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Original Article

Evaluating the Efficacy of Carbachol at Reducing Corvid Predation on Artificial Nests

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ABSTRACT Nest predation often limits recovery of threatened and endangered birds, especially ground-nesting species. Accordingly, a variety of techniques are used to reduce the impact of nest predation on listed species. We examined the efficacy of conditioned taste aversion, a nonlethal technique designed to induce avoidance behavior in predators after being exposed to prey items that have been treated, usually with a chemical emetic that causes predators to become ill within minutes of consumption. We used carbachol (carbamyl choline chloride) as a taste-aversive agent to condition corvids responsible for high levels of nest predation on 2 federally listed species—the western snowy plover (*Charadrius nivosus nivosus*) and California least tern (*Sternula antillarum browni*)—breeding at Marine Corps Base Camp Pendleton, California, USA. We conducted 2 separate experiments in 2013 and 2014, during which we deployed 772 artificial nests during the first experiment and 760 artificial nests during the second experiment. Each artificial nest contained 3 quail (*Coturnix* spp.) eggs. During the first stage of both experiments all of the nests only contained untreated quail eggs, and nest predation was high with >90% of nests failing within 1–2 days of deployment. In subsequent stages, we deployed carbachol-treated eggs in increasing proportion. We used nest survival models to evaluate daily survival rates (DSR) of artificial nests in all stages of both experiments. During both experiments, DSR increased concomitant with a greater proportion of carbachol-treated eggs. Common ravens (*Corvus corax*) accounted for 98.1% ($n = 471$) of all artificial nest predations in Experiment 1, and 95.6% ($n = 498$) of all artificial nest predations in Experiment 2. Using carbachol as a taste-aversive agent was effective at reducing predation on artificial nests as illustrated by increased DSR (0.47 to 0.98 in the first experiment and 0.00 to 0.99 in the second experiment); however, transferability of this technique to plover and tern nests was not fully realized. © 2018 The Wildlife Society.

KEY WORDS common raven, conditioned taste aversion, least tern, nest survival, predator management, snowy plover.

Nest predation has been reported as the primary cause of reproductive failure in birds, with implications for the recovery of threatened and endangered species (Ricklefs 1969, Martin 1993, Dowding and Murphy 2001, Pauliny et al. 2008). Accordingly, management efforts for many federally listed avian species often incorporate predator control methods that focus on reducing predation of eggs in an attempt to increase productivity (Côté and Sutherland 1997, Harding et al. 2001, Neuman et al. 2004, Bolton et al. 2007). Along the Pacific coast of the United States, the threatened western snowy plover (*Charadrius nivosus nivosus;* hereafter, "plover") and endangered California least tern (*Sternula antillarum browni;* hereafter, "tern") have been federally listed since 1993 and 1970, respectively (USFWS 2006, 2007). These 2 beach-nesting species experience high rates of nest loss due to predation (USFWS 2006, 2007). Nonlethal (e.g., deterrents and effigies) and lethal (e.g., shooting and poisons) predator-management techniques have been used to minimize these effects (Neuman et al. 2004, Dinsmore et al. 2014). Nonlethal methods are preferred to lethal control because they present a way to decrease predation while minimizing the need to remove native predators. Additionally, nonlethal methods are often supported over lethal control by the public for ethical reasons (Colwell 2010). Conditioned taste aversion (CTA) is a nonlethal management technique that can result in a reduction in specific predatory behavior after predators consume a chemically treated food item that mimics natural prey. As a consequence, predators exposed to this tainted food will generally develop an aversion during subsequent encounters (Nicolaus et al. 1983, Avery et al. 1995, Catry and Granadeiro 2006).

Since the late 1960s, CTA has been tested as a potential method for reducing nest predation by avian egg predators (Nicolaus et al. 1983, Conover 1984, Nicolaus 1987). Several

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chemicals including landrin (Nicolaus et al. 1983, Nicolaus 1987, Dimmick and Nicolaus 1990), methiocarb (Avery et al. 1995), and carbachol (Bogliani and Bellinato 1998, Gabriel and Golightly 2014) have been successfully used to develop CTA in avian predators such as common raven (*Corvus corax*) and American crow (*C. brachyrhynchos*), with carbachol being used more in recent studies. Corvids are known to be important nest predators of both terns and plovers (USFWS 2007, Marschalek 2011, Burrell and Colwell 2012), and corvid populations are increasing in the western United States in response to anthropogenic influences on the environment (e.g., supplemental food sources; Marzluff et al. 1994, 2001; Webb et al. 2004). Many studies have found CTA to be effective for inducing avoidance in corvids, which are intelligent and likely to associate the induced illness with consumption of eggs (Nicolaus et al. 1983, Nicolaus 1987, Dimmick and Nicolaus 1990, Avery et al. 1995, Gabriel and Golightly 2014).

We present experimental evidence using artificial nests to show that carbachol-induced CTA is an effective short-term method for reducing nest loss to corvids. Nest survival models provide a novel approach for evaluating effectiveness of CTA, and may allow researchers to determine proximate causes of nest failure through incorporation of covariates in the model selection process.

