OPEN ACCESS J. Zoo Biol. 04 (01) 2021. 09-19 DOI: 10.33687/zoobiol.004.01.3431

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Available Online at EScience Press

Journal of Zoo Biology

ISSN: 2706-9761 (Online), 2706-9753 (Print)

https://esciencepress.net/journals/JZB

Behavioral Changes Related to Stress in a Mexican Gray Wolf (*Canis lupus baileyi*) Pack

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ARTICLE INFO

Article History
Received: November 24, 2020
Revised: March 29, 2021
Accepted: June 15, 2021

Keywords
Group behavior
Environmental stress
Change priorities
Mother response

ABSTRACT

Stress is an interesting combination of biological responses to adverse environmental factors. Stress responses can be adaptive as a way individuals respond to potential life threats or situations potentially reducing reproduction success. Normally, it produces a change in an individual's priorities directing energy to solve immediate situations instead of long-term investments. In this work we report a two stress inducing situations for Mexican wolf packs at the old Wildlife Research Center (CIVS) of San Cayetano, México. In the first one, an attempt to provide artificial dens within the enclosure occurred just prior to the parturition date for the litter. The dam changed the described normal wolf-mother behavior giving birth in a small depression instead of a den and moving the puppies several times until they died, post-mortem analysis reveals that death of one pup was due to a pneumonia. In the second case accidental separation of one of the yearling females produced changes in behavioral patterns in the family pack, including increased exploratory, passing, watching, and smelling behaviors in comparison to the time when the entire pack was together. These two study cases document the behavioral responses to a stress situation.

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INTRODUCTION

Stress plays an important role in animals survival ability enabling them to rapidly respond before danger or potentially aggressive environmental situations (Álvarez & Pérez, 2009). Response to acute stress is made by threatening or danger detection by means of the central nervous system which triggers a glucocorticoids discharge through the hypothalamus-pituitary-adrenal stem (adrenaline, noradrenaline and related ones) (GC). GC release induces glucose release which allows a fast muscle contraction and stimulates sensory organs perception. This results in adaptive processes (Bonier et al., 2009), because those animals with fast responses increase their survival and, therefore their reproductive possibility (Bruisin & Romero, 2011). However, when stress conditions lengthen, they cause a negative effect provoking nervous stimulus exhaustion, muscles attrition and sensitive cells fatigue, causing a physiological process damage and even having psychological effects generating pathological and even self-destructive behaviors.

Stress has an important relationship in the animals' available resources distribution (Buchanan, 2000). Among other things stress makes a shift in those resources intended to go through long term processes to attend immediate needs. Several authors coincide in the fact that stress is a process that has diverse implications

in organisms' behavior selection, who react in different ways to stimulus, causing a different selective process (Korte et al., 2005), it has also been described that the GC effects produced by stress can modify important decisions in the couple's selection (Husak and More, 2008) or other reproductive processes (Moore and Jessop, 2003).

Stress relationship with other psychological reactions has been repeatedly proved in various mammals through different GC production that can be measured in the blood stream or in fecal feces (Keay et al., 2006). Response to stress could be considered as adaptive (Cote et al., 2001). However, it has been discussed those high levels of fecal GC presence can inhibit biological effects such as reproduction and the normal function of the immunological system (Saplosky et al., 2000; Martin, 2009). It has even been analyzed the role of stress in sexual selection through the testosterone interaction in the secondary sexual characters emergence (Moore et al., 2016). However, in some cases, it has been reported an increase in reproductive hormones with an increase in GC levels due to stress conditions such as the case of wolves (*Canis lupus* in Canada) (Bryan et al., 2015).

The Mexican gray wolf (*Canis lupus* baileyi) is an endangered subspecies. In Mexico it was classified as extinct in the wild within the Official Mexican Standard 059-ecol (INE, 2011).

The Mexican gray wolf recovery program, simultaneously managed by the Mexican and the United States governments (PACE) has the objective of increasing the number of births, puppy survival rates and population recovery, first in captivity and then it has supported recovery in the wild.

