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The Effect of a Yearling on Its Iberian Wolf (*Canis lupus signatus*) Parent Pair: Welfare Indicators in Captivity

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Maintaining biologically functional and compatible social groups is a primary welfare concern for curators of captive animals. The aim of this study was to determine the effect of a yearling's presence on the daily activity, use of space and inter-individual distance on its parental pair of Iberian wolves (*Canis lupus signatus*) housed at the Barcelona Zoo. Multifocal sampling methods were used for data collection, and instantaneous scans were made at 15-min intervals during 10-hr sessions. 432 sampling points were balanced for the daily periods – morning, midday, and afternoon - for two different phases: dyad and triad phases. The subjects studied during the dyad phase—from April to May 1999—were the adult mated pair during the adult female pregnancy. For the triad phase—from May to June 2000—we studied the mated pair together with its new yearling. When comparing these two study phases, there were statistically significant differences for the daily activity, particularly for exploration, locomotion, feeding, and inactivity of the mated pair. In addition, the pair used the space more homogenously during the triad phase. During this phase, the inter-individual distance of the mated pair was significantly smaller in the morning and in midday and the yearling was closer to the adult female than to the adult male. The mated pair also showed individualized results for their daily activity, use of space and inter-individual proximity during the triad phase. Increasing the understanding about the effects of pregnancy and the birth of a new pack member enables the improvement of captive management and helps providing wolf packs with the most appropriate social environments.

Keywords: animal welfare, daily activity, Iberian wolf, inter-individual distance, new pack member, space use

The Iberian wolf (*Canis lupus signatus*, Linnaeus 1758, Canidae, Carnivora) is an endemic subspecies from the Iberian Peninsula belonging to the Canidae family. The 2012-2014 census carried out by the Spanish Ministerio de Agricultura, Alimentación y Medio Ambiente, estimated a total Iberian population of 297 packs, which represents an increase when compared to the 250 packs estimated for the 2007 census (Blanco & Cortés, 2002; Grande del Brío, 2000; Sáez de Buruaga, 2018). In 2018, the International Union for Conservation of Nature (IUCN) Red List Species categorized the Iberian wolf as least concern, whereas the Red Book of Vertebrates in Spain categorizes this species as vulnerable because of the fragmentation of management regimes, the lack of a management plan at the population level, and the occurrence of largely unpredictable events of human reaction against wolves (e.g., poisoning, shooting) that may threaten the population at local levels (Iglesias et al., 2017). The small population of Sierra Morena is far apart from the main population in the North, and it should be classified as critically endangered (Blanco & Cortés, 2002). As a consequence, it is extremely important to ensure the survival of this species through habitat conservation and captive breeding programs.

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The basic social unit of a wild Iberian wolf population is the mated pair (Mech & Nelson, 1990) and its offspring, which functions in a tight-knit unit year-round (Olson, 1938; Young & Goldman, 1944). There are many variations on this process of pack formation and pack maintenance but, basically, packs are composed of a mated pair of wolves and their offspring. Packs originate when an unrelated male and female meet, pair up and produce pups (Smith et al., 1997). After dispersing from the families where each one was born, the members of a new pair travel together into an area not defended by other hostile packs in order to establish a new pack. Litter sizes range from one to 11, but on average five to six pups are born in a given year (Mech, 1970). The offspring usually remain with their parents for 10 to 54 months and, except under special circumstances, all offspring eventually disperse (Gese & Mech, 1991; Mech et al., 1998). Packs therefore may include the offspring of as many as four years. A wolf pack, therefore, is some variation on a mated pair plus offspring, and packs have been observed to contain as many as 42 members, although most include far fewer (Mech & Boitani, 2003), with average pack sizes ranging from three to 11 individuals (Carbyn et al., 1993). Theory holds that, in the wild, pack size should vary according to prey availability up to some optimum number; this optimum should meet the requirement of allowing predation with the least energy expenditure and the highest energy return (Macdonald & Moehlman, 1983). Furthermore, when prey availability is reduced, large packs can be reduced in size through lower reproduction and/or survival or through dispersal. In addition, as packs enlarge, they sometimes split or proliferate (Mech & Boitani, 2003).