STUDY AREA

We conducted this study on the beaches of Marine Corps Base Camp Pendleton (hereafter, "Camp Pendleton") near Oceanside, California, USA (Fig. 1). Camp Pendleton encompassed approximately 50,000 ha and provided habitat for 18 federally listed species, including 27 km of continuous beach and adjacent salt flats used by breeding plovers and terns (MCB Camp Pendleton 2007). During 2001–2009, Camp Pendleton hosted approximately 7% of the entire coastal breeding population of plovers (i.e., CA, OR, and WA, USA), and since 1993, approximately 20% of the breeding population of least terns in California (A. Breton et al., National Audubon Society, unpublished report). Tern and plover nests at Camp Pendleton typically occurred in the beach zone classified as "southern foredune," which was estimated to have 30%–60% vegetation cover, consisting of beach-bur (*Ambrosia chamissonis*), red sand verbena (*Abronia maritima*), sea fig (*Carpobrotus chilensis*), sea rocket (*Cakile maritima*), and beach evening primrose (*Camissoniopsis cheiranthifolia suffruticosa*; MCB Camp Pendleton 2007). Our study plots occurred primarily along the ocean-fronting beaches; however, Plot 1 was placed on a large tidal flat that abutted the eastern edge of the beach (Fig. 1).

METHODS

We conducted this study in 2 separate experiments, the first occurring in 2013 and the second in 2014, with experimental designs varying between years. We conducted experiments prior to the onset of egg laying (i.e., Feb–Mar) for plovers and terns to minimize the effects on their productivity. In both experiments, we used carbachol (carbamylcholine chloride; >99.0% pure, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) to develop an aversion to eggs in corvids. Carbachol is a water-soluble chemical that works as an emetic, causing predators to become ill within minutes of consumption (Bogliani and Bellinato 1998, Gabriel and Golightly 2014). It is also tasteless, colorless, and odorless, which reportedly makes it undetectable by predators and, therefore, a preferred aversive agent (Conover 1984, Nicolaus et al. 1989, Dimmick and Nicolaus 1990, Gabriel and Golightly 2014). We collected data using protocols approved by Humboldt State University's Institutional Animal Care and Use Committee (12/13.W.37-A; Brinkman 2015), and under a Migratory Bird Treaty Act depredation permit issued to Camp Pendleton (MB64809A-0).

Carbachol doses of 24–60 mg (57–209 mg/kg of body mass) have been found to induce aversive behavior in captive Steller's jays (*Cyanocitta stelleri*), hooded crows (*Corvus cornix*), and American crows (Nicolaus et al. 1989, Bogliani and Bellinato 1998, Gabriel and Golightly 2014). Doses used for hooded crows were greater because they do not consume the entire egg's contents (Bogliani and Bellinato 1998). Average body mass of American crows was 0.42 ± 0.03 kg ($n = 30$) and 0.38 ± 0.03 kg ($n = 20$) for males and females, respectively, based on museum records found primarily in California (Verbeek and Caffrey 2002). Common ravens collected at Camp Pendleton had an average mass of 0.81 ± 0.06 kg (0.70–0.88 kg, $n = 6$) and 0.75 ± 0.06 kg (0.68–0.80 kg, $n = 8$) for males and females, respectively (Linz et al. 1990). During the first experiment we used a carbachol dose of 30 mg/egg (i.e., 90 mg/artificial nest because we used 3-egg clutches) to attempt to condition ravens while not overdosing crows. Therefore, if corvids consumed the full 90 mg of carbachol available in a treated artificial nest, they would have ingested doses of 214–237 mg/kg (crows) or 111–120 mg/kg (ravens). Through allometric scaling, the dose we used for crows was similar to that used in Steller's jays (Gabriel and Golightly 2014). The dose we used for ravens was nearly twice the minimum dose found necessary to condition American crows (Nicolaus et al. 1989).

We mixed powdered carbachol with water to create a solution (100 mg/mL) to inject into treatment eggs. We removed approximately 1.5 mL of yolk and albumen from each egg using a 3-cubic-centimeter (cc) syringe with a 16-gauge needle, and then injected 0.3 mL of carbachol solution into the egg using a 1-cc syringe with a 22-gauge needle. We sealed the injection site on the eggs using a hot glue gun. We also pierced untreated (control) eggs with a 16-gauge needle and sealed the hole using a hot glue gun to ensure eggs in control plots appeared similar to treated eggs.

We deployed artificial nests in plots measuring approximately 500 m × 50 m (Fig. 1); beach width in some areas was constrained by dense vegetation and high tides, which resulted in some plots being narrower than 50 m. We positioned these plots on the landscape to allow artificial nests to be placed in areas with relatively sparse vegetation to mimic typical nests of terns and plovers. We deployed artificial nests in each plot at predetermined random

