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Fecal Pellet Morphometry of Barking Deer (Muntiacus vaginalis) in the Margalla Hills National Park, Islamabad, Pakistan

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Abstract

This study employed morphometric analysis of fecal pellets to accurately characterize the age and sex of barking deer (*Muntiacus vaginalis*) in the Margalla Hills National Park (33.7481° N, 73.0051° E), Islamabad, with implications for the species' conservation. Line transect sampling was employed to collect the samples. Fecal pellets were collected from three age/sex classes of barking deer: bucks, does, and yearlings. Fifty-eight pellets from yearlings, 112 pellets from bucks, and 142 pellets from does were chosen at random to be analyzed. The fecal pellets exhibited a diversity of morphometric attributes including the position and number of the tip and cleavage furrow patterns. A notable disparity was observed in the shape of fecal pellets between bucks and does. The fecal pellets of does were shorter and thicker than those of bucks in size. We calculated the weight, length, width, and volume of all selected fecal pellets. The findings regarding the estimated value of the length-to-width (L/W) ratio were higher for bucks (M=2.16) in contrast to does (M=1.67) and yearlings (M=1.66). The coefficient of variation of the (L/W) ratio exhibited a higher value of 3.334 for bucks, compared to does (2.64) and yearlings (0.121). The discriminant classification coefficient function and K-mean clustering methods were used for statistical analyses. The discriminant function correctly assigned 86.7% of bucks, 74.3% of does, and 98.1% of yearlings to their respective age classes. Additionally, the K-mean clustering analysis achieved 100% accuracy in differentiating individuals. The length of the fecal pellets in adults and the volume in yearlings were good indicators, and the overall results demonstrated a high level of accuracy in age and sex identification. A strong relationship (- 0.273) was observed between weight and volume of fecal pellets.

Keywords: age and sex determination, barking deer, fecal pellet, morphometry, Margalla Hills National Park

1. Introduction

Invasive methods used to determine the age and sex of various rare animal species hold value from both implementation and conservation perspectives. However, certain techniques, such as direct observation of the external genitalia (Morden et al., 2011), blood hormone analysis, and examination of pelvic morphology present practical challenges, especially when dealing with free-ranging wild species (Shaw et al., 2003). Moreover, capturing animals for these purposes not only poses difficulties but also harms wild populations (Han et al., 2009). To address the limitations associated with field observations, particularly for shy, solitary, and elusive species, an alternative approach for age and sex determination was the use of footprints (Wilson & Delahay, 2001) and fecal pellet assessments (Davidson et al., 2019; Putman, 1984; Rouco et al., 2012) can provide valuable clues for inferring age and sex without directly disturbing or capturing the animals.

Feces are a common 'calling card' left by animals in the wild, and such deposits have proven to be a valuable source of information regarding species (Barbosa et al., 2013; Hansson, 1971), sex (Eggert et al., 2003), diet, and age (Farrell et al., 2000). Collection of feces is less labor-intensive than methods to detect or capture live animals (Webbon et al., 2004); furthermore, feces may be the only material available to identify cryptic species (Barbosa et al., 2013; Webbon et al., 2004; Whisson et al., 2005). There is a scope for novel approaches to fecal analysis to supplement and support such methods, especially for new methods that are rapid and easily applied (Davidson et al., 2019). This alternative approach helps overcome the challenges associated with studying such species in their natural habitat while still obtaining crucial information for research and conservation efforts.

Previous studies have demonstrated the successful use of pellet morphometry to study habitat use, distribution, population estimates, and density in various deer populations (Collins & Urness, 1984; Ezcurra & Gallina, 1981; Galindo-Leal et al., 1993; Leopold & Krausman, 1991; Neff, 1968; Rowland et al., 1984; Sánchez-Rojas & Gallina, 2000a; Sanchez-Rojas et al., 2004b; Valdez & Ortega-S, 2014). Fecal pellets have been employed to identify the age and sex of individuals in the majority of the deer species: white-tailed deer, *Odocoileus virginianus* (Equihua, 1990); wapiti, *Cervus elaphus* (Thomas & Toweill, 1982); moose, *Alces alces*; mule deer, *Odocoileus hemionus* (Sánchez‐Rojas et al., 2004), reindeer, *Rangifer tarandus platyrhynchus* (MacCracken & Van Ballenberge, 1987); Sonoran pronghorn, *Antilocapra americana sonoriensis* (Wilson & Delahay, 2001; Woodruff et al., 2016); fallow deer, *Dama dama*; and roe deer, *Capreolus capreolus* (Furnas et al., 2018; Popp et al., 2020). The technique of counting groups of fecal pellets is rarely employed by wildlife ecologists, managers, conservationists, and hunters to assess the age structure and sex ratios of ungulates (Furnas et al., 2018; Prosekov et al., 2020).

Fecal pellets from deer species consist of undigested plant material, and their characteristics, such as size, shape, and color, vary depending on factors like age class, sex, food composition, water intake, and social status of the individual that produced them (Aranda Sánchez, 1981; Sánchez‐Rojas et al., 2004). Considering that the age of the individuals is an indirect indicator of body size, and that pellet shape is sex-specific in deer species (Eggert et al., 2003). By analyzing the morphological features of these pellets (Alvarez, 1994) we can determine the age and sex of individuals within a population, providing insights into their habitat preferences (Sánchez‐Rojas et al., 2004).

The barking deer (*M. vaginalis*) is native to Southeast Asia, including regions such as India, Sri Lanka, Southern China, Bangladesh, Taiwan, Japan, the Boso Peninsula, and various Indonesian islands (Alexiou et al., 2022). It is a nocturnal species that poses challenges for field studies, as it tends to remain hidden in dense scrub forests and becomes visible only when foraging (Singh et al., 2019). The species is commonly found in areas with thick vegetation, particularly *Acacia modesta* and *Olea ferruginea*, with an understory consisting of *Zizyphus mauritiana* and *Carisa opaca*, and it is very difficult to observe the ecology and the species directly (Roberts, 1997). However, there is currently no data on fecal pellet morphometry in barking deer (M. vaginalis), underscoring the importance of the current study. The primary aim of this study is to evaluate the feasibility of using morphometric attributes to classify the sex and age groups of barking deer (M. vaginalis) from their fecal pellets.

2. Method

2.1 Study Area

The study was carried out in the Margalla Hills National Park (MHNP), which encompasses an area of 14,786 hectares and is situated along the northeastern border of the federal capital city of Islamabad (Figure 1). The Margalla Hills represent the westernmost extension (Foothills) of the Indo-Himalayan ecosystem and serve as a transition zone with the arid Irano-Saharan ecosystem to the southwest. The MHNP is surrounded by more than 30 settlements, with a combined population of 70,000 individuals. The largest settlement, Nurpur, has a population of just over 15,000 people, followed by Chauntra with 12,000 people, and Kot Hathial, Gokena, Talhar, and Shah Allah Ditta settlements each having approximately 9,000 inhabitants (Aslam et al., 2024; Gulzar, 2019). The major threats to the national park include encroachments, woodcutting and grazing, wildfires, invasive plant species, quarries, and an increasing number of visitors (Fatima et al., 2021).