In captivity, maintaining biologically functional and compatible social groups is a primary welfare concern. Indeed, social living provides benefits other than simply finding food and avoiding predation; it is a major source of stimulation. The social milieu of many species represents a constant source of mental stimulation, the complexity and variety of which would never be replaced by any form of environmental enrichment in captivity. Indeed, appropriate management of social groups in some species is considered one of the most relevant, but difficult tasks to achieve in captivity (Schapiro et al., 1996; Young, 2003). Group composition and size are the most important factors influencing the formation and maintenance of successful social groups in captivity. For zoos, appropriate social groupings are of utmost importance in order to provide examples of species-typical behaviors, as well as for attaining captive breeding goals. In the wild, group-living evolved largely in response to the needs for predator avoidance and territory defense. The number of members in wild groups is a fundamental determinant of individual fitness, affecting net food intake and reproductive success. Captive animals encounter different environmental pressures as compared to their wild counterparts; food availability and predation are no longer concerns; however, they still face competition for mates and are unable to make the social adjustments necessary to decrease social tension. As such, group size has a large impact on the behavior, welfare, and reproductive success of captive animals. Depending on the species, suboptimal sizes can be associated with increased abnormal behaviors and a decrease in both reproductive success and infant survival in a range of captive mammals. However, in the absence of environmental constraints, many species can be housed in a greater diversity of social groups than observed in the wild. Thus, a key component of captive housing is the implementation of appropriate breeding programs to achieve flexibility in wolves' pack social structure (Price & Stoinski, 2007).

Different welfare ethological indicators are used to measure the appropriateness of social management in captivity, such as the rest/activity balance, the number of abnormal behaviors, the behavioral diversity, the use of space, and the inter-individual proximity, among others (Frézard & Le Pape, 2003; Maple & Perdue, 2013; Mason, 1991; Miller et al., 2020; Plowman, 2003; Ross et al., 2009; Veasey et al., 1996). In wolves in general, various studies have been performed on the social behavior in captivity using behavioral, spatial, and social welfare indicators (Frank, 1987; Frézard & Le Pape, 2003; Fox et al., 1974; Rabb et al., 1967; Schenkel, 1967; Zimen, 1982). For the particular case of the Iberian wolf, only four publications address social management in captivity through the use of different welfare indicators. These include discussions related to: (a) the type of social interactions (attention-aggression, submission, sexual-friendly contact, defense-appeasement, and play) in a pack of eight wolves (Colmenares, 1983), (b) scent and visual marking (urine marks, feces, scratching and rubbing marks) in two pairs that behaved in a distinctive way (Barja & De Miguel, 2000), (c) the effects of the death of the breeding male on the behavior and the use of space of the rest of the pack (Soriano et al., 2006), and (d) the inter-individual distance in different captive packs and its management applications (Soriano et al., 2021).

The aim of the present research was to determine the effect of a yearling on the daily activity, the use of space, and the inter-individual distance of its Iberian wolves' parents. This study used three types of welfare indicators to determine the effects of a new yearling on the Iberian wolf's captive social management.

Method

Animals and Housing

The studied subjects were three captive-born Iberian wolves (*Canis lupus signatus*) housed at the Barcelona Zoo (Table 1).

Table 1

Demographic Data for the Iberian Wolves

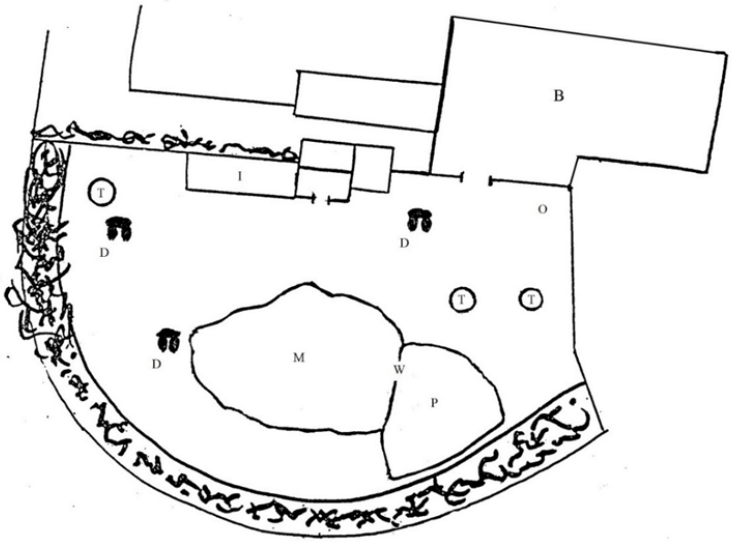
Age class and sex	Birth date	Rearing condition	Arrival date at zoo
Adult female	May 1996	Parent	October 1996
Adult male	May 1995	Hand	January 1999
*Yearling female	May 1999	Parent	May 1999

Note. *Yearling refers to an individual that is at least one year old and younger than two years old. Yearlings may not be considered fully mature (Mech & Boitani, 2003).

