

Behavioral Changes, Stress, and Survival Following Reintroduction of Persian Fallow Deer from Two Breeding Facilities

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Abstract: Reintroductions often rely on captive-raised, naïve animals that have not been exposed to the various threats present in natural environments. Wild animals entering new areas are timid and invest much time and effort in antipredator behavior. On the other hand, captive animals reared in predator-free conditions and in close proximity to humans may initially lack this tendency, but can reacquire some antipredator behavior over time. We monitored the changes in antipredator-related behaviors of 16 radio-collared Persian fallow deer (*Dama mesopotamica*) reintroduced to the Soreq Valley in Israel from 2 breeding facilities: one heavily visited by the public (The Biblical Zoo of Jerusalem, Israel) and the other with reduced human presence (Hai-Bar Carmel, Israel). We monitored each individual for up to 200 days after release, focusing on flush and flight distance, flight mode (running or walking), and use of cover. In addition, we compared fecal corticosterone (a stress-related hormone) from samples collected from known animals in the wild to samples collected in the breeding facilities. Reintroduced individuals from both origins exhibit increased flush distance over time; flush and flight distances were larger in individuals from Hai-Bar; use of cover increased with time, but was greater in Hai-Bar Carmel animals; corticosterone levels were significantly higher in fecal samples from reintroduced animals than in samples from captive animals; and Hai-Bar Carmel animals had an 80% survival rate over the 200 days, whereas no animals from the Biblical Zoo of Jerusalem survived. Reintroduced Persian fallow deer reacquired antipredator behaviors after the release, but the process was slow (months) and differences between conditions at the breeding facilities that were seemingly benign (e.g., number of visitors and other human related activities) influenced this process and consequently affected the success of the reintroduction. Captive breeding facilities for the purpose of reintroduction should minimize anthropogenic disturbances.

Keywords: behavior, corticosterone, *Dama mesopotamica*, Persian fallow deer, reintroduction, stress

Cambios Conductuales, Estrés y Supervivencia Después de la Reintroducción de *Dama mesopotamica* Proveniente de Dos Criaderos

Resumen: Las reintroducciones a menudo confían en animales inocentes, criados en cautiverio que no han sido expuestos a las diversas amenazas presentes en ambientes naturales. Los animales silvestres que entran en nuevas áreas son tímidos e invierten mucho tiempo y esfuerzo en conductas antidepredadores. Por otro lado, los animales cautivos criados en condiciones libres de depredadores y cerca de humanos inicialmente pueden

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carecer de esta tendencia, pero en el tiempo pueden readquirir algunas conductas antidepredadores. Monitoreamos los cambios en conductas antidepredadores de 16 individuos de gamo persa (*Dama mesopotamica*) marcados con radio collar reintroducidos en el Valle Soreq en Israel provenientes de dos criaderos: uno muy visitado por el público (El Zoo Bíblico en Jerusalén) y el otro con presencia humana reducida (Hai-Bar Carmel). Monitoreamos cada grupo individual hasta 200 días después de liberado, enfocando la distancia de alerta y huida, modo de huida (corriendo o caminando) y uso de cobertura. Adicionalmente comparamos la corticoesterona fecal (hormona relacionada con el estrés) de muestras recolectadas de animales silvestres conocidos con muestras recolectadas en los criaderos. Individuos reintroducidos de ambos orígenes mostraron incremento en la distancia de alerta, las distancias de alerta y huida fueron mayores en individuos de Hai-Bar, el uso de cobertura incrementó en el tiempo, pero fue mayor en animales de Hai-Bar Carmel; los niveles de corticoesterona fueron significativamente mayores en muestras fecales de animales reintroducidos que en animales cautivos; y los animales Hai-Bar Carmel tuvieron una tasa de supervivencia de 80% en 200 días, mientras que no sobrevivió ningún animal del Zoo Bíblico de Jerusalén. Los gamos persas reintroducidos readquirieron conductas antidepredadores después de su liberación, pero el proceso fue lento (meses) y las diferencias en las condiciones entre criaderos que aparentemente fueron benéficas (e.g., número de visitantes y otras actividades humanas relacionadas) influyeron en el proceso y consecuentemente afectaron el éxito de las reintroducciones. Los criaderos con objetivos de reintroducción deberían minimizar las perturbaciones antropogénicas.

Palabras Clave: conducta, corticoesterona, *Dama mesopotamica*, estrés, gamo persa, reintroducción

Introduction

Understanding the factors that affect the probability of establishment of reintroduced populations is central in reintroduction biology (Armstrong & Seddon 2008). Because captive animals are not subject to the threats and harsh conditions that exist in nature, reintroduced captive-born animals are commonly thought to lack the survival skills of wild-born animals, which hampers the success of reintroductions (Beck et al. 1994; McPhee 2003; Mathews et al. 2005). Thus, the ability of captive-born organisms to readapt to life in the wild is a major concern in animal conservation (Griffin et al. 2000; Letty et al. 2000; Banks et al. 2002). The performance of captive-born animals is poorer, in terms of survival and reproduction, than that of translocated wild-born animals (Ginsberg 1994; Shepherdson 1994; Banks et al. 2002). The environment in which the captive animals are raised may affect their behavior and plays an important role in the ability of reintroduced animals to adapt to the wild (Wielebnowski 1998).