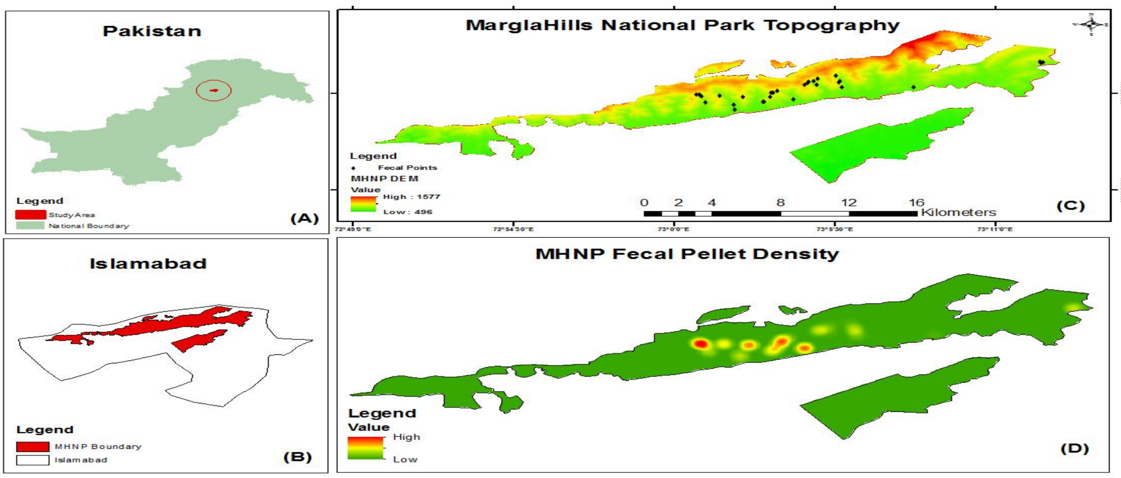

Figure 1. Map of the study area (A) Pakistan (B) Islamabad (C) location of the fecal sample collected along the elevation (D) Kernel density map showing the hotspots of fecal pellets of the barking deer (M. vaginalis) in Margalla Hills National Park, Islamabad, Pakistan.

The topography of the area is characterized by rugged terrain, with elevations ranging from 456 meters to 1580 meters. The overall orientation of the hills is southerly, and the landscape is interspersed with both large and small valleys. The rocks in the region date back to the Jurassic and Triassic periods. Despite being shallow, the soil in the area is dark, rich in minerals, and capable of supporting robust tree growth (Aslam et al., 2024; Khalid & Ahmad, 2016) . The general aspect is southerly, and the terrain is interspersed with both large and small valleys (GOP, 1992; Nawaz et al., 2007). The climate of the region is classified as sub-tropical semi-arid. It falls within the monsoon belt and experiences two distinct rainy seasons. The winter season lasts from January to March, while the summer season extends from July to September, with an average annual rainfall of 1900 mm (Khalid & Ahmad, 2016; Mazhar, 2022; Metelka, 2005) . Occasional light snowfall has been recorded during severe winters. The average minimum and maximum temperatures are -3.9 degrees Celsius and 46.6 degrees Celsius, respectively (Tahir & Asim, 2018).

2.2 Methodology

2.2.1 Field data collections

The study area was divided into four blocks: Bara Kahu range, Shadra range, Said Pur range, and Golra range. Fecal pellets were collected from barking deer individuals representing three age/sex classes: bucks (3-4 years old), does (3-4 years old), and yearlings (0-1.5 years old) during each of the four seasons: autumn (October-November), winter (November-January), spring (February-March), and summer (April-September) from all four blocks of the study area (see supplementary material). The collection of pellets occurred at three randomly selected sites within each of the four blocks, resulting in a total of 12 study sites. This data collection spanned from February 2019 to January 2022. To collect the samples, a line transects method of sampling of various lengths was established within the study sites. Fecal pellets were promptly collected from observed individuals immediately after defecation (the kernel density of the fecal pellet collection is illustrated in Fig. 1). For high-quality results, sample collection of fresh fecal pellets upon defecation of the deer should be ensured (Osborn and Ginnett, 2001; Bleke *et al*., 2022). Each pellet was labeled with relevant information (see supplementary material), including GPS coordinates, weather conditions, date, time, and sampling site, and then placed in airtight polythene bags for preservation and future reference according to their respective age/sex class (Sánchez‐Rojas *et al*., 2004). This approach enabled us to maintain a reliable record of the origin of each group of pellets. Subsequently, the fecal pellets were dried at room temperature within the laboratory and preserved at room temperature before undergoing morphometric measurements.

2.2.2 Lab methods

The study involved collecting five distinct groups of bucks' fecal pellets, totaling 187 pellets. Out of these, we randomly selected 112 pellets for calibration. Eight groups of does' fecal pellets were collected, totaling 247 pellets, from which 142 were randomly selected. Four groups of yearling pellets, totaling 94 pellets, from which we selected 58 for examination. The selection of intact and fresh fecal pellets was done based on the availability of defecated pellets in the wild (Gryz et al., 2022; Sánchez‐Rojas et al., 2004). Each pellet was subjected to various measurements (millimeters) to determine the weight (Wt) in grams, maximum length (L), maximum width (W), length-to-width ratio (L/W), and radius (r). The volume of each pellet (in square millimeters) was estimated using the formula $V = \pi (W/2)^2 L$, where W/2 represents the radius (r) and L is the height or length. All measurements were conducted by the same observer to avoid any inter-observer bias, utilizing a manual vernier caliper (Mitutoyos) with 0.05 accuracy class (Jabin et al., 2021). We visually examined the fecal pellets using a magnifying glass and classified them into different categories based on the number, position, and presence or absence of a tip. We also observed patterns of cleavage furrow in the fecal pellets of adult deer (Figures 7 & 8).

2.2.3 Data Analysis

In addition to discriminant analysis, a K-means fuzzy algorithm was used to allocate pellets to their respective age/sex classes, and its efficacy was compared with that of the discriminant analysis. The Fuzzy Clustering Tool program (Alberto, 2016; Equihua, 1990; Manley, 1994) was employed to apply this algorithm, providing membership values for each pellet group. To accurately assign hard-to-classify pellet groups to specific age and sex classes, we assigned each group to the class with the highest membership value. These techniques allowed us to assess the occurrence and abundance of fecal pellets in the study area. We conducted a paired t-test (95% confidence interval for the difference) to examine the disparity in mean fecal pellet volumes between bucks and does. The null hypothesis (H0) stated that the volume means of fecal pellets from bucks and does are equal, while the alternative hypothesis (H1) proposed that at least two volume means of fecal pellets from bucks and does are not equal. The linear multiple regression analysis was conducted to examine the relationship between the dependent variable, volume, and the independent variables, length and width, of fecal pellets from bucks, does, and yearlings. The mean values of pellet length, width, and volume of five pellet groups from each age/sex class (bucks, does, and yearlings) were examined to assess the null and alternative hypotheses. The null hypothesis (H0) stated that length and width do not have an impact on the pellet volume, while the alternative hypothesis (H1) proposed that the length and width influence the volume of pellets. The rejection region (RR) was defined as: t: $\{F_{cal}\}$(Ahmed et al., 2023) > $F_{(2,12.095)}$, which corresponds to F: $\{F_{cal}\} > 3.89$. The length of the explanatory variable and volume as a response variable in fecal pellets of bucks and does were depicted with percentile plots using PAST 4.030 (Hammer, 2001). The graphs for discriminant analysis and K-means fuzzy clustering were developed in SPSS 16.0 (IBM, 2007). The maps were created using ArcGIS 10.5 (Esri, 2019). using software programs 8.5 (Origin, 2010). Images of the morphology of fecal pellets were taken through a mobile phone, Infinix Note 11, with a front camera of 16 MP specification.

