



What do animals learn during anti-predator training? Testing for predator-specific learning in 'alalā (*Corvus hawaiiensis*)

Alison L. Greggor¹ · Bryce Masuda¹ · Anne C. Sabol^{1,2} · Ronald R. Swaisgood¹

Received: 19 July 2022 / Revised: 11 November 2022 / Accepted: 15 November 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Despite the growing use of conservation breeding and translocations in conservation, many translocation efforts fail due to predation post-release. Released animals often lack appropriate behaviors for survival, including anti-predator responses. Anti-predator training—a method for encouraging animals to exhibit wariness and defensive responses to predators—has been used to address this challenge with varying degrees of success. The efficacy of anti-predator training hinges on animals learning to recognize and respond to predators, but learning is rarely assessed, or interventions miss key experimental controls to document learning. We present an experimental framework for designing anti-predator training that incorporates suitable controls to infer predator-specific learning and illustrate their use with the critically endangered Hawaiian crow, 'alalā (*Corvus hawaiiensis*). Our training aimed to increase anti-predator behavior towards a natural predator, the Hawaiian hawk, 'io (*Buteo solitaries*). In addition to running live predator training trials, we included two control groups, to determine if responses could otherwise be due to accumulated stress and agitation or to generalized increases in fear of movement. We could not verify that the training induced anti-predator learning because responses were similar across all experimental groups. Therefore, without these control groups, we may have wrongly concluded that predator-specific learning occurred. Additionally, despite generations in human care that can erode anti-predator responses, 'alalā showed high levels of predatory wariness during baseline assessments. We discuss the implications of a learning-focused approach to training for managing endangered species that require improved anti-predator competence and the importance of understanding learning mechanisms in diagnosing behavioral problems.

Significance statement

Knowledge of animal learning has the potential to improve skills training for animals prior to release in the wild but only if animals learn from the stimuli provided in survival-relevant ways. Here, we test whether a setup used for anti-predator training actually leads to learning when applied with the critically endangered 'alalā (*Corvus hawaiiensis*) that struggle to survive alongside their native predator. We found that various experimental controls (e.g., capture net and live chicken) produce similar responses to the training itself, which questions the efficacy of training for inducing anti-predator learning. Such investigations are an important tool for improving the effectiveness of conservation behavior interventions that rely on learning outcomes to improve survival.

Keywords Anti-predator learning · Conservation behavior · Corvid · Learning mechanisms · Pre-release training · Reintroduction biology

Communicated by P. A. Bednekoff

✉ Alison L. Greggor
AGreggor@sdzwa.org

¹ Recovery Ecology, San Diego Zoo Wildlife Alliance, 15500 San Pasqual Valley Rd, Escondido, CA 92027, USA

² Department of Biological Sciences, Florida International University, Miami, USA

Introduction

Many conservation translocations—i.e., human-mediated relocations of wildlife to improve species' and habitat recovery—fail despite large commitments of resources (Hoffmann et al. 2010; Seddon et al. 2014). Many translocation failures can be attributed to predation after release (Fischer and Lindenmayer 2000; Moseby et al. 2011), yet the behavioral

mechanisms leading to increased predation are infrequently acknowledged or addressed (Berger-Tal et al. 2020). Therefore, conservation interventions that reduce behavioral vulnerability to predation have the potential to improve translocation outcomes widely (Berger-Tal et al. 2020). Deficiencies in released animals' anti-predator responses (Shier 2016; Berger-Tal et al. 2020), are a likely contributor to post-release predation and subsequent translocation failure, especially when source populations have been free from predation pressure (Ross et al. 2019). Just as other natural behaviors often erode in human care (Kraaijeveld-Smit et al. 2006; McPhee and Carlstead 2010), predator-free environments foster prey naivety, which results in ineffective anti-predator behavior (Cox and Lima 2006).

Anti-predator training—in which animals living in predator-free environments are provided opportunities to learn about predators—can be a useful tool to combat prey naivety across taxonomic groups (Griffin et al. 2000; Shier and Owings 2006; Teixeira and Young 2014; Moehrenschrager and Lloyd 2019), but its efficacy in translocation contexts often goes untested (Greggor et al. 2019; Ross et al. 2019; Rowell 2020). Accordingly, despite some successes (e.g., van Heezik et al. 1999; Shier and Owings 2006), training has often failed to adequately change anti-predator behavior (Campbell and Snowdon 2009; Jolly et al. 2020) or improve survival post-release (Moseby et al. 2012). Without being able to pinpoint where and why anti-predator training goes wrong, we lose the ability to address naivety and vulnerability to predators for translocated animals.

Anti-predator training requires manipulating animal learning. Many species naturally learn about predators during development, a process that is facilitated by experiencing a predatory cue (e.g., the sight, smell, or sound of a predator) alongside a conspecific signal of danger (e.g., an alarm call, scent, or evidence of attack) (Griffin et al. 2000). Some interventions expose animals to low levels of true predation to accurately replicate these cues and facilitate learning (e.g., Moseby et al. 2016; Ross et al. 2019). However, losing animals to predators pre-release is often not practical due to welfare concerns or when working with endangered species with few release candidates. Therefore, training efforts often try to mimic the natural learning process, by pairing an aversive stimulus (e.g., a conspecific alarm cue or physical restraint) with a predator or replica (Shier and Owings 2006; Teixeira and Young 2014). Ideally, these presentations utilize classical conditioning learning mechanisms, allowing animals to rapidly remember the predator, not simply the context where they encountered it (Griffin et al. 2000). When successful, pre-release training enables animals to distinguish predatory threats from non-threatening stimuli in the environment and respond to actual predators in the wild, despite never having been directly attacked. Such learning is necessary for training to be effective, but

measuring anti-predator learning over the course of training is not always straightforward.

A common framework for demonstrating learning compares behavior before and after training between a trained and a control, untrained group (Griffin et al. 2000). While this setup can successfully document changes to anti-predator behavior over the course of training, it does not expose the root causes of behavioral change. Specifically, two cognitive mechanisms—sensitization and generalization—can falsely present as predator learning in trained groups if not adequately addressed with experimental controls, each of which may have different downstream effects for post-release survival. Sensitization occurs when animals become more responsive to repeated presentations of stimuli and is especially likely after a mildly aversive stimulus (Shettleworth 2010). If animals cue into aspects of the training setup and anticipate danger, sensitization during repeated, fear-inducing training sessions could drive apparent anti-predator behavior during training, without target animals actually learning about the predator (e.g., Mathis and Smith 1993). Accordingly, sensitized animals would be unlikely to engage in anti-predator behavior when encountering a predator outside of the training setup. Second, animals may not learn about the predator itself but simply learn a generalized fear of animacy or animate stimuli in certain situations. While responding fearfully to a broad category of animate stimuli (including towards non-predators) may help with initial survival since predators would be avoided, animals can incur energy and resource costs if they consistently over-respond to false predatory threats (Carthey and Banks 2014). In both cases, animals that show a heightened response after training may not express optimal anti-predator behavior post-release. Therefore, by including experimental controls that offer repeated presentations of fearful (but not predatory) stimuli, and controls with non-fearful, animate stimuli, training designs can document true anti-predator learning while ruling out sensitization and generalization. These additional controls help diagnose why apparently trained animals may not experience the survival or fitness benefits training is expected to provide. Additionally, it can give managers and researchers an opportunity to assess the efficacy of training prior to release, which could prevent unnecessary deaths if training methods can be adjusted to facilitate predator-specific learning that reduces vulnerability to predators post-release.