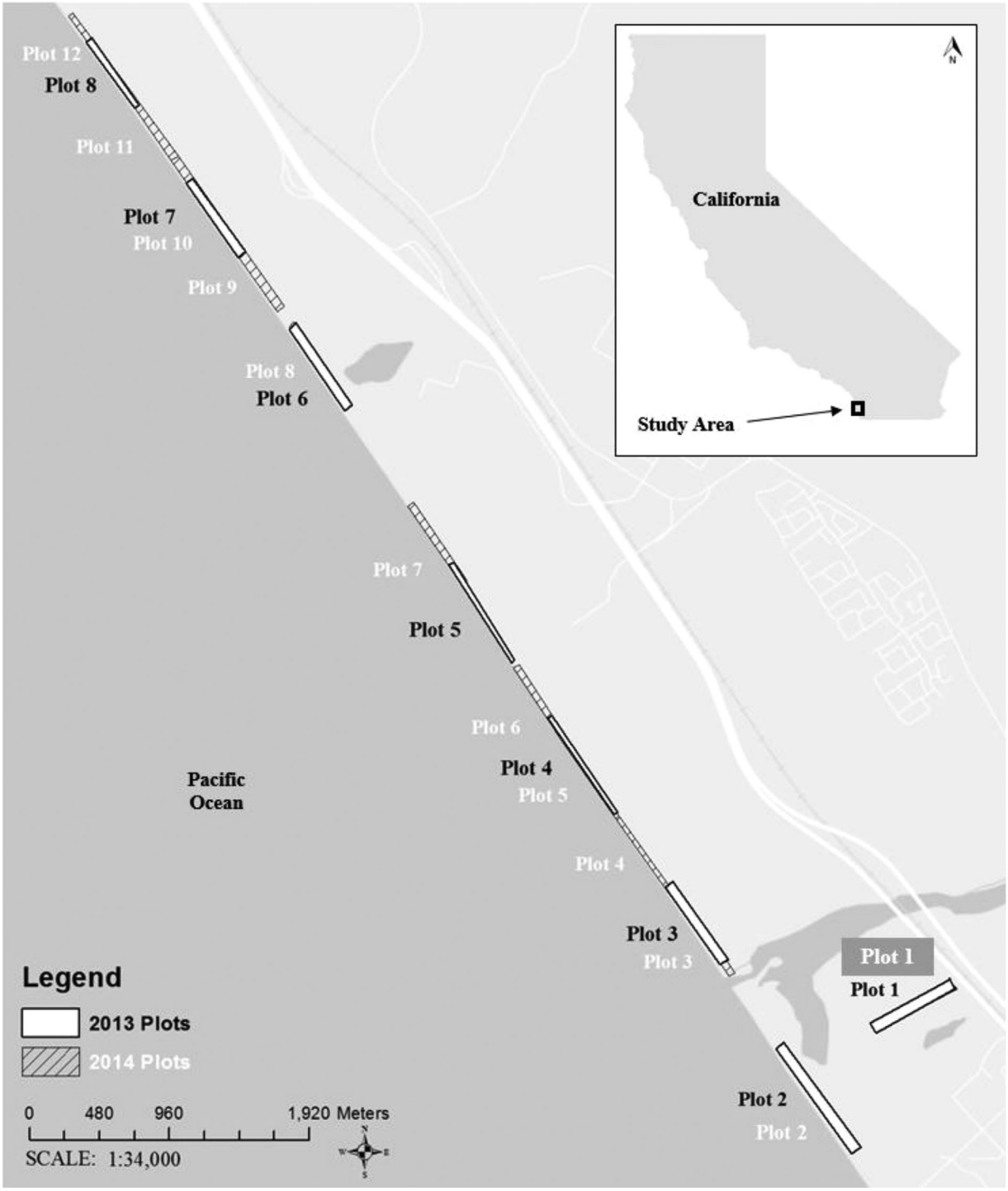

Figure 1. Locations of plots along 10 km of beach at Marine Corps Base Camp Pendleton, California, USA, where carbachol-treated quail eggs were used to evaluate effectiveness of conditioned taste aversion on daily survival of artificial nests in 2013 and 2014.

locations. We recorded Universal Transverse Mercator coordinates for each nest, and marked nests using a wooden stake placed approximately 1 m west of the nest; this method mimicked that used by another group monitoring tern and plover nests on Camp Pendleton during this study. We labeled each stake with the assigned plot and nest number, as well as "CTA" to designate it as an artificial nest rather than a tern or plover nest.

Each artificial nest contained 3 quail (*Coturnix* spp.) eggs, obtained from commercial game farms; eggs were similar in size and shape to those of plovers and terns. Coloration and patterning of quail eggs is highly variable; therefore, we selected eggs that were most similar to those of terns and plovers. We wore latex gloves while handling quail eggs to minimize human scent at the artificial nests. We created artificial nests by making a slight depression in the sand and

placing 3 quail eggs in this nest scrape. We did not deploy eggs if any corvids were within view. If we observed corvids while deploying eggs we immediately stopped and walked away from the plot while attempting to hide the flats of eggs we were carrying. We did not begin placing eggs again until the corvids were out of sight.

We checked each nest at 1–2-day intervals by approaching to within 5 m and inspecting the eggs, remaining at each nest just long enough to determine its status. For nest survival analysis we recorded artificial nests as "failed" only if all 3 eggs disappeared or were broken; we considered a nest "active" if $\geq$1 egg remained in the cup. We decided this method was appropriate for our study because each egg containing carbachol could potentially condition corvids; however, this decision was not necessarily practical in a biological sense because plovers usually abandon their nest after 1 or 2 eggs are predated (M. P. Brinkman, personal observation). We used predator tracks, puncture marks from teeth or beaks on the eggs, and disturbance of the nest cup to identify the predator (i.e., corvid, etc.) responsible for nest failure.