During the observations, the Iberian wolves were housed in a 940 m^2 naturalized outdoor enclosure surrounded by a metallic fence with vegetation (see Figure 1). The outdoor enclosure contains typical Mediterranean vegetation, three dolmens made of three big stones, and a waterfall that feeds into a pond, as well as different enrichment devices such as metallic boxes, an L-shaped metallic structure for hiding food, and a mechanical rabbit for chasing. In the middle of the outdoor enclosure, there is a mound measuring 2 m in height and 5 m in length. The indoor enclosure contains three cement cages out of sight from the public (7 m^2 each) and the breeding enclosure (300 m^2) which is a prolongation of the outdoor enclosure separated from it by a metallic fence and a door (Soriano et al., 2006, 2021, 2022).

Figure 1

Diagram of the Iberian Wolves' Enclosure



Note. B = breeding enclosure; D = dolmen; I = indoor enclosure; M = mound; O = outdoor enclosure; P = pond; T = tree; W = waterfall.

Daily Management

The studied animals were socially housed as advised given their social lifestyle (Grande del Brío, 2000). In November 1998, the adult female was the sole survivor from its original pack housed in the Barcelona Zoo since October 1996. In January 1999, the male Iberian wolf arrived at the Barcelona Zoo thanks to the donation by a private owner. In March 1999, the adult male and female wolves were put together after a progressive socialization program following the European Association of Zoos and Aquariums (EAZA) Ex- situ programmes (EEP). During the first month of this program, the adult female lived in the outdoor enclosure while the adult male was living in the breeding enclosure and they only had olfactory, non-physical, contact through the metallic fence and the separated door, which were both covered with black clothing. During the second month, both wolves had visual and olfactory, non-physical, contact as in this phase the black clothing was removed. Finally, during the third month the wolves were put together in the outdoor enclosure.

The management protocol allowed the Iberian wolves to go into their indoor enclosures voluntarily from 7:30 a.m. to 9:00 a.m.

The Iberian wolves' diet was seasonally unvaried and delivered twice daily. In the morning, each wolf ate 1 kg of raw horse meat at the outdoor enclosure and in the evening each wolf ate 1 kg of raw horse meat with bone at the indoor enclosure. All aspects of the animal husbandry described were the same for all phases of this study.

Procedure

Four different observers, trained in wolf behaviors, conducted the registers. These observers were psychology and biology students. They participated in the study on Iberian wolves' welfare in captivity through the observation of the daily activity patterns and the space use under different conditions. The observers spent four sessions per month to achieve the inter-observer reliability test, in which they were required to demonstrate an average agreement higher than 85% with another experienced observer (Lehner, 1998).

The dyad phase (DP) included the mated pair as the basic social unit, and it was conducted from April to May 1999 to study its daily activity, use of space, and inter-individual distance prior to the pup birth. This phase included the pregnancy period of the female. The triad phase (TP) included the mated pair and its offspring—a female—as the basic pack, and it took place from May to June 2000 to study the effect of the new yearling on these three variables.

Multifocal sampling methods were used for data collection given that there were two (DP) and three wolves (TP) to be observed—all-animal sampling—at the same time. Instantaneous scans were made at 15-min intervals during 10-hr sessions (Altmann, 1974). Both phases consisted of 432 sampling points balanced for the daily periods to obtain the daily patterns for each Iberian wolf. The variables recorded were registered by pencil on paper with check sheets.

The variables recorded for the Iberian wolves at each observational session were: (a) the phase of study (DP or TP); (b) the period of the day (morning, from 9:00 a.m. to 1:00 p.m.; midday, from 1:00 p.m. to 4:00 p.m.; and afternoon, from 4:00 p.m. to 7:00 p.m.); (c) the daily activity patterns (behavioral categories were defined as comprehensive and mutually exclusive; see Table 2) (Rees, 2015); (d) the space use, by graphing the whole enclosure, that is, the outdoor enclosure (divided into seven similarly sized zones—Zones 1-6 and mound), the indoor and the breeding enclosures, and an undetermined zone—when the animal location was unknown (see Figure 2); and (e) the inter-individual distance (ID) measured in meters and defined as the space that separates two animals. This was calculated by using the distance formula between two points from the Pythagorean Theorem in the enclosure plan (1:10) and assuming that animals were in the barycenter of each zone. The Cartesian values of each zone were calculated through the enclosure plan (Barlow, 2016; Soriano et al., 2021).

