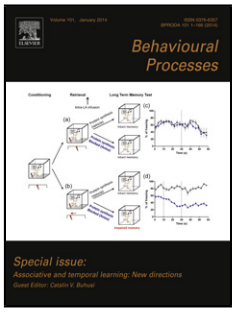

Title: Predator avoidance training of the endangered lizard
from El Hierro (Canary Islands): A new management strategy
before reintroduction into the wild

Authors: Gisela Burunat-Pérez, Mercedes Suárez-Rancel,
Miguel Molina-Borja

PII: S0376-6357(18)30297-3
DOI: https://doi.org/10.1016/j.beproc.2018.09.010
Reference: BEPROC 3739

To appear in: *Behavioural Processes*

Received date: 13-7-2018
Revised date: 17-9-2018
Accepted date: 25-9-2018

Please cite this article as: Burunat-Pérez G, Suárez-Rancel M, Molina-Borja M, Predator avoidance training of the endangered lizard from El Hierro (Canary Islands): A new management strategy before reintroduction into the wild, *Behavioural Processes* (2018), https://doi.org/10.1016/j.beproc.2018.09.010

Predator avoidance training of the endangered lizard from El Hierro (Canary Islands): a new management strategy before reintroduction into the wild

Short running title: Antipredator training in endangered lizards

Gisela Burunat-Pérez [a], Mercedes Suárez-Rancel[b], Miguel Molina-Borja [c, 1*]

[a] Centro para la Reproducción e Investigación del lagarto gigante de El Hierro, Frontera, El Hierro, Canary Islands, Spain.

[b] Depto. de Matemáticas, Estadística e Investigación Operativa, Facultad Ciencias, Univ. La Laguna, Tenerife.

[c] Grupo de investigación "Etología y Ecología del Comportamiento", Depto. Biología Animal, Facultad Ciencias, Sección Biología, Univ. La Laguna, Tenerife, Canary Islands, Spain.

Gisela Burunat-Pérez: e-mail: gburunat@gmail.com
Mercedes Suárez-Rancel, e-mail: msuarez@ull.edu.es

[*]**Corresponding author:** Miguel Molina-Borja
Depto. Biología Animal, Facultad Ciencias, Sección Biología,
Univ. La Laguna, Tenerife, Canary Islands, Spain.
e-mail: mmolina@ull.edu.es

Highlights

- Highlights:
- Endangered lizards trained for enhancing antipredator capacities
- Individuals reacted both to a kestrel and cat stuffed models
- Main changes after training were reduction in Basking and Locomotion activities
- Differences responses were found to the kestrel and to the cat models
- Antipredator training recommended for individuals to be released into the wild

Abstract

Animals raised in captivity during several generations may not express appropriate antipredator behaviour when reintroduced into the wild. Here we present the results of experiments to enhance behavioural responses to predators in adult males of the endangered lizard Gallotia simonyi (El Hierro, Canary Islands). Individuals were subjected to a training procedure (control, pre-training, training and post-training phases) using stuffed specimens of a kestrel and a cat as predators. We filmed all trials and compared relative durations of the more common behaviour patterns shown by lizards, both among experimental phases and before and after presentation of the stuffed predator. Locomotion and Basking were significantly reduced in the training and post-training trials and also after stimulus presentation, suggesting that the training protocol induced lizard avoidance over both predator models. To our knowledge, this is the first time lizards have been trained to show antipredator avoidance and our results provide the basis for a new management strategy that could be useful for reintroduction of captive-bred individuals of endangered species.

Keywords: antipredator training, endangered lizard, Gallotia simonyi, reintroduction, El Hierro,

1. Introduction

Many animal species are in danger of extinction due to human-induced habitat alteration or destruction (IUCN, 2016). Therefore, many recovery programs are carried out to assist declining populations (Morris Gosling & Sutherland, 2000). Some of these recovery programs include breeding of individuals from the endangered populations in controlled situations in laboratory or outdoor enclosures. For some endangered populations, individuals are bred in captivity during several generations before a number of them are released into the wild. There is evidence that, after a few generations in captivity, animals may lose certain behavioural capacities that are fundamental for survival (Wallace, 2000; Brokordt et al., 2006). The effect of experience on the development of antipredator behaviour, for example, can have practical importance when captive-bred individuals participate in reintroduction programs (Kleiman, 1989). Therefore, it is important to implement management actions to help those individuals to be released to acquire or enhance behavioural capacities that will increase their chances of surviving once in the wild (see Morris Gossling & Sutherland, 2000, for a review). This is particularly important when introduced predators have been the main reason of the previous decline as it is the case here.

Especially important for reintroduced individuals is the ability to enhance their foraging and their anti-predator behaviour. For example, increasing the opportunities for locomotion on natural substrates pre-release improves survival in young lion tamarins released into the wild (Stoinski & Beck, 2004). Providing individuals with predator training has also become a fundamental part of many recovery programs for endangered species (Griffin et al., 2000; Griffin, 2004) as for example in black-footed ferrets (Miller et al., 1994), bustards (van Heezik et al., 1999), fish (Mirza & Chivers, 2000; Brown & Laland, 2001; Kelley & Magurran, 2003), and prairie dogs (Shier & Owings, 2007).

