

The role of habituation in the adjustment to urban life: An experimental approach with burrowing owls

M. Cavalli*, A.V. Baladrón, J.P. Isacch, L.M. Biondi, M.S. Bó

Laboratorio de Vertebrados, Instituto de Investigaciones Marinas y Costeras (IIMyC), CONICET - Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata, B7602AYJ, Argentina

ARTICLE INFO

Keywords:

Flight initiation distance
Burrowing owls
Urban habitats
Rural habitats
Habituation

ABSTRACT

Birds exhibit variation in fear behaviour in response to an approaching human within and between species and across different habitat contexts. We analyze urban and rural burrowing owls' variation in risk perception along separate but consecutive days (Treatment 1) and risk perception within the same day (Treatment 2). Fear behaviour was measured as flight initiation distances (FIDs) and aggressiveness level when a pedestrian approached repeatedly to an owl individual. We predict that the attenuation in fear response along treatments should add support to the habituation hypothesis (decrease the response to a repeatedly stimulus after verifying that it is irrelevant) while consistency in behaviour might be indicative of a personality trait. We found that FID decreased for measurements made on both treatments in rural owls and also in urban owls for Treatment 2. These results are compatible with a habituation process. We found that aggressiveness remained invariable along treatments in both habitats suggesting that different mechanisms underlie these behavioural responses. Our results suggest that owls' risk perception to humans can be adjusted based on environmental risk perception and that individuals are able to recognize and learn when a stimuli stops being a threat.

1. Introduction

Animals living in urban habitats often show traits that are different from those of animals living in surrounding non-urban habitats (Møller, 2008, 2010; Sih et al., 2010). These modifications include changes in behaviour, breeding density, an extended breeding season, stress response, among others (Partecke et al., 2006; Sih et al., 2010; Tuomainen and Candolin, 2011). One remarkable difference between urban and rural animals is related to their risk perception and anti-predatory behaviour (Blumstein et al., 2006; Møller, 2008), revealed by the phenotypic traits that an animal displays in response to a menace in order to avoid being detected, attacked, and killed (Caro, 2005; Lima and Dill, 1990). In urban habitats, human presence is a main disturbance factor, since it is taken as a potential threat by many animal species (Blumstein, 2014; Frid and Dill, 2002; Lima, 2009). Then urban animals need to adjust their behaviour to balance the trade-off between respond to the threat (energetic cost) and ignore the risk (potential death) (Frid and Dill, 2002). Through learning animals can reduce these costs by assessing risk and responding appropriately. Many species adjust their response to different levels of risk through varying flight initiation distances (FID, i.e. the distance between an individual and an approaching human when the individual flees, Blumstein et al., 2006;

Tätte et al., 2018). Several studies have reported that more urbanized species show shorter FIDs and are fearless toward humans compared to those inhabiting rural habitats (e.g.: Isaksson et al., 2018; Samia et al., 2017; Vincze et al., 2016).

Different hypothesis have been proposed to explain differences in FID between individuals dwelling at urban and non-urban habitats regarding their ability to tolerate human disturbance. One of these hypotheses proposes a fear reduction or habituation to humans by urban individuals over time (Blumstein, 2016; Cooke, 1980; Li et al., 2011; Rodríguez-Prieto et al., 2009; Vincze et al., 2016), whereas alternative hypotheses suggest a selection process or non random distribution of animals based on their tolerance to humans (Carrete and Tella, 2011, 2013; Møller, 2010). According to the habituation hypothesis, animals living in urban areas would benefit by decreasing their fear response to humans, given that becoming alert to every human stimuli might be energetically too costly (Blumstein, 2014, 2016). Habituation would be adaptive if contributes to the reduction of predation risk but also decreases unnecessary responses to non-threatening stimuli (Blumstein, 2014, 2016; Rankin et al., 2009). Alternative hypotheses postulate that within the same species individuals that exhibit lower levels of fear or bolder behaviours would be able to settle closer to humans (towns, cities) while individuals showing higher levels of fear would settle

* Corresponding author.

E-mail address: mcavalli@mdp.edu.ar (M. Cavalli).

<https://doi.org/10.1016/j.beproc.2018.10.011>

Received 18 June 2018; Received in revised form 20 September 2018; Accepted 18 October 2018

Available online 22 October 2018

0376-6357/ © 2018 Elsevier B.V. All rights reserved.

farther (i.e. differential colonization hypothesis, Carrete and Tella, 2010, 2011; Møller, 2010). This idea proposes that urban habitats act as filters that contribute to accentuate differences in animal behavioural phenotypes between urban and surrounding habitats (Møller, 2010; Sprau and Dingemanse, 2017). In line with this idea, other hypothesis also proposes that differences in fear behaviour would be the result of a selection process on bold or fearless behaviours of animals living close to humans (Møller, 2008). Unlike the habituation process which involves a change in behaviour, the latter two hypotheses assume that behavioural response is consistent within individuals (Dingemanse et al., 2010; Mathot and Dingemanse, 2015). However, since environment is variable in space and time, it is to be expected that selection may act favoring not only a certain behavioural type but also individuals with some degree of behavioural flexibility (Brown, 2012; Lowry et al., 2013; Sol et al., 2013).