Two interrelated factors are likely to affect the survival of reintroduced animals: lack of experience-based survival skills and rate at which these skills are acquired following the reintroduction (Shepherdson 1994). Box (1991) describes 5 skills required for survival in the wild: orientation and locomotion; feeding and foraging; obtaining suitable places to rest and sleep; intraspecific interactions; and interspecific interactions, including predator avoidance. Many authors (Griffith et al. 1989; Bright & Morris 1994; Wolf et al. 1996; Fischer & Lindenmayer 2000) point to the lack of predator avoidance skills as a major factor in the failure of reintroductions.

Prey reared in a predator-free environment may lose their antipredatory behavior and become predator naïve

(Diamond 1990; Berger et al. 2001; Blumstein & Daniel 2005), but can reacquire antipredatory behavior when exposed to predators (Byers 1997; Laundré et al. 2001). Accordingly, reintroduced animals are believed to slowly reacquire some antipredatory behavior after they are released in the wild and exposed to predators (Banks et al. 2002), yet no work has shown this empirically (Seddon et al. 2007). The rate at which reintroduced animals reacquire antipredatory and other threat-related behaviors directly affects the animals' ability to survive and is, therefore, critical to the success of the reintroduction.

Conditions under which animals are kept in captive-breeding facilities vary. Prey species often view humans as predators (Andersen et al. 1996), but high levels of exposure to humans may induce habituation, which affects the animals' timidity and amount of time devoted to vigilant behavior (Tyler 1991). Furthermore, the constant exposure to a multitude of stimuli may dampen the animal's attention to novel situations. We focused on the process of reacquiring antipredator-related behaviors—including flight responses and habitat use—of reintroduced Persian fallow deer (*Dama mesopotamica*) from 2 breeding facilities in Israel, the Biblical Zoo of Jerusalem and Hai-Bar Carmel. The facilities are generally similar, but differ considerably in terms of levels of exposure to humans and human-related activities. We hypothesized that individuals with high exposure to human activities exhibit dampened antipredator behaviors (e.g., flush and flight distance, mode of flight, use of cover) and will take longer to reacquire these traits. We complemented our behavioral study with a comparison of stress responses (as determined from fecal corticosterone) and survival of reintroduced deer from the 2 sources.

Methods

Study Area and Species

The Soreq Valley Nature Reserve is in the Judean Mountains, Israel (coordinates: 31°45'N 35°04'E). The terrain is rugged, with elevations ranging from 450 to 800 m. Vegetation is heterogeneous. There are open areas with annual herbs, garigue with short bushes, stream vegetation, and dense Mediterranean woodland (maquis) dominated by common oak. The stream in the Soreq Valley is perennial, consisting mostly of treated water suitable for drinking. The reserve is surrounded by roads and villages and is crossed by an active railway. The golden jackal (*Canis aureus*) is the dominant predator in the region and is abundant throughout the reserve. Climate is mild, with cold wet winters and dry summers. The perimeters of the study area were delineated according to the movement of the reintroduced animals.

The Persian fallow deer is a medium to large deer (females: 50–70 kg; males: 80–100 kg.). They eat grass, leaves, and fruits and use dense Mediterranean woodland (maquis) for cover and open areas for feeding. Most of the time they move alone, but occasionally they are found in small groups of 2 to 8 individuals (Dolev et al. 2002; Perelberg et al. 2003; Bar-David et al. 2005). The species is listed as endangered (<http://www.iucnredlist.org>).

For this reintroduction, deer came from 2 sources—the Biblical Zoo of Jerusalem (JBZ) and Hai-Bar Carmel (HBC). The zoo is at the edge of the city of Jerusalem, is open to the public, and is visited by approximately 700,000 people annually (S. Yedvab, personal communication). It has many entertainment activities, including a tram that regularly and frequently passes by the deer enclosure. About 60 deer roam (with a few mountain gazelles [*Gazella gazelle*] and 20 scimitar-horned oryx [*Oryx dammah*]) in a 2.5-ha enclosure. The pen is traversed by a wooden bridge 1.5 m above the ground, which allows the public to observe the deer from above and at very short distances. An intercity railroad track is adjacent to the zoo. All fallow deer at JBZ are descendants of individuals received from HBC since 1997.

The HBC facility is operated by the Nature and Parks Authority for the breeding of threatened species intended for reintroduction. The facility is located within the Carmel Nature Reserve in a relatively pristine environment and consists of numerous fallow deer enclosures (2–3 ha) that hold groups of various sizes (15–40 individuals). Although it is open to the public, visiting hours are limited and visits are allowed only in groups escorted by a Nature and Parks Authority guide. Many of the enclosures are not accessible to the public. The number of visitors per year is approximately 30,000 (D. Rotem, personal communication). Except for a few trees that provide shade, the enclosures in both facilities are void of vegetation.

Table 1. The number of released individuals, their age, and sex in each of the release cycles in the Soreq Valley reintroduction of fallow deer.*

Source	1st release cycle	2nd release cycle	3rd release cycle	4th release cycle	Total released
JBZ					
female	2A, 1S	2A	2A	0	7
male	1S	4A	2S	0	7
HBC					
female	0	2A	1A	1S	4
male	0	2S	2S	2S	6
Total released	4	10	7	3	24

*Abbreviations: JBZ, Biblical Zoo of Jerusalem; HBC, Hai-Bar Carmel; A, adult; S, subadult.

Reintroduction Procedure

Animals were transported by truck from the breeding facilities to a ~2-ha habituation enclosure that bordered the stream at the bottom of the Soreq gorge. The animals remained in the enclosure for about 2 months and were then released by removing a section of the fence and allowing the animals to exit at will (Dolev et al. 2002). During the research period (May 2005–August 2006), 24 individuals were released in 4 cycles: 14 animals (7 male and 7 female) from the JBZ and 10 animals (6 male and 4 female) from HBC (Table 1). All animals were either young adults (2–4 years old) or subadults, and age and gender were roughly equally represented for both facilities. Twenty-one animals were radio collared: 10 male (4 from JBZ and 6 from HBC) and the rest female. All radios included mortality sensors.