Recognising predators and reacting to them with appropriate behaviour is crucial as an antipredator strategy in lizards (Greene, 1988). For example, hiding from an approaching predator or running and finding a suitable refuge may mean the difference between surviving and succumbing to a predatory attack (Cooper & Blumstein, 2015). Animals kept in captivity are rarely exposed to their natural predators, even when kept in outdoor enclosures. If those animals are candidates for reintroduction into the natural environment, their anti-predator behaviour may be affected. In fact, a common finding in several species is that individuals kept in captivity for several generations may lose the ability to recognise and react to predators (Caro & Sherman, 2012). The loss of predator avoidance and other behaviour patterns over generations in captivity might be due to relaxed selection, phenotypic plasticity and/or domestication (Price, 1999;

Relyea, 2002). Several captive breeding centres have developed methodologies to expose endangered species to models of predators to appropriately shape their anti-predator behaviours (van Heezik et al., 1999; Jule et al., 2008). These procedures imply use of classical conditioning (Griffin et al. 2000) where, after several paired presentations, presentation of a conditioned stimulus (CS) elicits the same response as an unconditioned stimulus (UCS) (Griffin, 2003).

Gallotia simonyi, Steindachner 1889, is an extremely endangered (IUCN, 2016) lacertid species, endemic to El Hierro Island, the smallest and westernmost of the Canary Islands. A small wild population lives in a north-western high inland cliff (Pérez-Mellado et al., 1997) and the reasons for the declining population in the past have been, namely, introduced predators, competitors and harvesting (Mateo & López-Jurado, 1999); However, most lizards currently alive have been born and raised in captivity (Rodríguez-Domínguez & Molina-Borja, 1998). Groups of captive bred lizards have been reintroduced at several localities in El Hierro Island over the last few years (Consejería Medio Ambiente, Canarian Government, 2004). However, the available evidence suggests that reintroduction of G. simonyi in two localities of El Hierro has not been successful (Trujillo, 2008). This may be due to several reasons, but mortality due to predation is likely an important factor. Common kestrels –Falco tinnunculus– that are native predators, and feral cats –Felis silvestris catus–, introduced in the past, are considered the main predators of G. simonyi (Nogales et al., 2004; Medina & Nogales, 2008; Bonnaud et al., 2010). Survival of reintroduced G. simonyi will depend crucially on how the lizards behave when confronted with those predators; kestrels are common throughout the island and cats are present in some localities where lizards have been reintroduced.

Here, we test whether training young G. simonyi lizards raised in semi-captive conditions with model predators (a stuffed kestrel and a stuffed cat) affects their anti-predator behaviour. If trained animals were better able to survive, those individual lizards showing the most effective anti-predator behaviour in captivity could be selected for reintroduction. Our prediction was that lizards trained to respond to predator models would devote less time to behaviours outside shelters and/or higher frequencies of alarm and/or flight behaviours when confronted with a predator. If lizards effectively change their behaviour, the procedure would be recommended as a new management strategy in the breeding centre.

2. Methods

2.1. Individuals and maintenance

We used 20 adult males of El Hierro giant lizard (G. simonyi) raised in the Centre for the Reproduction and Research in Frontera (North West of El Hierro Island) as part of the recovery program for this species. A few adult females were available at the time of the study, but they had recently been used for reproduction what induces poor body condition that could have modified their behavioural priorities; therefore, we decided not to use females as experimental individuals to avoid the potential influence of sex and recent reproductive activities. The males were born between 2006 and 2008 in a former breeding centre, and then transferred to the new breeding and maintenance facilities in El Palmeral (Frontera). They were candidates for release into the wild and were marked with a microchip inserted under the neck skin. Prior to the experiments described here their snout-vent length (SVL) and body mass (BM) were measured using standard procedures (Table 1).

Lizards were held together in a large outdoor terrarium (7 x 5 m; surrounded by a concrete wall 1 m high) located inside the new breeding centre. The terrarium had a wire mesh covering its entire surface. The breeding centre did not allow us to hold and train each individual lizard separately as it would have been appropriate, and therefore we were forced to conduct experiments on group-housed lizards. The terrarium had a mix of soil and lapilli as substrate, natural plants (mainly “verode”, Kleinia neriifolia; “calcosa”, Rumex lunaria and “tedera” Psoralea bituminosa) and at least a tile as shelter per lizard; one end of the tiles was in contact with the walls, and palm leaves were placed on top of them to avoid excessive heat inside during summer months. Hollow logs were also available inside the terrarium. Temperature at sunny sites and below a tile was recorded with a digital thermometer (Alecto 100-WS, 0.1 ºC precision). Air temperature (at ground level) did not vary between different trials of each experimental phase and predator model, but slightly varied among the different phases (Table 2); temperature under the tiles was on average 8.7ºC lower than in a sunny patch. However, there was no significant correlation between environmental temperature and behaviour pattern durations recorded in the experimental trials (Spearman rho, $p > 0.05$ in all cases, $n = 64$).

Lizards were fed three times weekly with natural food including leaves and fruits of local plants (mainly leaves from “tedera”, Bituminaria bituminosa and “verode”, Kleinia neriifolia; these species are part of the natural vegetation in lizard reintroduction areas). To minimise contact with humans, staff personnel supplied food from outside the terrarium before the lizards emerged from their night shelters. To encourage the lizards to search for food, the plant leaves were cut into pieces and dispersed in small clumps around the central part of the terrarium. As wild lizards do not usually have available water in the field (own observations in other Gallotia species), water was not permanently provided inside the terrarium; however, vegetables and fruits were sprayed with water before being introduced to the terrarium and in hot days a small pot with water was supplied. During their stay in captivity and during the experiments the animals were cared for in accordance with guidelines published by Animal Behaviour (ASAB/ABS 2012; Anim. Behav. 83:301– 309).