3. Results

Across all variables, bucks exhibited the highest estimated values, while yearlings showed the lowest, with does falling in between. The outcomes of the Shapiro-Wilk W test for the explanatory variables' length and width, the length of fecal pellets of bucks (0.685), and does (0.807) depict normal distribution, but yearlings (0.030) did not show normality. The width of fecal pellets of does (0.216) and yearlings (0.301), shows normal distribution, but that of bucks (0.000) did not do so. However, the number of fecal pellets for bucks (0.685), does (0.501), and yearlings (0.967) exhibited normal distribution (Table 1).

Table 1. Results of the Shapiro-Wilk W test for normal data distribution of barking deer fecal pellets.

	Bucks		Does		Yearlings	
Measurement	**W**	**p value**	**W**	**p value**	**W**	**p value**
Length	.906	.685	.960	.807	.751	.030
Width	.566	.000	.856	.216	.878	.301
No. of Pellets	.943	.685	.915	.501	.987	.967

Note. P value = 0.05

For the paired t-test with a degree of freedom value of 4.0, the rejection region (RR) was determined as; t: $> t_{(4;\ 0.0975)}$, which translates to t: $\{t_{cal}\} > 2.7763$. Since the calculated result ($t_{cal} = 0.170$) falls within the rejection region, we reject the null hypothesis (H0) and conclude that the volume means of fecal pellets from bucks and does are not the same (Table 2).

Table 2. Paired t-test results for volume means of fecal pellets between bucks and does.

	Mean	**Std. Deviation**	**Std. Error Mean**	**Lower**	**Upper**	**T**	**df**	**Sig. (2-tailed)**
Buck - Does	-1.04591E2	139.81573	62.52749	-278.19470	69.01362	-1.673	4	0.170

The result of linear multiple regression shows that the calculated ($F_{cal} = 115.915$) exceeds the critical F value of 3.89; we reject the null hypothesis (H0) and conclude that both length and width influence the volume of the pellets (Table 3).

Table 3. Linear Multiple Regression analysis for volume as the Dependent Variable and length and width as independent variables of fecal pellets from bucks, does, and yearlings.

Model		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
	Regression	26685.264	2	13342.632	115.915	.000[a]
1	Residual	1381.284	12	115.107		
	Total	28066.548	14			

Note. a. Predictors: (Constant), Width (mm), Length (mm). b. Dependent Variable: Volume (mm^2)

Figure 2 illustrates a comparison of morphometric measurements, including pellet length, width, length-to-width ratio, and volume, for five groups of fecal pellets randomly selected from each pellet group of barking deer (bucks). For the length (mm), the median value was 10.75, and the maximum and minimum values were 13.0 and 0.10, respectively. The width (mm) had a median value of 5.0, with maximum and minimum values of 7.0 and 0.10, respectively. The L/W Ratio (mm) indicated a median value of 2.16, with maximum and minimum values of 6.11 and 0.10, respectively. Volume (mm^2) of fecal pellets had a median value of 2.09, with maximum and minimum values of 327.42 and 111.65, respectively.

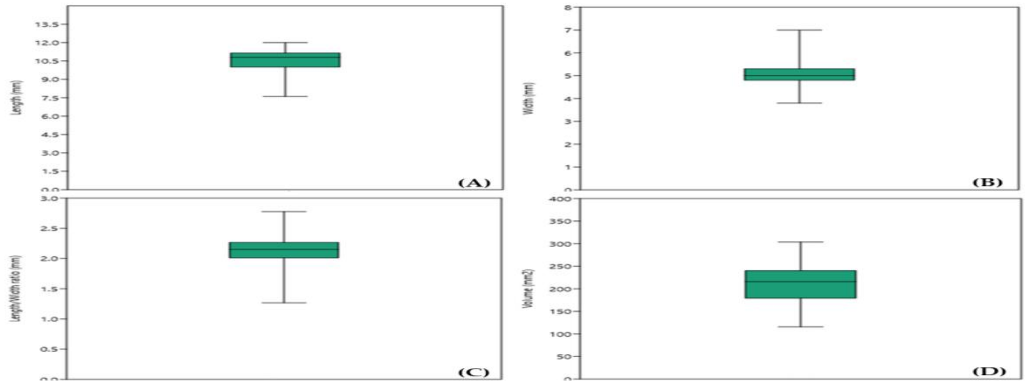

Figure 2. Comparison of morphometric measurements of fecal pellets from bucks. A horizontal line inside each box represents the median value, while the 25th and 75th percentiles are shown by the box boundaries. Additionally, short horizontal lines indicate the minimum and maximum values observed for each measurement.

Figure 3 illustrates a comparison of morphometric measurements, including pellet length, width, length-to-width ratio, and volume, for five groups of fecal pellets randomly selected from each pellet group of barking deer (does). For the length (mm), the median value was 9.30, the maximum and minimum values were 14.0 and 6.0, respectively. The width (mm) had a median value of 5.80, with maximum and minimum values of 9.0 and 4.0, respectively. The L/W Ratio (mm) indicated a median value of 1.67, with maximum and minimum values of 6.11 and 0.98, respectively. Volume (mm^2) of fecal pellets had a median value of 2.37, with maximum and minimum values of 703.36 and 111.00, respectively.

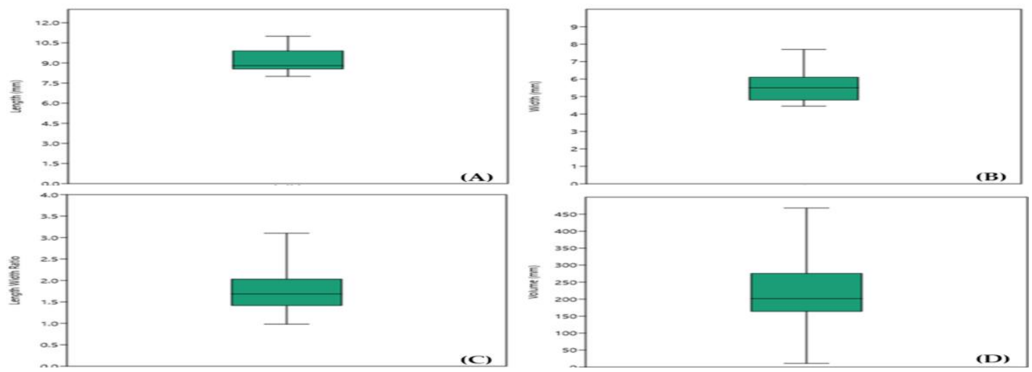

Figure 3. Comparison of morphometric measurements of fecal pellets from does.

Figure 4 illustrates a comparison of morphometric measurements, including pellet length, width, length-to-width ratio, and volume, for five groups of fecal pellets randomly selected from each pellet group of barking deer (yearlings). For the length (mm), the median value was 8.45, and the maximum and minimum values were 11.0 and 5.30, respectively. The width (mm) had a median value of 5.0, with maximum and minimum values of 7.0 and 3.40, respectively. The L/W Ratio (mm) indicated a median value of 1.66, with maximum and minimum values of 2.59 and 1.09, respectively. Volume (mm^2) of fecal pellets had a median value of 1.63, with maximum and minimum values of 330.80 and 58.08, respectively.