Field Methods and Data Analyses

We used telemetry to visually locate the deer. We attempted to relocate all individuals in the reintroduced population 4 to 6 times a week and obtained data on each individual for up to 200 days following its release from the enclosure. Observations from the first release cycle were excluded because these animals were released in an area without conspecifics and all released deer were from JBZ.

Each time an animal was sighted, we documented 3 components of flight behavior (estimated visually by approaching the animal): flush (escape) distance (Andersen et al. 1996), the distance at which the individual began moving away from the observer; flight distance, how far the animal moved away before it stopped or resumed its previous behavior (this parameter was quantified as long as it was not over 10 meters; for larger distances it was labeled over 10 m because after 10 m the animals usually disappeared into the bush); flight mode, running or walking. To assess habitat preference as it related to

antipredator behavior, we noted where the animal was located in terms of cover type (i.e., open areas and open woodland or dense Mediterranean woodland). We also recorded other conditions under which the animal was sighted and which may be flight related, including group size, animal posture when sighted (standing or lying), and whether the animal was feeding. These conditions and the animal's responses were recorded immediately when the animal was first sighted. For each animal we recorded no more than one sighting per day.

We used an information-theoretic approach (Burnham & Anderson 2002) based on multiple-linear regression (PROC REG, SAS Inc., Cary, North Carolina) to test for the effect of animal source and time since release (days) on the 2 dependent variables, namely flush distance and flight distance. In this approach, a set of regressions is carried out for each dependent variable and all possible combinations (subsets) of predictors are used. Thus, if there are k predictors, all 2^k possible regressions are considered, one with all k predictors, k with $k-1$ predictors, \dots , $\binom{k}{i}$ with only $k-i$ predictors, \dots , and one without any of the predictors (the null model). An estimator for the fit of each regression to the true model (Akaike's information criterion [AIC]) is derived from the residual sum of squares; thus, the best model is the one with the lowest AIC. The relative support for other models is indicated by the difference of their AIC from that of the best model (ΔAIC). Specifically, alternative models have tentative support if their ΔAIC is small enough, and the convention is to consider all models with $\Delta\text{AIC} < 2.0$. We used an AIC corrected for small sample size, AIC_c .

In addition to the source-of-animals and time-since-release predictors, there were 5 covariates: release cycle, gender, cover type, posture, and group size. Because multiple sightings were available for each animal, individual animal variation had to be accounted for. Therefore, the full model would include our 2 main predictors, 5 covariates, and 16 individual animals for which we had sufficient data. This would generate a huge amount (2^{23}) of subsets that would have to be ranked according to their AIC_c . To overcome this we carried out our analysis in 2 stages.

First, we ran the model-selection procedure for each individual separately, regressing its flush or flight distance on its predictors (time since release, cover type, posture, and group size)—altogether 2^4 regressions for each individual). From these 16 regressions, we considered those with a $\Delta\text{AIC}_c < 2.0$ and selected the model with the smallest ΔAIC_c that included time since release as a predictor. If the best model that included time since release had a $\Delta\text{AIC}_c \geq 2$, we considered the slope for that specific individual as zero (i.e., no change in behavior over time). From the selected model, we derived the slope (coefficient) for the changes in flush or flight distance as a function of time since release and the associated

intercept. The slope of time since release reflected the rate at which a specific behavior (flight or flush distance) changed since release. The intercept reflected the flight or flush distance at the time of release with all other predictors equalized and as such represented the loss of antipredator behavior due to captive conditions. Thus, for each of the 2 dependent variables, we had a set of 16 pairs of slope and intercept, one pair for each individual.

In the second stage, we regressed the 16 individual slopes of time since release and the 16 intercepts, derived from the first stage for the flight distance, on the population-related predictors (source of animals, release cycle, and gender). We then selected the best model with an information-theoretic approach. We did the same for flush distance.

To assess the effect of source of animals and time since release on flight mode, we used the proportion of sightings in which the animal escaped by running. To evaluate the time-since-release effect, we divided the data into 2 equal periods: from day of release up to day 30 after release and from day 31 up to day 60 after release. We followed a similar procedure for use of cover type in which we compared the proportion of times an animal was sighted in dense Mediterranean woodland. Proportions and standard errors (SE) were estimated with a cluster sampling technique (Cochran 1953), in which the independent clusters were the aggregates of non-independent observations for each individual. We used a 2-tailed t test to compare flight mode and use of cover between the sources of animals for each of the time periods. We carried out a third t test to determine animals from which source exhibited a greater change between the 2 periods. Because 3 tests were performed on the same data set for each of the dependent variables (flight mode and cover type), we used a Bonferroni corrected alpha of 0.017.

Corticosterone Analyses

Fecal corticosterone is a reliable indicator of social and environmental chronic or long-term stress in deer (Millsaugh et al. 2001; Creel et al. 2002). Over 2 weeks in April 2006, we collected fecal pellets from individuals at JBZ (5 female, 5 male); reintroduced individuals in the wild originating from JBZ (4 female); individuals at HBC (5 female, 7 male); reintroduced individuals in the wild originating from HBC (5 female, 4 male). Pellets were collected only from identified individuals by following a detected individual until it defecated. With this method, it was not possible to collect samples at a given time during the day; however, diurnal changes in fecal corticosterone are considered negligible (Brockmann 1998). Each sample, recovered within 30 min after it was voided, was placed in an individually labeled plastic tube and stored in a freezer (-40°C) to sustain concentrations of

corticosterone (Berg et al. 2005) until the analysis was performed.