2.2 Experimental procedure

Experiments were conducted during July-August 2013. Two weeks before the trials, each individual was marked with non-toxic acrylic paint to facilitate recognition from outside the terrarium. Observations were made from behind a cloth barrier with small holes outside of one of the terrarium walls. Experimental trials were conducted when at least six out of the 20 lizards were active –i.e. outside the tiles (see below).

We used the method of classical conditioning (reviewed in Griffin et al., 2000) with stuffed models of a kestrel (in flight position) and a domestic cat (walking position) as conditioned stimuli (CSs) and a noise as unconditioned stimulus (UCS, see below). Live natural predators could not be used and though using stuffed predators has some disadvantages (Griffin et al., 2000), these models allowed us to standardise stimulus presentation across trial sessions. Trials were performed firstly for the kestrel and secondly for the cat model. The stuffed kestrel was flown above the terrarium tied to a nylon string pulled with an electric device coupled to a pulley system, allowing the kestrel to be moved downwards, from one corner over to the diagonally opposed corner and beyond the terrarium wall. The kestrel was 2.6 m above the ground at the beginning of the run and 1.3 m when it arrived to the distal corner. The distance travelled was 9 m and each run lasted 10 s (Fig. 1). All trials were filmed with two GoPro video cameras suspended above the terrarium for detailed analysis of lizard behaviours; each video camera recorded the lizards in one half of the terrarium.

Training proceeded in four consecutive phases in the following order: 1) Control: only the nylon string was moved without the stuffed kestrel; this allowed us to assess the potential effect of unwanted noises (or other stimuli) made by the researcher while manipulating the devices used to move the predator models; 2) Pre-training (CS only): only the kestrel model was flown over the terrarium; 3) Training (US-CS): the kestrel model was flown again and immediately after this the researcher/observer (GB) produced an aversive stimulus (hitting a log twice against the ground outside the terrarium); 4) Post-training (CS only): the kestrel model was flown again but this time without the aversive sound stimulus. Trials were usually conducted between Monday and Friday along a 20 day period throughout July and August 2013 in 2-3 morning trials (between 10:00 and 13:00 h). However, trials were not performed in cloudy or very hot days; the number of trials for each experimental phase is shown in Table 3. Inter-trial interval was 15-30 min. Trials from different experimental phases were always conducted on different days. As not all lizards were visible in a single trial, and to record behaviour from the largest number of individuals, we repeated the trials in successive days (see Table 3) but never later than 9 days after the first trial (we could not perform trials in some cloudy days). For analyses we considered only data from the first trial in which a given lizard could be recorded (it was visible).

Three days after all trials for the kestrel experiment were completed, we performed a similar experiment using a stuffed cat as predator (Table 2). For this, the stuffed cat was attached to a wooden platform on wheels and pulled by a researcher (out of sight of the lizards) from outside the terrarium (Fig. 2). These trials were performed along 11 days, during August 2013.

2.3. Data analyses

From the recorded videos, and for each experimental phase, we quantified the behavioural activities of the lizards during stimulus presentation and during the five minutes before and after each stimulus presentation. Observations of the anti-predator behaviour in the wild have shown that, after a simulated predatory attack, lizards stay in their shelters for a variable time before re-emerging and resuming their routine activities (Cooper & Blumstein, 2015). Therefore, we considered that five minutes was long enough to allow lizards to resume activity outside their shelters. For the classification of the behavioural patterns, we used the ethogram previously published for Gallotia galloti (Molina-Borja, 1981). Using focal animal sampling (Altman, 1974) and continuous recording we quantified for every visible lizard in the video the duration they spent in every state-type behaviour (Basking, Locomotion, Eating, Flight) during the five minutes before and after presentation of the stimulus. To control for the different time each lizard was on sight, we calculated the duration of each behaviour category relative to the total time each individual was on sight during each five minute period. During stimulus presentation, we noted only the behaviour shown by each lizard at that moment (scan sampling). In addition to the state-type behaviours listed above, we also recorded the occurrence of Alarm (event-type behaviour). In a few cases, we recorded two behaviour patterns occurring simultaneously during stimulus presentation, for example Alarm and Flight.

After viewing all videos, we noted eight lizards in the kestrel experiment and 7 in the cat experiment were recorded in all protocol phases, and both before, during and after stimulus presentation. Therefore, for statistical analyses we used data only from these individuals. We analysed data for Locomotion, Basking and Eating categories, as all other behaviours occurred at very low frequencies. Data were scored blind with regard to treatment by a single observer (GBP) (Burghardt et al. 2012).

As data did not fulfil parametric requirements (normality and homoscedasticity), we compared relative durations of behaviour patterns before and after the stimulus presentation with a Permutation Analysis of Variance (Permanova, within Primer v6 statistical package, Anderson 2001). As fixed factors we used the experimental phases and recording periods (before and after the stimulus; hereafter b-a). We also performed post-hoc analyses to compare relative durations of behaviour categories among the four phases.

