti-predator training) and to test if displayed behaviors differed between distances (less than 1.3 m and more than 1.3 m) (Zar 1998). One-way ANOVA was used to test for differences in boldness score variation between treatments [Boldness Score after anti-predator training minus Boldness Score before anti-predator training (BSa - BSb)] (Zar 1998)].

The correlation between mean percentage of predator aversion behaviors (= average of 30-day and 60-day shy behaviors × 100 / total recorded behaviors) and personality was tested using non-linear correlation analysis with quadratic function. Cluster analysis was used to determine the similarity in personality between individuals before and after anti-predator training sessions. The distance measure used was the difference between boldness scores and amalgamation rule was UPGMA (Zar 1998). Statistical tests were run using Minitab 12, Mystat 12 and Past. For all statistical analyses, the confidence level was 95% ($\alpha = 0.05$).

RESULTS

Anti-predator training

Parrots behaved similarly during the conditioning phase 2 of the anti-predator training sessions, only differing in the expression of inactivity, with the parrots of the control group more inactive than the parrots of the hawk and ocelot groups (Fig. 2). Parrots trained with the ocelot hid more than the control group, and parrots trained against all predators (mixed group) flew more than the parrots of the control group (Fig. 2). Parrots of the control group paced and slept more than the other groups; parrots of the mixed group nodded their heads more than the parrots of the hawk group (Fig. 2). Relaxing behaviors, like yawning, self and allopreening, were not exhibited during the conditioning phase.

Parrots expressed more anti-predatory behaviors with all predator models during phase 3 (hiding behind the shrub, and flying). The control group behaved in a more relaxed way, expressing more behaviors like walking on the perch, inactivity, and sleeping (Fig. 3).

Aversion behaviors increased significantly after the presentation of the predator models, including with the human model (Fig. 3). Relaxing behaviors decreased during the presentation of the predator models, but increased significantly during phase 3, especially during the last 5 min of phase 3 (Fig. 3). Although aversion behaviors increased in phase 3 in the control group, it showed the lowest increase when compared to the predator models (Fig. 3).

No differences in the behaviors of the parrots were observed 30 and 60 days after the anti-predator training for all predator models, except for the behaviors inactive in the hawk group, and walking on perch in the mixed group. Parrots trained against the hawk were more inactive 30 days after the end of the anti-predator training sessions (mean ± SE: 30 days: 25.22 ± 6.14; 60 days: 9.39 ± 4.11; $Z = 2.29$, $P = 0.01$, $n = 18$; $df = 1$). Parrots trained in the mixed group walked more on the perch 30 days after the end of the anti-predator training sessions (mean ± SE: 30 days: 1.94 ± 0.57 records; 60 days: 0.83 ± 0.35; $Z = 2.37$, $P < 0.01$ $n = 18$; $df = 1$).