For analysis, fecal samples (1.5 g) were crushed, dried overnight at 60 °C, homogenized with a blender. We used 0.5 g of the processed sample for extraction with 10 mL of 80% methanol for 24 h with shaking (Shore et al. 1993). One-third of the extracted sample was centrifuged at 3000 rps for 10 min, and 3 mL of the supernatant was mixed with 4 volumes of 0.1 M sodium acetate. We extracted the mixture on C-18 maxi-extraction columns (Mega BE-C18 1 g, 6 mL PN 12256001 Varian, Middelburg, Netherlands). We washed the column with 10% methanol and dried and eluted the sample with 2-mL 100% methanol. The elutants were evaporated to dryness under air, residue was redissolved in 3 mL of the buffer used for steroid analysis, and 100 µL aliquots were taken for assay. We used a commercial Elisa with a detection limit of 0.3 ng/mL (DRG GmbH, Marburg, Germany) to determine concentration of corticosterone. The corticosterone results were analyzed with an analysis of variance (ANOVA) test.

Results

Environmental and Behavioral Parameters

We obtained 442 observations on 16 reintroduced individuals (7 individuals from JBZ and 9 individuals from HBC) from the 3 release cycles (first cycle excluded from the analysis). Individuals were monitored for an average (SD) of 100 days (33) for JBZ and 108 days (28) for HBC. The minimum number of monitoring days was 39 (one individual from JBZ), and all others were monitored at least 60 days. The number of observations per individual ranged from 11 to 46 with an average of 31 days (9) for JBZ and 25 days (11) for HBC.

In 9 of the 16 individual regressions on flush distance, the best model included a positive effect of time since release. Three regressions included time since release in one of the top models ($\Delta AIC_c < 2.0$), 2 with a positive response and one with a negative. In 4 cases, time since release was not included in one of the top models. Overall, the mean coefficient for flush as a function of time was 0.111 m per day (SE 0.027, $n = 16$) and was significantly greater than zero ($p < 0.002$). Although the mean slope (SD) was larger for HBC animals (0.146 [0.042], $n = 9$ versus 0.057 [0.028], $n = 7$ for HBC and JBZ, respectively) and the leading model with the slope for the individual animal regressions as the dependent variable included the source of animals (Table 2), this model was not better than the null model ($\Delta AIC_c = 0.22$). With intercepts from the individual animal regressions as the dependent variable, flush distance was approximately 23 m greater in HBC animals at the time of release than in JBZ animals (Table 3). Given that the increase in flush distance over time was similar for animals from both sources and that HBC animals had a shorter flush distance at the outset than JBZ animals, the latter exhibited flush distance similar to the former with roughly 200 days delay (23 divided by 0.111).

For flight distance, time since release was included in the leading model for only one individual and was not among the leading models ($\Delta AIC_c \geq 2$) for any of the other animals. Consequently, we found no evidence of a time effect on flight distance, and there was no effect of source of animals on flight distance as a function of time since release (Table 4). When comparing the intercepts of the individual animal regressions on flight distance, the source of the animals was included in both leading models (Table 5), and on the basis of model averaging (Burnham & Anderson 2002) the intercept was 2.70. Thus, HBC animals ran on average 2.7 m farther than JBZ animals. Because the data were right censored at 10 m this

Table 2. Parameter estimates of all possible subsets (models) of a linear multiple regression on the breeding facility from which the deer originated (source), the release cycle (release), and gender.*

Subset	Parameter estimates			AIC_c	ΔAIC_c	Model likelihood	Akaike weights
	source	release	gender				
1	0.089			-68.56	0	1	0.33
2				-68.34	0.22	0.9	0.3
3		0.023		-66.10	2.46	0.29	0.1
4			-0.005	-65.77	2.78	0.25	0.08
5	0.086	0.014		-65.70	2.85	0.24	0.08
6	0.093		0.017	-65.67	2.88	0.24	0.08
7		0.025	0.006	-63.12	5.43	0.07	0.02
8	0.091	0.019	0.025	-62.43	6.12	0.05	0.02

*The dependent variable is the slope of flush distance over time calculated for each of 16 reintroduced individuals (see text for details). Models are sorted vertically from best to worst based on Akaike's information criterion corrected for small sample size (ΔAIC_c). Parameter estimates indicate which predictors were used in each subset. Parameter estimates (regression coefficients) with a value indicate the predictors included in a specific regression (e.g., subset 8 with values for all 3 parameters is the full model and subset 2 with no values is the null model intercept only). Source indicates source of animals (JBZ = 1, HBC = 2). Release is release cycle (2, 3, or 4).

Table 3. Parameter estimates of all possible subsets (models) of a linear multiple regression on the breeding facility from which the animals originated (source), the release cycle (release), and gender in which the dependent variable is the intercept derived from multiple regressions carried out for each of 16 reintroduced individuals of flush distance on time-specific parameters.*

Subset	Parameter estimates ^a			AIC _c	ΔAIC _c	Model likelihood	Akaike weights
	source	release	gender				
1	23.18			79.11	0.00	1.00	0.67
2	23.39		0.90	82.07	2.96	0.23	0.15
3	23.29	-0.54		82.08	2.97	0.23	0.15
4	23.43	-0.38	0.74	85.55	6.44	0.04	0.03
5				89.70	10.59	0.01	0.00
6			-4.67	91.90	12.79	0.00	0.00
7		2.00		92.14	13.03	0.00	0.00
8		0.98	-4.22	94.86	15.75	0.00	0.00

*Models are sorted vertically from best to worst based on Akaike's information criterion corrected for small sample size (ΔAIC_c). Parameter estimates (regression coefficients) with a value indicate the predictors that were included in a specific regression; thus, subset 4 with values for all 3 parameters is the full model and subset 5 with no values is the null model (intercept only). Source indicates source of animals (JBZ = 1, HBC = 2). Release is release cycle (2, 3, or 4).

suggests a near 30% difference within this limitation, but we were unable to assess the actual difference.