To establish if there was any significant association between the experimental phase (1 to 4) and numbers of lizards exhibiting each behaviour pattern during stimulus presentation, we used a G-test of independence (Sokal & Rohlf, 1995). For all tests, we used an alpha level of 0.05.

3. Results

3.1. Experiment with kestrel as model predator

Permanova analysis of repeated measurements applied to relative durations of the most frequent behaviour patterns (Locomotion, Basking and Eating) showed a significant effect of the experimental phase (pseudo-F = 2.5748; df = 3; p = 0.032), recording period (b-a) (pseudo-F = 25.283; df = 1; p = 0.001), and a significant interaction of phase x b-a (pseudo-F = 5.2619; df = 3; p = 0.001). Univariate analyses for each behaviour pattern showed there was a significant effect of phase, b-a, and their interaction on relative duration of Basking, but not on the other two behaviour patterns (Table 4, Fig. 3). Post-hoc comparison showed that Locomotion lasted significantly less during training than in control period (t = 2.386, p = 0.02); basking was reduced in pre-training in comparison with control period (marginal significance, t = 2.125, p = 0.058), while Eating did not significantly change between any pair of periods (p > 0.05 in all cases).

During the presentation of the stimulus, a larger number of lizards showed Flight significantly more often than the other behaviour patterns in the pre-training, training and post-training phases (G-test of independence, G = 57.78, df = 15, p = 0.005; Fig. 5 a).

3.2. Experiment with cat as model predator

There was a significant effect of experimental phase (pseudo-F = 2.923; df = 3; p = 0.018), b-a (pseudo-F = 10.938; df = 1; p = 0.0005), and a significant interaction of phase x b-a (pseudo-F = 2.716; df = 3; p = 0.027). Univariate analyses for each behaviour pattern showed there was a significant effect of phase on relative duration of Locomotion, and of both factors and their interaction on the relative duration of

Basking; there was no significant effect of b-a or its interaction with phase on Locomotion, nor of any of the two factors or their interaction on Eating (Table 4, Fig. 4). Post-hoc comparisons showed that Locomotion lasted significantly less in the pre-training and training periods in comparison with control period (t = 2.053, p = 0.01 and t = 2.182, p = 0.003, respectively) while increased again in post-training period (t = 2.063, p = 0.04). Basking was significantly reduced in the pretraining and training periods in comparison with control period (t = 2.067, p = 0.05 and t = 2.360, p = 0.03, respectively). We did not find any significant difference for Eating (p > 0.05 in all cases).

During the presentation of the stimulus, a larger number of lizards showed Alarm and Flight significantly more often than the other behaviour patterns in pre-training, training and post-training phases (G-test of independence, G = 65.91, df = 15, p = 0.0007; Fig. 5 b).

4. Discussion

Our results show that training trials with both predator models reduced the duration of activities outside the shelters (e.g. Basking) after stimulus presentation and increased the frequency of Alarm and Flight behaviours during stimulus presentation in the pre-training, training or post-training phases in comparison with the control and pre-training phases. As a similar reduction in the time spent in Basking (and Locomotion in cat trials) did not take place before stimulus presentation, we contend the behavioural changes observed are due to the association between the fake predator (CS) and the aversive stimulus (US). Nevertheless, the effects of each predator were different. When using the kestrel, the effects were evident in all phases except in Control, mainly as a significant reduction in Locomotion during training than in control phase but also a decrease in Basking. The appearance of the passing kestrel made the lizards search for a refuge and reduced their activity outside the shelter in the ensuing five minutes. However, in the case of the cat model, Locomotion was significantly reduced in the training and post-training phases and Basking during training in comparison with control phase. Moreover, there were also significant effects during the presentation of the predators, which were manifested by increased frequencies of Alarm and/or Flight behaviours in the pre-training, training and post-training stages. These results support our initial prediction that these behaviours should increase and that lizards should reduce their activities outside shelters. Pseudoreplication is still under debate (Schank & Koehnle, 2009) and has been considered a source of error in antipredator training experiments (Johnston & Freeberg, 2016); in our case, we did not have access to different stuffed kestrels or cats to avoid a potential effect of using the same model predator.

That the lizards responded to the stuffed kestrel already during the pre-training phase suggests innate recognition of at least some avian predators. Our findings with the stuffed cat are no so clear-cut. These results make sense considering that lizards have been evolving with kestrels for a very long time in the Canary Islands (less than one million years in El Hierro), while only for around 500 years with cats (Medina & Nogales, 2009). Alternatively, it is possible that the different responses to the two predators could be due to the way they were presented; in the case of kestrel, it was moving from above, coming down and crossing diagonally the terrarium and therefore could be perceived by the lizards as highly threatening. In experiments with Jacky dragon (Amphibolurus muricatus), lizards were more responsive to a realistic raptor silhouette than to other stimulus shapes (Carlile et al., 2006). The cat model instead was moved along one of the terrarium walls and was not heading directly to the lizards. Different behaviours of an approaching predator have been shown to affect lizard behaviour in other species (Burger & Gochfeld, 1990; Cooper & Sherbrooke, 2016). As cat trials were conducted after those with the kestrel, the order of stimulus presentation could also have had an influence on the results with stuffed cat.