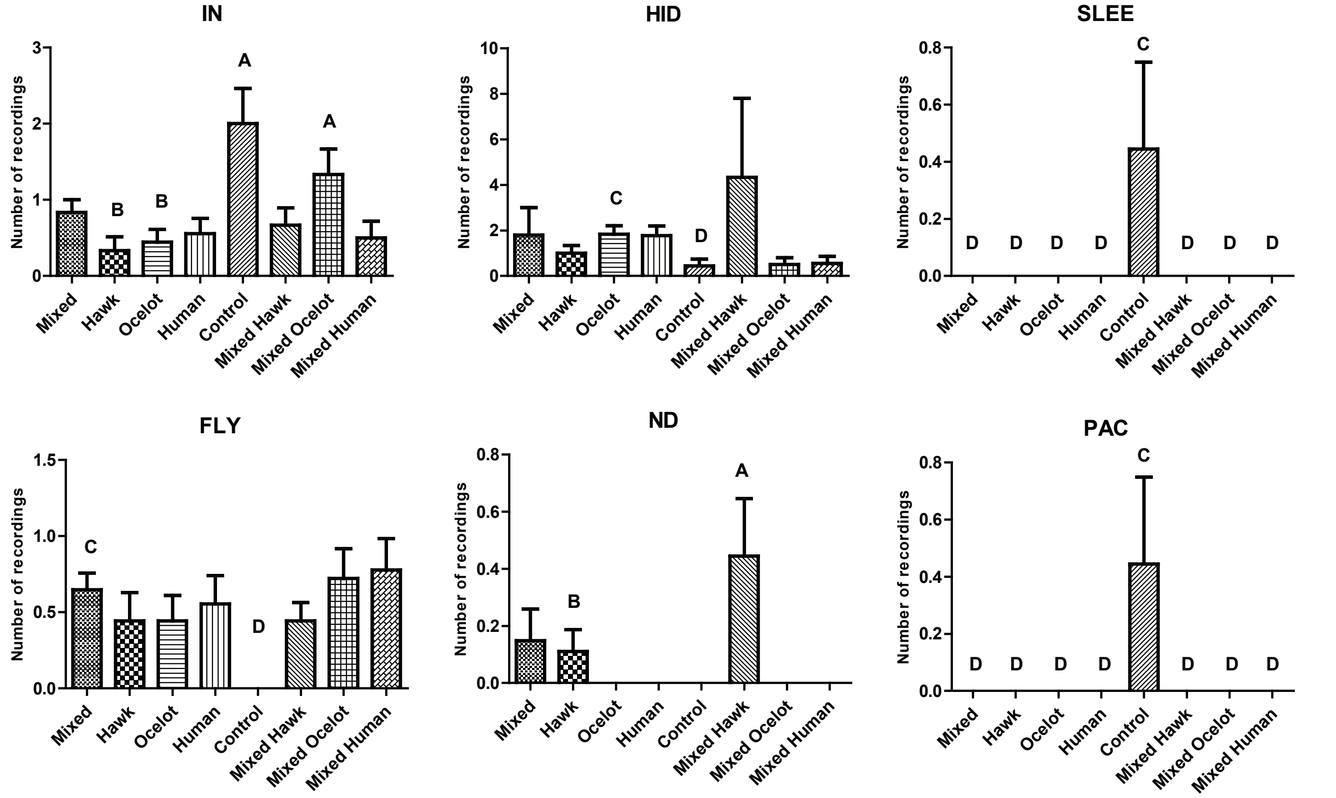

Figure 2. Behaviors displayed by Amazon Parrots during phase 2 of the anti-predator training sessions. IN = inactive; HID = hiding behind tree; FLY = flying; ND = nodding head; PAC = pacing; SLEE = sleeping; Superscript letters: CD = $P < 0.05$; AB = $P < 0.01$.

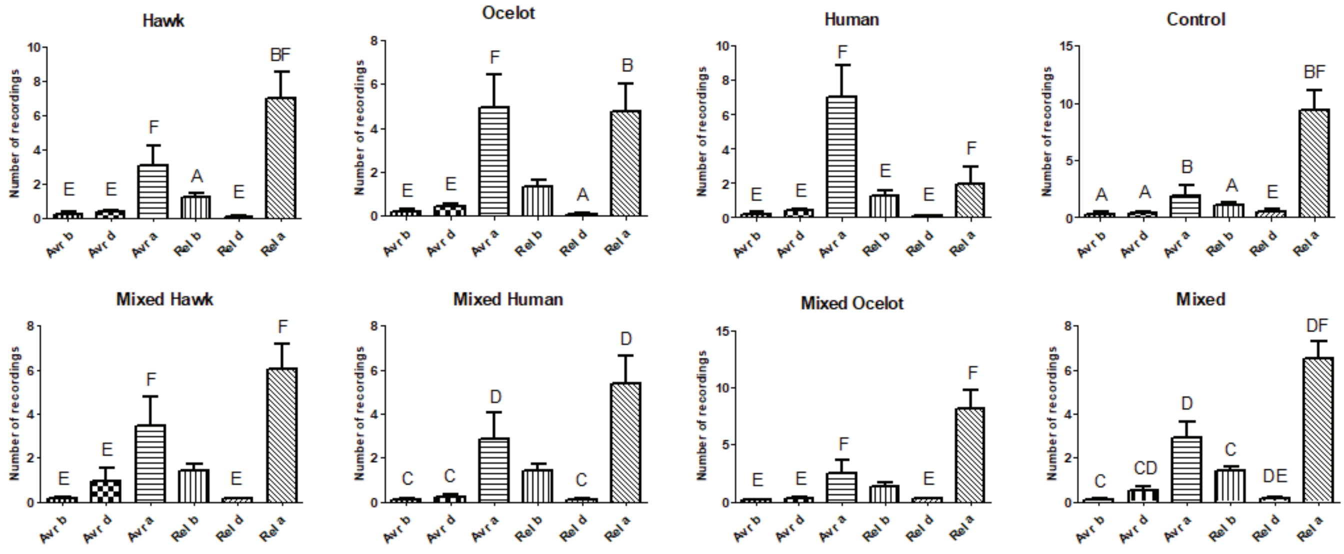

Figure 3. Behaviors displayed by the Amazon Parrots during phases 1, 2 and 3 of the anti-predator training sessions. Avr = aversion behaviors; Rel = relaxing behaviors; b = before the appearance of the predator model (phase 1); d = during the appearance of the predator model (phase 2); a = after the appearance of the predator model (phase 3); *AB* $P < 0.05$; CD $P < 0.01$; EF $P < 0.001$.