The wolf and coyotes are the America´s canine member that has a defined social behavior and it is found in packs, particularly the Mexican subspecies tends to form smaller family nucleus than that found among the larger wolf subspecies packs with a northern distribution (INE, 2010). These groups typically include: the reproductive couple, the breeding litter, and one or two previous year litter individuals. They establish hierarchical relationships which are the basis of their social organization, and which facilitates cooperative hunting (McIntryre, 1996) as well as a high parental investment by pack members (Servín, 1990).

Wolves present a seasonal reproduction at the end of winter (Soto et al., 2013). As the reproductive season approaches couples begin their courtship and breeding takes place normally in February (McBride, 1980), females go through the estrous period which is a weeklong (Asa, 1997).

Dens are excavated by females, and they usually consist of a dig in slope underground tunnel, trunks and stones may be used as a roof or support (Bernal et al.,1990), with a flat, round cavity at the bottom used for delivery and rearing of the litter. Females may use the same dens for several years, making seasonal adaptions or corrections when required. This structure allows to keep a stable temperature and relative humidity and provides security to the offspring against possible predators, thus the mother, can watch out the entrance access way (Mech, 1970). Once pups are born female stays as much time with them, however she can go out to drink, feed, pee are defecated, normally male brings food for her and leave it outside near den´s entrance (Malcom, 1985).

During reproductive and parenting seasons females (also guarding males) are sensitive to the environment stimuli. In nature these changes could be predators' presence, fire, or any other signal or change that threatens the pack's success, therefore they are very prone to modify their behavior.

In mammals stress can lead to different behavioral and physiological reactions. Normally the female will try to protect her pack increasing this behavior as more energy and resources she has invested. It is common that the females move their whole pack to another den if, for any reason, she considers it is no longer secure (Nelson, 2000).

Once puppies are born the pack's social structure is strengthened parents take control and become leaders, thus establishing the feeding disposition, and directing movements (González-Gomez, 2004; Peterson et al., 2002). These sons or daughters who stay there from the previous year follow their parents and they help with the new puppies' care and surveillance.

The use of wolf's behavioral skills to adapt to a particular housing, as well as the pack's hierarchy helps to maintain an equilibrium even under non-optimal or stressful conditions.

Due to the lack of wild populations most of these predators' behavioral studies come from animals in captivity (Fentress & Ryon, 1982). The Mexican gray wolf unfortunately was extirpated before any studies in the wild could be conducted so much of what we know of its behavior is extrapolated from captive studies and observations of wild packs since reintroduction began in

recent years in the United States and North part of Mexico. The Conservation and Research Wildlife Center in San Cayetano (from Wildlife General Direction SEMARNAT) is located at a pine forest in the center of a conservation facility of 10 Ha in a rural area far from the nearest town, where not public attendance is allowed a conducive site for behavioral patterns involved in the wolves' wellness and their adaptation in similar conditions to the wild environment.

This work describes a case study of the behavioral response presented by a wolf pack before two stress situations during their reproductive and non-reproductive seasons.

METHODOLOGY

Area of Study

This work was done at the Wildlife Research Center (CIVS) in San Cayetano, which belongs to Wildlife General Direction at the Environment Natural Resources Secretary (SEMARNAT) which is located Northwest of the State of Mexico, at 60 Km from the city of Toluca (19°22'50" N, 100°05'22" W) at 2785 m.o.s.l.

San Cayetano has a sub-humid with summer rainfall climate, with an average temperature of 18°C and a 1000 mm rain precipitation. In this zone *Pinus-Quercus* Forest domains, followed by the *Quercus-Pinus* Forest, *Pinus montezumae* and *P. patula* forests, with the presence of tree species such as *P. leophilla, P. patula, Q. craucifolia, Q. laurina* and *Cupressus lusitánica*, among others (Reyes, 2012).

San Cayetano CIVS has a total of 700 ha. The wolf's enclosure the study's wolf family pack has a triangle-rectangle like shape (Figure 1), with a 10,200 m^2 surface, delimited by a 2.15 m high cyclonic mesh. The enclosure is approximately in the geographical center of the CIVS, surrounded for a dense pine tree forest, the main idea from the CIVS construction was to reduce as much as possible human contacts, Wolves enclosures was 2km from CIVS offices This enclosure is located next to the one that shelters several fallow deer (*Dama dama*) individuals (González-Gómez et al., 2004).