Experiment 1

We deployed artificial nests in 8 plots in the first experiment (Fig. 1). We predicted mammalian predators would consume eggs in artificial nests; therefore, we placed plots 500 m apart to reduce the probability a single mammalian predator would impact adjacent plots. We placed artificial nests on the beach in 3 stages, with each stage lasting approximately 14 days. During the first (Control) stage, we placed quail eggs lacking carbachol in all artificial nests, which provided a baseline estimate of daily survival rate (DSR) to compare with subsequent stages of the experiment. After the first stage, we removed all eggs and deployed new eggs at randomly determined locations within each plot to begin the second stage. We did not refill the same nest locations that were used in the first stage, although some of the same nest locations may have been reused if that nest location was randomly selected. In the second stage, we randomly selected 4 plots to receive a treatment and 4 plots to remain as controls. We placed carbachol-treated eggs in all nests within the treatment plots, and untreated eggs in the control plots. The third and final stage began immediately after the conclusion of the second stage. We removed all remaining eggs from the second stage and once again deployed nests in randomly determined locations. The third stage comprised 7 treatment plots, along with 1 control plot to provide a temporal comparison of DSR between control and treatment nests within this stage. To prevent attracting predators to highly productive plover nesting locations immediately prior to the plover breeding season, we placed the control plot (Plot 8 in Fig. 1) on the northernmost end of the study area rather than randomly selecting its location. During each stage, we replenished the number of nests in a plot to 20 if >50% of the nests in that plot had failed before the end of that stage. We did this by placing new nests at new locations and did not refill nests that had already failed.

Table 1. Methodology of 2 experiments using carbachol-treated quail eggs to induce conditioned taste aversion in corvids at Marine Corps Base Camp Pendleton, California, USA, during 2013 and 2014.

Variable	Experiment 1 (2013)	Experiment 2 (2014)
Carbachol dose	30 mg	60 mg
Number of nests	772	760
Nest density per plot	20	10 nests (low); 20 nests (high)
Number of plots	8	12
Number of stages	3	4
Eggs painted	No	Yes
% Nests predated	61.0%	65.5%

Experiment 2

For the second experiment, we modified some of our methodology based on results from the first experiment, including 1) increasing the number of plots, 2) adjusting the proportion of carbachol-treated eggs deployed, 3) adding a fourth stage, 4) adjusting the density of nests within plots, 5) increasing the carbachol dose, and 6) improving the mimicry of quail eggs by painting them to more closely match those of plovers (Table 1). We increased the number of plots from 8 to 12 in the second experiment because we had little evidence of mammalian predation in the first experiment. The additional plots were created using the 500-m buffer areas originally used to minimize effect of mammalian predators between plots (Fig. 1). Utilizing the buffer areas allowed us to maintain the same beach extent and plot size from the first experiment while increasing the number of plots.

We adjusted the percentage of nests treated within plots to examine whether there was a minimum threshold of treated nests required to improve survivability of artificial nests. We increased this ratio for each treatment stage and also added a fourth stage to the experiment. The first stage was a control, similar to the first experiment, where all 12 plots contained untreated eggs. In the second and third stages of the experiment, we randomly selected 8 plots in which 30% (Stage 2) and 70% (Stage 3) of the artificial nests contained treated eggs. The remaining 4 plots during the second and third stage contained only untreated eggs. During the fourth stage, we deployed carbachol-treated eggs in 100% of the nests in 11 of 12 plots because we expected plovers to begin nesting and wanted to condition any predators that may not have been exposed to carbachol during previous stages. We again selected the northernmost plot (Plot 12 in Fig. 1) to be a control to account for temporal variation during this final stage. We also altered the density of artificial nests in plots to determine whether corvids foraged more in areas with greater nest densities. Prior to each stage, we randomly selected 6 of 12 plots to have high nest density (20 nests/plot) and the remaining 6 to have low density (10 nests/plot). We maintained 6 plots of low and high density during each stage, but randomly determined the density of each plot prior to the initiation of each stage.

We also increased the dose of carbachol from 30 mg/egg in the first experiment to 60 mg/egg in the second experiment because our observations suggested that the carbachol dose

used in the first experiment may have been too low to dissuade ravens from consuming eggs (Table 1). We concluded this because ravens occasionally predated 1 or 2 eggs from a 3-egg clutch, presumably sampling to determine whether it was safe to eat the remaining eggs in that nest. Increasing the dose to 60 mg/egg (i.e., 180 mg/nest) meant that ravens consuming a full clutch could be exposed to 222–240 mg/kg carbachol based on mean raven body mass (Linz et al. 1990). This increased dose was approximately 10% greater than that found to be effective for conditioning Steller's jays (209 mg/kg mean body mass; Gabriel and Golightly 2014).

The last modification we made to the methodology between experiments was painting the quail eggs to better imitate plover eggs. Previous CTA studies have experimented with different colored eggs to represent treatment and control groups. In these studies, corvid species showed avoidance to eggs of the treatment color even when they were subsequently presented with nontoxic eggs (Nicolaus et al. 1983, Bogliani and Bellinato 1998, Gabriel and Golightly 2014). Corvids rely on visual cues to locate food, so the treated food item should appear as similar as possible to the resource that we are attempting to protect. Painting the quail eggs for the second experiment allowed us to mimic the patterning and coloration of plover eggs with relative consistency. We placed untreated and treated eggs on a makeshift table made of chicken wire and lumber, and used a paint sprayer (Wagner Spraytech Inc., Plymouth, MN, USA) to coat the eggs in a base color. We used exterior acrylic paint with a flat sheen to coat the quail eggs, and selected the color "Jungle Camouflage" because it closely resembled the base color of plover eggs (Color code #N350-4; Behr Corp., Santa Ana, CA, USA). After the base coat had dried, we used a toothbrush to speckle black paint on the eggs to attempt to replicate the speckling pattern on plover eggs. We kept painted eggs refrigerated for a minimum of 2–3 days before deploying them on the beach. This allowed time for the paint to dry and odor to evaporate. It is unlikely that corvids could discern these eggs by smell because we could not detect a paint odor <1 day after painting.