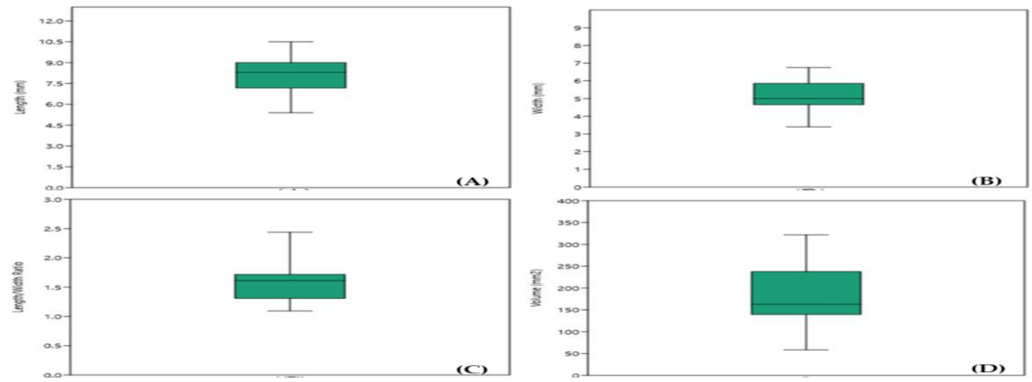

Figure 4. Comparison of morphometric measurements of fecal pellets from yearlings.

Results of length measurements as an explanatory variable in fecal pellets of does and bucks show that does tend to have a higher length value (11.0 mm) compared to bucks (10.0 mm). This shows a slight deviation from descriptive statistical analysis, for example, 14.0 mm and 13.0 mm for the does and bucks, respectively (see supplementary material). The volume as a response variable in the same set of fecal pellets of does exhibits a higher value, 703 mm^2, compared to 327 mm^2 for bucks (Fig. 5b). These results of both length and volume measurements consistently demonstrate that does tend to possess larger fecal pellets than bucks.

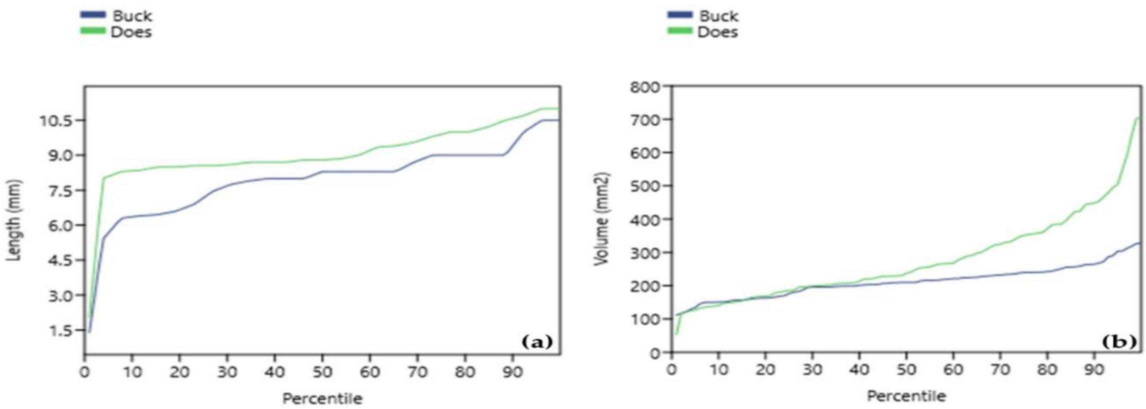

Figure 5. Percentile plots for (a) Length as an explanatory variable and (b) Volume as a response variable in fecal pellets of bucks and does.

In Fig. 6 (a), the K-means cluster analysis was performed on all variables, revealing an interesting pattern related to length as a predictive or explanatory variable. Specifically, in cluster 2 of fecal pellets from male bucks, the length value is found to be approximately 60 mm. This particular length measurement falls within the range of approximately 63 mm, indicating a good predictability of length as a variable in this cluster. It is worth noting that while length shows a clear predictive relationship, the other variables analyzed in the cluster, such as weight, width, ratio, radius, and volume, did not reach the first interval (10 mm). This suggests that these variables may not have as strong an impact or predictability as length in this particular cluster of fecal pellets from bucks.

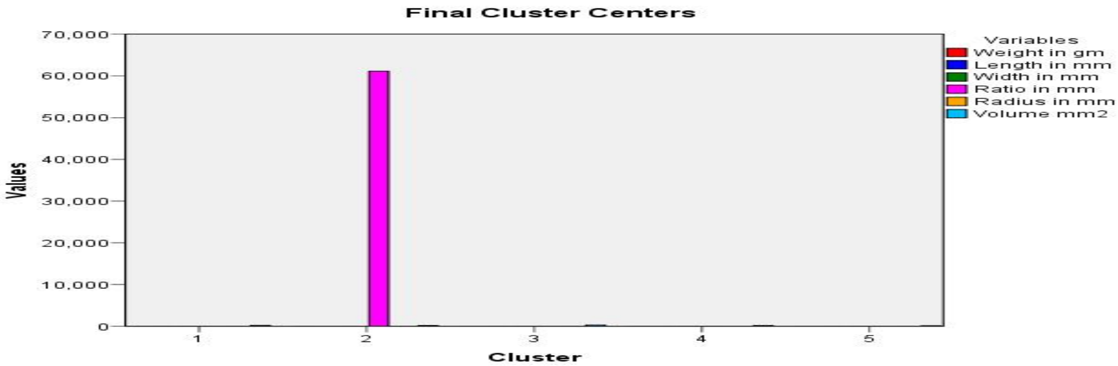

Figure 6. (a) K-means cluster analysis of all variables, highlighting length as an explanatory variable in cluster 2 of fecal pellets from the bucks.

In Fig. 6 (b), the K-means cluster analysis of all variables reveals a similar pattern to Figure 6 (a), but this time focusing on fecal pellets from female does. In cluster 5, length once again emerges as a good, predictable variable. The length value in this cluster is approximately 63 mm, while the other variables analyzed, such as width and volume, do not reach the first interval of 10 mm. These results indicate that length demonstrates a strong predictability in cluster 5 of fecal pellets from female does, while the other variables show less significant associations.

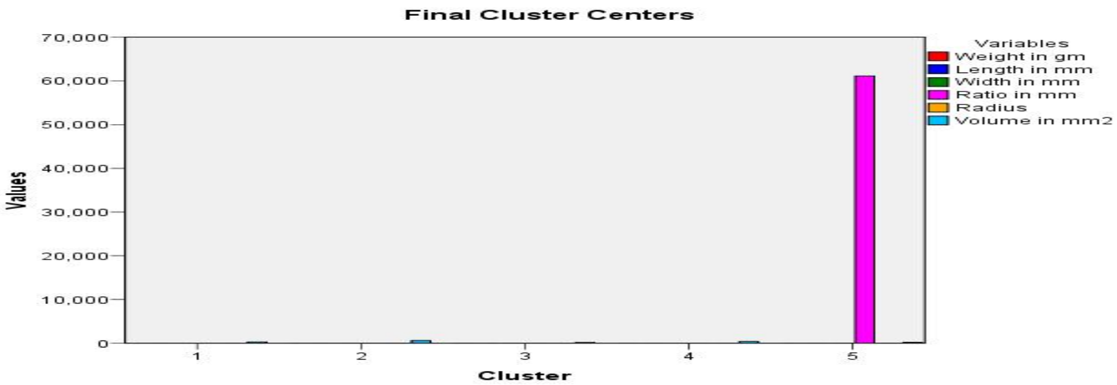

Figure 6. (b) K-means cluster analysis of all variables, highlighting length as an explanatory variable in cluster 2 of fecal pellets from the does.