In the case of the stuffed kestrel, behavioural responses of lizards probably involved fine-tuning of an innate anti-predator behaviour –probably to aerial predators (Carlile et al., 2006)- rather than acquisition of a novel fear response. In the classical conditioning method, the CS presented alone elicits the same response as the UCS after several paired presentations. As lizards hid on first seeing the kestrel (previous to the presentation of the noise –UCS-), this suggests they were initially exhibiting an unlearned (innate?) fear response to the predator; nevertheless, their response could be subsequently reinforced as shown by further reduction of durations of behavioural activities outside shelters in training or post-training stages. However, in cat trials the frequency of Alarm decreased while of Flight increased after stimulus presentation.

Therefore, it seems the cat model induced a more intense response during the post-training stage. We do know that individual differences are common in squamate reptiles of many species and individual differences in behaviour may influence the result of any experimental study as non-human animals, including some reptiles, have several “personalities” (Mark Waters et al., 2017). In our study, some experimental lizards did not show any flight reaction to predator stimuli even when other lizards did. Although social groups of Gallotia simonyi do not occur in the wild (MMB, personal observations), the possibility of social facilitation, shown in hatchling iguanas (Burghardt et al. 1977) or social learning (Wilkinson et al., 2010) cannot be ruled out. As unavoidable logistic restrictions/constraints posed by the breeding centre limited the capacity to ameliorate our experimental design, future improvements should consider the training of single individuals or small groups of lizards in their facilities.

Learning effects caused by using predator models have been shown in several vertebrates (Miller et al., 1990; McLean et al., 1996; Griffin et al., 2001; Gaudioso et al., 2011). For example, common rheas (Rhea americana) increased their vigilance behaviour following training with a stuffed predator or even with a real one (dog) (De Azevedo & Young, 2006).

To our knowledge, there are no previous reports of antipredator training in any lizard species. However, the available information suggests that rapid detection and flight in the face of stimuli associated to a potential predator increase survival probability and fitness in several lizard species (Schwarzkopf & Shine, 1992; Cooper, 2003; Plasman et al., 2007). As loss of adequate antipredator capacities may occur as a consequence of keeping individuals in captivity for several generations (Wallace, 2000; Caro & Sherman, 2012), training individuals to develop or increase their antipredator skills may be a crucial step before releasing them into the wild (IUCN, 1998; Griffin et al., 2000). Though it is difficult to simulate a realistic encounter with a predator by using a model, we have shown that lizards bred in captivity during several generations increase their antipredator behaviour after training with a moving predator model. Thus, antipredator training should reduce the effect of local natural predators; however, demonstrating that antipredator training has had an effect on the survival probability of reintroduced individuals requires a post-release control (Van Heezik et al., 1999; Jule et al., 2008; Gaudioso et al., 2011). Logistic limitations have not allowed us until now to obtain data on the post-release survival of trained G. simonyi lizards. However, to establish experimentally that there is an effective differential survival of trained lizards would imply releasing both trained and un-trained individuals; if, as it is well established in the literature, individuals with antipredator skills increase their survival probabilities, to release un-trained lizards would be a way to increase the number of lost individuals after reintroduction. Therefore, we have instructed the staff of the breeding centre to implement the antipredator training as a new management strategy for all lizards to be reintroduced.

Declarations of interest

None

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

We thank Enrique Font for the review of an earlier version of the manuscript, including the English text edition; Daniel Jesús García Cedrés for building the electronic device to move the stuffed kestrel and Gonzalo Albaladejo for preparing the stuffed cat. The Area de Medio Ambiente of Cabildo Insular de El Hierro (local island institution) gave permission to perform the experiments in the new facilities of the Breeding Centre at Frontera. Thus, we were coordinated with the official agency responsible for the conservation effort for this particular species. We are grateful for the technical collaboration of Dr. Miguel A. Rodríguez-Domínguez, and also Juan P. Pérez and Alfonso Quintero that helped with routine lizard maintenance.

References

Altmann, J., 1974. Observational Study of Behavior: Sampling Methods. Behaviour 49, 227–267.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Bonnaud, E., Medina, F.M., Nogales, M., Tershy, B., Zavaleta, E., Donlan, C.J., Keitt, B., Le Corre, M., Horwath, S.V., 2010. The diet of feral cats on islands: a review and a call for more studies. Biol. Invasions 13, 581–603.

Brokordt, K.B., Fernández, M., Gaymer, C.F., 2006. Domestication reduces the capacity to escape from predators. J. Exp. Mar. Biol. Ecol. 329, 11–19.

Brown, C., Laland, K.N., 2001. Social learning and life skills training for hatchery reared fish. J Fish Biol. 59, 471–493.

Burger, J., Gochfeld, M., 1990. Risk discrimination of direct versus tangential approach by basking black iguanas (Ctenosaura similis) -variation as a function of human exposure. J. Comp. Psychol. 104, 388–394.

Burghardt, G. M., Bartmess-LeVasseur, J.N., Browning, S.A., Morrison, K.E., Stec, C.L., Zachau, C.E., Freeberg, T.M., 2012. Perspectives -minimizing observer bias in behavioral studies: a review and recommendations. Ethology 118, 511–517.

Burghardt, G.M., Greene, H.W., R.S., 1977. Social behavior in hatchling green iguanas: life at a reptile rookery. Science 195, 689–691.

Carlile, P.A., Peters, R.A., Evans, C.S., 2006. Detection of a looming stimulus by the Jacky dragon: selective sensitivity to characteristics of an aerial predator. Anim. Behav. 22, 553–562.