To illustrate the importance of these cognitive considerations, we tested the efficacy of anti-predator training with the critically endangered 'alalā or Hawaiian crow (*Corvus hawaiiensis*). 'Alalā went extinct in the wild in 2002 and have been the subject of intensive reintroduction efforts since 2016. Previous attempts to re-establish the species faced many challenges, including predation by their natural predator, the 'io (Hawaiian hawk, *Buteo solitaries*) (U.S.

Fish and Wildlife Service 2009; Greggor et al. 2021). Therefore, future planning efforts incorporated the use of anti-predator training to improve the chances of survival (VanderWerf et al. 2013). We examined the efficacy of training in breeding facilities with ‘*alalā* that were not designated for imminent release, allowing us to evaluate methods that had been used on the release cohorts (Greggor et al. 2021), with a larger, more robust sample. We measured the anti-predator responses of ‘*alalā* towards a predator model before and after a classical fear conditioning training, across birds that received one of three treatments: a live predator, a control fear stimulus (net), and a control animate object (live chicken) (Fig. 1). These learning-focused control treatments were designed to help identify sensitization (fear-inducing, non-predatory net) or generalization (non-fearful, animate chicken), to determine if factors other than anti-predator learning contribute to increases in anti-predator behavior.

Our experiment was designed to differentiate anti-predator learning from these alternative learning processes that all could otherwise produce heightened anti-predator behavior during training. Crucially, the comparison between the baseline (day 1) and evaluation (day 3) trials across all three treatment groups should reveal what the ‘*alalā* may have learned during training. If the live predator group responded with greater anti-predator responses to the model in the evaluation trial than the other two groups, there would be strong evidence that the training produced predator-specific learning and therefore may reduce the probability of predation if these birds were released. In contrast, if ‘*alalā* showed little increase in anti-predator behavior in the live predator group or showed increases across any other group, the question of training effectiveness would be more complex. If ‘*alalā* responded with greater anti-predator behavior in all

three groups, we would infer that training caused the birds to become sensitized to threatening/novel stimuli, instead of producing predator-specific learning. With this result, we would predict that training offers little advantage in helping animals avoid predators since they did not learn to fear the predator itself. Meanwhile, if ‘*alalā* showed increases in anti-predator behavior in the live predator and chicken groups only, we would conclude they acquired a learned fear response to general animacy. From a conservation management standpoint, this last outcome, which would not show predator-specific learning, could still be beneficial, depending on the energetic or resource costs ‘*alalā* may incur by responding unnecessarily to harmless avian or other animate stimuli. However, there are clear advantages to developing anti-predator training programs that facilitate appropriate anti-predator behavior only toward predators and not to other animate or inanimate objects that present no risk.

Materials and methods

Study species

‘*Alalā* are the only remaining corvid species native to the Hawaiian Islands. They are a generalist forager, with one surviving native predator, the ‘*io*. Populations of ‘*alalā* declined precipitously during the late twentieth century due to habitat degradation, disease, human conflict, and predation by non-native mammals (U.S. Fish and Wildlife Service 2009). Despite supplementation translocations in the 1990s, ‘*alalā* went extinct in the wild in 2002. Since then, conservation breeding has increased the population from fewer than 20 to over 110 in human care today. Reintroduction

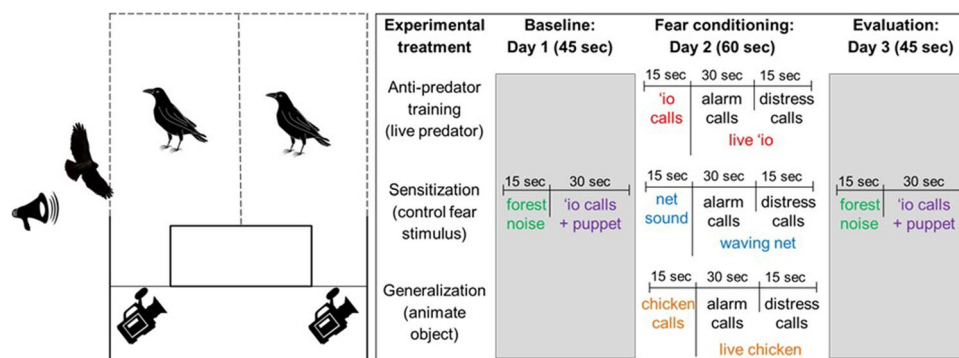


Fig. 1 Experimental setup and schedule. The setup (left panel): in 13 of the 19 multi-chambered aviaries, individuals could move freely between the two large chambers. The speaker (~2 m from aviary wall) and fear stimulus (‘*io* pictured) were placed on the outside of the aviary. The behavior of birds was video recorded and observed live from inside the aviary’s observation corridor. The dotted lines indicate areas of mesh that offer visual access; areas without dotted lines are solid walls through which the birds could not see. The

experimenter presented the stimuli while standing out of sight, up against the solid wall. The schedule (right panel): during each trial, 3 min of behavioral data was also taken immediately prior to and after the stimuli presentation. The fear conditioning day did not contain forest noise to prevent ‘*alalā* from associating the forest noise with the ensuing alarm and distress calls. The puppet refers to a taxidermy ‘*io* model with flapping wings. Ultimately, behavior on day 3 should reflect birds’ experiences on day 2

translocations were initiated in 2016 and are currently ongoing. However, translocation efforts have faced similar challenges over time. Predation by ‘io has been a primary cause of post-release losses in historical (U.S. Fish and Wildlife Service 2009) and ongoing translocations (Greggor et al. 2021). Therefore, the development of anti-predator training to reduce mortality from ‘io figures prominently in recovery planning documents (VanderWerf et al. 2013). Despite the need for effective training, the small size of release cohorts and critically endangered status of the species meant that training for the release birds could not contain untrained control groups with later measures of survival, in case it resulted in the deaths of untrained birds (Greggor et al. 2021). Therefore, the need for further research arose within the conservation breeding flock, where control groups would not be released and thus face no adverse survival impacts, to improve training methods in future recovery efforts.