The results show different patterns based on clusters. Specifically, volume emerges as a good, predictable variable in this analysis. Cluster 5 displays the highest volume value, reaching approximately 30.3 mm², indicating a larger pellet size within this cluster. On the other hand, cluster 4 exhibits the lowest volume value of 8 mm², suggesting smaller-sized pellets. Meanwhile, clusters 1, 2, and 3 demonstrate intermediate volume values, indicating varying pellet sizes within these clusters. These results highlight the variability in volume and its predictability as a variable in different clusters of fecal pellets from yearlings.

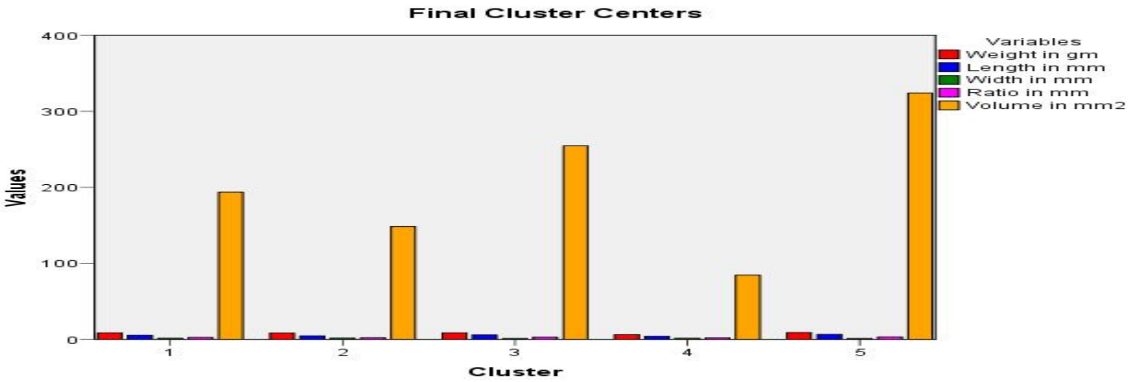

Figure 6. (c) K-means cluster analysis of all variables, highlighting volume as an explanatory variable in cluster 2 of fecal pellets from the yearlings.

In the stepwise discriminant coefficient function analysis, we obtained an estimated U (Wilks' Lambda) value of 0.011 by using the median. This value was found to be close to the F statistic [3, 1] of 24.00, indicating a significant difference between the age/sex classes ($P \leq 0.0001$). The combination of volume and length variables explained 91.4% of the variation observed in the data. By employing the discriminant classification function, we achieved accurate identification of 74.3% of does, 86.66% of bucks, and 98.1% of yearlings. This demonstrates the effectiveness of the classification model in correctly assigning the age/sex classes based on the given variables. Furthermore, the fuzzy k-means algorithm was utilized to determine the membership value for each pellet variable group. The results were plotted to illustrate the relationship between the age/sex classes. Using this technique, we identified the most influential variable, which in this case was length, for each pellet group in bucks, does, and yearlings.

We achieved a perfect assignment rate of 100% for bucks, 100% for does, and 100% for yearlings using the discriminant classification function. This indicates the high accuracy of the classification model in correctly assigning the age classes based on the available information. During the examination of morphometric attributes and visual aspects of fecal pellets from barking deer, we classified the pellets into different groups based on the length-width (L/W) proportion, cleavage furrow patterns, and apical texture.

A difference in the shape of fecal pellets was observed between bucks and does. Bucks' pellets were relatively thin, slender, and longer than the thicker, oblong pellets of does (Figs 7 & 8). However, yearling pellets displayed a more rounded shape compared to adult pellets. The classification of fecal pellets from barking deer involved several criteria to distinguish between different categories based on their morphology. One criterion considered was the relative position of the tip, resulting in the classification of pellets into two categories: those with a marginal tip and those with a medial tip. Additionally, the presence or absence of a distinct tip on the pellet was another criterion used for sorting the pellets. Regarding the tip structure, there were two categories of fecal pellets. The first category consisted of pellets with a rudimentary monoapical tip, while the second category comprised pellets with a prominent tip (Fig. 9).

Figure 7. Fecal pellets from (a) bucks and (b) does, respectively.

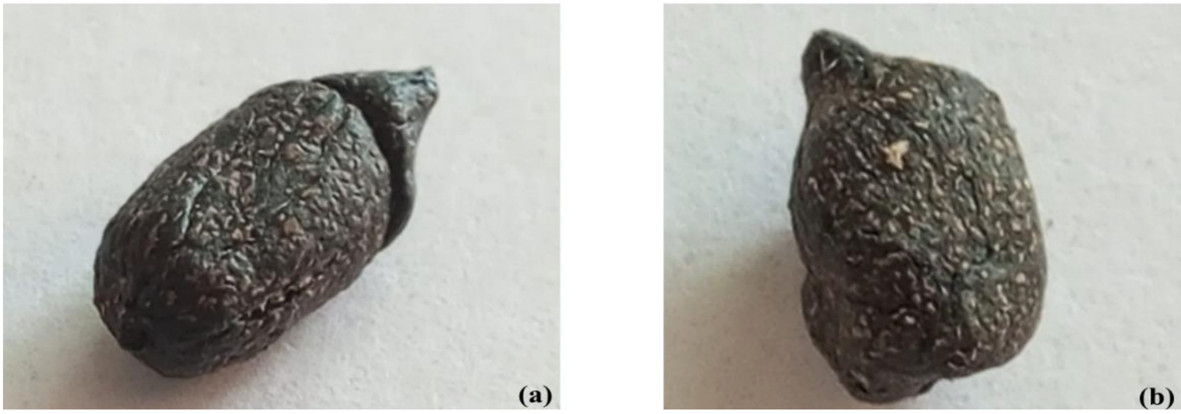

Figure 8. Morphology of a single fecal pellet from (a) bucks and (b) does with a magnification of 6X.

The cleavage furrow pattern was another criterion used for classification. Fecal pellets were divided into four categories based on the cleavage furrow pattern: sub-apical cleavage furrow, abdominal diagonal cleavage furrow, abdominal medial cleavage furrow, and abdominal radial cleavage furrow. Fig. 9 (j, k, i, n, and m) illustrates these different cleavage furrow patterns. Furthermore, some fecal pellets exhibited unique attributes. These included a cylindrical pellet with two cleavage furrows (Fig. 9n), a bullet-shaped pellet with a minor vertical fissure and a medial pit (Fig. 9o), a pellet with a rudimentary tip showing a "T" shaped cleavage furrow (Fig. 9p), two fused pellets (Fig. 9q), and normal or non-cleavage furrow pellets (Fig. 9r). These classifications and distinct characteristics contribute to a better understanding of the morphological diversity observed in fecal pellets from barking deer.