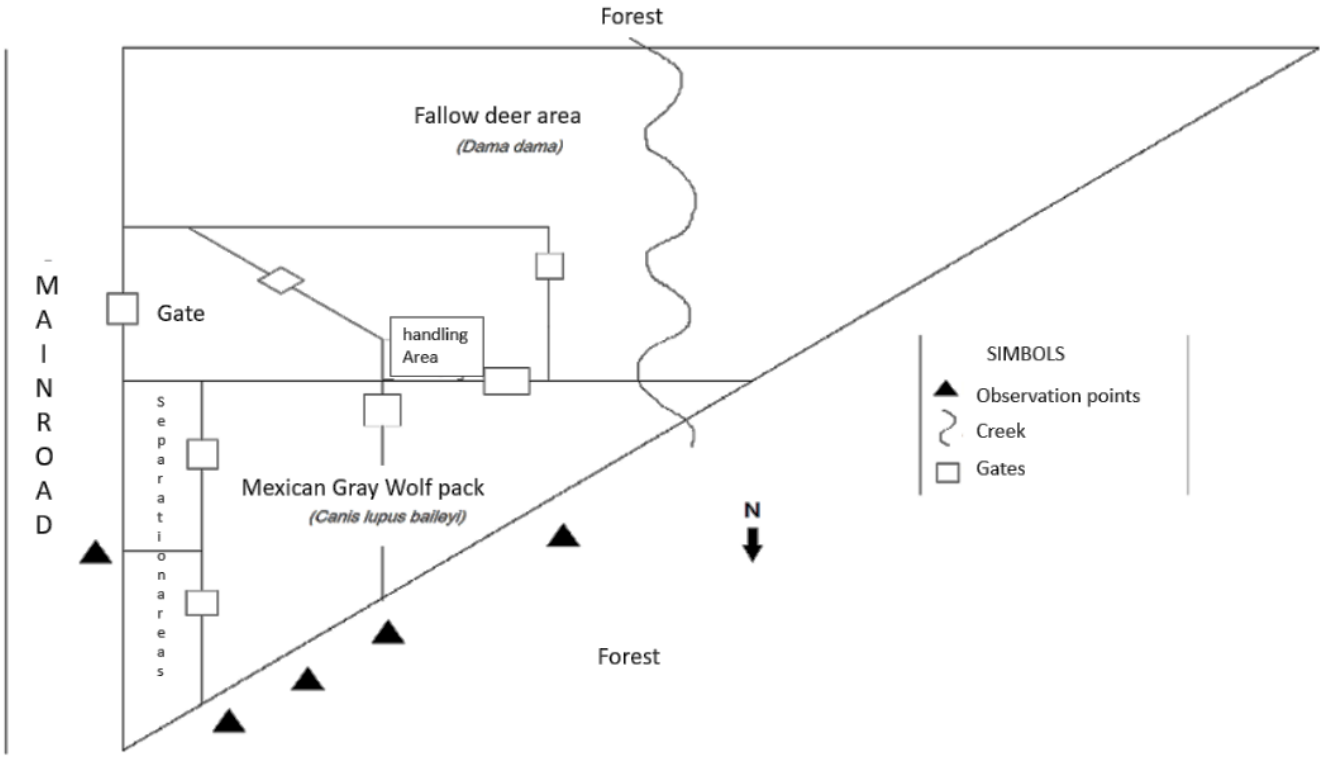

Figure. 1. Enclosures where the family Mexican gray wolf group is found at CIVS, San Cayetano, showing observations spots used in this study.

Study Subjects

Family group consisting of a reproductive pair (F-909 and M-983) and three one-year old daughters (F-1264, F-1265, and F1266). Animals were fed once a day with Mazuri® Exotic Canine and *ad libitum* water. And follow all the criteria established by the Mexican Wolf Husbandry manual (2009).

Observations

They were made from outside the enclosure, in morning periods (7:00 through 11:00 am), intermediate (11:00 through 15:00 hours) and evening (15:00 through 20:00 hours) during 20 consecutive weeks between December 2013 and May 2014. A Sony® DSC-W350 photographic camera and a Sony® CCD-TRV68video camera was used. An ethogram was developed, and pack behaviors were recorded without measuring time between behaviors of interest. Afterwards each of the described behavior relative frequencies were calculated and graphed.