Birds and housing

We tested ‘alalā housed in an ongoing conservation breeding program at the Keauhou Bird Conservation Center (KBCC) in Volcano, Hawaii. Our sample comprised male ($N=23$) and female ($N=20$) birds that were hand-reared ($N=35$) or partially or fully parent-reared birds ($N=8$). We tested ‘alalā in their home enclosures, with their mate, family group, or juvenile social group. Since ‘alalā are a social species, testing individuals alone can cause stress and compromise the reliability of anti-predator responses. Moreover, training is more effective in natural social groups (Shier and Owings 2007). Therefore, data were taken on each individual within their group, and the potential for social effects was accounted for statistically with mixed effects models (see below). No ‘alalā tested in this study had previously been trained to fear ‘io as part of release efforts, but all had the occasional opportunity to observe wild ‘io that are resident in the area. It is possible they may have observed predation events by ‘io on other forest birds, but these events were never observed by staff in the years leading up to this study.

All birds had access to food and water and were exposed to ambient light and weather conditions. Each aviary contained areas of covered perching, an indoor feeding chamber, and open areas. See Greggor et al. (2018) for a detailed description of husbandry, enrichment, and housing practices. While the dimensions of each aviary differed slightly, the basic setup was the same (Fig. 1). Aviaries included in this study were spaced between 242 and 538 m from their closest neighboring aviary, with no visual access between them, due to the surrounding forest. While it was unlikely that birds were aroused by hearing other aviaries receive stimuli for this study prior to their trials, no neighbors received the same experimental condition, and efforts were made to

temporally space out trials at closer aviaries, to reduce the potential for impacting later trials.

Stimuli and trials

We conducted all trials from July 7 to 20, 2018, between 08:00 and 16:00. We assigned each aviary to one of three experimental treatments, exposing each bird group to a series of three trials. The three trials (baseline, fear conditioning, and evaluation) occurred on consecutive days at the same time of day. Each trial, regardless of treatment, consisted of three periods: (1) a 3-min pre-trial observation, (2) a period of stimuli exposure (whose duration and stimuli depended on the treatment and trial day), and (3) a 3-min post-trial observation (Fig. 1). Including these pre- and post-trial periods for each trial allowed us to eliminate the potential that we accidentally induced fear while setting up the trial, prior to the presentation of stimuli, which would muddy the results. The experimenter set up the trials by putting out the speaker and camcorders, allowing the birds to settle for ten minutes and then beginning the pre-trial observation period. The experimenter then presented experimental stimuli, which lasted either 45 s or 1 min, depending on the trial type. Once the stimuli were removed, the 3-min post-stimuli period began. All trials followed this same schedule, but the stimuli differed by trial type and treatment group (Fig. 1).

We exposed each experimental group to the same stimuli for baseline (day 1) and evaluation (day 3) trials: an audio recording and a flapping model ‘io. These recordings contained 15 s of ambient forest noise (recorded at KBCC)—reducing the likelihood ‘alalā would be startled by a sudden sound from the speaker—followed by 30 s of ‘io territory calls. We created two exemplar audio recordings to address pseudoreplication, each with a similar number and timing of calls. Subjects randomly received one exemplar for the baseline and the other for the evaluation. During trials, the hidden experimenter presented the model ‘io next to the aviary as the recording started playing ‘io calls. The model was an ‘io taxidermy, mounted on a pole with wings extended, containing a mechanism that tipped the body forward on command. The experimenter moved the model similarly for each presentation, making the dipping motion every 2 s and holding for 2 s.

During fear conditioning trials (day 2), the experimenter presented one of three stimuli, depending on the experimental treatment: a live ‘io predator (anti-predator training), a net (sensitization control), or a live chicken (generalization control). The experimenter played sounds specific to the fear stimulus for 15 s and then presented the stimulus alongside an audio track containing ‘alalā alarm and distress calls. We previously recorded ‘alalā alarm calls during husbandry-related disturbance (e.g., nest-checks) and distress calls

during routine veterinary procedures. The alarm calls were overlaid on each other to mimic a flock of birds (increasing the perceived risk of danger) (Coomes et al. 2019), followed by a single individual emitting a series of distress calls. Like other passerines, ‘*alalā* make distress calls when faced with imminent danger, such as during physical capture and restraint, potentially offering information about threats to others (Griffin 2008). No calls from experimental subjects in this study were used in the audio files.

For the live ‘*io* treatment, we borrowed a glove-trained ‘*io* from the Panaewa Rainforest Zoo and Gardens in Hilo, Hawai‘i. He was maintained at the KBCC in an outdoor enclosure between trials. He voluntarily stepped onto a falconer’s glove for each presentation and was encouraged to flap at the ‘*alalā*, in response to gentle motion of the glove. The handler remained hidden behind the side of the aviary, extending the glove into the area of visual access for the ‘*alalā*. The audio track used prior to the live ‘*io* presentation was a compilation of ‘*io* territorial calls not used in either the baseline or evaluation trials.

For the sensitization control treatment, we presented a large black recapture net during the fear conditioning stimuli. As the primary recapture method at the facility, the net served as a known, but artificial, fear-inducing stimulus. Presenting a stimulus which had a high likelihood of inducing fear allowed us to be confident we could detect an effect of sensitization, which requires repeated fearful stimuli. Before presenting the net, the experimenter clanked two net poles together repeatedly (every 2 s) for 15 s, simulating the sound of staff removing nets from work trucks during recapture. Immediately after the net sounds, the hidden experimenter played the ‘*alalā* alarm and distress calls while waving the net in a similar range of movement to the ‘*io*’s flapping wings.

For the generalized animacy control treatment, we presented a live chicken during the fear conditioning trial. The ‘*alalā* had never seen a live adult chicken and have no evolutionary history of predation by ground-based birds, so we predicted that it would not elicit anti-predator responses (although its novelty could still elicit neophobia) (e.g., Greggor et al. 2020). Prior to the presentation of the chicken, the experimenter played a series of non-fear-related chicken

calls. The hidden experimenter then played the ‘*alalā* alarm calls and held the chicken out on the side of the aviary, encouraging him to flap. Regardless of whether ‘*alalā* could attend to the flapping motion or the presence of a live bird, the chicken offered an opportunity to determine if they would generalize responses to other animate, flapping birds.

We edited sound files using Audacity® software and broadcast them from an Altec Lansing Bluetooth speaker connected to an iPhone 6S. We broadcast sound files at the same maximum volume level (80 dB), verified with the Decibel X Power Meter app for iPhones from the same 2-m distance of the speaker from the aviary wall. Unless otherwise specified, we collected sound recordings using a Roland R-05 acoustic recorder and a Sennheiser microphone with a Rycote softie wind cover.