Analytical Methods

We used the "RMark" package in Program R to analyze nest survival and estimate daily survival rate (DSR) of artificial nests during each stage of the experiment (Laake 2013, R Core Team 2014). The RMark package is an interface that allowed us to utilize nest-survival model designs developed in Program MARK (White and Burnham 1999, Dinsmore et al. 2002, Dinsmore and Dinsmore 2007, Rotella 2014). We created a set of competing models and compared them using Akaike's Information Criterion corrected for small sample sizes (AIC_c; Burnham and Anderson 2002). Program MARK requires a single number to be entered for "encounter occasions"; therefore, we set this number to 40 in the first experiment and 44 in the second experiment because this was the total length of each experiment. We included covariates in the models that we anticipated might influence variation in DSR of artificial nests (Table 2). These variables were included to eliminate the possibility of missing an important component influencing DSR of artificial nests (i.e., to evaluate whether CTA was the driving factor behind increased DSR).

RESULTS

Experiment 1

We placed 772 artificial nests among 8 plots during 3 stages of the first experiment (Table 1; Appendix A, in Supporting Information available online). All nests ($n = 298$) failed within 2 days of deployment in Stage 1, so there did not appear to be a difference in predation pressure among plots. Of the total nests deployed, 513 (66.5%) were untreated control nests and 259 (33.5%) nests contained eggs treated with carbachol. Overall, 471 nests (61.0% of total nests) failed due to predation, with a larger percentage of control nests (80.3%) predated than treatment nests (22.8%; $\chi^2_1 = 368.57$, $P \leq 0.001$). We determined that common ravens were the primary predators of artificial nests based on tracks found at failed nests (462 nests; 98.1%); remaining predated nests had tracks of American crows (5 nests; 1.1%), coyotes (*Canis latrans*, 3 nests; 0.6%), and a striped skunk (*Mephitis mephitis*, 1 nest; 0.2%). Additionally, 85 control

Table 2. Variables hypothesized to explain variance in daily nest survival of artificial nests deployed as part of a conditioned taste aversion study conducted at Marine Corps Base Camp Pendleton, California, USA, in 2013 and 2014.

Variable	Abbreviation	Justification
Control or treatment nest	TREATMENT[a]	Many studies found that predators exposed to carbachol treated eggs were conditioned to avoid that prey source on future visits to those sites (e.g., Nicolaus et al. 1989, Prescott et al. 1997, Gabriel and Golightly 2014)
Stage of experiment	STAGE[a]	We predicted that artificial nests occurring in the second and third stage of the experiment would have a greater daily survival rate than those in the first stage, because predators would become conditioned to avoid nests after being exposed to eggs treated with carbachol.
Plot	PLOT[a]	Another study at Camp Pendleton concluded that ravens were able to discern between sites that had treated eggs and sites that did not make them ill (Avery et al. 1995). Including plot as a covariate provided us a spatial component to evaluate daily survival rate.
Density of nests within a plot	DENSITY[b]	We randomly selected whether a plot would contain 10 or 20 artificial nests upon initial deployment to determine if ravens would focus on sites with greater prey availability

[a] Variable was used to evaluate models during both 2013 and 2014 field experiments.
[b] Variable was only used to evaluate models during 2014 field experiment.

Table 3. Results of nest survival analyses evaluating the effects of covariates on daily survival rate of artificial nests with carbachol-treated eggs at Marine Corps Base Camp Pendleton, California, USA, in 2013.

Model name[a]	K[b]	AIC_c[c]	ΔAIC_c[d]	w_i[e]	Deviance[f]
S(TREATMENT + STAGE)	4	1,982.93	0.00	0.84	1,974.92
S(TREATMENT + STAGE × TREATMENT)	6	1,986.20	3.26	0.16	1,974.18
S(STAGE)	3	2,086.81	103.87	0.00	2,080.80
S(TREATMENT + PLOT)	3	2,120.49	137.56	0.00	2,114.48
S(TREATMENT)	2	2,152.97	170.04	0.00	2,148.96
S(PLOT)	2	2,825.84	842.90	0.00	2,821.83
S(.)	1	2,839.43	856.50	0.00	2,837.43

[a] Model name including covariates used. "S" indicates survival.
[b] Number of parameters.
[c] Value of Akaike's Information Criterion corrected for small sample size calculated for each model.
[d] Difference in AIC_c value from top-ranked model.
[e] Proportion of total wt calculated for each model.
[f] Quality of fit.

nests and 25 treatment nests failed for reasons unrelated to predation (tidal over-wash, human-caused, and unknown cause of failure).

We evaluated 7 nest survival models for the first experiment (Table 3). The top-ranked model included covariates TREATMENT and STAGE, and a second-ranked model that contained an interaction between those 2 variables also carried some model weight (Table 3). The top 2 models had similar AIC_c scores; therefore, we used model-averaging to estimate values of DSR for each stage and treatment (Burnham and Anderson 2002, Symonds and Moussalli 2011). Treatment nests had a greater estimated DSR than control nests; however, DSR of control nests also increased between stages (Table 4). There was a smaller difference in DSR between control and treatment nests during the third stage compared with the second stage (Table 4). Estimates of DSR extrapolated (by raising the estimated DSR value to an exponent defined by the 28-day incubation period for snowy plovers) to hatching success of 17.7% and 56.8% for treatment nests, and 0.0%, 0.0%, and 7.1% for control nests for each stage of the experiment.