Table 2

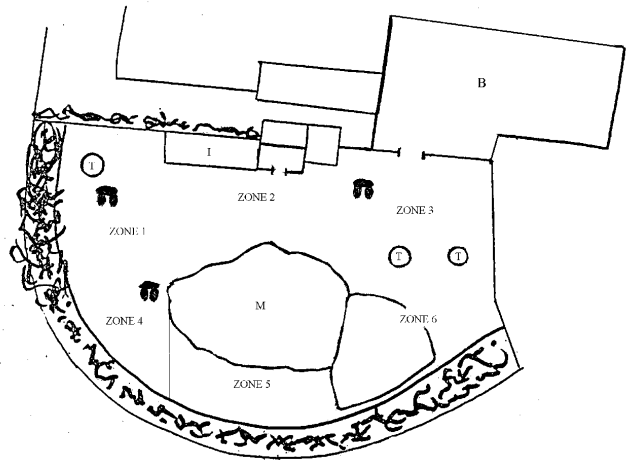
Definitions of the Iberian Wolves' Daily Activity Patterns

ACTIVITY: means any behavior not classified as inactive, which includes:	
Exploration	The wolf sniffs the air, the substrate, food or objects. It also includes when the wolf is alert with the head up and the eyes open.
Locomotion	The wolf moves around the enclosure without exploration. It also includes chasing the mechanical rabbit.
Scent marking	The wolf defecates or urinates using different leg postures. It also includes substrate scratching after urination or defecation and rubbing against odors.
Feeding	The wolf consumes food items; this also includes drinking and nibbling on herbs.
Solitary play	It mainly involves the wolf's movements; vigorous, rigorous, exaggerated like jumping or running.
Maintenance	The wolf self-grooms and scratches with the mouth and/or the paws. It also includes when the animal shakes.
Manipulation	The wolf claws at, swipes at, nibbles at, picks up the food and the non-food items with the mouth and/or the paws. It also includes digging a deep burrow.
Human interaction	The wolf sits up while orienting to humans. The wolf tries to communicate with humans in different forms (e.g., following the same path as the humans).
Social interaction	This includes affiliation, play, howling or agonistic behavior.
INACTIVITY: the wolf rests seated or lain with relaxed musculature.	
OUT OF VISUAL RANGE: the wolf or its' behavior is not observable because the Iberian wolves' enclosure design allowed wolves to hide (i.e., behind the mound or the dolmen or inside the indoor or the breeding enclosures).	

Note. The daily activity patterns were classified into three macro categories.

Figure 2

The Iberian Wolves' Enclosure Showing the Division of the Area



Note. I = indoor enclosure; M = mound; B = breeding enclosure.

Data Analyses

All data analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL 60606, USA) Version 23.0 for Windows. Normality was assessed using the Kolmogorov-Smirnov test. The chi-square test and the Fisher's exact test were used to determine which categories of the daily activity and space use showed statistically significant differences across the periods of the day. The adjusted residuals statistic used had an absolute value of 1.96 for a normal distribution, assuming that the significance level is $p = .05$ (Forthman & Bakeman, 1992; Haberman, 1978).

In order to analyze the effect of the new yearling on homogeneous space use, a Modified Spread-of-Participation index (SPI) for observed frequencies was used. A value of 1 indicated minimum use of the enclosure, and a value of 0 indicated that the use of the space was totally homogeneous (Plowman, 2003; Rose & Robert, 2013).

The Mann-Whitney *U* test was used to determine if there were statistically significant differences in inter-individual distances for the Iberian wolves across both study phases.

Results

Effect of the New Yearling on the Daily Activity Patterns

The daily activity pattern of the Iberian wolf mated pair showed statistically significant differences—except for the male in midday—in each period of the day when both phases were compared (Table 3).