Data collection and analysis

We collected behavioral data via live observer and video recorded from multiple angles. It was not possible to record data blind because live observers could see and hear the experimental stimuli. We recorded the number of anti-predator behaviors (alarm calls and rapid escape flights across the length of the aviary) and non-fear behaviors (affiliative begging calls) across each trial period (pre-stimuli, during stimuli, post-stimuli). Additionally, we classified birds’ level of engagement with the stimuli into one of several categories (Table 1). An independent observer, blind to the experimental questions and original data, recoded a subset of videos (20%) to assess inter-observer reliability, which was calculated with an intraclass correlation coefficient (ICC).

Non-fear behavior occurred too infrequently (3.6% of trial periods) to merit formal analysis. Therefore, we focused on anti-predator behavior and level of engagement with the stimuli to answer three main questions. First, to confirm that all treatment groups began with similar levels of fear towards the ‘*io* model, we compared anti-predator behaviors across treatments on the baseline trial (day 1). Second, to determine if the live predator, net, and chicken stimuli elicited different responses during the fear conditioning trial, we compared anti-predator behavior and engagement levels across treatments during

Table 1 Definitions of behavioral categories used to explain the levels of approach behavior during exposure to the experimental stimuli

<i>Engagement level</i>	<i>Definition</i>
Curiosity	Bird makes a close approach (<2 m) to the stimulus (or the point closest in their chamber to the stimulus) without any audible fear response
Distant alarm calling	Bird makes one or more alarm calls but does not approach the stimulus
Mobbing	Bird alarm calls while approaching the stimulus < 2 m (or 2 m of the closest point in their chamber to the stimulus). Can involve active aggression (hammering on enclosure) or persistent, close alarm calling
Vigilance only	Bird makes no audible alarm response and no approach to the stimulus but makes visible, alert changes to head position, such as sky-scanning or directing attention towards the stimuli

fear conditioning (day 2). Finally, we compared changes between baseline trials (day 1) and evaluation trials (day 3) across treatments to determine if anti-predator behavior increased over trials to indicate predator-specific learning.

We compared anti-predator behavior for each question with a generalized linear mixed model (GLMM) in R version 4.0.4 (R Core Team 2021) using either the lme4 package for binomial distributions (Bates et al. 2015) or the glmmTMB package for negative binomial distributions (Brooks et al. 2017). Our main variables of interest were treatment (anti-predator training, sensitization control, and generalization control) and trial period (pre-stimuli, stimulus, post-stimuli), to help us detect evidence for anti-predator learning. However, we also included sex, number of birds present in the aviary, and whether the bird had access to the stimuli side of the aviary in each model to account for other potential sources of variation. Models examining changes to anti-predator behavior over time also contained an interaction between trial number (baseline = 1, evaluation = 3) and treatment. Subject ID was included as a random factor for all models. Accounting for this source of non-independence was necessary since each individual contributed data for the three trial periods, across all three trials. Age was not included since only one aviary contained birds younger than breeding age, and their data were not outliers. To confirm the validity of our model interpretations, we conducted model selection with the MuMIN package (Barton 2020) and evaluated factors based on their impact to the global model's AICc value when removed. Coefficients are reported from global models, and model fit and assumptions, including dispersion, outliers, uniformity, and zero inflation, were checked with the DHARMA package on the model with the lowest AICc score (Hartig 2021).

We transformed the response variable (count of anti-predator behaviors) to a binomial variable (presence of anti-predator behavior = 1, absence = 0) for comparing responses across treatments in the baseline trials. Compared to the baseline, there was greater variation in responses in the fear conditioning and evaluation trials, so we converted both from behavioral sums (alarm calls plus full flights) to a count per 3-min period, to account for the different trial period durations, and analyzed them with a negative binomial error distribution to account for zero inflation. For the third question evaluating changes in anti-predator behavior from the baseline to evaluation trials, we focused on the “during” stimuli period. Since the during-stimuli comparison of baseline and evaluation trials served as the ultimate test of effectiveness for the training, we conducted a post hoc power analysis on this GLMM using the simr package (Green and Macleod 2016). We used this approach to explore different sample size scenarios, given the existing variation in our data, by subsampling from our dataset.

In addition to analyzing anti-predator responses, we conducted separate analyses to investigate birds' engagement levels with the fear stimuli. We examined whether birds were more likely to respond within a certain category (Table 1) in the fear conditioning trial (day 2) with an exact multinomial test, assuming an equal 25% chance of any behavior occurring. We ran post hoc binomial tests with Bonferroni corrections to investigate categories of interest. We also compared birds' response between baseline and evaluation trials with a marginal homogeneity test, using the coin package (Hothorn et al. 2008).

Results

We tested 43 ‘alalā ($N=13$ chicken, 13 live predator, 17 net) across 19 aviaries. Of these, two aviaries were excluded for fear conditioning and evaluation trials (days 2 and 3) because birds in one aviary started breeding (net treatment) and in another the speaker malfunctioned midway through the alarm and distress calls (chicken treatment). Inter-observer reliability was high for the composite measure of alarm calls and full flights ($ICC(1)=0.91$, $p<0.001$, $CI=0.86-0.94$) and for the level of engagement (96.4% concurrence). One bird was not reliably visible on video during the fear conditioning trial (day 2), and her data were removed for that day.

Treatment effect during baseline trials

In baseline trials (day 1), all treatment groups showed increased anti-predator behavior during the ‘io model presentation and post-stimuli time periods in comparison to pre-stimuli anti-predator rates (binomial GLMM, $N=129$ observations, 43 birds across 3 periods; during, $\beta \pm SE = 2.39 \pm 0.67$, $z = 3.54$, $p < 0.001$; post-stimuli, $\beta \pm SE = 1.50 \pm 0.63$, $z = 2.37$, $p = 0.018$) (Fig. 2). Birds tested in larger groups had higher levels of anti-predator responses ($\beta \pm SE = 0.77 \pm 0.32$, $z = 2.43$, $p = 0.015$). There was no effect of treatment, as expected, since all groups were exposed to the same stimuli and no effect of sex or bird access to the stimuli side of the aviary (Table S1).