The burrowing owl (*Athene cunicularia*) represents an example of a bird species that has managed to live in human proximities. This species is an underground nesting bird that can be found in open landscapes such as treeless plains, grasslands, prairies, savannah, golf courses, road verges, airports, and also in urban and peri-urban areas (Poulin et al., 2011). This owl is a year-round resident at its southernmost distribution and excavates its own burrows. Mating pairs are territorial and highly conspicuous in the daylight and are easily located near their nests (Marks et al., 1994). The presence of burrowing owls in a great array of habitat types has been associated to its adaptability and behavioural flexibility to environmental change (Berardelli et al., 2010; Cavalli et al., 2016a). In this sense, previous studies have reported that urban burrowing owl populations show shorter FIDs than rural populations (Carrete and Tella, 2013; Cavalli et al., 2016a, 2016b; Rebolo-Ifrán et al., 2017).

In this study we aim to investigate the extent to which previously described differences in FID among urban and rural populations reflect flexible responses in the form of habituation. For this we design a protocol carefully adjusting the time between the stimuli of a person approaching to burrowing owls to assess if fear response changes when exposed to very repetitive and controlled human stimuli. Previous studies regarding burrowing owl fear response towards a human approaching stimulus have discarded habituation as a relevant process, however it is important to note that such studies were conducted considering a human stimulus that was spaced in time (between years or several times along a breeding season; Carrete and Tella, 2010, 2013). Even when those previous studies allow detecting a behavioural type or personality within owl population, a habituation process cannot be dismissed if response to a stimuli occurring at short intervals exposition is not tested. Further, there are evidences that demonstrate certain degree of behavioural flexibility on FID behaviour by burrowing owls, for example, it has been demonstrated that fear behaviour vary according to breeding stage (Cavalli et al., 2016a) and with stimuli type (Cavalli et al., 2016b).

Although the human presence in urban areas can be randomly distributed, it is expected that the exposure to humans of individuals in such habitats will be repeated throughout time and will show a higher frequency than in rural habitats. Consequently, the possibility that urban owls could become habituated to the frequent presence of humans in that habitat should be thought as an alternative hypothesis. We predict that attenuation in fear response along the experiment trials should add support to the habituation hypothesis while no variation in this behaviour would be indicative that only more tolerant individuals with a fixed behaviour would dwell to urban habitats (i.e., urbanization acts as a filter) or that it is the outcome of an adaptation process. The results of this study may contribute to understand the mechanisms by which bird species can inhabit and succeed in human environments.

2. Methods

2.1. Study area

This study was conducted in rural and urban habitats in south-eastern Pampas region of Argentina. This area dominated in the past by grasslands, wetlands and dunes is now a mosaic of different land-uses where agroecosystems (grazing fields, croplands and pasturelands) and urban areas dominate the landscape (Isacch et al., 2016). Different localities and rural areas were sampled between Mar Chiquita village (37° 44.6' S, 57° 25.7' W) and Mar del Plata city (38° 00.8' S, 57° 33.1' W) in Buenos Aires province. In this area, burrowing owls inhabit rural habitats, sand dunes, and urban habitats (Baladrón et al., 2016; Martínez et al., 2017). We defined urban habitats as built-up areas where owls will regularly encounter humans. We considered as urban owls those individuals whose nests were surrounded by more than 15 houses in a 200 m radius. Rural habitats comprise open farmlands, grazing fields, and croplands. Human presence is low in this habitat and restricted to unpaved roads or farm houses and ranches in most cases.

2.2. Experimental design and data collection

As part of a study developed during 6 years with populations of burrowing owls, we monitored 410 nests. For this study we specifically selected a pool of nests that were enough apart to make samples independent but to be relatively near to perform trials in the same day by the same person. This work was carried out in mid-late September of 2013 and 2014. At each nest we performed trials that consisted on a person walking in a straight line (pedestrian) at a constant speed (5 km/h) towards owls that were standing outside its nest. Males were distinguished from females by their lighter plumage coloration (Baladrón et al., 2015). All trials started approximately 200 m from the nest to avoid variability in owl fear response associated with varying starting distance (Mikula, 2014; Rodríguez-Prieto et al., 2009) and all trials were performed during the morning to reduce bias due to daytime. No trial was carried out during days with precipitation, fog or extreme wind (Andersen, 1990; Sproat and Ritchison, 1993).

During each trial, we evaluated burrowing owls' fear behaviour by measuring flight initiation distance (hereafter FID) and behaviour aggressiveness. FIDs were measured by counting the number of steps between the pedestrian and the owl at the moment the owl flew away. Behaviour aggressiveness was recorded with a digital voice recorder while the pedestrian was walking to each nest. Behaviour aggressiveness was classified into five categories of rank 0–4 (adapted from Fisher et al., 2004; Galeotti et al., 2000); 0: Owl flies away or enters the nest 1: owl bows and/or vocalizes, 2: owl raises feathers and spreads wings in order to appear larger, usually meant to distract a predator from a nest, 3: owl displays threat flights and 4: owl displays dive attacks. Only the most aggressive response during each trial was used for statistical analyses. Owl behaviour was always measured by the same person (MC) who wore clothes with the same color pattern during all trials.

We evaluated variation in owl fear response to humans by performing two treatments that differed in the frequency of human stimuli:

2.2.1. Treatment 1: risk perception along separate but consecutive days

We sampled owls at 27 nests (13 rural and 14 urban). We performed successive trials to each owl during nine consecutive days (1 per individual per day). All trials were performed in the morning (6:00–11:30AM). This protocol was specifically designed to evaluate if a daily stimulus of a human who does not represent a threat causes habituation, sensitization (increase of the response), or does not cause a change in owl fear behaviour over time. We extended this treatment during 9 days since the longer the treatment, the fewer random errors in sampling (Bell et al., 2009).