Experiment 2

We placed 760 artificial nests among 12 plots throughout 4 stages of the second experiment (Table 1; Appendix A, in Supporting Information available online). Of the total nests deployed, 481 (63.3%) were untreated control nests and 279 (36.7%) contained eggs treated with carbachol. Overall, 498 nests (65.5% of total nests) failed due to predation, with a larger percentage of control nests (84.4%) predated than treatment nests (33.0%; $\chi^2_1 = 183.07$, $P \leq 0.001$). As with the first experiment, we determined the primary predators of artificial nests to be common ravens based on tracks found at the predated nests (476 nests; 95.6%), whereas remaining predated nests had tracks of American crows (22 nests; 4.4%). Fewer artificial nests failed for reasons unrelated to predation (12 control and 19 treatment nests) in the first experiment than the second, although these causes of failure were the same in each experiment.

We evaluated 8 nest survival models for the second experiment (Table 5). The top 3 models had an AIC_c score <2.0 from the top model, so we used model-averaging to determine estimates of survival for each stage and treatment within stage (Burnham and Anderson 2002, Symonds and Moussalli 2011; Table 5). The top-ranked model contained only STAGE as a covariate (Table 5). The second-ranked model contained an interaction term between STAGE and TREATMENT, and the third model also contained the STAGE and TREATMENT variables. Although the second and third models carried some weight, this was likely due to the explanatory power of the STAGE variable because models containing an effect of treatment without the STAGE variable did not contain any weight (Table 5). Nest density within plots did not appear to influence DSR; the model containing the DENSITY covariate was ranked low and had a high ΔAIC_c value (Table 5). The estimate of DSR

Table 4. Results of 2 experiments using carbachol-treated quail eggs to induce conditioned taste aversion in corvids at Marine Corps Base Camp Pendleton, California, USA, during 2013 and 2014. DSR, daily survival rate.

	Experiment 1 (2013)			Experiment 2 (2014)		
	No. nests	DSR estimate (95% CI)	% treated nests	No. nests	DSR estimate (95% CI)	% treated nests
Stage 1 (control)	298	0.47 (0.43–0.52)	No treatment	180	0.00 (0.00–0.00)	No treatment
Stage 2 (control)	185	0.78 (0.75–0.81)	All 20 nests were treated	178	0.30 (0.25–0.35)	30% of nests were treated in
(treatment)	113	0.94 (0.92–0.96)	in 4 of 8 plots	42	0.29 (0.22–0.38)	8 of 12 plots
Stage 3 (control)	30	0.91 (0.84–0.95)	All 20 nests were treated	103	0.90 (0.88–0.92)	70% of nests were treated in
(treatment)	146	0.98 (0.97–0.99)	in 7 of 8 plots	77	0.90 (0.88–0.92)	8 of 12 plots
Stage 4 (control)				20	0.99 (0.97–1.00)	100% of nests were treated in
(treatment)				160	0.99 (0.98–0.99)	11 of 12 plots

Table 5. Results of nest survival analyses evaluating the effects of covariates on daily survival rate of artificial nests with carbachol-treated eggs at Marine Corps Base Camp Pendleton, California, USA, in 2014.

Model name[a]	K[b]	AIC_c[c]	ΔAIC_c[d]	w_i[e]	Deviance[f]
S(STAGE)	4	1,072.58	0.00	0.55	1,064.57
S(TREATMENT + STAGE × TREATMENT)	8	1,074.31	1.73	0.23	1,058.27
S(TREATMENT + STAGE)	5	1,074.42	1.84	0.22	1,064.41
S(TREATMENT + PLOT)	3	2,154.60	1,082.02	0.00	2,148.60
S(TREATMENT)	2	2,170.40	1,097.82	0.00	2,166.40
S(DENSITY)	2	2,652.79	1,580.21	0.00	2,648.78
S(.)	1	2,657.56	1,584.98	0.00	2,655.56
S(PLOT)	2	2,659.50	1,586.92	0.00	2,655.50

[a] Model name including covariates used. "S" indicates survival.
[b] Number of parameters.
[c] Value of Akaike's Information Criterion corrected for small sample size calculated for each model.
[d] Difference in AIC_c value from top-ranked model.
[e] Proportion of total wt calculated for each model.
[f] Quality of fit.

for Stage 1 of the second experiment was essentially nonexistent because every nest ($n = 180$) failed within 2 days of deployment (Table 4). Model-averaged estimates of DSR were similar for treated and control nests during stages containing a treatment effect (Table 4). These estimates of DSR extrapolated to hatching success of 0.0%, 5.2%, and 75.5% for treatment nests, and 0.0%, 0.0%, 5.2%, and 75.5% for control nests during each stage of the experiment.