Figure 9. (a) A monoapical fecal pellet with a rudimentary tip, (b) a mono-apical pellet with a marked depression, (c) a biapical fecal pellet, (d) a fecal pellet with a marginal tip, (e) a bi-apical pellet with a marked depression, (f) a curved mono-apical oblong-shaped pellet with debris scar, (g) a slender biapical penguin shaped pellet (h) a pical pellet with a sub-apical depression (i) a rudimentary mono-apical pellet with the transverse cleavage furrow, (j) a mono-apical oblong-shaped pellet with diagonal cleavage furrow, (k) a pellet with the longitudinal cleavage furrow, (l) a biapical oblong-shaped pellet, (m) a pellet with the abdominal cleavage furrow, (n) a pellet with the sub-apical diagonal cleavage furrow, (o) a pellet with the abdo-diagonal cleavage furrow, (p) a pellet with "T" shaped cleavage furrow, (q) two fused pellets, (r) non-apical pellet, (s) monoapical with subapical cleavage pellet, (t) monoapical bullet shaped pellet.

4. Discussion

Invasive methods for determining the age and sex of deer species pose significant challenges, especially when dealing with free-ranging threatened wildlife taxa. Techniques such as direct observation of external genitalia, examination of pelvic morphology, and analysis of different age groups are impractical and difficult to implement in ecological studies, particularly for shy, solitary, and elusive species. These invasive methods not only require capturing the animals, which can be harmful and stressful for the wild species, but they also lack reliability (Zemanova, 2020). To overcome these limitations and to find non-invasive alternatives for age and sex determination, we have, for the first time, turned to fecal morphometry of *M. vaginalis*. This non-invasive approach is particularly valuable in ecological studies focused on threatened wildlife taxa, where minimizing disturbance and preserving the natural behaviors of the species are paramount. Therefore, fecal morphometry serves as an ecologically sound and practical alternative to invasive methods, providing researchers with a valuable tool to study and understand age and sex in a non-intrusive and sustainable manner (Lafferty et al., 2022; Pukazhenthi & Wildt, 2003). Fecal samples are frequently the most easily collected source of information for studying rare and elusive species. However, to extract valuable information, the species must be properly identified. Our findings imply that age estimation based on fecal morphology is feasible, at least for barking deer, although more information would be required to establish valid age estimation criteria. Although it is premature, we think our data can provide important baseline information for identifying ungulate species and estimating the age of barking deer.

The data analysis revealed distinct variations in the attributes of fecal pellets, including weight, length, width, and volume, among different age classes of barking deer (bucks, does, and yearlings). The median value of the length-to-width (L/W) ratio was higher for bucks (M=2.1620) in contrast to does (M=1.6785) and yearlings (M=1.6600). The coefficient of variation (C.V.) in relation to the (L/W) ratio exhibited a higher value of 3.334 for bucks compared to does (2.648) and yearlings (0.121). This indicates a greater degree of variability among individual pellet groups for bucks. Notably, each measurement displayed a varying level of variance, ranging from high to small, across the different pellet groups. For volume-related variables, all classes demonstrated a minimal degree of overlap, while weight exhibited the lowest coefficient of variation (See supplementary material).

These findings are consistent with previous studies conducted on other deer species, supporting the notion that fecal pellet characteristics can provide valuable insights into age and sex differences (Bowyer et al., 1998; Eggert et al., 2003; Equihua, 1990; Ezcurra & Gallina, 1981; MacCracken & Van Ballenberge, 1987; Sanchez-Rojas et al., 2004b). When analyzing the collected fecal pellet samples, we took into consideration all possible age variations. However, it is worth noting that there may be some overlapping estimates for yearlings based on sex. On the other hand, this was not observed for bucks or does. It is important to acknowledge that factors such as diet composition and morphology of the large intestine can influence fecal pellet attributes (Maurer et al., 2021). The observed dimensional variations among pellet groups in relation to sex can be attributed to the physical differences between bucks and does. In the case of the barking deer, a species known for its sexual dimorphism, bucks tend to be heavier (34 kg) compared to does (27 kg) (Roberts, 1997).

Seasonal differences in vegetation availability strongly influence diet composition in barking deer (Muntiacus vaginalis), as determined by micro-histological analysis of fecal pellets. Summer fecal pellets in Pakistan's Himalayan foothills showed indications of a higher diversity of plant species, including trees (37.0%), shrubs, grasses, and herbs, which reflects the greater availability of food items during this season. On the other hand, because there was significantly less green grass available during the colder months, tree leaves (42.1%) and shrubs dominated winter diets found in fecal pellets. These results show that diet composition is directly influenced by seasonal plant availability, which can be identified using fecal pellet analysis (Habiba et al., 2021).

While there is some overlap in the values of the variables between sexes, the overlap is relatively low for the volume and width variables, as indicated by the significant results from the step-by-step discriminant analysis. According to these measurements and the statistical procedure, bucks exhibited the highest values, followed by does, and then yearlings. To further explore and characterize the morphological criteria that can distinguish between different species, a discriminant analysis was performed using the mean measurements of pellet groups from ten sympatric species of ungulates. The mean diameter of pellets within each pellet group emerged as the most valuable variable for species segregation, while the second axis separated species based on the mean indent depth (Hibert et al., 2008). In a related study conducted by (Costa et al., 2017) on deer species in neotropical regions, discriminant analysis of fecal pellets achieved accuracies ranging from 56% to 92% for species identification. These findings highlight the utility and effectiveness of fecal pellet analysis in differentiating between deer species and further support the significance of this approach in ecological research.

Both the discriminant classification function and the fuzzy cluster analysis methods successfully recovered most of the age and sex classes within each fecal group. However, the fuzzy cluster analysis method has a distinct advantage over the discriminant classification function method. Unlike the discriminant method, the fuzzy cluster analysis does not require prior knowledge of the age or sex of the origin of each pellet group during the calculation stage. This makes the fuzzy cluster analysis technique particularly valuable for field studies where the collection of fecal pellets involves animals of unknown age or sex. Determining the age and sex structure of a barking deer population based on pellet morphological attributes is a crucial step for effective management and conservation strategies, especially in areas such as the MHNP where the species is facing serious challenges to its survival due to overhunting by local communities. The methods employed in this study have been widely used in various research endeavors, and the clustering methods, in particular, have proven to be highly useful depending on the nature of the field data collections and study objectives (Lasch et al., 2004; Niknam & Amiri, 2010). The utilization of these statistical tools offers several advantages that make them highly beneficial for ecological studies on the age and sex classes. These methods are affordable, which makes them accessible for researchers with limited resources. The statistical tools employed in this study are noninvasive, meaning that they do not require direct contact or interference with the animals themselves. These methods yield valuable results regarding the age and sex structure of the population using a suitable sample size of fecal pellets. By collecting an appropriate number of pellets, researchers can obtain representative data that provides insights into the age and differentiation.