Personality tests

Boldness scores of parrots ranged between 178 and 240. The personality of most individuals changed after the treatments. The scores increased significantly in 50% of cases (t = -4.47, n = 16, df = 15, P < 0.001) and decreased significantly in 33% of cases (t = 2.35, n = 16, df = 15, P = 0.02).

The most fearful individuals before training based on boldness scores were those that were trained against the Harri's Hawk (boldness score's mean: 224; Fig. 4), and the most fearful individuals after training were those that were trained against the Ocelot (boldness score's mean: 212; Fig. 4). In general, there was a tendency of increased boldness after training, except in parrots trained against ocelots, where the boldness score decreased (Fig. 4).

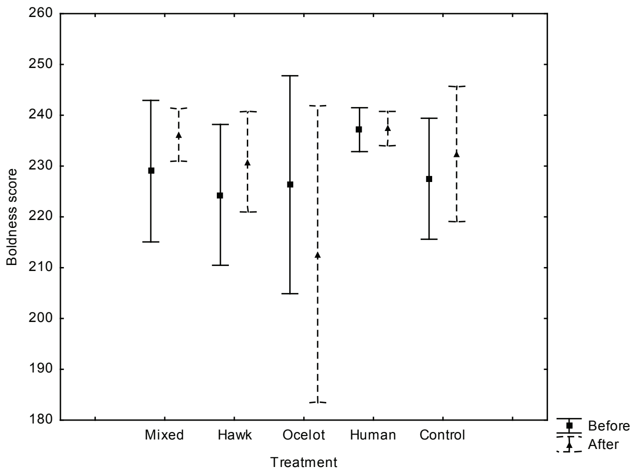

Figure 4. Means and confidence intervals (95%) of Blue-fronted Parrot boldness scores before and after anti-predator training sessions. All individuals underwent anti-predator training (except control ones), either against all models (mixed) or one predator only (hawk, ocelot, and human).

There were no significant differences in the personality of parrots that underwent different training and controls (F = 1.7, n = 28, df = 4, P = 0.177), before and after treatment (F = 0.1, n = 28, df = 4, P = 0.814), and no significant interaction between these factors was observed (F = 1.7, n = 28, df = 4, P = 0.186). Additionally, there were no significant differences in mean differences of boldness scores (BSa - BSb) between treatments (F = 1.7, P = 0.186). However, parrots displayed behaviors more frequently in the distances greater than 1.30 m from the objects (t = -6.45, P < 0.001, n = 56, df = 1).

Cluster analysis did not group parrots from different treatments or separate control parrots from individuals who underwent anti-predator training. For instance, before the treatment, bird 18 was separated from the other individuals and had a boldness score indicative of shyness (BSb = 188). After the treatment, the same individual was grouped with other parrots (27, 4, 9, 11, and 12), and exhibited a higher boldness score (BSa = 205). Parrots that exhibited intermediate boldness scores after training belonged mostly to the group trained with the Harris's Hawk (9, 11, and 12), but also to the control (25), ocelot (18), and mixed groups (4).

Boldness scores after training had a significant quadratic relationship with mean percentage of predator aversion, measured 30 and 60 days after the treatments (Aversion = -16.0 + 0.16 × BSa – 0.0004 x BSa^2) (Fig. 5), which means that parrots with intermediary boldness scores showed higher aversion to the predators.

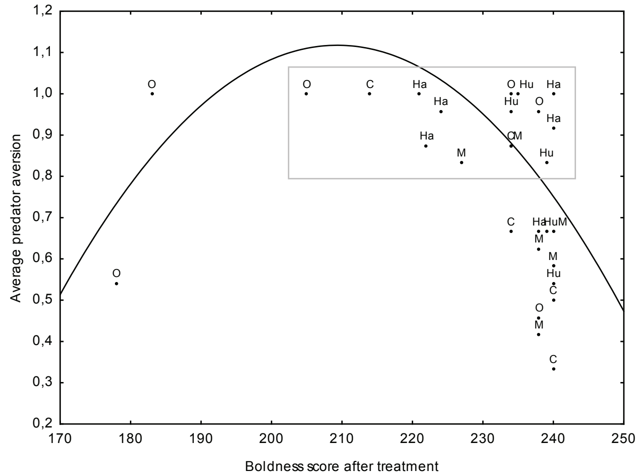

Figure 5. Relationship between boldness scores after anti-predator training and average aversion to three predator types 30 and 60 days after training. Bolder individuals only exhibited lower aversion values. The box indicates the region of the graph with intermediate and bold individuals that exhibited high aversion to predators and that would be good candidates for release. The letters indicate the different training regimes: C – control, Ha – trained against Harris's Hawk, O – trained against ocelot, Hu – trained against human, and M – mixed training against all predator models.