Treatment effect during fear conditioning trials

Similar to baseline trials, during the fear conditioning trials (day 2), ‘alalā displayed higher rates of anti-predator behavior during the stimuli presentation and post-stimuli period relative to the pre-stimuli period (negative binomial GLMM, $N=111$ observations, 37 birds across 3 periods, during, $\beta \pm SE = 4.39 \pm 0.48$, $z = 9.16$, $p < 0.001$; post-stimuli, $\beta \pm SE = 2.80 \pm 0.48$, $z = 5.90$, $p < 0.001$). All treatment groups were indistinguishable in their rates of anti-predator behavior, despite receiving either a live predator, net,

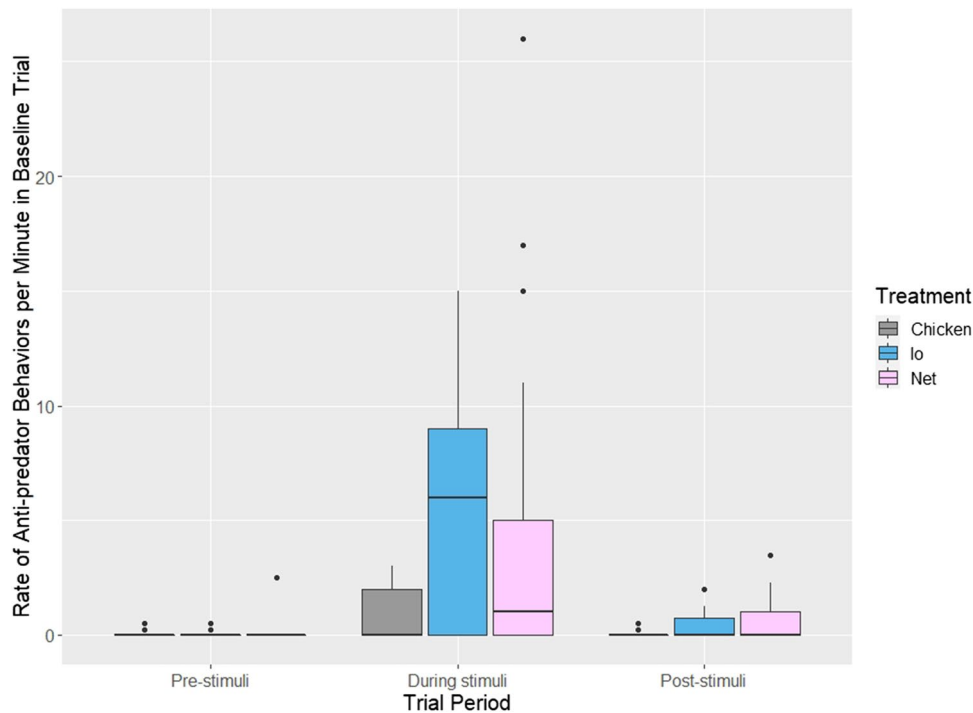


Fig. 2 Boxplots depicting raw rates of individual anti-predator behavior across trial periods of baseline trials (day 1). Boxes depict the interquartile range (IQR), the horizontal line in each box depicts the median value, the vertical lines depict values extending to 1.5*IQR, and outlying data points are pictured. Anti-predator behavior is measured as the rate of alarm calls and pace flies per minute of trial period. All treatments received the same stimuli during these baseline

trials: a 3-min pre-stimuli period, 45 s of exposure to an ‘io model and ‘io calls, and a 3-min post-stimuli period. There was no effect of experimental treatment, and all conditions show the same pattern: little anti-predator behavior during the pre-trial increased fear while the model and calls were present and reduced fear once stimuli were removed in the post-trial period

or live chicken (Table S2). However, birds in larger social groups had slightly higher rates of anti-predator behavior ($\beta \pm SE = 0.37 \pm 0.15$, $z = 2.44$, $p = 0.015$). There was no effect of sex or whether birds had access to the stimuli side of the aviary (Table S2). Additionally, while stimuli were present, ‘alalā were more likely to mob (binomial test, Bonferroni correction applied, $p < 0.001$) and less likely to exhibit curiosity ($p < 0.001$) (Fig. 3) than engage in vigilance or distant alarm calling.

Treatment effect on predator-specific learning (baseline versus evaluation)

There was no interaction between treatment and trial (Table 2). Across all treatments, ‘alalā increased their anti-predator behavior towards the ‘io model during the evaluation trials, relative to baseline trials ($\beta \pm SE = 0.55 \pm 0.26$, $z = 2.10$, $p = 0.036$) (Fig. 4). ‘Alalā tested in larger group sizes showed higher levels of anti-predator behavior ($\beta \pm SE = 0.58 \pm 0.17$, $z = 3.37$, $p < 0.001$). Including sex and side of the aviary did not improve overall model fit (Table S3). Additionally, more ‘alalā mobbed the ‘io model during evaluation than baseline trials (marginal homogeneity test; $\chi^2 = 8.027$, $df = 3$, $p = 0.045$) (Fig. 3). Power

analyses revealed a low likelihood of finding an effect during the stimuli presentations with our sample size, even for relatively large effect sizes ($\beta \pm SE = 0.635$, power = 64.7%,

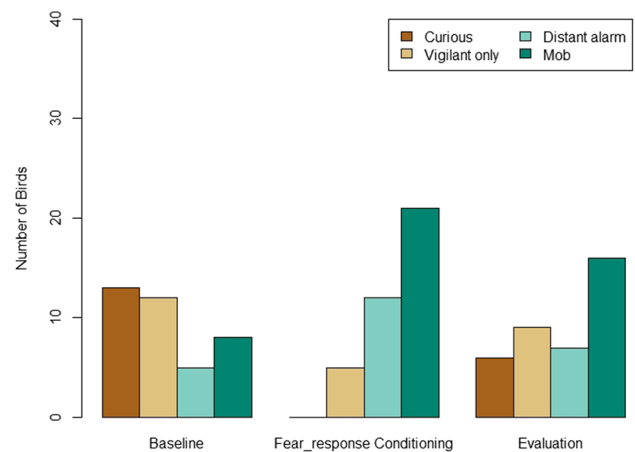


Fig. 3 Number of ‘alalā exhibiting response categories to the stimuli during each trial type. During the fear conditioning trials, birds were more likely than chance to mob and less likely than chance to show a curious response. In comparing between trial types, more ‘alalā mobbed the taxidermy ‘io model during the evaluation than baseline trials

Table 2 Analysis of anti-predator behavior during the presentation of the ‘io model on the baseline and evaluation trial (day 1 and 3), GLMM coefficients from global model. Fixed effects are listed from the model output for all terms except those included in the interaction term. Since the main effects cannot be interpreted when also included in an interaction term, the coefficients listed for treatment and trial are reported from the model without the interaction. The reference category for experimental treatment was the anti-predator training (live ‘io) condition. The model term access denoted whether birds were housed in the chamber adjacent to the experimental stimuli. A random effect of bird ID was used in all models. See Table S3 for model selection

Fixed effects	<i>B</i>	SE	<i>z</i>	<i>p</i>
(Intercept)	0.10	0.75	0.13	0.897
Stimuli access, yes	1.13	0.60	1.90	0.057
No. birds	0.58	0.17	3.37	<0.001
Sex, male	0.42	0.34	1.24	0.216
Treatment				
Generalization (chicken)	−0.40	0.44	−0.89	0.372
Sensitization (net)	−0.18	0.40	−0.45	0.652
Trial (day 3)	0.55	0.26	2.10	0.036
Treatment: trial				
Generalization (chicken): day 3	0.45	0.79	0.65	0.519
Sensitization (net): Day 3	0.14	0.58	0.24	0.812