Caro, T., Sherman, P.W., 2012. Vanishing behaviors. Conserv. Lett. 5, 159–166.

Cooper, W. E., Jr., 2003. Risk factors affecting escape behaviour by the desert iguana, Dipsosaurus dorsalis: speed and directness of predator approach, degree of cover, direction of turning by a predator, and temperature. Can. J. Zool. 81, 979–984.

Cooper, W.E., Jr., Blumstein, D.T., 2015. Escaping from predators: an integrative view of escape decisions. Cambridge University Press, New York.

Cooper, W.E., Jr., Sherbrooke, W.C., 2016. Strategic escape direction: orientation, turning, and escape trajectories of zebra-tailed lizards (Callisaurus draconoides). Ethology 122, 542–551. doi:10.1111/eth.12501

De Azevedo, C.S., Young, R.J., 2006. Behavioural responses of captive-born greater rheas Rhea americana Linnaeus (Rheiformes, Rheidae) submitted to antipredator training. Rev. Bras. Zool. 23, 186–193.

Gaudioso, V.R., Sánchez-García, C., Pérez, J.A., Rodríguez, P.L., Amenteros, J.A., Alonso, M.E., 2011. Does early antipredator training increase the suitability of captive red-legged partridges (Alectoris rufa) for releasing? Poult. Sci. 90, 1900–1908.

Greene, H.W., 1988. Antipredator mechanisms in reptiles, in: Gans, C., Huey, R.B. (Eds.), Biology of the Reptilia, Vol. 16. Alan R. Liss, New York, pp. 1-152.

Griffin, A.S., 2003. Training tammar wallabies (Macropus eugenii) to respond to predators: a review linking experimental psychology to conservation. J. Comp. Psychol. 16, 111–129.

Griffin, A.S., 2004. Social learning about predators: a review and prospectus. Learn. Behav. 32, 131–140. doi:10.3758/BF03193188

Griffin, A.S., Blumstein, D.T., Evans, C.S., 2000. Training captive-bred or translocated animals to avoid predators. Conserv. Biol. 14, 1317–1326. doi:10.1046/j.1523-1739.2000.99326.x

Griffin, A.S., Evans, C.S., Blumstein, D.T., 2001. Learning specificity in acquired predator recognition. Anim. Behav. 62, 577–589. doi:10.1006/anbe.2001.1781

IUCN, 1998. Guidelines for Re-introductions, prepared by the IUCN/SSC re-introduction specialist group. IUCN, Gland, Switzerland and Cambridge.

IUCN, Red List of Threatened Species. Version 2016-3.

Johnson, I.T., Freeberg, T.M., 2016. Pseudoreplication in use of predator stimuli in experiments on antipredator responses. Anim. Behav. 119, 161–164. doi:10.1016/j.anbehav.2016.07.006

Jule, K.R., Leaver, L.A., Lea, S.E.G., 2008. The effects of captive experience on reintroduction survival in carnivores: A review and analysis. Biol. Conserv. 141, 355–363. doi:10.1016/j.biocon.2007.11.007

Kelley, J. L., Magurran, A.E., 2003. Learned predator recognition and antipredator responses in fishes. Fish Fish. 4, 216–226.

Kleiman, D.G., 1989. Reintroduction of captive mammals for conservation: guidelines for reintroducing endangered species into the wild. Bioscience 39, 152–161.

Mark Waters, R., Bowers, B.B., Burghardt, G.M., 2017. Personality and individuality in reptile behaviour, in: Vonk J., Weiss, A.R., Kuczaj, S.A. (Eds.), Personality in Nonhuman Animals. Springer, Switzerland, pp. 300–314.

Mateo, J.A. & López-Jurado, L.F. 1999. El Lagarto Gigante de El Hierro. Bases para su conservación. Monografías de Herpetología. Asociación Herpetológica Española. Las Palmas de Gran Canaria.

McLean, I. G., Lundie-Jenkins, G., Jarman, P.J., 1996. Teaching and endangered mammal to recognise predators. Biol. Conserv. 75, 51–62.

Medina, F.M., Nogales, M., 2008. A review on the impacts of feral cats (Felis silvestris catus) in the Canary Islands: implications for the conservation of its endangered fauna. Biodivers. Conserv. 18, 829–846. doi:10.1007/s10531-008-9503-4

Miller, B., Biggins, D., Wemmer, C., Powell, R., Calvo, L., Hanebury, L. & Wharton, T., 1990. Development of survival skills in captive raised Siberian polecats (Mustela eversmanni) II. Predator avoidance. J. Ethol. 8, 95-104.

Miller, B., Biggins, D., Honebury, L. & Vargas, A., 1994. Reintroduction of the black-footed ferret (Mustela nigripes), in: Olney, P.J.S., Mace, G.M., Feistner, A.T.C. (Eds.), Creative Conservation. Chapman Hall, London, pp. 455-464.

Mirza, R.S., Chivers, D.P., 2000. Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. Can. J. Zool. 78, 2198–2208. doi:10.1139/z00-164

Molina-Borja, M., 1981. Etograma del lagarto de Tenerife, Gallotia galloti galloti. Doñana Acta Vert. 8, 43–78.

Morris Gosling, L., Sutherland, W.J., 2000. Behaviour and Conservation. Cambridge University Press, Cambridge.