RESULTS

Stress Effects on Maternal Behavior

During December, January, and February no evident disturbance within the enclosure could be detected, therefore animals displayed what was considered "unaltered behaviors" or baseline behavior, among these behaviors we found alert rest, indifferent rest, marking, running (Table 1). There were also sporadic sexual behaviors such as: mating and copulation trials (Figure 2), which are typical behaviors for individuals F-909 and M-983 during the reproductive period. Some environmental disturbances in March and April, first one was the appearance of dogs, dogs come to the fence and wolves come to them some time either the dogs or the wolves were caught showing "seeking for play" behavior (Bekoff and Wells, 1981, 1982). Deforestation activities by local people, with loud motor saw machines near the enclosure.

These disturbances occurring during the reproductive season. Some evidence suggested wolves have mated in February and female had good chance to be pregnant.

The den was a hole excavated and enlarged by females previously hosted in San Cayetano, it had a single entrance close to a pine tree based and latter could be measured it has a single chamber of approximately 1.25 x 1.42 m in a semicircular base with an entrance of 1.3 m in length, the entrance was a semicircular of approx. 50 cm in diameter. Female F-909 was enlarging the entrance and going in and out several time, presumably working on that before her due date.

Since the mate date was not registered properly, due date was not well determined. A civil work was authorized inside the enclosure it consisted in a 2 X 2 m and a one-meter-deep excavation. Work continued throughout the day and several times even until late night utilizing a portable power plant. The excavation was located less than a meter from the entrance of the den, since the idea was to serve as control room to put video-cameras into den´s interior. Footprint of at least three people were around dens entrance coming in and out of the enclosure. In those days, close to the delivery, the female showed stress behavioral signs, she gave birth to a litter of apparently 3 puppies in a small, uncovered hollow rather than in the den. Lately the female excavated a new den, far from original where she moved the puppies.

As a response to the mentioned stress the female excessively carried the pups taking them from one side of the enclosure to another, back and for caring them within her mouth.

Numerical data about the couple's behavior indicate that there was an increase in running, pacing, and moving and a significant decrease in indifferent rest during reproductive season (Figure 3, Table 1).

The Effect of Stress due to a Pack's Member Separation

Once the wolf pack were joined together, they had behaviors such as alert rest, indifferent rest, exploratory locomotion, watching, jogging, howling, and body contact playing (Figure 4). However, by mid-December an animal handling was made which caused one of the daughters (f-1265) to jump the fence that divided the enclosures and staying in the next enclosure where fallow deer (*Dama dama)* were found (Figure 1). During the time the female stayed in the enclosure with the fallow deer she spent a lot of time close to the fence that separates both enclosures, and frequently howled.

Familiar group break caused an increase in behaviors such as exploratory locomotion, stereotypic locomotion, watching, smelling, and jogging while they tried to locate her (Table 1, Figure 4). Once the young reintegrated to the group her parents showed pin and other aggressive behaviors toward her, while she showed Passive submission end active Submission (Table 1) behaviors, Once the family group was joined again behaviors like body contact, playing and indifferent rest increased (Figure 4). Upon group separation howling, exploratory locomotion, and stereotyped locomotion behaviors increased (Figure 4).

Table 1. Behavioral Patterns in each of the analyzed processes relation.