DISCUSSION

Anti-predator training modified the behaviors of the parrots, increasing their awareness about their predators and diminishing relaxing behaviors. Additionally, the anti-predatory behaviors persisted for at least 60 days after the end of the anti-predator training sessions. Anti-predator behaviors have to be effective in the very first time a prey encounters its predator, but these responses can be improved with experience (McLean & Rhodes 1991, Griffin *et al.* 2000). Although the parrots of this study responded to the predators in the first session, they exhibited stronger responses after two training sessions; a similar result was observed for the Noisy Friarbird *Philemon corniculatus* (Latham, 1790) trained by Curio *et al.* (1978).

Relaxing behaviors were more frequent in the parrots of the control group during phase 2 (predator model appearance), and this response reflects the fact that the parrots were able to recognize that the model of a chair was not a threatening stimulus. New Zealand Robins [*Petroica australis* (Sparrman, 1788)], Greater Rheas [*Rhea americana* (Linnaeus, 1758)] and Tropical Screetch-owls [*Megascops choliba* (Vieillot, 1817)] also responded to a control stimulus expressing relaxing behaviors (Maloney & McLean 1995, Azevedo & Young 2006, Specht 2007). The abnormal behavior pacing was exhibited by one parrot of the control group (number 26); this individual displayed such behavior in all phases of the study, and should be eliminated from the group of parrots destined to be reintroduced.

The behavior hiding behind the shrub was more frequently exhibited in response to the mixed hawk group. This response was also observed in a study with Hispaniolan Amazon Parrots [*Amazona ventralis* (Müller, 1776)] trained against falcons (White-Jr. *et al.* 2005), and when training owls against hawks (Alonso *et al.* 2011). Nodding head was exhibited mainly against the hawk model. The most frequent anti-predator response of Rock Partridges *[Alectoris graeca* Meisner, 1804)] to a hawk model was to crouch (Zaccaroni *et al.* 2007) which, according to the authors, is a cryptic behavior, very effective against predatory birds. Maybe this behavior, associated to the green plumage of the parrots (they become cryptic immerse in the vegetation), constitute a good way to avoid being located by flying predators (Alcock 2013).

Inactive and flying were the most recorded behaviors displayed by parrots in association with Ocelot and human models. Possibly, the best tactic against terrestrial predators, which use a silent and furtive approach toward prey, is to remain inactive in order to monitor and evaluate the behavior of the predator (Zaccaroni *et al.* 2007). This strategy allows prey to save energy, since it will only exhibit escape behaviors if necessary (risk-disturbance hypothesis; Frid & Dill 2002). The Ocelot and the human models elicited both behaviors, but for the human model, these responses were stronger than for the Ocelot, where parrots flew away in almost all sessions. Perhaps this stronger response to the human model could be a result of the perception of a greater risk of predation by the parrots, which corroborates the risk-disturbance hypothesis of Frid & Dill (2002).

Memory tests showed that the responses of parrots to the predators lasted for at least 60 days after the end of the training sessions. Similar results were found in a study of Greater Rheas (Azevedo & Young 2006). Predator encounters probably occur within a period of two months in the wild, and the persistence of the proper anti-predatory responses is important, since it can increase the survival rate of the parrots when reintroduced.

It is difficult to establish the exact number of training sessions necessary to elicit the right anti-predatory behaviors and to avoid habituation, but it is suggested that the least number of training sessions is best to avoid habituation (Griffin *et al.* 2000, Hemmi & Merkle 2009). For example, Houbara Bustards [*Chlamydotis undulata* (Jacquin, 1784)] habituated to a fox model after two training sessions (van Heezik *et al.* 1999), and Greater Rheas habituated to predator models after five training sessions (Azevedo & Young 2006). Parrots in this study did not show signs of habituation since the responses to the predator models were consistent over all trials; in fact, in the first two trials they were already responding strongly to the predators.

Anti-predator training changed the personality of Blue-fronted Amazon Parrots, and in most cases it promoted an increase in the boldness scores. A study with Greater Rheas, a study with tropical Screech-owls, and a study with Trinidadian Guppies [*Poecilia reticulata* (Peters, 1859)] found significant decreases in the boldness scores of the individuals after anti-predator training sessions (Azevedo & Young 2006, Specht 2007, Smith & Blumstein 2012). In the present study, boldness scores decreased after anti-predator training sessions in only 33% of sampled parrots.