2.2.2. Treatment 2: risk perception within the same day

We sampled owls at 39 nests (21 rural and 18 urban). On the same day, we performed 5 successive trials to each owl. All trials were performed during the morning (6:00–11:30AM) and interstimulus interval was of approximately 50 min. We only performed 5 trials to each owl since that was the maximum logistically possible number of trials during the morning by the same person. Individuals sampled during this treatment were different from those sampled during Treatment 1.

2.3. Statistical analyses

We tested the effect of habitat and trial and their interaction on FID behaviour for each Treatment (1 and 2) by adjusting linear mixed effects models (lme function, nlme package, [Pinheiro and Bates, 2000](#)) with Gaussian error distribution and identity link function. For Treatment 1 analyses explanatory variables were Day number (numeric) and Habitat (categorical with two levels: urban, rural), their interaction (Habitat x Day number), and Sex (categorical with two levels: male, female), whereas FID (sqrt transformed) was the response variable (continuous). For Treatment 2 analyses, predictors of the response variable were similar to Treatment 1. To be able to express the rate of habituation over consecutive trials, we used Day number in Treatment 1 and Trial number in Treatment 2 as continuous variables (thus assuming a linear change over time).

We tested the effect of the random term by comparing models including owls identity nested with nest identity as random effect, models including only nest identity as random effect, and models without a random effect. In order to find the optimal structure of the random component we compared models using Restricted Maximum Likelihood (REML) estimators and performing a likelihood-ratio test ([Zuur et al., 2009](#)). Such test is based on the comparison of the logarithm values of the restricted likelihood of mixed linear models and is distributed as a Chi-square function, being degrees of freedom similar to the number of terms between models ([Zuur et al., 2009](#)). Models with random structure including only nest identity as random effect fitted better for both treatments, thus subsequent analyses were performed including this random effect structure.

We used information-theoretic procedures to evaluate models performance ([Burnham and Anderson, 2002](#)). We considered models with all possible combinations of predictor variables. This resulted in 10 candidate models for the analysis of variation in FID behaviour for each treatment. We calculated the Akaike's information criterion corrected for small sample size (AICc) for each model ([Burnham and Anderson, 2002](#)). Then we performed model selection by comparing ΔAICc , which is the difference between the lowest AICc value (i.e. best of suitable models) and AICc from all other models. The AICc weight of a model (W_i) signifies the relative likelihood that the specific model is the best of the suite of all models. Further, we calculated the parameter estimates using model-averaged parameter estimates based on W_i from all candidate models ([Burnham and Anderson, 2002](#)). To supplement parameter-likelihood evidence of important effects, we calculated 95% confidence interval limits (CL) of parameter estimates. Model adjustments were assessed graphically for the assumption of normality and homoscedasticity. Heteroscedasticity was corrected by using the varLm function in R ([Pinheiro and Bates, 2000](#)).

2.4. Behaviour aggressiveness

To test the effect of Habitat, Sex, Trial number or Day number, and their interaction (explanatory variables) on burrowing owls' behaviour aggressiveness (ordinal response variable) we fitted a cumulative link mixed model for each treatment (clmm function, ordinal library; [Christensen, 2012](#)). We built models including nest identity and a model including nest and owl identities as random factors and fitted them by probit link function and equidistant threshold parameters. We evaluated the importance of the random term structure by performing a

Table 1

Summary of model-selection results for models explaining variation in FID behaviour of the Burrowing owl in relation to Habitat (HAB), Experimental Day number (DAY), Individual sex (SEX) and the interaction between Day number and Habitat (DAY x HABITAT) during Treatment 1. k is the number of estimated parameters. See methods for details. Models are listed in decreasing order of importance.

Models	k	ΔAICc	W_i
DAY HAB DAYxHAB	7	0.0	0.505
DAY SEX HAB DAYxHAB	8	0.05	0.493
DAY HAB	6	13.22	0.001
DAY SEX HAB	7	13.83	0.001
HAB	5	16.42	0.000
SEX HAB	6	17.15	0.000
DAY	5	57.31	0.000
DAY SEX	6	58.40	0.000
Null Model	4	60.62	0.000
SEX	5	61.80	0.000

likelihood-ratio test ([Christensen, 2015](#)). Cumulative link mixed models are models for ordinal response variables and are fitted with the Laplace approximation ([Christensen, 2012](#)). All statistical analyses were carried out using R software, Version R-3.4.4 ([R Development Core Team, 2018](#)).

3. Results

We recorded FID and behaviour aggressiveness of burrowing owls in 343 instances during Treatment 1 (170 in rural and 173 in urban habitats) and in 256 instances during Treatment 2 (146 in rural and 110 in urban habitats).

3.1. Fear response of burrowing owls

3.1.1. Treatment 1: risk perception along separate but consecutive days

Variation in owls FID was best described by a model including Habitat, Day number, and the interaction between these variables ([Table 1](#)). FIDs were shorter for urban owls compared to rural ones. Rural owls tended to decrease their FIDs along time, while urban owls did not show variations in this behaviour ($T_{\text{rural}, 156} = -4.653$, $p < 0.005$, $T_{\text{urban}, 158} = -0.252$, $p = 0.801$; [Table 2](#), [Fig. 1a](#)).

