

# Stress Responses in Captive African Painted Dogs (*Lycaon pictus*) After Removal of a Pack Member and its Relation to Pack Cohesiveness

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## Abstract

African painted dogs, *Lycaon pictus*, have a highly complex social structure characterized by an alpha-breeding pair, a high degree of social cohesion, and group living. This research examines whether this social system is significantly impacted by the removal and reintroduction of a single pack member by conducting a comparative analysis of pack cohesiveness and stress responses before and after the removal period. Behavioral observations of captive African painted dogs were assessed over this timeline to determine the degree of similarity in frequencies of targeted behaviors. The African painted dog pack at the Audubon Zoo served as the subject of analysis in both the pre-removal and post-removal observation. Results indicate that the removal of the beta female was a stressful event that increased stress levels in the pack as a whole. Stress levels and the frequency of stress-response behaviors significantly decreased following the individual's reintroduction. Furthermore, pack cohesiveness improved throughout the duration of the study. The African painted dogs likely used contact resting as a coping mechanism for the stress caused by the removal. Zoo management should model the protocols carried out through the pack's reintroduction process as a guide for future removal events to increase the probability of successful removal and reintroduction.



## Introduction

Social management of captive animals whose dynamics feature a dominant breeding pair is challenging and can have significant health impacts on the entire group (Langan & Jankowski, 2018). While extensive research has investigated the implications for removal and translocation into natural wild environments, current literature lacks clear guidelines for management decisions regarding priorities when an individual is removed and reintroduced into the same captive pack. Recent suggestions for managing separation describe subdividing the pack and reintroducing all members simultaneously. Alternatively, studies note that dividing the pack and reintegrating subordinate individuals before dominant pairs may be an effective method of successful reintroduction (Langan & Jankowski, 2018). Besides minimal suggestions, no conclusive guidelines exist for approaching temporary removals in captivity. More detailed recommendations are urgently needed to ensure smooth transitions, reduce stress and aggression, and promote normal behaviors in order to avoid potential rejection interactions upon reintroduction.

To gain a deeper understanding of the effects of the temporary removal of a pack individual on group dynamics, the study was conducted on a captive pack of African painted dogs (*Lycaon pictus*). African painted dogs live in cooperative packs with a distinct dominance hierarchy in each sex, constituting a dominant breeding pair of one female and one male (Creel et al., 1997). Wild packs of African painted dogs are closely bonded and display highly complex social behaviors; demonstrations of such behaviors are a crucial indicator of the pack's social and physical well-being and are displayed to reinforce relationships, demonstrate cooperation with pack rank, and communicate information. (Langan & Jankowski, 2018; Chen, 2018; de Villiers et al., 2003). Establishing a pack hierarchy and maintaining stability is required to avoid excessive stress and aggression. Studies have shown that the permanent or temporary removal of an established pack member can have profound social impacts, including eliciting a change in social hierarchy, substantial levels of aggression, and increases in stress despite a high degree of bonding (Langan & Jankowski, 2018).

In the wild and captivity, African painted dogs often rest together as a pack unit, representing an integral part of the social structure of these animals (Chen, 2018). Previous research suggests that spatial relationships mirror the relative strength of social bonds and, therefore, can be used to quantify the degree of social integration between the males and females of the pack (McCreery, 2000; de Villiers et al., 2003). A study evaluating the changes in social relationships found a pattern of increased stress behaviors in the months following pack separation but observed a homogenous distribution of social relationships between pack members (Zijlmans & Duchateau, 2019). During this period, Zijlmans and Duchateau theorized that resting in physical contact served as a coping mechanism to reduce the stress associated with pack separation. A high frequency of contact resting positions would indicate a high degree of social bonding between those individuals.

Monitoring an emergent captive removal and reintroduction process in real-time is a rare opportunity to gather invaluable information on integrating new pack members for zoo management. This research examines whether the removal and reintroduction of a single member significantly impacts the social system of a previously stable pack. To do so, this study investigated

pack cohesiveness and stress-response behaviors before and during a removal event and following the subsequent reintroduction. Expanding upon the information gained in this study can aid in providing recommendations and further information for the captive management of African painted dogs and other pack animals with similar social structures. The objective was to evaluate whether a temporary removal and reintroduction (1) significantly affected stress levels within the pack and (2) altered pack cohesiveness and social stability. Prior knowledge formed an expectation that removing and reintroducing the beta female would lead to a rise in stress levels, as seen through increased frequencies of stress-response behaviors after the removal, with a continuation after reintroduction. Furthermore, pack cohesiveness would improve over time as the frequency of contact resting positions of all members increase to cope with stress levels.