During the first 30 days after release, HBC animals used running as an escape mode more often than JBZ animals (mean [SE] 0.827 [0.030], $n = 8$ vs. 0.463 [0.071], $n = 7$, respectively, $t = 4.747$, $df = 8.13$, $p = 0.001$). Animals from both sources increased the frequency of running during days 31–60, but HBC animals still ran more often than JBZ animals (0.873 [0.039], $n = 9$ vs. 0.660 [0.063], $n = 7$, respectively, $t = 2.859$, $df = 10.37$, $p = 0.017$). However, the improvement was greater in the JBZ animals (2-tailed t test, $t = 2.834$, $df = 13$, $p = 0.014$).

In terms of cover type, during the first 30 days after release, HBC animals were found in dense Mediterranean woodland more often than JBZ animals (mean [SE] 0.505 [0.093], $n = 8$ vs. 0.198 [0.026], $n = 7$, respectively, $t = 3.173$, $df = 8.07$, $p = 0.013$). During days 31–60, HBC

animals still spent more time in this cover type than JBZ animals (0.606 [0.063], $n = 9$ vs. 0.220 [0.071], $n = 7$, respectively, $t = 4.064$, $df = 13.04$, $p = 0.001$). We found no improvement over time in use of cover in both groups ($p > 0.05$).

Hormone Levels

Although a 2-way ANOVA displayed no significant difference in mean corticosterone levels between JBZ and HBC individuals ($F_{1,31} = 0.16$, $p = 0.693$), corticosterone levels were significantly higher in samples collected in the wild (mean [SE] 95.3 ng/g [15.0]) than in those from captivity (69.7 ng/g [6.2]) ($F_{1,31} = 4.31$, $p = 0.046$). The mean corticosterone level in pellet samples from captivity was lower for JBZ than HBC, whereas in the wild this feature was reversed (Fig. 1) and was not significant ($F_{1,31} = 1.50$).

Table 4. Parameter estimates of all possible subsets (models) of a linear multiple regression on the breeding facility from which the animals originated (source), the release cycle (release), and gender in which the dependent variable is the slope of flight distance over time calculated for each of 16 reintroduced individuals.*

Subset	Parameter estimates ^a			AIC _c	ΔAIC _c	Model likelihood	Akaike weights
	source	release	gender				
1			0.027	-109.25	0.00	1	0.34
2	-0.020			-107.77	1.48	0.48	0.16
3				-107.26	1.99	0.37	0.13
4	-0.014		0.023	-107.27	2.02	0.36	0.12
5		-0.009		-106.77	2.48	0.29	0.10
6		-0.003	0.025	-106.34	2.91	0.23	0.08
7	-0.018	-0.007		-105.22	4.03	0.13	0.05
8	-0.014	-0.002	0.022	-103.79	5.46	0.07	0.02

*Models are sorted vertically from best to worst based on Akaike's information criterion corrected for small sample size (ΔAIC_c). Parameter estimates (regression coefficients) with a value indicate the predictors that were included in a specific regression; thus, subset 8 with values for all 3 parameters is the full model and subset 3 with no values is the null model (intercept only). Source indicates source of animals (JBZ = 1, HBC = 2). Release is release cycle (2, 3, or 4).

Table 5. Parameter estimates of all possible subsets (models) of a linear multiple regression on the breeding facility from which the animals originated (source), the release cycle (release), and gender in which the dependent variable is the intercept derived from multiple regressions carried out for each of 16 reintroduced individuals of flight distance on time-specific parameters.*

Subset	Parameter estimates ^a			AIC _c	ΔAIC _c	Model likelihood	Akaike weights
	source	release	gender				
1	2.64	0.76		5.43	0.00	1.00	0.53
2	2.80			6.70	1.26	0.53	0.28
3	2.71	0.83	0.33	8.48	3.05	0.22	0.12
4	2.79		-0.03	9.68	4.25	0.12	0.06
5		1.05		19.76	14.33	0.00	0.00
6				20.12	14.69	0.00	0.00
7			-0.69	22.05	16.62	0.00	0.00
8		0.99	-0.24	22.67	17.24	0.00	0.00

*Models are sorted vertically from best to worst based on Akaike's information criterion corrected for small sample size (ΔAIC_c). Parameter estimates (regression coefficients) with a value indicate the predictors that were included in a specific regression; thus, subset 3 with values for all 3 parameters is the full model and subset 6 with no values is the null model (intercept only). Source indicates source of animals (JBZ = 1, HBC = 2). Release is release cycle (2, 3, or 4).

Survival

Of the 24 reintroduced individuals (all releases including the first), one transmitter on a JBZ male failed immediately after the release, and this individual was never sighted again. Of the 10 HBC individuals, 8 survived at least 200 days. By contrast, none of the 13 individuals (including those from the first release) from the Biblical Zoo survived to 200 days ($p = 0.009$, 2-tailed Fisher's exact test). Of the 15 mortalities, 13 were due to collisions with trains (11 individuals from JBZ and 2 from HBC), and the remains of the other 2 suggested they were preyed upon.