3.1.2. Treatment 2: Risk perception within the same day

Variation in owls FID was best described by a model including Habitat, Day number, and the interaction between these variables ([Table 3](#)). FIDs were shorter for urban owls compared to rural ones. Both rural and urban owls tended to decrease their FIDs along time, the variation in this behaviour was more pronounced for rural owls ($T_{\text{urban}, 86} = -2.272$, $P = 0.025$, $T_{\text{rural}, 116} = -3.845$, $p < 0.005$; [Table 4](#),

Table 2

Parameter likelihoods, estimates (\pm SE) and 95% confidence interval limits (CL) for explanatory variables describing variation in FID behaviour during Treatment 1. Explanatory variables with CL excluding zero are in bold. See methods for details.

Explanatory variable	Parameter Likelihood	Parameter estimate \pm SE	CL	
			Lower	Upper
Intercept		7.607 \pm 0.325	6.969	8.245
Day	1.00	-0.212 \pm 0.045	-0.302	-0.122
Habitat ^a urban	1.00	-4.272 \pm 0.412	-5.080	-3.464
Day x habitat	1.00	0.206 \pm 0.051	0.104	0.456
Sex ^b	0.49	0.191 \pm 0.135	-0.072	0.456
male				

^a Relative variable to value of habitat type (rural).

^b Relative variable to value of sex (female).

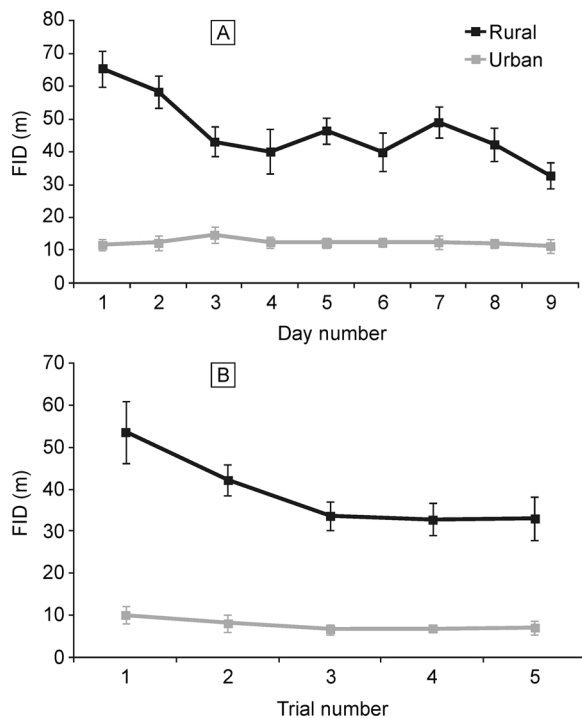


Fig. 1. Burrowing owls' FID behavior in urban and rural habitats during (A) Treatment 1 (single human approaches performed during nine days in a row) and (B) Treatment 2 (five human approaches performed on a single day). Points represent mean values \pm standard errors.

Table 3

Summary of model-selection results for models explaining variation in FID behaviour of the burrowing owl in relation to Habitat (HAB), Experimental Trial number (TRIAL), Individual sex (SEX) and the interaction between Trial number and Habitat (TRIAL x HABITAT) during Treatment 2. k is the number of estimated parameters. See methods for details. Models are listed in decreasing order of importance.

Models	k	$\Delta AICc$	Wi
TRAIL HABITAT TRAIL x HABITAT	7	0.00	0.494
TRAIL HABITAT SEX TRAIL x HABITAT	8	0.89	0.316
TRAIL HABITAT	6	2.88	0.117
TRAIL HABITAT SEX	7	3.83	0.073
HABITAT	5	15.22	0.000
HABITAT SEX	6	17.02	0.000
TRAIL	5	45.91	0.000
TRAIL SEX	6	46.96	0.000
Null Model	4	58.00	0.000
SEX	5	59.84	0.000

Fig. 1b).

3.2. Behaviour aggressiveness

Aggressiveness of burrowing owls did not vary significantly according to Day number, Habitat, or their interaction for Treatment 1. However, females tended to be more aggressive than males (Table 5). For Treatment 2, aggressiveness did not vary with respect to Trial number, Habitat, their interaction, or Sex (Table 5).

4. Discussion

Animals react in response to a threat according to the level of the risk perceived. This response is, in turn, modulated by their previous life-time experience (i.e., learning) and by innate recognition of a threat (Frid and Dill, 2002; Sol et al., 2013). In this study, we predicted that if

Table 4

Parameter likelihoods, estimates (\pm SE) and 95% confidence interval limits (CL) for explanatory variables describing variation in FID behaviour during Treatment 2. Explanatory variables with CL excluding zero are in bold. See methods for details.

Explanatory variable	Parameter Likelihood	Parameter estimate \pm SE	CL	
			Lower	Upper
Intercept		6.815 \pm 0.420	5.987	7.642
Trial	1.00	−0.314 \pm 0.107	−0.526	−0.102
Habitat ^a	1.00	−3.973 \pm 0.525	−5.029	−2.916
urban				
Trial x Habitat	0.81	0.229 \pm 0.102	0.027	0.432
Sex ^b	0.39	0.206 \pm 0.187	−0.163	0.577
male				

^a Relative variable to value of habitat type (rural).

^b Relative variable to value of sex (female).