Nogales, M., Martin, A., Tershy, B.R., Donlan, C.J., Veitch, D., Puerta, N., Wood, W., Alonso, J., 2004. A review of feral cats eradication on islands. Conserv. Biol. 18, 310–319.

Pavlov, I.P., 1927. Conditioned reflexes. Oxford University Press, New York.

Pérez-Mellado, V., Romero-Beviá, M., de la Torre, A., Vicedo, M., García-Sirvent, J., 1997. Hábitat, distribución actual y tamaño de la población de Gallotia simonyi en la isla de El Hierro (Islas Canarias), in: López-Jurado, L.F., Mateo-Miras, J.A. (Eds.), El lagarto gigante de El Hierro: bases para su conservación. Jorcry S.L., Las Palmas de Gran Canaria, pp. 27-42.

Plasman, M., Duchateau, M.J.H.M., 2007. Anti-predation behaviour of Dickerson’s collared lizard, Crotaphytus dickersonae 57, 231–246.

Price, E.O., 1999. Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. 65, 245–271.

Relyea, R., 2002. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. Ecol. Monogr. 72, 77–93.

Rodríguez-Domínguez, M.A., Molina-Borja, M., 1998. Reproduction of the endangered Hierro giant lizard Gallotia simonyi machadoi. J. Herpetol. 32, 498–504.

Schank, J.C., Koehnle, T.J., 2009. Pseudoreplication is a pseudoproblem. J. Comp. Psychol. 123, 421–433.

Schwarzkopf, L., Shine, R., 1992. Costs of reproduction in lizards: escape tactics and susceptibility to predation. Behav. Ecol. Sociobiol. 31, 17–25.

Shier, D.M., Owings, D.H., 2007. Effects of social learning on predator training and post-release survival in juvenile black-tailed prairie dogs, Cynomys ludovicianus. Anim. Behav. 73, 567–577.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edition. W.H. Freeman and Company, New York.

Stoinski, T.S., & Beck, B.B., 2004. Changes in locomotor and foraging skills in captive-born, reintroduced lion tamarins (Leontopithecus rosalia rosalia). Behaviour 56, 801–809.

Trujillo, D., 2008. Censo y distribución de las poblaciones reintroducidas del lagarto gigante de El Hierro Gallotia simonyi (Steindachner, 1889) en La Dehesa, el Julan y el Roque Chico de Salmor. Unpublished Technical Inform. Consejería Medio Ambiente, Gobierno Autónomo de Canarias.

van Heezik, Y., Maloney, R.F., Seddon, P.J., 1999. Helping reintroduced houbara bustards avoid predation: effective anti-predator training and the predictive value of pre-release behaviour. Anim. Conserv. 2, 155–163. doi:10.1111/j.1469-1795.1999.tb00061.x

Wallace, M.P., 2000. Retaining natural behaviour in captivity for re-introduction programmes. Behav. Conserv. Morris Gosling, L, Sutherl, 300–314.

Wilkinson, A., Kuenstner, K., Mueller, J., Huber, L., 2010. Social learning in a non-social reptile (Geochelone carbonaria). Biol. Lett. 6, 614–616.

Fig. 1.- Diagram of the terrarium used for the experiments showing: its dimensions, the positions of the stuffed kestrel used in the trials, the length of the path traversed by the kestrel and the hollowed barrier from which the investigator could ascertain the visible lizards.

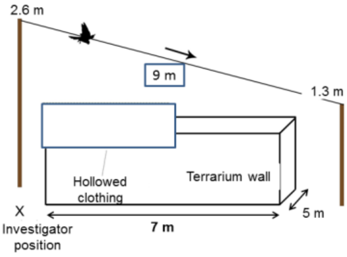

Fig. 1.- Burunat-Pérez et al

Fig. 2.- Diagram of a top view of the same terrarium showing: the positions of the tiles used as lizard shelters (covered by palm leaves), the place of the two videocameras and the position of the wheelbarrow used for moving the stuffed cat during experimental trials.

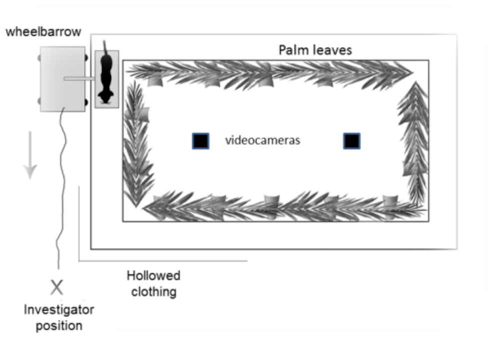

Fig. 2.- Burunat-Pérez et al

Fig. 3.- Mean (+ 95% CI) relative durations of the three more common behavioural categories expressed by the lizards (N = 8) during the four phases of the experiment, both before and after passing the kestrel as a potential predator.

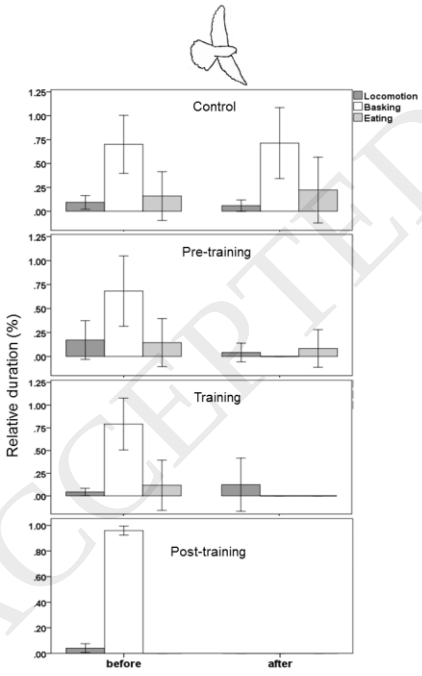

Fig. 3.- Burunat-Pérez et al.