Table 3

Chi-Square Test and Fisher's Exact Test for Daily Activity and Use of Space

		Adult male			Adult female		
		Test value	*df*	*p*	Test value	*df*	*p*
Daily activity	Morning	42.16[b]	1, 10	.001**	34.00[b]	1,7	.001**
	Midday	7.33[b]	1,7	.35	75.29[b]	1,7	.001**
	Afternoon	47.93[a]	9	.001**	92.21[a]	8	.001**
	Total	22.37[a]	10	.01*	146.69[a]	9	.001**
Space use	Morning	132.46[b]	1,6	.001**	48.95[b]	1,7	.001**
	Midday	110.36[b]	1,6	.001**	61.83[a]	7	.001**
	Afternoon	60.39[a]	7	.001**	12.40[b]	1,9	.17
	Total	264.4[a]	8	.001**	81.59[a]	9	.001**

Note. [a]Chi-square test; [b]Fisher's exact test; statistically significant differences: *$p < .05$ and **$p < .001$

During the TP the adult male showed a statistically significant increase in exploration and inactivity in the morning, locomotion and feeding in the afternoon and a decrease in locomotion in the morning and inactivity in the afternoon. In the same phase, the adult female showed a statistically significant increase in locomotion in the three periods of the day, exploration and social interaction in the midday and afternoon, and feeding in the afternoon. The adult female also showed a statistically significant decrease in out of visual range in the three periods of day, inactivity in the midday and afternoon, and maintenance in the morning.

The daily activity patterns for the yearling female were only registered during the TP. Most of the activity patterns (exploration, locomotion, feeding, maintenance, manipulation, and social interaction) were more frequently observed during the afternoon. Scent marking, inactivity, and out of visual range were more frequently observed during the morning while solitary play was more frequently observed during midday (Table 4).

Table 4

Observed Frequencies of the Daily Activity Behavioral Categories in Each Period of the Day for the Two Phases of the Study

	Adult male						Adult female						Yearling female		
	Morning		Midday		Afternoon		Morning		Midday		Afternoon		Morning	Midday	Afternoon
	DP	TP	DP	TP	DP	TP	DP	TP	DP	TP	DP	TP		TP	
Exploration	6	15*	13	17	17	18	19	27	3	27*	14	31*	20	21	22
Locomotion	111	70*	47	46	22	44*	7	21*	8	29*	8	37*	16	29	37
Scent marking	1	1	0	0	0	2	1	0*	0	0	0	0	1	0	0
Feeding	3	0	3	2	4	24*	4	0	0	2	5	25*	2	4	23
Solitary play	1	1	0	1	0	2	0	1	0	2	1	3	0	1	0
Maintenance	0	1	0	1	2	2	7	0*	8	0	9	0	1	2	5
Manipulation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Human interaction	2	0	0	2	2	3	0	0	0	1	1	1	0	1	1
Social interaction	7	7	3	8	5	9	8	7	3	17*	5	19*	8	12	17
Inactivity	13	47*	78	67	84	38*	84	88	109	66*	94	27*	85	74	38
Out of visual range	0	2	0	0	0	2	14	0*	13	0*	7	1*	11	0	0

Note. DP = dyad phase (the mated pair); TP = triad phase (the mated pair and the yearling); statistical significance with adjusted residuals: *r > I1.96I

Effect of the New Yearling on Space Use

The use of space in the Iberian wolf mated pair showed statistically significant differences—except for the female in the afternoon—in each period of the day when both phases are compared (Table 3).

During the TP, the adult male showed a statistically significant increase in the use of Zone 4 and a decrease in the use of Zone 2 for the three daily periods. In addition, the male showed a statistically significant increase in the use of Zones 1 and 5 in the morning and Zone 5 in the afternoon. The adult male also showed a statistically significant decrease in the use of the mound during the afternoon. During the same phase, the adult female showed a statistically significant increase in the use of Zones 1 and 4 in the morning and Zone 4 in midday. This female also showed a statistically significant decrease in the use of the mound and the breeding enclosure during the morning and midday, Zone 2 in midday and the indoor enclosure during the afternoon.

The spatial use for the yearling female was only registered during the TP. The majority of spatial categories (Zones 2, 3, 4, 5, and 6) were more frequently used by the yearling female during the afternoon. In the morning, the mound and the undetermined zone were more frequently used while in midday Zone 1 was more frequently used by the yearling female. It was never observed using the indoor and breeding enclosures (Table 5).