Table 5

Parameter likelihoods, estimates (\pm SE) and 95% confidence interval limits (CL) for explanatory variables describing variation in Behaviour aggressiveness of burrowing owls during Treatment 1 and Treatment 2. Explanatory variables with CL excluding zero are in bold. See methods for details.

Explanatory variable	Parameter estimate \pm SE	CL	
		Lower	Upper
Treatment 1			
Day	0.017 \pm 0.032	−0.046	0.081
Habitat ^a	0.381 \pm 0.289	−0.187	0.949
urban			
Sex ^b	−0.948 \pm 0.135	−1.214	−0.682
male			
Habitat x Day	−0.013 \pm 0.045	−0.102	0.076
Treatment 2			
Trial	−0.072 \pm 0.075	−0.220	0.075
Habitat ^a	0.275 \pm 0.426	0.560	1.111
urban			
Sex ^b	0.483 \pm 0.342	−0.188	1.155
male			
Habitat x Trial	0.085 \pm 0.109	−0.130	0.300

^a Relative variable to value of habitat type (rural).

^b Relative variable to value of sex (female).

the burrowing owls response to human stimuli is in line with habituation hypothesis (i.e., owls cease to perceive humans as a menace after repeated harmless encounters), then we would observe a reduction in their fear response along time (decrease in FID and/or behaviour aggressiveness). Our results showed that, when urban and rural owls are exposed to two different frequencies of human stimuli (within and among days), rural owls decreased their FID for both treatments while urban owls decrease their fear response only when stimuli intensify (within days). These results would at a first glance indicate that, after a repeated stimulus of an approaching human, owls would cease to perceive it as a threat. Aggressiveness was invariable along time for both rural and urban owls.

Previous studies on this species suggested that burrowing owls distribute throughout territory according to their tolerance to human disturbance. Hence, some behavioural types would be prone to settle in human proximities while others would avoid them (i.e., differential colonization hypothesis, Carrete and Tella, 2010, 2013). In this sense, based on high consistency of certain behaviours, it has been suggested that the idea that owls living in contact with humans reduce FID through habituation would lack of support (Carrete and Tella, 2013). However, our results evidencing FID decreasing pattern by rural and urban owls, strongly suggests a habituation process. Habituation to human and reduction in fear response has been previously reported in studies where time between stimuli was carefully controlled. For

example, Pellitteri-Rosa et al., (2017) found that the common wall lizard (*Podarcis muralis*) tended to progressively habituate to simulated but not effective predatory attack, Blumstein (2016) showed that marmots (*Marmota flaviventris*) allowed people to approach them more closely after repeated approaches, and González et al., (2006) found that fear behaviour of nesting spanish imperial eagles (*Aquila adalberti*) was related to the frequency of human activities. Our results suggest that, even when individuals with certain behavioural types might be more prone to settle in urban habitats (Carrete and Tella, 2013; Moroni et al., 2017; Rebolo-Ifrán et al., 2017), learning cannot be dismissed as an intervening factor in the adjustment of burrowing owls to different levels of human disturbance.

It was suggested that the ability to habituate to human presence could represent a benefit for urban birds, in terms of time and costs saved for other activities such as foraging or mating (Lima, 2009; Samia et al., 2015). In line with this, recent studies have shown that populations of a same species living in different environments can exhibit distinctive learning capacities. For example, a study on house sparrows demonstrated that urban individuals habituated faster to human disturbance than their rural conspecific which reflects their behavioural plasticity (Vincze et al., 2016). Similarly, common wall lizard from urban habitats seems to habituate faster than rural individuals (Pellitteri-Rosa et al., 2017).

In our study, rural owls showed greater FIDs than urban owls in both treatments which is in accordance with all the work done in burrowing owls on this matter (e.g., Carrete and Tella, 2013; Cavalli et al., 2016a,b; Rebolo-Ifrán et al., 2017). Moreover, one of the predictions of this study was that no changes in fear behaviour would be found in urban populations because individuals are fully habituated to human presence (i.e., ceiling effect, Oosten et al., 2010) or because only bold individuals would select this habitat (Carrete and Tella, 2011, 2013). Surprisingly and contrary to our expectations for urban owls, we found that when we intensified the stimuli frequency (as we did during Treatment 2), urban owls also tend to decrease FID. This could mean that even in situations of relaxation against frequent stimuli, it may be adaptive to maintain certain flexibility in their risk-taking responses. More evidences supporting individual behavioural flexibility when facing a threat have been recently given by DeVault et al. (2017) in their studies with cowbirds suggesting that animals might learn to anticipate a repeated and consistent disturbance associated to urbanization. Behavioural flexibility observed in urban owls on FID, a trait in which we did not expect variation, would represent an advantage specially to cope with changing environments such urban environments. In this context, learning may allow animals to improve behaviour based on previous experience and offer advantages to face those possible changes in the environment (Sol et al., 2013).