## Methods

### *Study Species and Study Site*

Field observations occurred at the African painted dog exhibit at the Audubon Zoo from September to November of 2023. The pack comprises three members with a known alpha-breeding pair and a single beta female. Samara and Kashyyk constitute the dominant pair, and Macite is the only subordinate pack member. In March 2023, the Audubon veterinary staff spayed Samara due to a reproductive tract disease. Samara's relatively young age and the veterinary staff's early recognition of symptoms contributed to the successful recovery and subsequent return to the exhibit after one night of isolated holding. In late September, zoo staff noticed increased lethargy and a decrease in appetite from Macite and later found her lying in the exhibit unresponsive in early October. Macite was diagnosed with pyometra, and veterinary staff performed an emergency spay. As a result of a rapid escalation of her intense symptoms as well as catching the disease at a highly progressed state, Macite's recovery was not linear, and her health struggled following the procedure. Audubon Zoo staff separated Macite from the exhibit for ten days- the most prolonged period the females had ever been separated. Reintroduction occurred on October 20th by introducing Macite and Samara together first in the den box holding while Kashyyk maintained visual contact, and then Kashyyk was allowed to join.

### *Experimental Design*

Observational studies included *focal sampling* of stress behaviors and *instantaneous scan sampling* to examine resting positions (Altman, 1974). Over the duration of five-minute continuous sample periods, the frequency of displayed stress behaviors of each individual was recorded (Table 1).

**Table 1.** Ethogram of Observed Stress-Response Behaviors

Behavior	Definition
<i>Stress Behaviors</i>	
Vocalization	“Hoo call”; a high pitched whining or whimpering sound indicative of distress; used in the wild to call out to A missing pack member
Solitude	An alteration of typical behavior in which the individual isolates themselves from the pack
Scent gather	Sniffing at the den box area where the removed individual was held; heightened alertness and an attempt to Information about the environment
Pacing	walking and/or trotting back and forth over a consistent and repeated area (Rafcaz & Santymire 2014)

*Stress Response Behaviors*

Analysis employed various vocal and behavioral indicators to quantify the dogs' stress levels. A "hoo call" is the only known long-distance contact call displayed by African painted dogs. The sound is a barking vocalization most frequently displayed when the animal is distressed. In the wild, the dogs will hoo to reconnect with other members after becoming separated during a hunting event in an attempt to return all individuals to the pack (Robbins, 2000). Repetitive pacing is a stereotypic behavior exhibited by many captive species but is particularly prevalent in carnivores with large wild-home ranges, such as the African painted dog (Bernstein-Kurtycz et al., 2022). Seeking solitude is not a typical behavior of these animals; when a dog seeks isolation, it is likely distressed and looking for its packmates (Creel, 2001). African painted dogs utilize scent as a communication device and a means of investigating their environment (Apps et al., 2022). During her isolation period, keepers temporarily placed Macite in a holding area near the den box stall bordering the exhibit's exterior. Continued sniffing represented an effort to seek and locate the removed individual.

**Data Collection**

If, during this sample period, two or more pack members began to lie for rest, the previous sampling temporarily paused to record these instances. At the start of rest, observation occurred with five-minute sample periods, and the proximity of resting positions of each individual was recorded at 30-second intervals. The measures of proximity are marked in **Table 2**.

**Table 2.** Social Distance During Rest (measured in full adult body lengths) (Curto 2018).

Behavior	Definition
<i>Resting Positions</i>	
Contact	Physically touching another individual
Proximate	Within one adult body length of another individual
Out of Reach	One to three adult body lengths of separation from another individual
Distant	More than three adult body lengths of separation from another individual