The distribution of fecal pellet data in the study area was checked using a normality test. We applied a non-parametric Shapiro-Wilk W test, statistical results show our data was not normally distributed in the study area (Table 1). Fecal pellets of Roe and Fallow deer were not distributed normally in the fenced study area when the length, width, and number of pellets in a group were chosen for assessment by applying the Shapiro-Wilk W test. The p values for the length, width, and number of pellets in a group for 100 groups for each species were: Fallow deer 5.79E-10, 1.60E-14, and 1.20E-06 and Roe deer 2.79E-12, 1.99E-16 and

0.00131, respectively (Gryz et al., 2022). Before data analysis, distribution was tested for normality using the nonparametric Shapiro-Wilk W test and Friedman's test (Lee Foote & Rice Hornung, 2005). They estimated the abundance and diversity of odonates as biological indicators of grazing effects on Canadian prairie wetlands. The impact of thinning type on the intensity of bark-stripping damage caused by red deer (*Cervus elephas*). Data was verified using the Shapiro-Wilk test and also by common probability charts (Turek et al., 2016).

We found a strong negative relationship (-.273) between fecal pellet volume and the body weight of bucks and does because fecal pellet size is proportional to body size and weight (See supplementary material; Table S5). The protein and energy status of winter-fed white-tailed deer was compared to check the relationship between pellet size and the diet of the deer (Page & Underwood, 2006). They found that pellet size would approximately correlate to deer size and determined a positive relationship between fecal nitrogen and fresh pellet mass. The age/sex classes of Caribou were successfully identified based of fecal pellet dimensions (Ball, 2010).

Barking deer (*M. vaginalis*) is a medium-sized ungulate with distinct dimorphism, and fecal pellet volume of bucks and does also differ in statistical values. Our result of the paired t-test shows a difference in the mean volume of pellets (Table 2). A significant difference in pellet dimensions, volume, and width among bucks, does, and yearlings was found in mule deer (Sánchez - Rojas et al., 2004). We determined the contribution of explanatory variables, length, and width for the volume as the response variable in the sex classes of barking deer (*M. vaginalis*) through linear multiple regression (Table 3). Our result shows that the volume of fecal pellets depends on the combined effect of length and width. Fecal pellet morphometry has been successfully used as a statistical tool to distinguish two sympatric deer species: Roe deer and Fallow deer in Poland. Calibration of pellets, length, width, and length-width ratio provided the base for differentiation between these two species based on mean values of length and number of pellets in each group of 100 groups of both species (Gryz et al., 2022).

The visual aspects (relative position of tip/apex and cleavage furrow patterns) of fecal pellets, which we identified during laboratory examination, gave useful information on the morphology of the pellets (Jameel et al., 2019; Morden et al., 2011). We consider these visual aspects to be mainly based on the constriction of internal and external anal sphincters during egestion. No such superficial patterns were observed in the fecal pellets of yearlings, which were round and smaller than those of adults. We found fecal pellets in all types of habitats related to both vegetation and elevation, visited by the barking deer; in the scrub thorn forest, mixed deciduous forest, and alpine forest; ascending and descending slopes of trails used by the public, at the banks of the stream, open air ground, under the tree canopy, partially concealed under rock stones, and even post forest fire affected areas. Fecal pellets were found in small numbers (fewer than 7-10) to large numbers (more than 80-90) per cluster, depending on the animal's egestion rate at the different sampling sites in the study area. Barking deer choose mature shrubs or trees with canopies as a cover used for both night stay and latrine in the scrub forest of MHNP. Fecal pellets of the barking deer exhibited resistance to decay in the wild; we found more than one-year-old fecal pellets in intact form, with a dull, sloughed-off outer surface. Field observations also revealed that freshly defecated fecal pellets are not decomposed by the soil-dwelling invertebrates due to the presence of glossy mucilage covering.

We observed variations in the morphometry of fecal pellets among bucks, does, and yearlings of barking deer in their habitat. This indicates that there are differences in the characteristics of pellets based on age and sex. Both the discriminant function and fuzzy clustering analyses employed in this study proved to be highly useful and precise for examining the morphometric attributes of pellets. However, it is important to note that these methods provide gross estimates, as there is a possibility of data loss or disposal during their execution. One limitation of the discriminant function method is the requirement of prior knowledge regarding the age or sex of the animal that produced the pellets. This reliance on a priori information can be considered a drawback of the method. Nevertheless, these statistical tools offer valuable optimization for data gathered from field surveys, enabling us to obtain gross estimates of population parameters and explore differences in habitat utilization between does and bucks of barking deer. Fecal pellet survey methods provide a means of acquiring such data. Additionally, our study successfully identified a unique morphometric feature of fecal pellets, the presence or absence of tips and various superficial cleavage furrow patterns in both bucks and does. This finding adds to our understanding of the diverse characteristics exhibited by fecal pellets and complements the estimation of pellet weight.

Our study findings suggested that fecal morphometry serves as an ecologically sound and practical method to understand age and sex in a non-intrusive and sustainable manner. The non-invasive fecal sample method combined with prior knowledge of environmental factors may be valuable in the study of endangered ungulate species.

5. Conclusions

Variations in fecal pellets among bucks, does, and yearlings were observed. The minimum and maximum values of fecal pellet length in the case of bucks were 0.10 and mm13.0mm. Does showed the minimum and maximum values of fecal pellet length, 6.0 mm and 14.0 mm. Bucks exhibited a range of fecal pellet volumes from 111.65 mm^2 to 327.42 mm^2. The fecal pellet volume for does range from 111.00 mm^2 to 703.36 mm^2. Bucks' pellets were relatively thin, slender, and longer than the thicker, oblong pellets of does. Both length and width contribute to the volume of fecal pellets. Kernel density depicts hot spots lie in the Saidpur range of the MHNP. In bucks and does, fecal pellet length emerged as an explanatory variable, whereas in yearlings, pellet volume was a significant predictor of their characteristics. Visual aspects, a morphometric feature of fecal pellets, mainly the presence of tips and various superficial cleavage furrow patterns in both bucks and does, have been reported. Both the discriminant function and fuzzy clustering analyses employed in this study proved to be highly useful and precise for age/sex determination based on fecal pellets.

Recommendations

By accurately discerning various age and sex categories through fecal pellet morphometry, it will be possible to study the habitat preferences, food composition, population density, gut parasitology, and reproductive potential of barking deer (*M. vaginalis*) using this non-invasive approach. The outcomes of this research will hold considerable significance for researchers, wildlife managers, and wildlife observers to facilitate effective conservation and sustainable management practices.

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Author contributions

Shahzad Aslam (SA) was responsible for study design and revision. Shahzad Aslam (SA), Kiran Sahar (KS), Sanober Sahar (SS), and Ahmer Riaz (AR) conducted the field study. Kiran Sahar (KS) and Sanober Sahar (SS) calibrated fecal pellets in the laboratory. Ahmer Riaz (AR) performed statistical data analysis and interpreted the results. Shahzad Aslam (SA) and Kiran Sahar (KS) edited the manuscript. All authors read and approved the final manuscript.

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References

Ahmed, H., Jallat, H., Hussain, E., Saqib, N. u., Saqib, Z., Khokhar, M. F., & Khan, W. R. (2023). Quantitative assessment of deforestation and forest degradation in Margalla Hills National Park (MHNP): employing landsat data and socio-economic survey. *Forests*, *14*(2), 201. https://doi.org/10.3390/f14020201

Alberto, J. A. N. (2016). *Multivariate statistical methods: a primer.* Chapman and Hall/CRC.