Learning provides animals the opportunity to adapt their behaviour to changing environments and is the primary mechanism animals use to cope with shifting environmental variables (Brown, 2012). Changes in the environment such as urbanization often occurs too rapidly for evolutionary process to respond, thus, learning may represent one of the ways to deal with environmental variation (Brown, 2012; Sol et al., 2005). However, when environment is stable, there will be no need for the individual to display behavioural flexibility and innate behaviours should prevail. Further, it has been proposed that learning should prevail under intermediate levels of disturbance (Brown, 2012). Several studies have linked larger brains with enhanced learning abilities in mammals and birds (Brown, 2012; Griffin et al., 2017; Lefebvre et al., 1997, 2004; Sol et al., 2002), and also with their success invading novel environments like urban habitats (Griffin et al., 2017; Sol et al., 2005). Moreover, relative brain size is also associated to the rate of behavioural innovations in the field and also linked to the ecologically relevant test for innovation, that is, the ability to solve novel feeding problems (Griffin et al., 2017; Sol et al., 2013, 2018). These attributes are, in turn, considered as key components of behavioral flexibility in animals (Reader and Laland, 2001). In this sense, previous studies

regarding burrowing owls' brain size and urbanization, suggested an association between enlarged brains and the evolution of behavioural variability among individuals of this raptor. These studies predict that owls would not only modify their behaviour in potentially adaptive ways, but also through increased differences in individual traits (Carrete and Tella, 2011). This suggests inter-individual variation in response to urban stimuli more than intra-individual variation in behaviour, as we found in our study. As we state above, even when individuals with certain behavioural traits may be more prone to settle in urban habitats, behavioural flexibility cannot be dismissed in urbanization processes, and our results support this last idea.

Burrowing owls FID and aggressiveness did not show the same pattern in both treatments. Many studies have found that fear behaviour and aggressiveness are often correlated, thus indicating a behavioural syndrome such that more aggressive individuals are also bolder or less fearful (Hardman and Dalesman, 2018; Scales et al., 2011). However, we found that aggressiveness remained invariable along trials and no difference between urban and rural environments. Previous studies have shown that urban owls tend to be more aggressive than rural owls when they face predators associated with urban life such as dogs (Cavalli et al., 2016b), but also that both urban and rural owls similarly increase their aggressive behaviour according to breeding stage (Cavalli et al., 2016a). Although our results do not allow us to draw strong conclusions about mechanisms underlying aggression response, it became clear that different and independent mechanisms might be involved in modulating owls' fear response and aggressiveness toward an approaching human.

Our results based on identified owls monitored along two frequencies of exposure to humans allow us to state that fear response to humans may be modulated based on environmental perception and that individuals are able to learn when a stimulus can be considered as low risk. Differences between urban and rural burrowing owl populations has been previously explained as the consequence of a habitat selection process (Carrete and Tella, 2013; Rebolo-Ifrán et al., 2017), however our results support the idea that individual behavioural flexibility mediated by learning processes should be considered as a main process modulating fear behaviour and explaining urban colonization.

Acknowledgements

We thank J. Guido for her assistance during fieldwork and an anonymous reviewer for helpful comments on an earlier version of the manuscript. We appreciate the improvements in English usage made by Peter Lowther through the Association of Field Ornithologists' program of editorial assistance. This study was funded by Universidad Nacional de Mar del Plata Grant (15-E317), Agencia de Promoción Científica y Técnica (PICT 12-461), Consejo Nacional de Investigaciones Científicas y Técnicas. The authors adhered to guidelines for the use of animals in research and to the legal requirements in Argentina.

References

- Andersen, D.E., 1990. Nest-defense behavior of red-tailed hawks. *Condor* 92, 991–997.
- Baladrón, A.V., Isacch, J.P., Cavalli, M., Bó, M.S., 2016. Habitat selection by Burrowing Owls *Athene cunicularia* in the Pampas of Argentina: a multiple-scale assessment. *Acta Ornithol.* 51, 137–150.
- Baladrón, A.V., Cavalli, M., Bó, M.S., Isacch, J.P., Martínez, G., Madrid, E.A., 2015. Body size and sexual dimorphism in the southernmost subspecies of the Burrowing Owl (*Athene cunicularia cunicularia*). *J. Raptor Res.* 49, 479–485.
- Bell, A., Hankison, S.J., Laskowski, K.L., 2009. The repeatability of behaviour: a meta-analysis. *Anim. Behav.* 1–13.
- Berardelli, D., Desmond, M.J., Murray, L., 2010. Reproductive success of Burrowing Owls in urban and grassland habitats in southern New Mexico. *Wilson J. Ornithol.* 122, 51–59.
- Blumstein, D.T., 2014. Attention, habituation, and antipredator behaviour: implications for urban birds. In: Gil, D., Brumm, H. (Eds.), *Avian Urban Ecology. Behavioural and Physiological Adaptations*. Oxford University Press, Oxford, pp. 41–53.
- Blumstein, D.T., 2016. Habituation and sensitization: new thoughts about old ideas. *Anim. Behav.* 120, 255–262.
- Blumstein, D.T., Bitton, A., DaVeiga, J., 2006. How does the presence of predators