Table 5

Observed Frequencies of use of the Spatial Categories in Each Period of the Day for the Two Phases of the Study on the Iberian Wolves

	Adult male						Adult female						Yearling female		
	Morning		Midday		Afternoon		Morning		Midday		Afternoon		Morning	Midday	Afternoon
	DP	TP	DP	TP	DP	TP	DP	TP	DP	TP	DP	TP		TP	
Zone 1	13	26*	40	38	39	34	21	45*	33	33	38	35	31	33	22
Zone 2	110	27*	88	20*	72	27*	5	12	5	14*	11	12	9	15	19
Zone 3	1	0	1	2	4	2	1	5	2	7	12	5	1	3	6
Zone 4	1	50*	6	70*	10	33*	8	25*	9	46*	28	27	21	22	26
Zona 5	10	50*	6	70	4	33*	4	6	3	7	14	15	3	7	19
Zona 6	1	35	0	10	2	30	1	2	1	3	1	7	0	2	6
Mound	8	1	3	1	4	3*	90	48*	78	32*	33	31	68	62	46
Indoor enclosure	0	5	0	3	0	13	0	0	0	0	2	9*	0	0	0
Breeding enclosure	0	0	0	0	0	0	14	1*	13	2*	5	3	0	0	0
Undetermined zone	0	0	0	0	0	2	0	0	0	0	0	0	11	0	0

Note. DP = dyad phase (the mated pair); TP = triad phase (the mated pair and the yearling); statistical significance with adjusted residuals: *r > I1.96I

The modified SPI values determined that male and female Iberian wolves used the space more homogenously during the TP than during the DP. The female yearling SPI was identical to that of the adult male (Table 6).

Table 6

Modified SPI for the Two Phases of Study in the Iberian Wolves

	Adult male	Adult female	Yearling female
DP	.59	.46	
TP	.43	.32	.43

Note. DP = Dyad phase (mated pair or basic unit);
TP = Triad phase (mated pair and yearling or basic pack)

Effect of the New Yearling on the Inter-individual Distance

During the TP, the inter-individual distance between male and female Iberian wolves was significantly smaller ($U = -4.62$, $p = .00$). Moreover, during this phase and in terms of the daily periods, the inter-individual distance between male and female was significantly smaller in the morning ($U = -2.34$, $p = .02$) and in midday ($U = -4.00$, $p = .00$) but there was not a statistically significant difference in the afternoon ($U = -1.41$, $p = .15$) (Table 7).

The inter-individual distance for the yearling female was only registered during the TP. The yearling female was closer to the adult female than to the adult male for the three periods of the day. Moreover, the yearling female was closer to its parents during the afternoon than during the morning and midday (Table 7).

Table 7

Inter-individual Distances (m) for the Two Phases of Study and the Periods of the Day for the Three Iberian Wolves

	Mean ± SD					
	Adult male-Adult female		Adult male-Yearling female		Adult female-Yearling female	
	DP	TP	DP	TP	DP	TP
Morning	9.44 ± 5.85	7.95 ± 6.08		10.01 ± 8.12		9.09 ± 8.87
Midday	11.13 ± 6.76	8.40 ± 7.84		10.56 ± 7.72		9.43 ± 7.67
Afternoon	10.43 ± 7.29	9.30 ± 7.90		9.25 ± 7.94		7.98 ± 7.79
Total	10.33 ± 6.68	8.55 ± 7.32		9.95 ± 7.93		8.83 ± 8.14

Note. DP = Dyad phase (mated pair or basic unit); TP = Triad phase (mated pair and yearling or basic pack)

Discussion

Both male and female of this study showed an individual response to the new yearling which could easily be attributed to the sex of the wolves, parenting roles, type of rearing, and so on. These individual behavioral profiles are a possible way of describing variation in temperament, analogous to "personality" in humans, which can be a very useful tool for the welfare management of these wolves (Mech & Boitani, 2003). Several researchers have attempted to use multivariate statistical techniques to determine the basic dimensions of variation in personality among captive wolves (Bekoff et al., 1975; Colmenares, 1979; Derix, 1994; Lockwood, 1979; Packard, 1980; Van Hoof & Wensing, 1987).

When the new yearling was studied, the mated pair increased the exploration and feeding activities and decreased the inactivity. This pair increased the exploration of external and internal environments and decreased inactivity probably in order to raise and protect the yearling properly. Feeding also increased when the female yearling was present, probably aiming at recovering from the high energetic cost that the care of the pup implies (Malcolm, 1985).