Discussion

Reacquisition of antipredator behaviors by reintroduced animals is a time-dependent process. If the length of this process is too long, it will be detrimental to the success

of a reintroduction (Seddon et al. 2007). How much of this behavior is lost during captivity and how long it takes to reacquire it after reintroduction are important factors.

By definition, reintroductions involve the translocation of animals to a novel area. In the wild, novelty is synonymous with danger (Grandin 1997), and animals entering new areas are often timid and invest much time and effort in antipredatory behavior (Grandin 1997; Welp et al. 2004; Wierzbicki et al. 2005). Animals reintroduced from captive-breeding facilities, however, may lose their antipredator reactions (Lima & Dill 1990). In our study both HBC and JBZ deer had lost some of their antipredator behaviors and were naïve compared with wild-born animals (e.g., flight-initiation distance is remarkably higher in European fallow deer [*Dama dama*] [de Boer et al. 2004]). Nevertheless, all responses at the time of release (intercept of flush and flight distances, the proportion of use of dense Mediterranean woodland, and flight mode during the first 30 days after release) indicated this loss was considerably greater in animals from the heavily visited JBZ, which suggests that HBC animals were better prepared for life in the wild than their JBZ counterparts.

The breeding facilities were very similar in terms of size, animal treatment, and the physical conditions inside the pens. The main difference was the number of visitors frequenting them and the associated commotion. Human activities, in general, pose a disturbance to animals. If human activity is frequent enough and of no actual consequence (nuisance disturbance), animals will ignore the activity, and a general reduction in antipredator behaviors results (vigilance, Shochat et al. 2004). Although the poorer flight-related responses of JBZ animals could be attributed to a weaker response to humans specifically, their tendency to spend more time in open habitat indicated a weaker response to potential threat and higher susceptibility to predation (Pierce et al. 2004). Thus, intense anthropogenic disturbance at breeding facilities makes reintroduced animals more prone to risk,

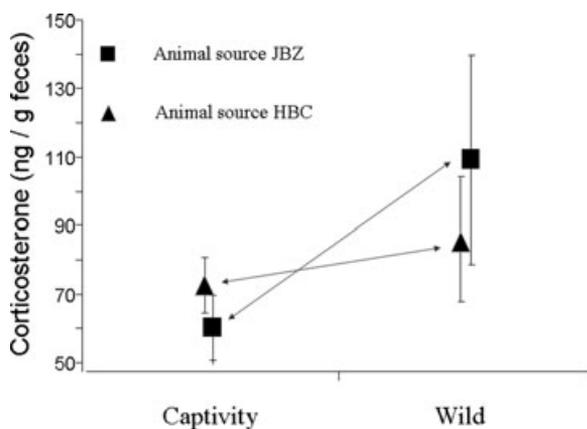


Figure 1. Average (SE) corticosterone level in feces of individuals from the Biblical Zoo ($n = 10$, \hat{A}) and from Hai-Bar Carmel ($n = 12$, \hat{B}) collected in the facilities and in the wild, respectively. Arrows highlight the interaction.

not only from human-related elements, but also in terms of general predator avoidance. Thus, the level of loss of antipredator traits due to captive breeding depends, at least in part, on the general conditions in the breeding facilities and on the absence of predators or antipredator training. Furthermore, differences in captive conditions that are seemingly of little consequence in terms of risk of predation (e.g., number of visitors to the breeding facility and the associated commotion) cause significant differences in the antipredator behavior of reintroduced animals.

When exposed to predators, animals can reacquire antipredator behaviors (Laundré et al. 2001). In our study the rate at which antipredator behaviors were reacquired varied depending on the specific behavior and source of the animals. For example, flush distance increased over time, but at a slow rate, and did not differ between HBC and JBZ. Nevertheless, given the initial differences in flush distance between animals from the 2 sources and the rate of change in this response, it would take 200 days in the wild before JBZ individuals achieved a flush distance similar to that of HBC animals at the time of release. By contrast, JBZ animals, which tended to use walking as a mode of flight during the first month after release, shifted to mostly running in the second month, tending to behave more like HBC animals. In terms of use of cover, HBC animals were nearly 3 times more likely than JBZ animals to be found in thick cover, and this did not change over the course of the first 60 days in the wild.

Reintroduced animals from both sources showed elevated levels of corticosterone when compared with their captive counterparts. Thus, the reintroduced animals responded to the new environment physiologically as well. The response was stronger (albeit insignificant, possibly due to lack of statistical power) in JBZ animals, which suggests that although JBZ individuals were less cautious after the release, the reintroduction procedure was marginally more stressful for them, perhaps because the change was more extreme. Because fecal corticosterone is associated with reduced immunity and reproductive success (Millspaugh et al. 2001; Creel et al. 2002), we point, with caution, to the possibility that captive conditions may influence stress levels in reintroduced animals.

The major cause of mortality was collisions with trains, which mostly affected JBZ animals. The deer were attracted to the tracks by invasive plant species (*Chenopodium album*, *Conyza canadensis*, *Solanum nigrum*, and *Polygonum lapathifolium*) growing alongside it. The tracks were in open habitat, which the JBZ animals, in contrast to their HBC counterparts, did not avoid. Furthermore, the increased level of visitors and commotion at the JBZ and proximity to the intercity railroad may have resulted in deer habituating to mechanical sounds and noises and, thus, their lack of response

to approaching trains in the wild. Therefore, the low survival of JBZ animals was not due to a single factor, but rather an assemblage of behaviors all stemming from the conditions at the breeding facility. Two of the 15 mortalities were due to predation, and both were from the JBZ.