It is known that fear responses can vary according to the personality of the individuals (Verbeek *et al.* 1994, Wilson 1998, Carere *et al.* 2005), and according to the individual's life history (early experiences) (Levine *et al.* 1993, Fox & Millam 2004). For instance, bolder Rainbow Trout [*Onchorhyncus mykiss* (Walbaum, 1792)] became shyer after watching shy individuals being presented to novel objects (Frost *et al.* 2007). In the current study, the early experiences of the parrots could not be determined, since all individuals were recovered from traffic. Although interviews were conducted with the parrot owners, they did not provide any information about the origins of the birds, fearing legal punishment. The only information about the early experiences of parrots was that all individuals studied had lived in captivity for at least five years. The boldness of the parrots trained against humans achieved the highest mean-scores and the minimum treatment variation (236 before training to 237 after training; corroborating the hypothesis that they may be hand-reared. This result indicates that these individuals may not be ideal for reintroduction, since the chance of being recaptured by humans may be great or that training against humans should be more intense. Feenders *et al.* (2011) showed that hand-reared Starlings [*Sturnus vulgaris* (Linnaeus, 1758)] had greater latency time to move in novel environments than wild-caught ones, but found no difference in the behavioral responses between both groups of birds in a novel object experiment.

The later experiences of the parrots, however, could be determined since the parrots stayed in the IBAMA facilities for at least four months prior to experiments. All parrots received a routine of environmental enrichment, and it has been shown that environmental enrichment diminishes neophobia in Amazon Parrots (Meehan *et al.* 2003, Fox & Millam 2007). The consistency of lack of variation in responses of parrots to the novel objects before-after-anti-predator training may be reflecting this routine.

The tendency of parrots to exhibit bold behaviors in the personality test even after the training sessions may be explained by their remarkable cognitive ability (Emery 2006). The ability to differentiate non-predators from predators ensures that animals do not generalize their responses to non-predators (Griffin *et al.* 2000, Azevedo *et al.* 2012), as the objects used in the tests. Probably parrots perceived that the objects presented did not pose danger because their shape differed from that of their predators (Bremner-Harrison *et al.* 2004, Kurvers *et al.* 2010, Lendvai *et al.* 2011). Parrots in the control group exhibited a similar response, remaining calm and showing bold behaviors during training, in which a harmless object (a chair) was presented to them. This corroborates the idea that the parrots were able to discriminate between the objects used during the personality tests and the predators used during the anti-predator training.

In conclusion, anti-predator (aversion) behaviors increased significantly with the training sessions showing that the parrots adjusted their responses according to the new situation. Additionally, the adequate anti-predatory response persisted for 60 days, showing that the conditioning technique was successful. The anti-predator conditioning program also affected the personalities of the parrots, making parrots bolder or shyer. The anti-predator training protocol should be included in all conservation programs dealing with parrots, since this can enhance the survival skills of the birds after reintroduction. Personality tests combined with anti-predator training may help to select accurately the individuals more suited for release, and intermediate individuals or bold ones that recognize potential predators and exhibit aversive behaviors toward predators should be preferred.

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REFERENCES

Alcock J. 2013. *Animal behavior, an evolutionary approach*, 10th Edition. Ann Arbor: Sinauer Associates.

Alonso R., Orejas P., Lopes F. & Sanz C. 2011. Pre-release training of juvenile Little Owls *Athene noctua* to avoid predation. *Animal Biodiversity and Conservation* 34: 389–393.

Altmann J. 1974. Observational study of behavior: sampling methods. *Behaviour* 49: 227–267.

Andrade A.A. & Azevedo C.S. 2011. Efeitos do enriquecimento ambiental na diminuicão de comportamentos anormais exibidos por Papagaios-verdadeiros (*Amazona aestiva,* Psittacidae) cativos. *Revista Brasileira de Ornitologia* 19: 56–62.

Azevedo C.S. & Young R.J. 2006. Behavioural responses of captive-born Greater Rheas *Rhea americana* Linnaeus (Rheiformes, Rheidae) submitted to antipredator training. *Revista Brasileira de Zoologia* 23: 186–193

Azevedo C.S., Young R.J. & Rodrigues M. 2012. Failure of captive-born Greater Rheas (*Rhea americana*, Rheidae, Aves) to discriminate between predator and nonpredator models. *Acta Ethologica* 15: 179–185.

Beck B.B., Kleiman D.G., Dietz J.M., Castro I., Carvalho C., Martins A. & Rettberg-Beck B. 1991. Losses and reproduction in reintroduced Golden Lion Tamarins *Leontopithecus rosalia*. *Dodo* 27: 50–61.

Begon M., Townsend C.R. & Harper J.L. 2006. *Ecology: from individuals to ecosystems*, 4th Edition. Oxford: Blackwell Publishing.

Beissinger S.R. & Bucher E.H. 1992. Sustainable harvesting of parrots for conservation, p. 73–115. In: Beissinger S.R. & Snyder N.F.R. (eds.). *New World parrots in crisis, solutions from conservation biology*. Washington: Smithsonian Institution Press.