- influence the persistence of antipredator behavior? *J. Theor. Biol.* 239, 460–468.
- Brown, C., 2012. Experience and learning in changing environments. In: Candolin, U., Wong, B.B.M. (Eds.), *Behavioural Responses to a Changing World. Mechanisms and Consequences*. Oxford University Press, Oxford, pp. 46–62.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference: a Practical Information-theoretic Approach*. Springer-Verlag, New York.
- Caro, T., 2005. *Antipredator Defenses in Birds and Mammals*. University of Chicago Press.
- Carrete, M., Tella, J.L., 2011. Inter-individual variability in fear of humans and relative brain size of the species are related to contemporary urban invasion in birds. *PLoS One* 6, e18859.
- Carrete, M., Tella, J.L., 2010. Individual consistency in flight initiation distances in burrowing owls: a new hypothesis on disturbance-induced habitat selection. *Biol. Lett.* 6, 167–170.
- Carrete, M., Tella, J.L., 2013. High individual consistency in fear of humans throughout the adult lifespan of rural and urban burrowing owls. *Sci. Rep-UK* 3 3524–3524.
- Cavalli, M., Baladrón, A.V., Isach, J.P., Biondi, L.M., Bó, M.S., 2016b. Differential risk perception of rural and urban Burrowing Owls exposed to humans and dogs. *Behav. Process.* 124, 60–65.
- Cavalli, M., Isach, J.P., Baladrón, A.V., Biondi, L.M., Bó, M.S., 2016a. Differing nest-defence behaviour in urban and rural populations of breeding Burrowing Owls. *Emu* 116, 428–434.
- Christensen, R.H.B., 2012. *Regression Models for Ordinal Data R Package Version 2015*. pp. 6–28. <http://CRAN.R-project.org>.
- Christensen, R.H.B., 2015. *Analysis of Ordinal Data With Cumulative Link Models-Estimation With the R-package Ordinal*. <http://CRAN.R-project.org>.
- Cooke, A.S., 1980. Observations on how close certain passerine species will tolerate an approaching human in rural and suburban areas. *Biol. Conserv.* 18, 85–88.
- DeVault, T.L., Seamans, T.W., Blackwell, B.F., Lima, S.L., Fernández-Juricic, E., 2017. Individual variation in avian avoidance behaviours in response to repeated, simulated vehicle approach. *Can. J. Zool.* 96, 441–446.
- Dingemanse, N.J., Kazem, A.J., Réale, D., Wright, J., 2010. Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol. Evol. (Amst.)* 25, 81–89.
- Fisher, R.J., Poulin, R.G., Todd, L.D., Brigham, R.M., 2004. Nest stage, wind speed, and air temperature affect the nest defence behaviours of burrowing owls. *Can. J. Zool.* 82, 707–713.
- Frid, A., Dill, L.M., 2002. Human-caused disturbance stimuli as a form of predation risk. *Avian Conserv. Ecol.* 6, 11.
- Galeotti, P., Tavecchia, G., Bonetti, A., 2000. Parental defence in Long-eared Owls *Asio otus*: effects of breeding stage, parent sex and human persecution. *J. Avian Biol.* 31, 431–440.
- González, L.M., Arroyo, B.E., Margalida, A., Sánchez, R., Oria, J., 2006. Effect of human activities on the behaviour of breeding Spanish imperial eagles (*Aquila adalberti*): management implications for the conservation of a threatened species. *Anim. Conserv.* 9, 85–93.
- Griffin, A.S., Netto, K., Peneaux, C., 2017. Neophilia, innovation and learning in an urbanized world: a critical evaluation of mixed findings. *Behav. Sci.* 16, 15–22.
- Hardman, S.I., Dalesman, S., 2018. Repeatability and degree of territorial aggression differs among urban and rural great tits (*Parus major*). *Sci. Rep-UK* 8, 5042.
- Isach, J.P., Bó, M.S., Vega, L.E., Favero, M., Baladrón, A.V., Pretelli, M.G., Stellatelli, O., Cardoni, D.A., Copello, S., Block, C., Cavalli, M., Comparatore, V.M., Mariano-Jelicich, R., Biondi, L.M., García, G., Seco Pon, J., 2016. Diversidad de Tetrápodos en un mosaico de ambientes del sudeste de la ecorregión Pampeana como herramienta para planificar en conservación. *Rev. Mus. Argent. Cienc. Nat.* 18, 211–233.
- Isaksson, C., Rodewald, A.D., Gil, D., 2018. Behavioural and ecological consequences of urban life in birds. *FEVO* 6, 50.
- Lefebvre, L., Reader, S.M., Sol, D., 2004. Brains, innovations and evolution in birds and primates. *Brain Behav. Evol.* 63, 233–246.
- Lefebvre, L., Whittle, P., Lascaris, E., Finkelstein, A., 1997. Feeding innovations and forebrain size in birds. *Anim. Behav.* 53, 549–560.
- Li, C., Yang, X., Ding, Y., Zhang, L., Fang, H., Tang, S., Jiang, Z., 2011. Do Pere David's deer lose memories of their ancestral predators? *PLoS One* 6, e23623.
- Lima, S.L., Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68, 619–640.
- Lima, S.L., 2009. Predators and the breeding bird: behavioral and reproductive flexibility under the risk of predation. *Biol. Rev.* 84, 485–513.
- Lowry, H., Lill, A., Wong, B.B., 2013. Behavioural responses of wildlife to urban environments. *Biol. Rev.* 88, 537–549.
- Marks, J.S., Canning, R.J., Mikkola, H., 1994. Family strigidae (typical owls). In: In: del Hoyo, J., Elliot, A., Sargatal, J. (Eds.), *Handbook of the Birds of the World*. Vol 5. Barn-owls to Hummingbirds, Lynx Edicions, Barcelona, pp. 76–242.