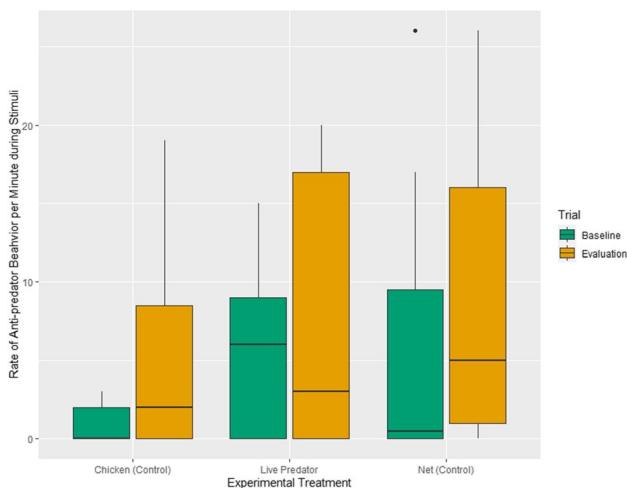


Fig. 4 Boxplots depicting rates per minute of anti-predator behavior on the baseline (day 1) and evaluation (day 3) trials across experimental treatments while stimuli were present. Anti-predator behavior is depicted here as the rate of alarm calls and full aviary flights per minute during the stimuli period. Birds in all experimental conditions demonstrated an increase in anti-predator behavior during the evaluation in comparison to baseline trials, and no interaction effect was detected statistically). Boxes depict the interquartile range (IQR), the horizontal line in each box depicts the median value, the vertical lines depict values extending to 1.5*IQR, and outlying data points are pictured

CI = 61.65–67.68%). However, the data illustrate similar upward trends from baseline to evaluation in each treatment group (Fig. 4), showing little support for an interaction between treatment and trial, which would have indicated predator-specific learning. Based on the variation present within our dataset, a sample size of at least 18 birds per treatment is likely needed for sufficient (80%) power (Fig. S1).

Discussion

Had we not conducted multiple learning controls, we may have wrongly concluded that predator-specific learning occurred in ‘alalā exposed to live predator training. Many published accounts of training include only a non-training control to support their efficacy (Crane and Mathis 2011; Teixeira and Young 2014). Yet, we have shown that several alternative cognitive hypotheses could explain a similar increase in fear after exposure to training and control stimuli. ‘Alalā displayed more anti-predator behavior towards an ‘io model after the live predator and either control treatment, suggesting that they became sensitized to the setup and anticipated the appearance of dangerous stimuli. In other words, the initial model ‘io may have primed the birds to find the next presentation of the model scarier than before (the opposite of habituation) (Shettleworth 2010). Alternatively, experiencing multiple days of fear stimuli may have put the birds in a sustained agitated state (e.g., McIvor et al. 2018), causing them to react more strongly to the predator model over time. In future, work with ‘alalā and other species being trained for reintroduction could extend the time between baseline and evaluation trials or conduct the evaluation trials in a different enclosure (e.g., Mathis and Smith 1993), potentially reducing the likelihood that animals carry over motivational effects between trials. This would remove one potential source of sensitization and also reduce the likelihood that other non-learning factors, such as neophobia, contribute to anti-predator responses (Abudayah and Mathis 2016).

We faced a challenge that is common to many translocation programs; there are often few animals available for testing. Even with the relatively large sample size for studies of this nature ($N = 43$, representing approximately 1/3 of the world’s population), our analysis lacked sufficient power to confirm that birds responded statistically similarly to live predator and control treatments. However, we remain confident in our general findings because numerical trends suggested similar increases in anti-predator behavior across treatments. By adding multiple controls to our setup, we increased the effort and sample size needed but were able to better assess the efficacy of our training. Had we just included the live chicken control, we would have been unable to determine if ‘alalā generalized their responses to animate avian

stimuli or if they sensitized to the setup, and either conclusion could lead to different survival outcomes. Specifically, if birds were merely sensitized to the training, they would not likely respond with anti-predator behavior to actual predators post-release, because the diverse contexts where they encounter predators would not mirror the exact training setup.

Even if conducting two types of controls is not possible, there are other benefits to approaching training with learning in mind. For example, anti-predator training relies on tapping into social cues and reliable predator-relevant cues (Griffin 2004; Shier and Owings 2007), but the social environment and types of cues used can both influence learning outcomes. The social environment contributed to the expression of anti-predator behavior we documented, with larger groups of birds demonstrating more anti-predator behavior in all trials. However, our findings do not indicate that group size influenced learning. Whether the training was more effective in larger ‘*alalā* groups requires further evaluation, but in other species, matching natural social groupings improves training outcomes (Shier and Owings 2007). Additionally, alarm calls are potent cues for corvids (Coomes et al. 2019), and the calls we played during fear conditioning trials proved highly effective in producing fear, even without predators present. For instance, the chicken was intended to be a non-threatening, animate stimulus but was unexpectedly fear-inducing for ‘*alalā*. Other birds have learned to fear novel and otherwise non-threatening animals and inanimate objects when paired with alarm calls (Curio 1978), which may have occurred in our trials.

Documenting learning requires a baseline measure of behavior, which can also help assess training needs. ‘*Alalā* showed high levels of anti-predator behavior towards the model predator during baseline assessments, corroborating and strengthening similar evidence from a separate study on a smaller set of juvenile released ‘*alalā* (Greggor et al. 2021). Together, these results suggest that much of the species is not naïve to ‘*io as a threat, despite their generations in human care. It is unclear whether their baseline predatory wariness stems from observing the few resident ‘*io near the facility or has been maintained through multiple generations in conservation breeding (contrary to other species’ declines in anti-predator behavior) (e.g., Kraaijeveld-Smit et al. 2006; Dixon-MacCallum et al. 2021). Although vulnerability to predators may have contributed to losses seen in historical (U.S. Fish and Wildlife Service 2009) and recent translocations (Greggor et al. 2021), the high baseline anti-predator responses that ‘*alalā demonstrated suggest they recognize ‘*io as a threat. Other aspects of predatory evasion—such failing to act appropriately after recognizing a predator, vigilance levels in the absence of a detected predator, or using habitat in ways that minimize vulnerability—may present greater issues (Greggor et al. 2021). Failures to respond adequately to predators can stem from differing mechanisms, based on****

the evolved relationship with the predator/prey in question and the current predator landscape (Carthey and Blumstein 2018). Systematically addressing these components of predation risk and other threats post-release contributes to an adaptive management approach while also improving the theory and application of translocation biology (Seddon et al. 2007; Taylor et al. 2017). An additional advantage of documenting baseline behavior in anti-predator training is that it can be a screening tool to evaluate individual competency, can be compared with target behaviors of wild individuals, and allows for assessing inter-individual variation.