Béjcek V. & Stastný K. 2002. *Enciclopédia das Aves: as várias espécies e seus habitats.* Lisboa: Livros Livros.

Bell A.M. & Stamps J.A. 2004. Development of behavioural differences between individuals and populations of Sticklebacks, *Gasterosteus aculeatus Animal Behaviour* 68: 1339–1348.

Biro P.A. & Stamps J.A. 2008. Are animal personality traits linked to life-history productivity? *Trends in Ecology and Evolution* 23: 361–368

Bremner-Harrison S. Prodohl P.A. & Elwood R.W. 2004. Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred Swift Fox (*Vulpes velox*). *Animal Conservation* 7: 313–320.

Cade T.J. 1988. Using science and technology to reestablish species lost in nature, p. 279–288. In: Wilson E.O. & Peter F.M. (eds.). *Biodiversity*. Washington: National Academic Press.

Carere C., Drent P.J., Privitera L., Koolhaas J.M. & Groothuis T.G.G. 2005. Personalities in Great Tits, *Parus major*: stability and consistency. *Animal Behaviour* 70: 795–805.

Carlstead K. 1999. *Constructing behavioural profiles for zoo animals: incorporating behavioral information into captive population management*. Oregon: AZA Behaviour and Husbandry Advisory Group and Oregon Zoo.

Carter A.J. & Feeney W.E. 2012. Taking a comparative approach: analysing personality as a multivariate behavioural response across species. *PLoS ONE* 7: e42440.

Collar N.J. 1997. Family Psittacidae (parrots), p. 280–479. In: del Hoyo J., Elliot A. & Sargatal J. (eds.). *Handbook of the Birds of the World, v.* 4 (sandgrouse to cuckoos). Barcelona: Lynx Editions.

Curio E. 1988. Cultural transmission of enemy recognition by birds, p. 75–97. In: Zental T.R. & Galef-Jr. B.G. (eds.). *Social learning, psychological and biological perspectives*. New Jersey: Hilldalf.

Curio E., Ernst U. & Vieth W. 1978. The adaptive significance of avian mobbing: II. Cultural transmission of enemy recognition in Blackbirds: effectiveness and some constraints. *Ethology* 48: 184–202.

Dingemanse N.J. & Réale D. 2005. Natural selection and animal personality. *Behaviour* 142: 1159–1184.

Eilam D. 2005. Die hard: a blend of freezing and fleeing as a dynamic defense-implications for the control of defensive behavior. *Neuroscience and Biobehavioral Reviews* 29: 1181–1191.

Emery N.J. 2006. Cognitive ornithology: the evolution of avian intelligence. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 361: 23–43.

Feenders G., Klaus K. & Bateson M. 2011. Fear and exploration in European Starlings (*Sturnus vulgaris*): a comparison of hand-reared and wild-caught birds. *PLoS ONE* 6: e19074.

Foose T.J. 1986. Riders of the lost ark: the role of captive breeding in conservation strategies, p. 141–165. In: Kaufman L. & Mallory D. (eds.). *The last extinction*. Cambridge: The Mit Press.

Fox R.A. & Millam J.R. 2004. The effect of early environment on neophobia in Orange-winged Amazon Parrots (*Amazona amazonica*). *Applied Animal Behaviour Science* 89: 117–129.

Fox R.A. & Millam J.R. 2007. Novelty and individual differences influence neophobia in Orange-winged Amazon Parrots (*Amazona amazonica*). *Applied Animal Behaviour Science* 104: 107–115.

Frid A. & Dill L.M. 2002. Human-caused disturbance stimuli as a form of predation risk. *Ecology and Society* 6:11.

Frost A.J., Winrow-Giffen A., Ashley P.J. & Sneddon L.U. 2007. Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of the Royal Society of London B: Biological Sciences* 274: 333–339.

Gilardi J.D. & Munn C.A. 1998. Patterns of activity, flocking, and habitat use in parrots of the Peruvian Amazon. *Condor* 100: 641–653.

Griffin A.S., Blumstein D.T. & Evans C.S. 2000. Training captive-bred or translocated animals to avoid predators. *Conservation Biology* 14: 1317–1326.

Griffin A.S., Evans C.S. & Blumstein D.T. 2001. Learning specificity in acquired predator recognition. *Animal Behaviour* 62: 577–589.

Groothuis T.G.G. & Carere C. 2005. Avian personalities: characterization and epigenesis. *Neuroscience & Biobehavioral Reviews* 29: 137–150.

Hemmi J.M. & Merkle T. 2009. High stimulus specificity characterizes anti-predator habituation under natural conditions. *Proceedings of the Royal Society B: Biological Sciences* 276: 4381–4388.