We conclude that the reacquisition of antipredator behavior patterns in the wild is a continuous process. The process is influenced by conditions in the breeding facility, and the specific influence varies among the various components of the antipredator behavior (e.g., cover use and flight behavior). Individuals that exhibit a greater loss of specific antipredator behaviors will require more time to reacquire them. Thus, although the train, as the main cause of mortality, was a site-specific condition, the behavioral tendencies that exposed the JBZ deer to the train are global and make them, generally, more prone to risk.

Conservation Implications

Reintroductions of captive-bred animals are an important tool for preventing species loss (IUCN 1987). Nonetheless, the survival in the wild of captive-bred animals is often low (Shepherdson 1994; Letty et al. 2000). Our results indicate that general conditions at the breeding facility affect the response of the reintroduced animals to potential threats. Captive-breeding programs should, therefore, be evaluated in terms of population dynamics (Ostermann et al. 2002) and in terms of the possible effects of captivity on behavior of the animals when reintroduced.

The tendency of many reintroductions to fail early (Beck et al. 1994) points to the possible need to extend reintroduction programs to enable research focusing on the probable causes of failure and effective mitigation programs (Seddon 1999; Armstrong et al. 2007). Our findings support the notion that these failures may be due, in part, to the increased susceptibility of the animals immediately following release while they reacquire the necessary behavioral tools for surviving in the wild (as opposed to specific permanent condition at the release site). Thus, the speed at which certain behaviors are reacquired is critical. Prerelease antipredator training is one way to increase the speed of reacquiring antipredator behaviors (Miller et al. 1994; Maloney & McLean 1995; van Heezik et al. 1999; Griffin et al. 2000); however, reducing the overall level of anthropogenic-related disturbances at the breeding facilities may be no less important and perhaps easier to implement.