The new yearling in the pack caused more effects on the female's daily activity—exploration, locomotion, scent marking, feeding, maintenance, social interaction, inactivity, and out of visual range—than on the male's—exploration, locomotion, feeding, and inactivity—probably due to the female wolf contributing more directly to yearling care in the form of teaching the types of social interactions. This teaching implied the development of daily activities such as scent marking, maintenance, and social interactions as a form of social learning. On the other hand, the adult male contributes indirectly to the learning of defense of home sites, hunting, and provisioning the adult female under the form of daily activities such as exploration, locomotion, and feeding (Malcolm, 1985).

In terms of space use, the breeding enclosure was more intensely used by the female during the DP than during the TP as opposed to the indoor enclosure that was used more by her during the TP, when she left the breeding enclosure because the yearling started to feed on its own. This assertion could also be valid for the homogeneity of space use as the Iberian wolves used the space more homogenously during TP because it was the time when the female and the yearling left the breeding enclosure and all the subjects used more intensely the outdoor exhibit. These results agree with observations in the wild where the pregnant female may be located near a den for up to a month before parturition, generally accompanied by the male (Mench & Boitani, 2003).

During the TP, the inter-individual distance between male and female Iberian wolves was significantly smaller than during pregnancy because this period covered the breeding season which means more male implication in yearling care, protection, and the teaching of hunting (Grande del Brío, 2000). The female wolf of this study was closer to the yearling than the male, similar to the results observed in the field that show that females are reported to spend more time than males in the vicinity of yearlings, probably due to a greater implication of the female in taking care of the pups (although the total time either adult is present decreases with the pup's age; Malcolm, 1985).

The use of a distance formula in this study of inter-individual proximity is a new method to determine the spatial relationship among animals and it has noticeable advantages because it allows the calculation of proximity *ex-situ* and *a posteriori* through the record of the space use and the enclosure plan. This formula allows for recovering information about proximity once the recording period is over (Soriano et al., 2021).

Animal Welfare Implications and Conclusion

In wild conditions, average litter size was found to be five to six times higher than the single cub born in this study (Mech & Boitani, 2003). The reason why the mated female of this study gave birth to only one cub is open to different possible explanations: (a) unsuitable captive conditions (i.e., diet composition, pack composition, enclosure size, management, etc.), (b) inappropriate reproductive male (i.e., hand reared), and/or (c) mother's age and experience (Grande del Brío, 2000).

All the information about group size will be significant in order to achieve the optimal pack composition considering that its social dynamics are particularly important: birth, death, introduction, or removal of pack members. One of the most important challenges for wolf welfare is to achieve the highest possible similarity between wild and captive wolf packs. From our point of view, there are a lot of captive species with overly complex social behaviors for which the majority of captive centers cannot recreate adequate conditions, similar to those in the wild, for many important reasons (e.g., territory size, hunting, inbreeding avoidance, or pack budding and splitting). In these cases, it would be better to resign from maintaining these animals in such inadequate conditions (Soriano et al., 2021).

In terms of animal welfare, a currently unanswered question is whether the needs of social species can be adequately met within captive environments. Understanding how social structures affect captive animals enables opportunities to examine the constraints acting upon group composition. Identification of factors that impact social groupings and subsequent welfare issues may allow improved management as well as the identification of target areas for change in designing captive environments and management (Williams et al., 2018).

This study is the first of its kind in calculating the behavioral, spatial, and proximity welfare indicators in order to compare the pregnancy period with the addition of a yearling period in a management care facility.

It is clear that much more research needs to be conducted in order to identify the driving factors behind the development and maintenance of wolf social relationships in captive centers. It would have been interesting to more closely study: (a) the bonding, courtship, copulation, and pregnancy periods over 24-hr because during the three daily periods considered here, these kinds of behaviors were not frequently observed. It well may be that these behaviors would be expressed in intimate conditions when the zoo is closed; (b) the activity inside the breeding enclosure with the use of video cameras in order to know if there is pup mortality, the duration of the lactation bouts, the male implication, or the number of times that the female leaves the breeding enclosure.

The needs of captive wolves are likely to be not just species—but also individual—specific. Historically, researchers looked at wild wolf packs in order to predict captive wolf pack wants and needs, but the captive environment is artificial and social packs are more stable than in the wild. In order to maintain good levels of welfare in captive wolves, captive centers must be prepared and capable of being flexible with social housing through the implementation of appropriate captive breeding programs (Williams et al., 2018). Further field and captive studies are required to be able to broaden the knowledge of this interesting species and to improve their captive breeding and conservation programs.

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