DISCUSSION

Similar to other CTA studies, we found carbachol to be an effective aversive agent for reducing predation of artificial nests (e.g., Bogliani and Bellinato 1998, Cox et al. 2004, Gabriel and Golightly 2014). We demonstrated the ability to condition ravens to avoid quail eggs in both experiments; however, we did not observe any evidence that the conditioning from the first experiment carried over into the following experiment. We lost all 180 artificial nests to ravens within 2 days of deploying eggs during the control stage of the second experiment. Although it would have been instructive to determine the effectiveness of CTA between experiments, this study was not designed to make this assessment. It would have required marking ravens present in the study area to determine if individuals potentially exposed to carbachol in the first experiment were still present at the start of the second experiment. We also lethally removed 22 ravens after completion of the first experiment because we found evidence that these ravens were predating plover nests (M. P. Brinkman and D. K. Garcelon, unpublished report). Vacant territories created by lethal removal would likely be filled by immigrant ravens that had not been previously conditioned to avoid eggs. Finally, retention times of CTA-facilitated avoidance behavior have been reported to last through the entirety of studies—as long as 8 weeks in jays (Gabriel and Golightly 2014), 66 days in crows (Dimmick and Nicolaus 1990), and our study showed avoidance of quail eggs for 40 and 44 days in ravens. It has also been suggested that avoidance behavior was retained for ≥1 year in crows after exposure to Landrin, but most crows did not seem to retain this behavior (Dimmick and Nicolaus 1990). It seems likely that CTA would need to be reinforced annually prior to the onset of breeding by the species of concern, but to determine the necessity of this it would be informative for additional studies to analyze retention of CTA over periods ≥1 year.

Daily survival rate of artificial nests increased during each consecutive stage of both experiments, suggesting that ravens began avoiding eggs after consumption of carbachol-treated eggs. We observed ravens foraging in our study area throughout the length of both experiments, which further supports the efficacy of CTA because these predators were still present but ceased predation of artificial nests. During the final stage of both experiments, daily survival rate of carbachol-treated nests was sufficiently high to result in acceptable hatching success had these been plover nests (56.8% in the first experiment and 75.5% in the second experiment). Daily survival rate also increased for control nests from the first to last stages, indicating that CTA positively influenced survival of untreated nests. This may be our most important result; control nests in this study served as surrogates for actual plover nests, and improvement of daily survival rate in untreated nests suggests CTA may reduce raven predation on plover nests.

In the first experiment, we observed variance in predation rates between treated and control nests occurring in the same stage, with lower daily survival rate for control nests. At first this may seem like an intuitive result; however, carbachol has been reported to be undetectable by sight, smell, and taste, so it is unlikely that ravens would be able to determine whether an egg was treated. It is possible instead, that ravens identified specific areas as unsafe to consume eggs based on previous experience and avoided sites with food items that made them ill. A CTA study conducted in 1991 and 1992 at Camp Pendleton used methiocarb to condition territorial ravens to avoid quail eggs, and results from that study also suggested there may be a site effect because ravens consumed eggs at a greater rate from untreated sites than those sites containing treated eggs (Avery et al. 1995). If ravens are relying on spatial cues to determine safe areas for egg consumption, then combining treated and untreated nests within plots, as we did in the second experiment, may make

ravens more risk averse with respect to sampling eggs. This may be the reason we observed similar daily survival rates between treated and untreated nests in the second experiment. Ensuring that all protected areas have adequate coverage of treated eggs will make it difficult for ravens to find areas where eggs are safe to eat, and could potentially discourage ravens from occupying those sites altogether. Use of CTA may also be more effective when targeting breeding ravens that will defend territories rather than nonbreeding individuals or groups of "floater" birds (Colwell 2010). By conditioning breeding ravens, not only will they no longer be affecting the protected species, but they will also defend their territory from other ravens that may not have been conditioned to avoid eggs (Nicolaus 1987, Avery et al. 1995). This is an important consideration when determining the amount of effort required to effectively utilize CTA at a given site. It would benefit managers to determine the territoriality of ravens at a given location prior to deciding whether CTA is an appropriate method for that site because the effort required to condition groups of floater ravens may be considerably greater than what is required for territorial individuals. Nonbreeding ravens may be 1) in larger groups and 2) ephemeral in their use of an area; therefore, employing CTA may be inefficient in conditioning them to avoid eggs.

In the later stages of both experiments, we noticed more nests being partially predated, losing 1 or 2 eggs rather than all 3. We only considered a nest "failed" when all 3 eggs were gone, so our models did not account for this partial-predation unless that nest ultimately failed before the end of the stage. Utilizing daily survival rate for individual eggs instead of individual nests may not be appropriate in this case, given the fate of an individual egg would rarely be independent from the fate of the other eggs in that clutch. For this study it may have been more suitable to code nests as failed when 1 egg was predated because birds may abandon their nest after a partial predation occurs (ducks [Anatinae]—Ackerman et al. 2003; plovers—M. P. Brinkman, personal observation); however, recoding nests and reanalyzing those data would not likely change these results because we did not observe any partial predations during the early stages of either experiment.

Our findings from the second experiment furthered our understanding of the effort required for effective CTA. We determined that increasing the treatment level from 30% to 70% tripled daily survival rate of artificial nests, and a treatment level of 100% resulted in a daily survival rate of nearly 1. Although daily survival rate increased to 0.90 for artificial nests at the 70% treatment level, this daily survival rate would result in low hatching success (5.2%) over a plover's 28-day incubation period. The daily survival rate at the 100% treatment level (0.99) would result in acceptable hatching success (75.5%), but this ratio of treated to untreated nests is only possible in the absence of plover nests. Though extrapolated, hatching success for plover nests was low at a 70% treatment level; this stage of the experiment followed the 30% treatment. This sequence likely resulted in nest failures during the initiation of the 70% treatment stage, prior to ravens achieving adequate conditioning to avoid artificial nests. Had ravens been conditioned during a previous stage, or we only tested daily survival rate for the second half of the 70% treatment stage, daily survival rate would likely have been greater than we observed. We did not evaluate treatment levels between 30%, 70%, and 100%, so we cannot say what the results would be with intermediate levels of treatment; however, we would expect daily survival rate to improve with increasing levels of treatment because this is the trend we observed during this experiment.