Ideally released animals respond fearfully to predators and only to predators. We have shown the difficulty, yet necessity of approaching anti-predator training from a cognitive standpoint to document this type of predator-specific learning. Concurrently, however, more work is needed to determine if other processes, such as sensitization or generalization, can still provide benefits, post-release. Even simple sensitization could make less-reactive animals more wary and vigilant if triggered close to release, which could be beneficial, depending on the prevalence of predators. However, generalized fear responses could levy serious costs if they divert attention from other fitness-enhancing activities (Carthey and Banks 2014). Training controls that allow managers to identify vulnerabilities to over-responding may explain unplanned losses of animals and allow release programs greater effectiveness in adaptively managing training and post-release conditions in future. Focusing on an evidence-based approach to translocation biology offers promise for improving outcomes and prioritizing interventions with higher probabilities of success, including for behavioral-based interventions such as pre-release training (Seddon et al. 2007; Berger-Tal et al. 2020). Therefore, testing the efficacy of actions involved in translocations, especially those that are labor intensive, such as anti-predator training, will help support greater conservation progress overall.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00265-022-03273-8>.

Acknowledgements We thank the ‘*Alalā Working Group for discussions about anti-predator training and are grateful to P. Mizuno for lending us “Kaponu” from Panaewa Zoo; to D. Alverson for lending us his chicken “Homie”; to K. Earnest, K. R. Bergfeld, and K. Whitaker for help with data collection and video coding; to J. Gaudiosa-Levita for helping acquire the model ‘*io; to P. Hart for lending audio recording equipment; and to the dedicated animal care team at KBCC for facilitating the project. Finally, we thank two anonymous reviewers for constructive feedback on the manuscript.**

Author contribution ALG, BMM, and RRS designed the study. ACS helped with data collection, troubleshooting, and video coding. BMM secured permits. ALG led the analyses and the first draft of the manuscript. All authors were involved in subsequent writing, and all approved of the final submitted version.

Funding No funding was received for this work specifically, but conservation breeding of ‘alalā was supported by the US Fish and Wildlife Service, Hawaii Division of Forestry and Wildlife, anonymous donors, and San Diego Zoo Wildlife Alliance.

Data availability All data and code used for the analysis in this paper are contained within the Supplementary information. A pre-print of this study can be found at [biorxiv.org/content/biorxiv/early/2021/12/02/2021.11.30.470590.full.pdf](https://doi.org/10.1101/2021.11.30.470590).

Declarations

Ethics approval All applicable international, national, and institutional guidelines for the use of animals were followed. Specifically, this work using animal subjects was approved by San Diego Zoo Wildlife Alliance’s IACUC committee (No. 16–009), and permits allowed conservation breeding of ‘alalā (USFWS Native Endangered Species Recovery Permit TE060179-5, State of Hawaii Protected Wildlife Permit WL19-16) and the possession of live ‘io (USFWS Special Purpose Miscellaneous permit MB09204C-1).

Conflict of interest The authors declare no competing interests.

References

- Abudayah WH, Mathis A (2016) Predator recognition learning in rainbow darters *Etheostoma caeruleum*: specific learning and neophobia. *J Fish Biol* 89:1612–1623. <https://doi.org/10.1111/jfb.13061>
- Barton K (2020) MuMIn: multi-model inference. R package version 1.43.17. <https://CRAN.R-project.org/package=MuMIn>. Accessed 23 Apr 2021
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48. <https://doi.org/10.18637/jss.v067.i01>
- Berger-Tal O, Blumstein DT, Swaisgood RR (2020) Conservation translocations: a review of common difficulties and promising directions. *Anim Conserv* 23:121–131
- Brooks ME, Kristensen K, Benthem KJ, Magnusson A, Berg C, Nielsen A, Skaug HJ, Mächler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *RJ* 9:378–400. <https://doi.org/10.32614/RJ-2017-066>
- Campbell MW, Snowdon CT (2009) Can auditory playback condition predator mobbing in captive-reared *Saguinus oedipus*? *Int J Primatol* 30:93–102. <https://doi.org/10.1007/s10764-008-9331-0>
- Carthey AJR, Banks PB (2014) Naïveté in novel ecological interactions: lessons from theory and experimental evidence. *Biol Rev* 89:932–949. <https://doi.org/10.1111/brv.12087>
- Carthey AJR, Blumstein DT (2018) Predicting predator recognition in a changing world. *Trends Ecol Evol* 33:106–115. <https://doi.org/10.1016/j.tree.2017.10.009>
- Coomes JR, McIvor GE, Thornton A (2019) Evidence for individual discrimination and numerical assessment in collective antipredator behaviour in wild jackdaws (*Corvus monedula*). *Biol Lett* 15:20190380. <https://doi.org/10.1098/rsbl.2019.0380>
- Cox J, Lima S (2006) Naïveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. *Trends Ecol Evol* 21:674–680. <https://doi.org/10.1016/j.tree.2006.07.011>
- Crane AL, Mathis A (2011) Predator-recognition training: a conservation strategy to increase postrelease survival of hellbenders in head-starting programs. *Zoo Biol* 30:611–622. <https://doi.org/10.1002/zoo.20358>
- Curio WE (1978) The adaptive significance of avian mobbing II: cultural transmission of enemy recognition in blackbirds effectiveness and some constraints. *Z Tierpsychol* 48:184–202
- Dixon-MacCallum GP, Rich JL, Lloyd N, Blumstein DT, Moehrenschrager A (2021) Loss of predator discrimination by critically endangered Vancouver Island marmots within five generations of breeding for release. *Front Conserv Sci* 2:718562. <https://doi.org/10.3389/fcsc.2021.718562>
- Fischer J, Lindenmayer DB (2000) An assessment of the published results of animal relocations. *Biol Conserv* 96:1–11. [https://doi.org/10.1016/S0006-3207\(00\)00048-3](https://doi.org/10.1016/S0006-3207(00)00048-3)
- Green P, Macleod CJ (2016) SIMR: an R package for power analysis of generalized linear mixed models by simulation. *Methods Ecol Evol* 7:493–498. <https://doi.org/10.1111/2041-210X.12504>
- Greggor AL, Vicino GA, Swaisgood RR, Fidgett A, Brenner D, Kinney ME, Farabaugh S, Masuda B, Lamberski N (2018) Animal welfare in conservation breeding: applications and challenges. *Front Vet Sci* 5:323. <https://doi.org/10.3389/fvets.2018.00323>
- Greggor AL, Price CJ, Shier DM (2019) Examining the efficacy of anti-predator training for increasing survival in conservation translocations: a systematic review protocol. *Environ Evid* 8:11. <https://doi.org/10.1186/s13750-019-0154-6>
- Greggor AL, Masuda B, Flanagan AM, Swaisgood RR (2020) Age-related patterns of neophobia in an endangered island crow: implications for conservation and natural history. *Anim Behav* 160:61–68. <https://doi.org/10.1016/j.anbehav.2019.12.002>
- Greggor AL, Masuda B, Gaudioso-Levita JM, Nelson J, White TH, Shier DM, Farabaugh SM, Swaisgood RR (2021) Pre-release training, predator interactions and evidence for persistence of anti-predator behavior in reintroduced ‘alalā. *Hawaiian Crow Global Ecol Conserv* 28:e01658. <https://doi.org/10.1016/j.gecco.2021.e01658>
- Griffin AS (2004) Social learning about predators: a review and prospectus. *Learn Behav* 32:131–140
- Griffin AS (2008) Socially acquired predator avoidance: is it just classical conditioning? *Brain Res Bull* 76:264–271. <https://doi.org/10.1016/j.brainresbull.2008.02.005>
- Griffin AS, Blumstein DT, Evans CS (2000) Training captive-bred or translocated animals to avoid predators. *Conserv Biol* 14:1317–1326. <https://doi.org/10.1046/j.1523-1739.2000.99326.x>
- Hartig F (2021) DHARMA: Residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.1. <https://CRAN.R-project.org/package=DHARMA>
- Hoffmann M, Hilton-Taylor C, Angulo A et al (2010) The impact of conservation on the status of the world’s vertebrates. *Science* 330:1503–1509. <https://doi.org/10.1126/science.1194442>
- Hothorn T, Hornik K, Wiel MA, Zeileis A (2008) Implementing a class of permutation tests: the coin package. *J Stat Softw* 28:1–23
- Jolly C, Webb JK, Gillespie GR, Phillips BL (2020) Training fails to elicit behavioral change in a marsupial suffering evolutionary loss of antipredator behaviors. *J Mammal* 101:1108–1116. <https://doi.org/10.1093/jmammal/gyaa060>
- Kraaijeveld-Smit FJL, Griffiths RA, Moore RD, Beebe TJ (2006) Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian: fitness of reintroduced toads. *J Appl Ecol* 43:360–365. <https://doi.org/10.1111/j.1365-2664.2006.01137.x>
- Mathis A, Smith RJF (1993) Fathead minnows, *Pimephales promelas*, learn to recognize northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike’s diet. *Anim Behav* 46:645–656
- McIvor GE, Lee VE, Thornton A (2018) Testing social learning of anti-predator responses in juvenile jackdaws: the importance of accounting for levels of agitation. *R Soc Open Sci* 5:171571. <https://doi.org/10.1098/rsos.171571>