IUCN (International Union for the Conservation of Nature). 2015. *The IUCN red list of threatened species*, Version 2015.1. http://www.iucnredlist.org. (access on 12 May 2016).

Kurvers R.H.J.M., Prins H.H.T., van Wieren S.E., van Oers K., Nolet B.A. & Ydenberg R.C. 2010. The effect of personality on social foraging: shy Barnacle Geese scrounge more. *Proceedings of the Royal Society of London B: Biological Sciences* 277: 601–608.

Lendvai A.Z., Bókony V. & Chastel O. 2011. Coping with novelty and stress in free-living House Sparrows. *Journal of Experimental Biology* 214: 821–828.

Levine S., Atha K. & Wiener S.G. 1993. Early experience effects on the development of fear in the Squirrel Monkey. *Behavioral and Neural Biology* 60: 225–233.

Macias C., Parás A., González J.J., Enkerlin E., Ritchie B., Stone E., Lamberski N. & Ciembor D. 2003. Release of confiscated Amazon parrots in Mexico. *Psittascene* 15: 2–4.

Maloney R.F. & McLean I.G. 1995. Historical and experimental learned predator recognition in free-living New Zealand Robins. *Animal Behaviour* 50: 1193–1201.

Martin, P. & Bateson, P. 2007. *Measuring behaviour: an introductory guide*, 3rd Edition. Cambridge University Press: Cambridge.

McDougall P.T., Réale D., Sol D. & Reader S.M. 2006. Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. *Animal Conservation* 9: 39–48.

McLean I.G. 1996. Teaching an endangered mammal to recognize predators. *Biological Conservation* 75: 51–62.

McLean I.G. & Rhodes G. 1991. Enemy recognition and response in birds, p. 173–211. In: Power D.M. (ed.). *Current Ornithology* v. 8, Santa Barbara: Plenum Press.

McLean I.G., Hölzer C. & Studholme B.J.S. 1999. Teaching predator-recognition to a naive bird: implications for management. *Biological Conservation* 87: 123–130.

Meehan C.L., Millam J.R. & Mench J.A. 2003. Foraging opportunity and increased physical complexity both prevent and reduce psychogenic feather picking by young Amazon Parrots. *Applied Animal Behaviour Science* 80: 71–85.

Miles W.T.S., Parsons M., Close A.J., Luxmoore R. & Furness R.W. 2013. Predator-avoidance behaviour in a nocturnal petrel exposed to a novel predator. *Ibis* 155: 16–31.

Miller B., Biggins D., Hanebury L. & Vargas A. 1994. Reintroduction of the Black-footed Ferret (*Mustela nigripes*), p. 455–464. In: Olney P.J.S., Mace G.M. & Feistner A.T.C. (eds.). *Creative conservation, interactive management of wild and captive animals*. London: Chapman and Hall.

MMA (Ministério do Meio Ambiente). 2014. *Portaria N° 444, de 17 de dezembro de 2014*. Brasilia: Ministério do Meio Ambiente.

Pitter E. & Christiansen M.B. 1997. Behavior of individuals and social interactions of the Red-fronted Macaw *Ara rubrogenys* in the wild during the midday rest. *Ornitología Neotropical* 8: 133–143.

Prestes N.P. 2000. Descrição e análise quantitativa do etograma de *Amazona pretrei* em cativeiro. *Ararajuba* 8: 25–42.

Ribeiro L.B. & Silva M.G. 2007. O comércio ilegal põe em risco a diversidade das aves no Brasil. *Ciência e Cultura* 59: 4–5.

Richards J. 1998. Return of the natives. *Australian Geographic* 50: 82.

Rosier R.L. & Langkilde T. 2011. Behavior under risk: how animals avoid becoming dinner. *Nature Education Knowledge* 2: 8.

Ryer C.H. & Olla B.L. 1998. Shifting the balance between foraging and predator avoidance: the importance of food distribution for a schooling pelagic forager. *Environmental Biology of Fishes* 52: 467–475.

Sanz V. & Grajal A. 1998. Successful reintroduction of captive-raised Yellow-shouldered Amazon Parrots on Margarita Island, Venezuela. *Conservation Biology* 12: 430–441.

Schunk F., Somenzari M., Lugarini C. & Soares E.S. 2011. *Plano de ação nacional para a conservação dos papagaios da Mata Atlântica*. Brasília: Ministério do Meio Ambiente.

Seixas G.H.F. & Mourão G.M. 2002. Nesting success and hatching survival of the Blue-fronted Amazon (*Amazona aestiva*) in the Pantanal of Mato Grosso do Sul, Brazil. *Journal of Field Ornithology* 73: 399–409.