Behavior	Description	Unaltered Period	Reproductive Stress	Separation Stress		
				Before	During	After
Alert rest (AR)	Lying individual with raised head and ears	X	X	X	X	X
Indifferent rest (IR) Relaxed?	Lying individual with head down and indifferent ears	X	X	X		X
Marking (Mr)	Just males. They rest their back foot, and they have a very short urination, <1 sec.	X	X			
Mating Trial (MT)	The male sets his frontal feet over the female's rump in a fast movement	X				
Intercourse (I)	After setting the front foot the male makes a penis introduction into the female's vulva	X				
Passive Submission (PS)	Wolf approaches another in crouch or semi-crouch position with body oriented sideways to partner; head typically rolled sideways. while looking at partner. May be accompanied by whimper/whine. and licking intentions towards partner.			X		X
Active Submission (AS)	In presence of another, wolf falls or lies on its side or back, often. with hind legs raised and ears back. May be accompanied by whimper/whine. Can follow passive submission.					X
Pin Pi	To lunge and bite at the neck or muzzle, forcing the wolf to the ground and holding it there					X
Running (R)	Individuals move fast	X	X			
Stereotypic Locomotion Pacing (SL)	Repetitive behaviors with a fixed model without any apparent objective		X	X	X	X
Exploratory Locomotion (EL)	Individual's shift making sure they know what happens around their habitat			X	X	X
Watching (W)	Watching something or somebody with much attention and with more detail to get some knowledge			X	X	X
Jogging (J)Trotting	Moving modality, it is about an accelerated walk			X	X	X
Crying (C)	A very acute and strident sound that the animal releases					X
Submission (S)	Exhibited behaviors to inhibit aggression through a series of body postures					X
Playing with body contact (PB)Play bout	Physical interactions, pushing, chasing, and slight biting among individuals	X				X
Howling (H)	A long voice	X	X			

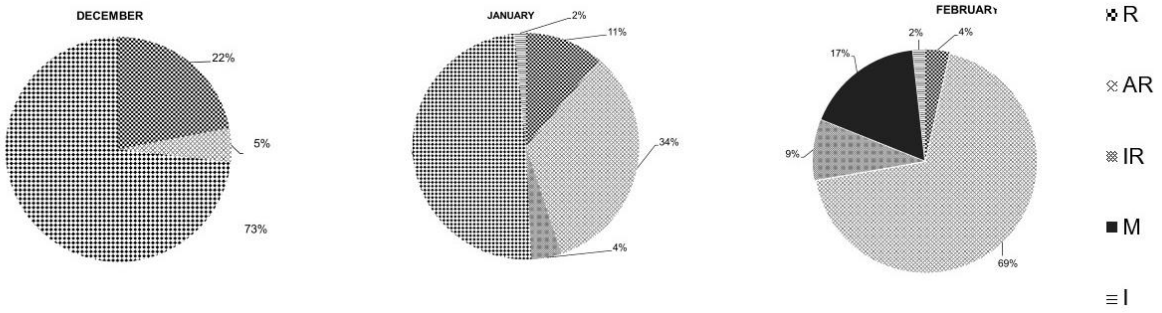

Figure 2. Wolves' activity percentage during the reproductive and pregnancy seasons in which undisturbed behaviors are shown (R=Running; AR=alert rest; IR=indifferent rest; M=marking, I=intercourse).

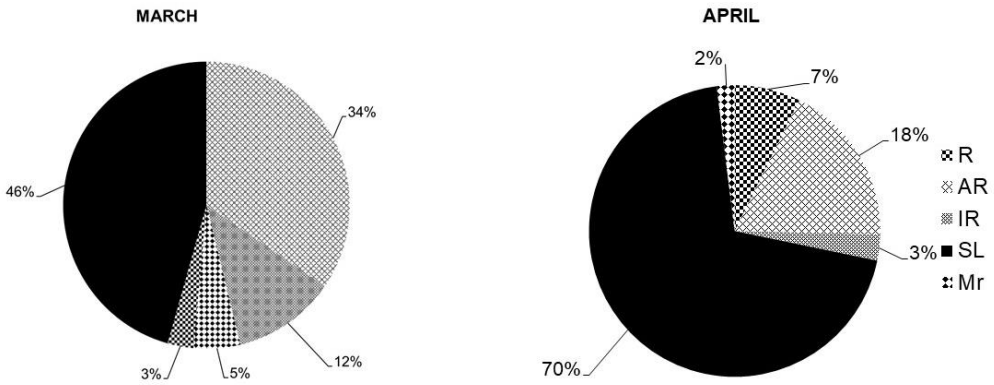

Figure. 3. Wolves' activity percentage during the months in which the reproductive stress was present, which coincide with the end of pregnancy and delivery (loc. est. =stereotyped locomotion (R=Running; AR=alert rest; IR=indifferent rest; M=marking, SL= Stereotypic Locomotion).