- McPhee ME, Carlstead K (2010) The importance of maintaining natural behaviors in captive mammals. In: Kleiman DG, Thompson KV, Baer CK (eds) Wild mammals in captivity. The University of Chicago Press, Chicago, pp 303–313
- MoehrenschlagerLloyd A (2019) Release considerations and techniques to improve conservation translocation success. In: Jachowski DS, Millspaugh JJ, Angermeier PL, Slotow R (eds) Reintroduction of fish and wildlife populations. University of California Press, Berkeley, pp 245–280
- Moseby KE, Read J, Paton D, Copely P, Hill B, Crisp H (2011) Predation determines the outcome of 10 reintroduction attempts in arid South Australia. *Biol Conserv* 144:2863–2872
- Moseby KE, Cameron A, Crisp HA (2012) Can predator avoidance training improve reintroduction outcomes for the greater bilby in arid Australia? *Anim Behav* 83:1011–1021. <https://doi.org/10.1016/j.anbehav.2012.01.023>
- Moseby KE, Blumstein DT, Letnic M (2016) Harnessing natural selection to tackle the problem of prey naïveté. *Evol Appl* 9:334–343. <https://doi.org/10.1111/eva.12332>
- R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>
- Ross AK, Letnic M, Blumstein D, Moseby KE (2019) Reversing the effects of evolutionary prey naïveté through controlled predator exposure. *J Appl Ecol* 56:1761–1769. <https://doi.org/10.1111/1365-2664.13406>
- Rowell T (2020) Predator-awareness training in terrestrial vertebrates: progress, problems and possibilities. *Biol Conserv* 252:108740. <https://doi.org/10.1016/j.biocon.2020.108740>
- Seddon PJ, Armstrong DP, Maloney RF (2007) Developing the science of reintroduction biology. *Conserv Biol* 21:303–312
- Seddon PJ, Griffiths CJ, Soorae PS, Armstrong DP (2014) Reversing defaunation: restoring species in a changing world. *Science* 345:406–412. <https://doi.org/10.1126/science.1251818>
- Shettleworth S (2010) Cognition, evolution, and behaviour. Oxford University Press, New York
- Shier DM (2016) Manipulating animal behavior to ensure reintroduction success. In: Berger-Tal O, Saltz D (eds) Applying behavioral ecology to wildlife conservation and management. Cambridge University Press, Cambridge, pp 275–304
- Shier DM, Owings DH (2006) Effects of predator training on behavior and post-release survival of captive prairie dogs (*Cynomys ludovicianus*). *Biol Conserv* 132:126–135. <https://doi.org/10.1016/j.biocon.2006.03.020>
- Shier DM, Owings DH (2007) Effects of social learning on predator training and postrelease survival in juvenile black-tailed prairie dogs, *Cynomys ludovicianus*. *Anim Behav* 73:567–577. <https://doi.org/10.1016/j.anbehav.2006.09.009>
- Taylor G, Canessa S, Clarke RH, Ingwersen D, Armstrong DP, Seddon PJ, Ewen JG (2017) Is reintroduction biology an effective applied science? *Trends Ecol Evol* 32:873–880
- Teixeira B, Young RJ (2014) Can captive-bred American bullfrogs learn to avoid a model avian predator? *Acta Ethol* 17:15–22. <https://doi.org/10.1007/s10211-013-0150-8>
- U.S. Fish and Wildlife Service (2009) Revised recovery plan for the ʻalala (*Corvus hawaiiensis*). https://ecos.fws.gov/docs/recovery_plan/090417.pdf
- van Heezik Y, Seddon PJ, Maloney RF (1999) Helping reintroduced houbara bustards avoid predation: effective anti-predator training and the predictive value of pre-release behaviour. *Anim Conserv* 2:155–163. <https://doi.org/10.1111/j.1469-1795.1999.tb00061.x>
- VanderWerf EA, Switzer RA, Lieberman AA, Swaisgood RR (2013) ʻAlalā restoration plan. Pacific Rim Conservation and San Diego Zoo Global, San Diego, pp 121

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.