### Statistical Analyses

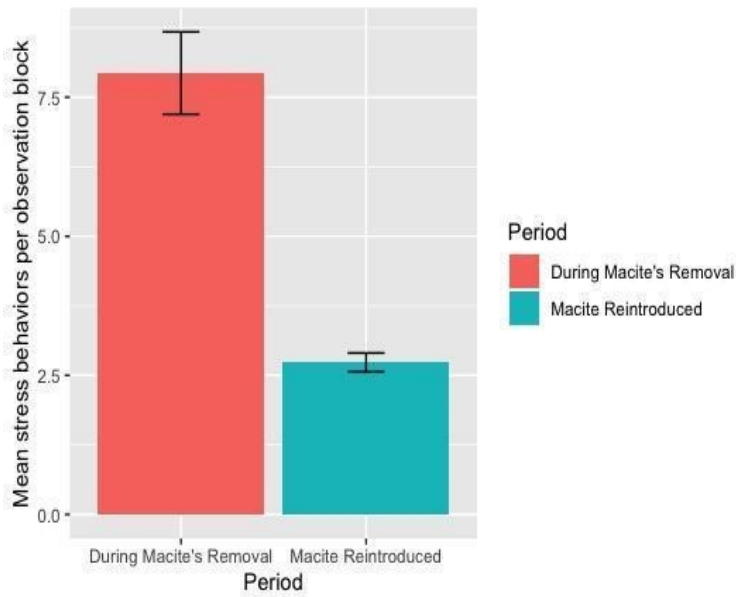
All statistical tests were conducted using R-Studio programming. A linear mixed-effects model was used to evaluate the prediction that stress-response behaviors would increase by the pack as a whole during the removal event and after the reintroduction ( $\alpha=0.05$ ). The lme4 package was installed to allow for the formulation of this multilevel model and account for random effects structures within the data (Speekenbrink, 2023). Given that the data contained the random variable of dog identity (observation was repeated on the same individual over time), the linear-mixed effects model contributes additive fixed (i.e.: time period) and random effects (i.e.: dog identity) to the outcome variable (i.e.: average frequency of stress behavior) (Starkweather, n.d.). Both the overall stress levels of the pack and the display frequency of each individual stress behavior were analyzed.

A one-way Analysis of Variance (ANOVA) test was used to evaluate the prediction that pack cohesiveness would improve throughout the study as the frequency of contact resting positions increases over time ( $\alpha=0.05$ ). A one-way ANOVA tests whether there is a difference in the means of the quantitative variable (frequency of contact rest) according to three independent categorical groups (time period). The null hypothesis for the ANOVA test states there are no statistically significant differences in the means at each level; the alternate hypothesis states that means at each level differ from each other (Bevans, 2020). A post hoc multiple comparison test (Tukey’s Honest Significant Difference test) was conducted under the condition that evidence was found indicating some group means are not equal. Tukey's test compared the means between the three periods to further investigate the groupwise differences.

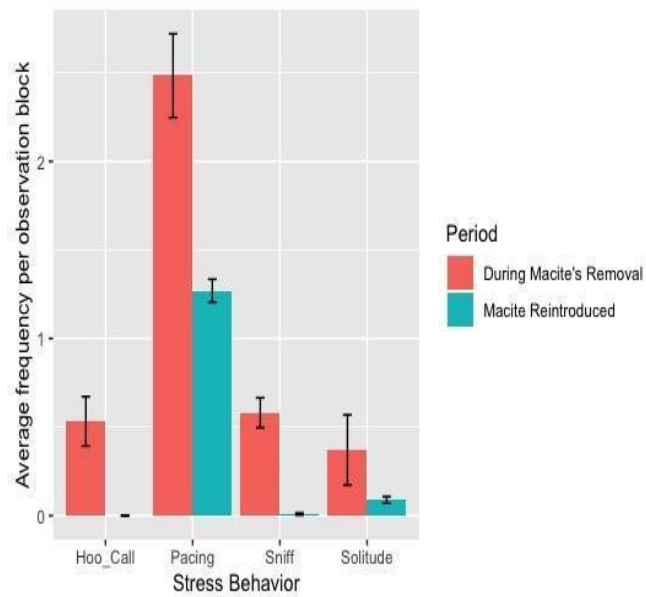
### Results

During the study period, roughly 23 total hours of observation occurred at the African painted dog exhibit across 11 days.

*Pack Stress-Response*



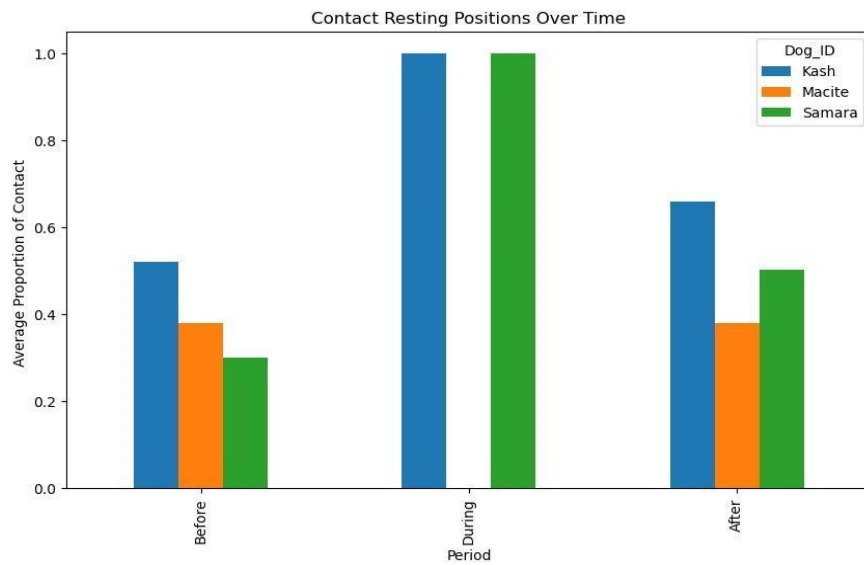
**Figure 1.** Overall Stress Levels of the African painted dog pack during the removal period and following the beta female's reintroduction.