- Martínez, G., Baladrón, A.V., Cavalli, M., Bó, M.S., Isach, J.P., 2017. Microscale nest-site selection by the burrowing owl in the pampas of Argentina. *Wilson J. Ornithol.* 129, 62–70.
- Mathot, K.J., Dingemanse, N.J., 2015. Plasticity and personality. In: Martin, L.B., Ghalambor, C.K., Woods, H.A. (Eds.), *Integrative Organismal Biology*. Wiley, New Jersey, pp. 55–69.
- Mikula, P., 2014. Pedestrian density influences flight distances of urban birds. *Ardea* 102, 53–60.
- Møller, A.P., 2008. Flight distance of urban birds, predation and selection for urban life. *Behav. Ecol. Sociobiol. (Print)* 63, 63–75.
- Møller, A.P., 2010. Interspecific variation in fear responses predicts urbanization in birds. *Behav. Ecol.* 21, 365–371.
- Moroni, E., Crivellaro, A.Z., Soares, T.L., Guillermo Ferreira, R., 2017. Increased behavioural responses to human disturbance in breeding Burrowing Owls *Athene cunicularia*. *Ibis* 159, 854–859.
- Oosten, J.E., Magnhagen, C., Hemelrijk, C.K., 2010. Boldness by habituation and social interactions: a model. *Behav. Ecol. Sociobiol. (Print)* 64, 793–802.
- Partecke, J., Gwinner, E., Bensch, S., 2006. Is urbanisation of European blackbirds (*Turdus merula*) associated with genetic differentiation? *J. Ornithol.* 147, 549–552.
- Pellitteri-Rosa, D., Bellati, A., Cocca, W., Gazzola, A., Martín, J., Fasola, M., 2017. Urbanization affects refuge use and habituation to predators in a polymorphic lizard. *Anim. Behav.* 123, 359–367.
- Pinheiro, J.C., Bates, D.M., 2000. *Mixed-Effects Models in S and S-PLUS*. Springer, New York.
- Poulin, R., Todd, L.D., Haug, E.A., Millsap, B.A., Martell, M.S., 2011. Burrowing owl (*Athene cunicularia*). In: Poole, A. (Ed.), *The Birds of North America Online*. Cornell Lab of Ornithology, New York.
- R Development Core Team, 2018. *R: a Language and Environment for Statistical Computing*. R foundation for statistical computing, Vienna, Austria. <http://www.R-project.org>.
- Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., Mc Sweeney, F.K., Wilson, D.A., Wu, C.F., Thompson, R.F., 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiol. Learn. Mem.* 92, 135–138.
- Reader, S.M., Laland, K.N., 2001. Primate innovation: sex, age and social rank differences. *Int. J. Primatol.* 22, 787–805.
- Rebollo-Ifrán, N., Tella, J.L., Carrete, M., 2017. Urban conservation hotspots: predation release allows the grassland-specialist burrowing owl to perform better in the city. *Sci. Rep. UK* 7.
- Rodríguez-Prieto, I., Fernández-Juricic, E., Martín, J., Regis, Y., 2009. Antipredator behavior in blackbirds: habituation complements risk allocation. *Behav. Ecol.* 20, 371–377.
- Samia, D.S., Blumstein, D.T., Díaz, M., Grim, T., Ibáñez-Álamo, J.D., Jokimäki, J., Tätté, K., Markó, G., Möller, A.P., 2017. Rural-urban differences in escape behavior of European birds across a latitudinal gradient. *FEVO* 5, 66.
- Samia, D.S., Nakagawa, S., Nomura, F., Rangel, T.F., Blumstein, D.T., 2015. Increased tolerance to humans among disturbed wildlife. *Nat. Commun.* 6, 8877.
- Scales, J., Hyman, J., Hughes, M., 2011. Behavioral syndromes break down in urban song sparrow populations. *Ethology* 117, 887–895.
- Sih, A., Ferrari, M.C., Harris, D.J., 2010. Evolution and behavioural responses to human-induced rapid environmental change. *Evol. Appl.* 4, 367–387.
- Sol, D., Timmermans, S., Lefebvre, L., 2002. Behavioural flexibility and invasion success in birds. *Anim. Behav.* 63, 495–502.
- Sol, D., Duncan, R.P., Blackburn, T.M., Cassey, P., Lefebvre, L., 2005. Big brains, enhanced cognition, and response of birds to novel environments. *Proc. Natl. Acad. Sci. U.S.A.* 102 5460e5465.
- Sol, D., Lapedra, O., González-Lagos, C., 2013. Behavioural adjustments for a life in the city. *Anim. Behav.* 85, 1101–1112.
- Sol, D., Maspons, J., Gonzalez-Voyer, A., Morales-Castilla, I., Garamszegi, L.Z., Möller, A.P., 2018. Risk-taking behavior, urbanization and the pace of life in birds. *Behav. Ecol. Sociobiol.* 72, 59.
- Sprau, P., Dingemanse, N.J., 2017. An approach to distinguish between plasticity and non-random distributions of behavioral types along urban gradients in a wild passerine bird. *Front. Ecol. Evol.* 5, 92.
- Sproat, T.M., Ritchison, G., 1993. The nest defense behavior of eastern screech-owls: effects of nest stage, sex, nest type and predator location. *Condor* 95, 288–296.
- Tätté, K., Möller, A.P., Mänd, R., 2018. Towards an integrated view of escape decisions in birds: relation between flight initiation distance and distance fled. *Anim. Behav.* 136, 75–86.
- Tuomainen, U., Candolin, U., 2011. Behavioural responses to human induced environmental change. *Biol. Rev.* 86, 640–657.
- Vincze, E., Papp, S., Preiszner, B., Seress, G., Bókony, V., Líker, A., 2016. Habituation to human disturbance is faster in urban than rural house sparrows. *Behav. Ecol.* 27, 1304–1313.
- Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. *Mixed Effects Models and Extensions in Ecology With R*. Springer Science and Business Media, New York.