Fig. 4.- Mean (+ 95% CI) relative durations of the three more common behavioural categories expressed by the lizards (N = 7) during the four phases of the experiment, both before and after passing the cat as a potential predator.

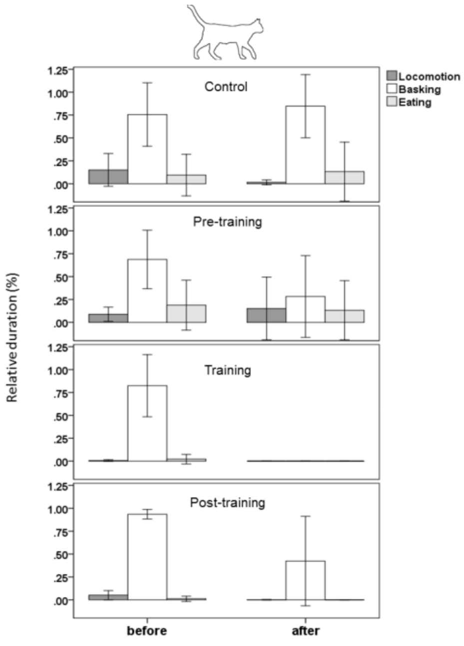

Fig. 4.- Burunat-Pérez et al.

Fig. 5.- Number of lizards showing each behaviour category during the specific moment when the potential predator: kestrel (a) and cat (b) was moved.

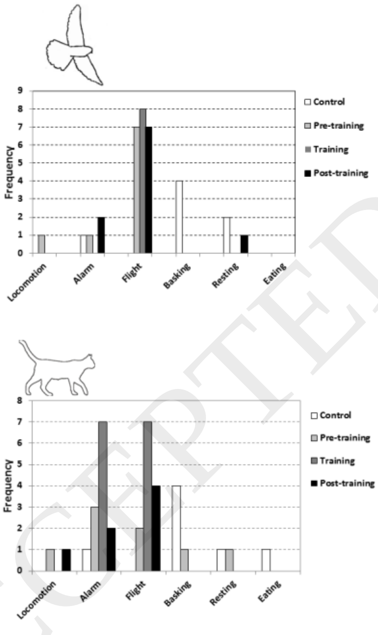

Fig. 5.- Burunat-Pérez et al.

Table 1.- Codes, year when they were born, snout-vent length (SVL) and body mass (BM) of lizards participating in the trials of the study.

Lizard code (microchip)	Born (year)	SVL (mm)	BM (g)
397F	2006	188	199
1C79	2006	176	188
6871	2006	166	149
7D42	2006	180	182
192C	2006	181	197
3810	2006	175	188
3370	2008	166	138
1548	2007	185	186
2E76	2008	167	145
4276	2008	159	122
4C12	2007	186	196
0351	2008	151	118
7725	2008	172	165
0803	2007	165	148
3927	2007	173	168
4479	2007	173	163
290B	2008	145	109
4279	2008	155	119
6F1D	2008	147	92
0918	2008	139	75

Table 2.- Temperatures (in º C) at ground level in a sunny patch of the terrarium in a typical day within each experimental phase with each predator model.

	Control	Pre-training	Training	Post-training
Kestrel	28.2	30.7	30.6	30.0
Cat	31.0	30.1	30.1	29.8

Table 3.- Number of trials performed in each of the four phases of kestrel and cat experiments.

Experimental phase	kestrel	Cat
Control	8	9
Pre-training	7	7
Predator + aversive stimulus	12	7
Post-training	12	8

Table 4.- Results from PERMANOVA of repeated measurements applied separately to relative durations of each of the three more frequent behaviour categories shown by lizards, during all phases and before and after passing the stimulus (b-a) of experimental trials with kestrel and cat models. In bold: significant results.

ES: effect size, calculated as (SS of each factor / SStotal) * 100.

		kestrel				cat			
Behaviour categories	Factors	Pseudo-f	df	p	ES	Pseudo-f	df	p	ES
Locomotion	phase	0.999	3	0.939	4.76	**3.427**	**3**	**0.004**	17.02
	(b-a)	0.999	1	0.994	1.58	0.319	1	0.69	0.05
	phase x (b-a)	1.0	3	0.435	4.76	0.601	3	0.72	2.98
Basking	phase	**3.39**	**3**	**0.025**	7.82	**3.302**	**3**	**0.031**	11.49
	(b-a)	**40.286**	**1**	**0.0001**	30.97	4.151	1	0.14	19.07
	phase x (b-a)	**7.862**	**3**	**0.0003**	18.13	**3.952**	3	**0.017**	13.75
Eating	phase	1.290	3	0.291	6.16	1.623	3	0.17	9.08
	(b-a)	0.162	1	0.826	0.25	1.292	1	0.33	0.39
	phase x (b-a)	0.924	3	0.374	4.41	0.164	3	0.92	0.92