**Figure 2.** Average frequencies of each stress-response behavior displayed during the removal period and following the beta female's reintroduction.

Variable	P-Value	t-value	DF
Pacing	0.0219*	-2.52	17
Scent	<0.00001*	-19.85	17
Solitude	0.03*	-2.32	17
Vocalization	0.0008*	-4.06	17
Overall Stress	<0.0001*	-7.47	17

**Table 3.** Frequency of Stress-response Behaviors During Removal and After Reintroduction. \*indicates  $p < 0.05$ .



**Figure 3.** Frequency of Contact Resting Positions Before and During Removal and Following Reintroduction.

Based on the linear mixed-effects model, there was a significant difference in the pack’s stress behavior frequencies during the removal period when compared to after the reintroduction period ( $t = -7.47$ ;  $p < 0.0001$ ;  $df = 17$ ; **Figure 1**). The display of stress behaviors decreased as a whole after the reintroduction of the beta female. In addition, every stress-response behavior analyzed significantly decreased throughout these time periods. Hoo calls ( $p = 0.0008$ ), solitude ( $p = 0.03$ ), and sniffing at the den box stall ( $p < 0.00001$ ) were rarely observed after the reintroduction, as supported by the error bars lacking overlap (**Figure 2, Table 3**). Pacing frequency, although still observed, significantly decreased following the beta female’s reintroduction ( $p = 0.0219$ ; **Table 3**).

*Pack Rest and Bonding*

There was a significant difference in the average frequency of contact rest positions across all three time periods for the dogs (ANOVA:  $F=15.53$ ;  $p= 6.6e-07$ ; **Figure 3**). The average combined pack frequency of contact resting positions taken before the beta female's removal was 0.3995; during the removal period was 1.0; following her reintroduction was 0.6679. The average frequency of contact rest increased from before removal to after reintroduction (Tukey HSD:  $p= 0.0379$ ). Further, the comparison of before removal to during reintroduction led to a significantly higher average frequency of contact rest (Tukey HSD:  $p= 0.0075$ ); however, the average frequency of contact rest did not significantly differ from during the removal to after reintroduction (Tukey HSD:  $p=0.519$ ).

**Discussion**

In the captive pack of African painted dogs studied at the Audubon Zoo, the removal of the beta female initiated a high-stress period that only subsided following her reintroduction. These results countered the initial hypothesis that stress would continue following her return to the pack. However, results provided support for the prediction that contact positions during rest would increase throughout the time periods. The frequency of contact rest increased throughout the study, peaking during the removal period and remaining statistically similar to this level following reintroduction.

*Stress Response*

The results of data analyses revealed that behavioral displays and overall stress levels decreased following Macite's reintroduction to the pack. Hoo calls, sniffing, and solitude were reduced almost entirely- these behaviors were nearly never observed following Macite's return to the pack. Although reducing in occurrence, pacing still occurred moderately frequently following reintroduction. It is plausible that the displays of hoo calling, sniffing at the den box, and seeking solitude resulted solely from the stress caused from Macite's removal, given that these behaviors were almost completely eradicated once the pack was restored. However, a similar conclusion cannot be drawn to interpret pacing behavior.

A captive environment contributes to the stereotypic displays of pacing behavior, regardless of other external factors (Clubb & Mason, 2003). Pacing is theorized to serve as a coping mechanism for blocking environmental-related stressors (Mason & Latham, 2004). Unpredictable events and alterations of an environment, such as the emergent removal of an individual, are recognized to upset an animal's response (Yasmeen et al., 2022). Likely, the stress caused during Macite's removal coupled with captive conditions contributed to a high pacing frequency by Kashyyk and Samara. We can conclude that Macite's removal did have a significant impact on stress levels, given that the group displayed pacing at a significantly lower frequency once she returned. It is intriguing to note that while Macite was removed, Kashyyk and Samara paced along the right side of the exhibit, closest to where Macite was temporarily held. Upon reintroduction, pacing resumed by all three members to the typical left side of the exhibit, where



pacing had always been observed prior to the removal. Kashyyyk and Samara likely shifted their pacing habits to the right side of the exhibit in search of or as a direct response to Macite's absence. Presumably, the dogs pace through their exhibit as a natural response to captivity; however, the elevated pacing frequency and overall stress increase are most likely correlated to the stress associated with the unpredictable change in environment.