Short J., Bradshaw S.D., Giles J., Prince R.I.T. & Wilson G.R. 1992. Reintroduction of macropods (Marsupialia: Macropodoidea) in Australia – a review. *Biological Conservation* 62: 189–204.

Sih A., Bell A. & Johnson J.C. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution* 19: 372–378.

Smith B.R. & Blumstein D.T. 2012. Structural consistency of behavioural syndromes: does predator training lead to multi-contextual behavioural changes? *Behaviour* 149: 187–213.

Smith B.R. & Blumstein D.T. 2013. Animal personality and conservation biology: the importance of behavioral diversity, p. 381–413. In: Carere C. & Maestripieri D. (eds.). *Animal personalities: behavior, physiology, and evolution*. Chicago: The University of Chicago Press.

Specht G.V.A. 2007. *Treinamento antipredação em Corujinha-do-mato (*Megascops [Otus] choliba*, (Vieillot, 1817) - Strigiformes – Aves) para programa de translocação.* MSc. Dissertation. Belo Horizonte: Pontifícia Universidade Católica de Minas Gerais.

Stamps J. & Groothuis T.G.G. 2010. The development of animal personality: relevance, concepts and perspectives. *Biological Reviews* 85: 301–325.

Stankowich T. & Blumstein D.T. 2005. Fear in animals: a meta-analysis and review of risk assessment. *Proceedings of the Royal Society of London B: Biological Sciences* 272: 2627–2634.

Taylor R.W., Boon A.K., Dantzer B., Réale D., Humphries M.M., Boutin S., Gorrell J.C., Coltman D.W. & McAdam A.G. 2012.

Low heritabilities, but genetic and maternal correlations between Red Squirrel behaviours. *Journal of Evolutionary Biology* 25: 614–624.

Toms C.N., Echevarria D.J. & Jouandot D.J. 2010. A methodological review of personality-related studies in fish: focus on the shy-bold axis of behavior. *International Journal of Comparative Psychology* 23: 1–25.

Valle A.L., Silva C.W., Teixeira R.A., Batista L.P. & Ramos B.C. 2010. Comparativo entre duas metodologias de soltura para *Amazona aestiva* observando-se poucos critérios de *soft-release*. *Reintrodução de Psitacídeos* 1: 48–50.

van Heezik Y., Seddon P.J. & Maloney R.F. 1999. Helping reintroduced Houbara Bustards avoid predation: effective anti-predator training and the predictive value of pre-release behaviour. *Animal Conservation* 2: 155–163.

van Oers K., Drent P.J., de Goed P. & van Noordwijk A.J. 2004. Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. *Proceedings of the Royal Society of London B: Biological Sciences* 271: 65–73.

van Oers K., de Jong G., van Noordwijk A.J., Kempenaers B. & Drent P.J. 2005. Contribution of genetics to the study of animal personalities: a review of case studies. *Behaviour* 142: 1185–1206.

van Oers K.V. & Naguib M. 2013. Avian personality, p. 66–95. In: Carere C. & Maestripieri D. (eds.). *Animal personalities: behavior, physiology, and evolution*. Chicago: The University of Chicago Press.

Veloso-Júnior, R.R., Sousa L.C. & Brito M.L.O. 2010. Soltura e monitoramento de Jandaia verdadeira na Ilha de São Luís. *Reintrodução de Psitacídeos* 1: 40–43.

Verbeek M.E.M., Drent P.J. & Wiepkema P.R. 1994. Consistent individual differences in early exploratory behaviour of male Great Tits. *Animal Behaviour* 48: 1113–1121.

Watters J.V. & Powell D.M. 2012. Measuring animal personality for use in population management in zoos: suggested methods and rationale. *Zoo Biology* 31: 1–12.

White-Jr. T.H., Collazo J.A. & Vilella F.J. 2005. Survival of captive-reared Puerto Rican Parrots released in the Caribbean National Forest. *Condor* 107: 424–432.

Wilson D.S. 1998. Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 353: 199–205.

Wilson D.S., Clark A.B., Coleman K. & Dearstyne T. 1994. Shyness and boldness in humans and other animals. *Trends in Ecology and Evolution* 9: 442–446.

Wolf M. & Weissing F.J. 2012. Animal personalities: consequences for ecology and evolution. *Trends in Ecology and Evolution* 27: 452–461.

Yorzinski J.L. & Platt M.L. 2012. The difference between night and day: anti-predator behavior in birds. *Journal of Ethology* 30: 211–218

Zaccaroni M., Ciuffreda M., Paganin M. & Beani L. 2007. Does an early aversive experience to humans modify anti-predator behaviour in adult Rock Partridges? *Ethology, Ecology & Evolution* 19: 193–200.

Zar J.H. 1998. *Biostatistical analysis*, 4th Edition. New Jersey: Prentice Hall.

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