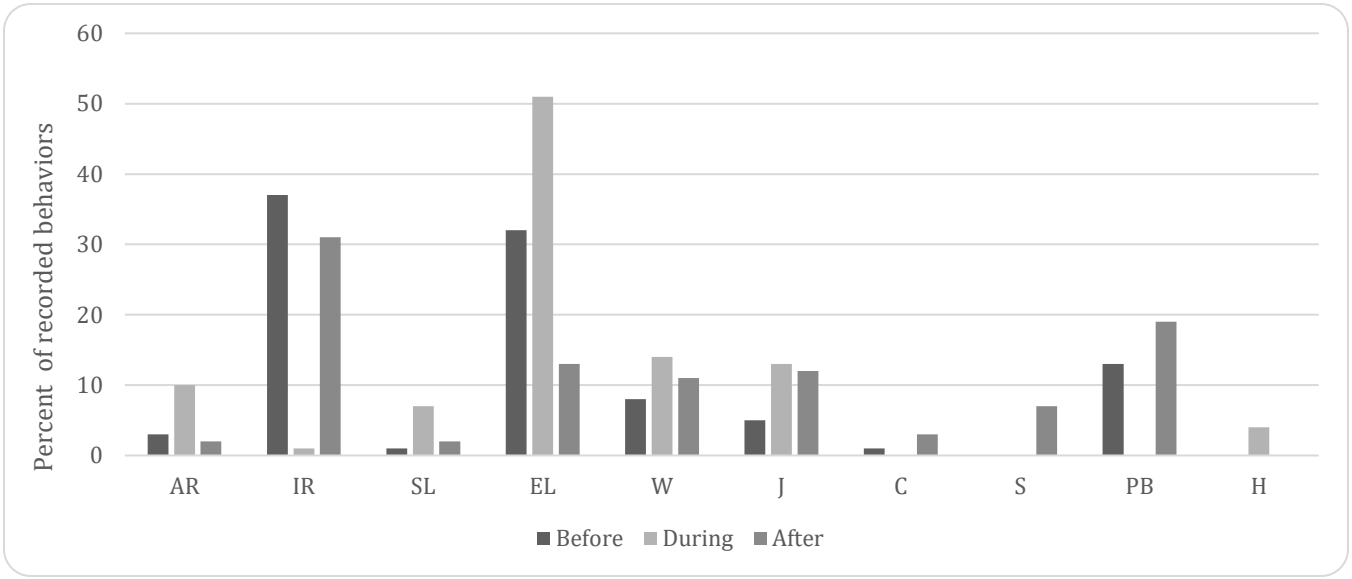

Figure. 4. Family group activity percentage during three phases: A. Observed behaviors before a member separation; B. Observed behaviors during a member separation, and C. Observed behaviors after an absent member re-introduction (AR = alert rest; IR = Indifferent rest; SL. = Stereotyped locomotion; EL. = exploratory locomotion; E= Watching J= Jogging; C. = Crying; S. = Submission; PB = body contact; H. = howl).

DISCUSSION

Wolf is a social species so he is quite sensitive to all those elements that could put into risk his group's stability (Jordan, 2014), thus, any disturbances that surround them during delivery and nursing are of great importance for the pack's survival. Like many other carnivores, wolves are altricial animals who depend on parental care in their first stage of life (Numan, 2010), therefore for their mother, den's security is essential because it is there where she will inhabit with the newborn at least during their first two weeks (Bernal et al., 1990; C. Vázquez-González, 2017). During this period puppies are blind, and she focuses on feeding them Between 2-4 hours Packard et al., 1992 hour, and therefore, they are very vulnerable to predators and sickness, in wildlife conditions the mother does not abandon the den; in the first two weeks it is the father that provides feed (Mech, 1970). The mother's exhibited behavior about carrying her puppies from one side to another in the enclosure strengthens what Buchanan (2000) expressed in the sense that stress obligates animals to take fast decisions at the expense of a long-term investment.

The use of open or shallow digs for whelping and stashing newborn pups as nest under trees has been reported (Mech et al. 1998 p.67), mother wolves use covered and then pups were moved to a formal den within few hours after been born. But normally birth takes places at least under shadow or cover areas in contrary what happened in this case, were mother, that find her den affected by the excavation nearby and the noise from the power plant, could not make another proper birthplace and move just born puppies.