### *Pack Cohesiveness*

Further analysis displayed a significant increase in the frequency of contact resting positions before Macite's removal and following her reintroduction. As a whole, results demonstrated a high degree of social integration within the pack was formed over the course of the removal and reintroduction period. The results of this study provide support for the suggestion by Zijlmans and Duchateau that African painted dogs rest in close contact as a coping mechanism for stressful events (Zijlmans & Duchateau, 2019). The Audubon Zoo pack likely bonded together as an adaptive strategy to reduce the stress levels associated with Macite's separation. Being the only individuals on exhibit for the duration of the removal, Kashyyyk and Samara were observed exclusively lying in contact with one another at nearly every instance of rest. Upon her reintroduction, the pack maintained a high frequency of contact rest, demonstrating an effort to strengthen personal bonds and re-form a socially stable pack.

Removals of this nature are unpredictable and give rise to sudden changes in the social hierarchy and the well-being of the pack; even if zoo staff have to temporarily remove a dog to conduct a medical procedure, it can potentially lead to positional shifts in the dominance hierarchy (Miller-Butterworth, 2021). Currently, there is no exhaustive framework for managing these removals, which often occur without warning. Conditions after a pyometra diagnosis can escalate rapidly. Without a reliable procedure established, it can be challenging for zoo staff to successfully and swiftly manage the well-being of all pack members. The Audubon Zoo's method of temporarily placing the removed individual nearby in the den box stall so that the remaining pack members could detect her presence via scent and a gradual two-step reintroduction led to a successful outcome. By evaluating what practices produced a favorable reintroduction result with this pack, the Audubon Zoo is now better equipped to handle these situations and optimistically obtain the same positive outcome experienced throughout the study. Pyometra and other reproductive tract diseases are especially prevalent in large captive canids (Asa et al., 2014); this situation or a similar case will likely occur again, whether in this specific pack or a future group. Zoos extending beyond Audubon can use the results found in this study as recommendations for creating an effective protocol and arranging a plan for a favorable removal and reintroduction process.

Due to time constraints and the necessity of prioritizing the health of the animals, this research could not fully explore the full-term effects of the removal event in relation to stress. Although significant differences were found in the frequency of stress behaviors during and after the removal, no data was collected prior to removal. Longer-term observations over this time period would aid in establishing what the "normal" stress levels are in this pack. The inability to draw significant conclusions from the period before removal creates difficulty in gathering the most accurate and effective interpretations of how pacing behavior was altered as a result of the stress

event versus the effects of captivity. If sufficient data was collected in the months prior to when removal-related stress began, a standard frequency of pacing solely as a result of captivity could be analyzed. Future research should ensure that more abundant information is gathered on the pack before a manipulation to create a foundational baseline for statistical analysis. Future studies should additionally consider incorporating an endocrinological approach to analyzing stress levels by investigating cortisol levels, the primary hormone associated with stress. Cortisol is a physiological marker of stress released as a bodily response to a stressful event. Utilizing physiological markers allows for a more precise and standardized measure of stress levels, ultimately providing the opportunity to draw more accurate conclusions. Considering both subjective visual measures of stress as well as objective biological levels can offer a more comprehensive understanding of the effects of removal on pack stress. Furthermore, future research should address if different results are obtained depending on the social status of the removed individual. Captive removal of an alpha-pair individual compared to a beta individual can provide essential information on whether the effects of reintroduction differ based on social status.

### Conclusion

The complexity of the social structure of African painted dog packs makes the reintroduction process's fragility even more challenging to direct. African painted dogs are currently considered an endangered species, and the wild population is rapidly declining due to the expansion of human populations and the associated habitat fragmentation this causes (Woodroffe & Ginsberg, 1999). Since the 1950s, multiple southern African institutions have created captive breeding programs to ensure the population's long-term survival; however, attempts at reintroduction into the wild have been largely unsuccessful (Frantzen et al., 2001; Mills et al., 1998). Understanding successful reintroductions in captive populations is imperative to preserving the endangered levels of wild African painted dog populations (Bouley et al., 2021). The approach of reconnecting wild populations stems from ensuring captive populations are prepared for reintroductions of this nature. It is of the utmost importance for wildlife management institutions to understand how removal events in captivity impact stress and pack cohesiveness and how this may affect long-term population recovery efforts; the potential restoration of the African painted dog population begins with the effective preservation of captive populations.

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