The number of treated nests could be modified with varying breeding activity throughout the season to maintain an appropriate ratio of treated to untreated nests. For example, if there were 20 plover nests in a given location, there should be $\geq$47 artificial nests containing carbachol-treated eggs to ensure 70% of total nests in that area are treated. Theoretically, the size of the study area should not matter as long as this minimum ratio is maintained. Of course, these results are predicated on the fact that ravens cannot discern between actual nests and artificial nests. If ravens can learn to distinguish real nests from artificial nests (even those with painted quail eggs), the value of using CTA becomes more limited.

Results from both experiments demonstrated the potential to use CTA as a nonlethal management technique to improve daily survival rate; however, although these results suggested that carbachol increases survival of artificial nests, this effect did not completely transfer to protection of plover and tern nests. Approximately 1 month after the end of first experiment, ravens at Camp Pendleton were deemed responsible for predation of >35 plover nests based on tracks found at the nest (M. P. Brinkman and D. K. Garcelon, unpublished report). Anecdotally, it appeared that the painted quail eggs improved transferability of CTA to plover nests; fewer ravens were observed predating plover nests in 2014 ($n = 4$) than 2013 ($n = 22$; M. P. Brinkman and D. K. Garcelon, unpublished report). Four ravens were able to discern plover nests from artificial nests in 2014; they were observed flying directly between plover nests while ignoring neighboring artificial nests (M. P. Brinkman, personal observation). Plover nests are relatively simple, consisting of a depression in the sand lined with small pebbles or shells; therefore, creating seemingly accurate representations of these nests was not difficult (Page et al. 2009). However, if ravens were focusing on the adult plover leaving the nest after incubating, as described by Burrell and Colwell (2012), ravens would then be able to associate adult plovers with nests that are safe to eat. It would be difficult to imitate this plover behavior at artificial nests. There may be other subtleties that ravens could use to discern between artificial nests and plover nests that we did not account for: 1) tracks from incubating adult plovers going to and from their nest; 2) without incubating adults, the quail eggs in artificial nests can begin to spoil in high temperatures, thus giving off a rotten odor; and 3) avian species can view color into the ultraviolet spectrum, so although our painted eggs looked similar to plover eggs to our eyes, they may appear different to a raven (Cuthill et al. 2000). There may be additional differences; but if ravens use any of these cues to discover

plover nests as safe to eat, it would make transferability of CTA to improve daily survival rate of plover nests challenging.

Both experiments were conducted over 6–7 weeks during February–March, prior to the onset of breeding by plovers, so it is unlikely that there was any significant seasonal variation in predation pressure by corvids. However, seasonal variation in predation pressure may be an important component to consider when determining the effectiveness of CTA. Similar to plovers, ravens breed in the spring, and their foraging rate and food requirements increase when they are feeding their nestlings (Kelly et al. 2005). This requirement may make ravens more willing to risk becoming ill by sampling eggs because the ability to discern "good" eggs equates to a rich food source to bring to their offspring. It is also plausible that resident ravens may be aware of the seasonal variation in the abundance of eggs once plovers and other beach-nesting species begin breeding. Therefore, for CTA to be effective, it may be important to condition resident ravens prior to the breeding season and continue deployment of treated eggs through the season to minimize the likelihood that ravens find enough untreated eggs to make egg predation worthwhile.

MANAGEMENT IMPLICATIONS

Finding effective nonlethal techniques will benefit the native predator community by reducing the need for their lethal control, concomitantly minimizing predator impact on the species of concern. Although we found CTA to be effective at increasing survival of artificial nests, transferability of this technique to plover and tern nests was not evaluated, and therefore, further research into applicability of CTA is necessary. It would be prudent to deploy treated eggs prior to the onset of each breeding season to condition new individual corvids and reinforce conditioning for residents that remained into the next season. To effectively employ CTA as a management technique, placing and maintaining treated nests for approximately 1 month prior to initiation of nesting by the target species would provide time for the resident corvid population to be conditioned. After the target species begins nesting, a ratio of >70% treated artificial nests to untreated nests (e.g., plover nests) should be maintained throughout the breeding season for the species of concern. To be most effective, artificial nests and eggs within those nests should appear as similar as possible to the species of concern. The length of time that ravens will avoid nests after exposure to carbachol varies and may depend on the size and territoriality of the local raven population. Conditioned taste aversion, as used in this study, would only condition ravens to avoid eggs, not nestlings or young chicks. Therefore, hatching success may be improved through CTA, but the benefit of this increase would not be transferred to the next life stage if plover chicks are eaten before fledging. Consequently, CTA may be one tool in the "chest" of multiple tools needed to manage negative effects of an intelligent synanthropic omnivore such as the raven.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site. Results of CTA experiments (2013 and 2014) showing percent of artificial nests in which eggs disappeared (i.e., unsuccessful and presumed predated) during 14-day intervals.