It has been reported puppy's movement out of the den as a response to stress (Fritts & Mech, 1981; Armella & Soto non-published data), however, in disturbed spaces, where sites availability is limited (as in the case of San Cayetano enclosures) the female might not consider any of them as a safe site except for exposing puppies to a movement excess. In the case of the female f-909, even when there are several dens from previous years, she did not choose any of them to re-accommodate her puppies, she ran with each one of them in her mouth from one site to another without an apparent destiny instead. Female f-909 was an experienced mother she had previous litters in the same enclosure in 2007, 2012, 2013 and then 2015 (Siminski 2007, 2012, 2013 2015) successfully in all those years she used dens to whelp therefore it is not likely she would use an uncover area in that particular year, therefore we can considered the disturbance near the den she had been working on probably produce stress and when labor stated select the shallow depression, then start looking for a safer place to the puppies, unfortunately she could not find a right place.

On the other hand, stress has been reported as a breast milk production inhibitor (Aguayo et al., 2009). According to an official necropsy, as reported by SEMARNAT (data obtained from SEMARNAT, number DGVS_387115), just one of the three puppies could be recovered to make such necropsy and the document indicates that the puppy presented a clinical picture compatible with bacterial pneumonia (Siminski, 2014), Besides, in the judgement it is indicated the presence of possible milk "residues" in the stomach, however, it is not possible to determine how long it remained there, neither the amount, so lactation inhibition cannot be discarded as indirect or direct cause of the puppies' death.

One of the data that supports the lack of puppies' feed hypothesis is the reported puppies' weight in the necropsy. Mech (1970) indicates that wolves use to have a great weight increase during the first stage. According to data of puppies retrieved from mothers at a young age (3-14 days old n=23) (Mossotti, Pers com 2021) and considering that the average weight at birth in Mexican gray wolf is of about 300 g (Servín, 1997), we run a simple regression model (NCSS, 2018) to estimate growth ratio is 106.71±9.54 g/day. Supposing that the studied puppy was a female – sex was not reported in the necropsy -, and in the report it is stated that the puppy died after 8 days of its birth, the puppy would have weighed 923.54 g (95% Confidence Interval 867.42, 979.46 g), however, the necropsy reports that it weighed 810 g, between 7 to 18% less than expected. This last data supports the possibility that, due to stress, the female will not feed the pack correctly. However, a large variation among birth weight as growth rate, has been known to exist in wolves therefore it is difficult to absolute blame this as dead cause.

Wolves' pack integration is very important (Mech, 1970; Jordan, 2014), in most of the cases, and particularly, in the case of the Mexican gray wolf, packs are formed by extended family groups, and that is why separation of some of their members causes stress for the rest of the members (Servín, 2002), due to the fact that one of the social structure features is help among them, so when one of the members in missing their survival as a group is decreased.

The change of behavioral patterns in which the time dedicated to "Indifferent rest" decreased and the increase in "exploratory behavior" is an evidence of the group's interest in finding the separated member, as does an increase in vocalization, all consistent with works reported for wild wolves (Mech, 1970). Submissive social behavior as well as the return to prior? behavioral patterns, which are like the ones above presented at the exit, are according to what Smith et al. (2011) observed in the pack re-construction, in which hierarchy is re-affirmed by means of stereotyped behaviors in which all the pack members participate.

Therefore, it may be determined that a pack member's absence generates stress modifying diverse behaviors' frequency which are especially important within the group, because they keep relationships among its members socially stable.

In conclusion, the environmental elements that alter conditions which wolves live result in acute stress behavioral patterns, which conditions in regular individual packs' regular behaviors changes independently of the environment in which they are located. These behaviors are evolutionary, destined to re-establish the individual or group homeostasis (Lorenz, 1980).

ACKNOWLEDGEMENT

Authors thank to the personal of San Cayetano CIVS at the time of the study, specially to Jorge Yáñez. To Regina H Mossotti Director of Animal Care and Conservation, and Tracy Rein Conservation Assistant and Registrar of the Endanger Wolf Center for allow us to use some of their data to estimate growth rate of puppies and to an anonymous reviewer for her/his useful comments of the manuscript.

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