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Literature Cited

- Andersen, R., J. D. C. Linnell, and R. Langvatn. 1996. Short term behavioural and physiological response of moose *Alces alces* to military disturbance in Norway. *Biological Conservation* **77**:169-176.
- Armstrong, D., P. Isabel Castro, and R. Griffiths. 2007. Using adaptive management to determine requirements of re-introduced populations: the case of the New Zealand hihi. *Journal of Applied Ecology* **44**:953-962.
- Armstrong, D. P., and P. J. Seddon. 2008. Directions in reintroduction biology. *Trends in Ecology & Evolution* **23**:20-25.
- Banks, P. B., K. Norrdahl, and E. Korpimäki. 2002. Mobility decisions and the predation risks of reintroduction. *Biological Conservation* **103**:133-138.
- Bar-David, S., D. Saltz, T. Dayan, A. Dolev, and A. Perelberg. 2005. Demographic models and reality in reintroductions: the Persian fallow deer in Israel. *Conservation Biology* **19**:131-138.
- Beck, B. B., L. G. Rapaport, M. R. Stanley Price, and A. C. Wilson. 1994. Reintroduction of captive-born animals. Pages 265-286 in P. J. S. Olney, G. M. Mace, and A. T. C. Feistner, editors. *Creative conservation: interactive management of wild and captive animals*. Chapman and Hall, London.
- Berg, N., Y. Zurovsky, L. S. Shore, A. Turkel, and Y. Steinberger. 2005. The correlation between social hierarchy and testosterone and corticosterone levels in male *Oryx dammah*. *Israel Journal of Zoology* **51**:60.
- Berger, J., J. E. Swenson, and I. L. Persson. 2001. Recolonizing carnivores and naive prey: conservation lessons from Pleistocene extinctions. *Science* **291**:1036-1039.
- Blumstein, D. T., and J. C. Daniel. 2005. The loss of antipredator behaviour following isolation on islands. *Proceedings of the Royal Society of London Series B - Biological Sciences* **272**:1663-1668.
- Box, H. O. 1991. Training for life after release: simian primates as examples. Pages 111-124 in J. H. W. Gipps, editor. *Beyond captive breeding: reintroduction of endangered mammals to the wild*. Clarendon Press, Oxford, United Kingdom.
- Bright, P. W., and P. A. Morris. 1994. Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. *Journal of Applied Ecology* **31**:699-708.
- Brockmann, D. K. 1998. Reproduction in free-ranging male *Propithecus verreauxi*: The hormonal correlates of mating and aggression. *American Journal of Physical Anthropology* **105**:137-151.
- Burnham, K. P., D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. 2nd Edition. Springer-Verlag, New York.
- Byers, J. A. 1997. *American pronghorn: social adaptations and the ghosts of predators past*. Chicago University Press, Chicago, Illinois.
- Cochran, W. G. 1953. *Sampling techniques*. Wiley, New York.
- Creel, S., J. E. Fox, A. Hardy, J. Sands, B. Garrot, and R. O. Peterson. 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. *Conservation Biology* **16**:809-814.
- de Boer, H. Y., L. van Breukelen, M. J. M. Hootsmans, and S. E. van Wieren. 2004. Flight distance in roe deer *Capreolus capreolus* and fallow deer *Dama dama* as related to hunting and other factors. *Wildlife Biology* **10**:35-41.
- Diamond, J. 1990. Biological effects of ghosts. *Nature* **345**:769-770.
- Dolev, A., D. Saltz, S. Bar-David, and Y. Yom-Tov. 2002. The impact of repeated releases on the space-use patterns of reintroduced Persian fallow deer (*Dama mesopotamica*) in Israel. *Journal of Wildlife Management* **66**:737-746.
- Fischer, J., and D. B. Lindenmayer. 2000. An assessment of the published results of animal relocations. *Biological Conservation* **96**:1-11.
- Ginsberg, J. 1994. Captive breeding, reintroduction, and the conservation of canids. Pages 365-381 in P. J. S. Olney, G. M. Mace, and A. T. C. Feistner, editors. *Creative conservation: interactive management of wild and captive animals*. Chapman and Hall, London.
- Grandin, T. 1997. Assessment of stress during handling and transport. *Journal of Animal Science* **75**:249-257.
- Griffin, A. S., D. T. Blumstein, and C. S. Evans. 2000. Training captive bred and translocated animals to avoid predators. *Conservation Biology* **14**:1317-1326.
- Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. *Science* **245**:477-480.
- IUCN (International Union for Conservation of Nature). 1987. *Translocation of living organisms: introductions, reintroductions, and restocking*. IUCN position statement. IUCN, Gland, Switzerland.
- Laundré, J. W., L. Hernández, and K. B. Altendorf. 2001. Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, U.S.A. *Canadian Journal of Zoology* **79**:1401-1409.
- Lety, J., S. Marchandea, J. Clobert, and J. Aubineau. 2000. Improving translocation success: an experimental study of anti-stress treatment and release method for wild rabbits. *Animal Conservation* **3**:211-219.
- Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**:619-640.
- Maloney, R. F., and I. G. McLean. 1995. Historical and experimental learned predator recognition in free-living New Zealand robins. *Animal Behaviour* **50**:1193-1201.
- Mathews, F., M. Orros, G. McLaren, M. Gelling, and R. Foster. 2005. Keeping fit on the ark: assessing the suitability of captive-bred animals for release. *Biological Conservation* **121**:569-577.
- McPhee, M. E. 2003. Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. *Biological Conservation* **115**:71-77.
- Miller, B. D., L. H. Biggins, and A. Vargas. 1994. Reintroduction of the black-footed ferret (*Mustela nigripes*). Pages 455-464 in P. J. S. Olney, G. M. Mace, and A. T. C. Feistner, editors. *Creative conservation: interactive management of wild and captive animals*. Chapman and Hall, London.
- Millspaugh, J. J., R. J. Woods, K. E. Hunt, K. J. Raedeke, G. C. Brundige, B. E. Washburn, and S. K. Wasser. 2001. Using fecal glucocorticoid assays to study the physiological stress of elk. *Wildlife Society Bulletin* **29**:899-907.
- Ostermann, S. D., J. R. DeForge, and W. D. Edge. 2002. Captive breeding and reintroduction evaluation criteria: a case study of peninsular bighorn sheep. *Conservation Biology* **15**:749-760.
- Perelberg, A., D. Saltz, S. Bar-David, A. Dolev, and Y. Yom-Tov. 2003. Seasonal and circadian changes in the home ranges of reintroduced Persian fallow deer. *Journal of Wildlife Management* **67**:485-492.
- Pierce, B. M., R. T. Bowyer, and V. C. Bleich. 2004. Habitat selection by mule deer: forage benefits or risk of predation? *Journal of Wildlife Management* **68**:533-541.
- Seddon, P. J. 1999. Persistence without intervention: assessing success in wildlife reintroductions. *Trends in Ecology & Evolution* **14**:503.
- Seddon, P. J., D. P. Armstrong, and R. F. Maloney. 2007. Developing the science of reintroduction biology. *Conservation Biology* **21**:303-312.
- Shepherdson, D. 1994. The role of environmental enrichment in the captive breeding and reintroduction of endangered species. Pages 167-177 in P. J. S. Olney, G. M. Mace, A. T. C. Feistner, editors. *Creative conservation: interactive management of wild and captive animals*. Chapman and Hall, London.

- Shochat, E., S. B. Lerman, M. Katti, and D. B. Lewis. 2004. Linking optimal foraging behavior to bird community structure in an urban-desert landscape: field experiments with artificial food patches. *The American Naturalist* **164**:232-243.
- Shore, L. S., E. Harel-Markowitz, M. Gurevich, and M. Shemesh. 1993. Factors affecting the concentration of testosterone in poultry litter. *Journal of Environmental Science and Health Part A* **28**:1737-1749.
- Tyler, N. J. C. 1991. Short-term behavioural responses of Svalbard reindeer *Rangifer tarandus platyrhynchus* to direct provocation by a snowmobile. *Biological Conservation* **56**:179-194.
- van Heezik, Y., P. J. Seddon, and R. F. Maloney. 1999. Helping reintroduced houbara bustards avoid predation: effective anti-predator training and the predictive power of pre-release behavior. *Animal Conservation* **2**:155-163.
- Welp, T., J. Rushen, D. L. Kramer, M. Festa-Bianchet, and A. M. B. de Passillé. 2004. Vigilance as a measure of fear in dairy cattle. *Applied Animal Behaviour Science* **87**:1-13.
- Wielebnowski, N. 1998. Contributions of behavioral studies to captive management and breeding rare and endangered mammals. Pages 130-162 in T. Caro, editor. *Behavioral ecology and conservation biology*. Oxford University Press, New York.
- Wierzbicki, H., P. Przysiecki, E. Gorajewska, and A. Filistowicz. 2005. Adaptation of arctic fox (*Alopex lagopus*) to the new environmental conditions. *Medycyna Weterynaryjna* **61**:914-918.
- Wolf, C. M., B. Griffith, C. Reed, and S. A. Temple. 1996. Avian and mammalian translocations: update and reanalysis of 1987 survey data. *Conservation Biology* **10**:1142-1154.