Alexiou, I., Abrams, J. F., Coudrat, C. N., Nanthavong, C., Nguyen, A., Niedballa, J., Wilting, A., & Tilker, A. (2022). Camera-trapping reveals new insights in the ecology of three sympatric muntjacs in an overhunted biodiversity hotspot. *Mammalian Biology*, *102*(2), 489-500. https://doi.org/10.1007/s42991-022-00248-0

Alvarez, G. (1994). Morphological variability and identification of deer pellets in central Spain. *Folia zoologica (Brno)*, *43*(1), 25-37. http://pascalfrancis.inist.fr/vibad/index.php?action=getRecordDetail&idt=4079700

Anwar, M., & Chapman, J. (2000). Distribution and population status of grey goral in the margalla hills national park. *Pakistan Journal of Agricultural Research*, *16*(2). https://www.cabi.org/GARA/FullTextPDF/2009/20093351049.pdf

Anwar, M., & Chapman, J. A. (2000). Distribution and population status of grey goral in the margalla hills national park. *pakistan Journal of Agriculture Research*, *16*(2), 147-150.

Aranda Sánchez, J. M. (1981). Rastros de los mamíferos silvestres de México: manual de campo.

Aslam, S., Kayani, A. R., Ashraf, M. I., Jameel, M. A., & Sahar, K. (2024). Food Preference of Rhesus Monkey (Macaca mulatta) in the Margalla Hills National Park, Islamabad, Pakistan. *Pakistan Journal of Zoology*, *56*(3), 1249-1262. https://doi.org/10.17582/journal.pjz/20210724200757

Ball, M. C. (2010). Faecal pellet size can be used to differentiate age-classes in caribou:

implications for non-invasive genetic studies. *Conservation Genetics Resources*, *2*(1), 239-241. https://doi.org/10.1007/s12686-010-9252-z

Barbosa, S., Pauperio, J., Searle, J., & Alves, P. (2013). Genetic identification of I berian rodent species using both mitochondrial and nuclear loci: Application to noninvasive sampling. *Molecular Ecology Resources*, *13*(1), 43-56. https://doi.org/10.1111/1755-0998.12024

Bowyer, R. T., Kie, J. G., & Van Ballenberghe, V. (1998). Habitat selection by neonatal black-tailed deer: climate, forage, or risk of predation? *Journal of Mammalogy*, *79*(2), 415-425. https://doi.org/10.2307/1382972

Collins, W. B., & Urness, P. J. (1984). The Pellet-Group Census Technique as an Indicator of Relative Habitat Use: Response to Leopold et al. *Wildl Soc Bul (1973-2006)*, *12*(3), 327-327.

Costa, E. B. V., de Oliveira, M. L., Peres, P. H. d. F., Grotta-Neto, F., Vogliotti, A., Piovezan, U., & Duarte, J. M. B. (2017). Low accuracy of identifying Neotropical deer species by scat morphology. *Stud on Neotrop Faun and Environ*, *52*(1), 37-42. https://doi.org/10.1080/01650521.2016.1263418

Davidson, N. B., Koch, N. I., Sarsby, J., Jones, E., Hurst, J. L., & Beynon, R. J. (2019). Rapid identification of species, sex and maturity by mass spectrometric analysis of animal faeces. *BMC Biology*, *17*, 1-14. https://doi.org/10.1186/s12915-019-0686-9

Eggert, L., Eggert, J., & Woodruff, D. (2003). Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. *Molecular Ecology*, *12*(6), 1389-1402. https://doi.org/10.1046/j.1365-294X.2003.01822.x

Equihua, M. (1990). Fuzzy clustering of ecological data. *The Journal of Ecology*, 519-534. https://doi.org/10.2307/2261127

Esri. (2019). *ARC GIS In (Version 10.5) Esri.* https://doc.arcgis.com/en/arcgis-online/reference/display-copyrights.htm

Ezcurra, E., & Gallina, S. (1981). Biology and population dynamics of white-tailed deer in northwestern Mexico. *Deer biology, habitat requirements, and management in western North America*, 78-108.

Farrell, L. E., Roman, J., & Sunquist, M. E. (2000). Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Molecular Ecology*, *9*(10), 1583-1590. https://doi.org/10.1046/j.1365-294x.2000.01037.x

Fatima, H., Mahmood, T., Hennelly, L. M., Farooq, M., Akrim, F., & Nadeem, M. S. (2021). Spatial distribution and dietary niche breadth of leopard cats (Prionailurus bengalensis) inhabiting Margalla Hills National Park, Pakistan. *Zoological Studies*, *60*. https://doi.org/10.6620%2FZS.2021.60-34

Furnas, B. J., Landers, R. H., Hill, S., Itoga, S. S., & Sacks, B. N. (2018). Integrated modeling to estimate population size and composition of mule deer. *The Journal of wildlife* *management*, *82*(7), 1429-1441. https://doi.org/10.1002/jwmg.21507

Galindo-Leal, C., Morales, A., & Weber, M. (1993). Distribution and abundance of coues deer and cattle in Michilia Biosphere Reserve, Mexico. *The Southwestern Naturalist*, 127-135. https://doi.org/10.2307/3672064

GOP. (1992). *Margalla Hills National Park Management Plan.* Islamabad: Government of Pakistan

Gryz, J., Krauze-Gryz, D., & Klich, D. (2022). Alien or Native? How to Distinguish Feces of Fallow and Roe Deer Using Central Poland as a Case Study. *Animals*, *12*(3), 290. https://doi.org/10.3390/ani12030290

Habiba, U., Anwar, M., Khatoon, R., Hussain, M., Khan, K. A., Khalil, S., Bano, S. A., & Hussain, A. (2021). Feeding habits and habitat use of barking deer (Muntiacus vaginalis) in Himalayan foothills, Pakistan. *Plos one*, *16*(1), e0245279.

Hammer, O. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontol electron*, *4*, 9. https://doc.rero.ch/record/15326/files/PAL_E2660.pdf

Han, S.-H., Lee, S.-S., Cho, I.-C., Oh, M.-Y., & Oh, H.-S. (2009). Species identification and sex determination of Korean water deer (Hydropotes inermis argyropus) by duplex PCR. *Journal of Applied Animal Research*, *35*(1), 61-66. https://doi.org/10.1080/09712119.2009.9706986

Hansson, L. (1971). Small rodent food, feeding and population dynamics: a comparison between granivorous and herbivorous species in Scandinavia. *Oikos*, 183-198. https://doi.org/10.2307/3543724

Hibert, F., Fritz, H., Poilecot, P., Abdou, H. N., & Dulieu, D. (2008). Morphological criteria to identify faecal pellets of sympatric ungulates in West African savanna and estimates of associated error. *African Journal of Ecology*, *46*(4), 523-532. https://doi.org/10.1111/j.1365-2028.2007.00889

IBM, I. (2007). *SPSS for Windows.* In (Version 16) IBM Incorporation. https://www.ibm.com/support/pages/how-cite-ibm-spss-statistics-or-earlier-versions-spss

Jabin, G., Ghosh, M., Dolker, S., Joshi, B. D., Singh, S., Chandra, K., Sharma, L. K., & Thakur, M. (2021). Faecal morphometry in assigning species identity of three Himalayan ungulates. *Proceedings of the Zoological Society,*

Jameel, M. A., Tabassum, S., Mehmood, S., Shah, T., Khan, M. F., Kabir, M., & Awan, M. N. (2019). Does Trophy Hunting of Kashmir Markhor Really Contributing in Its Conservation? *International Journal of Conservation Science*, *10*(3), 525-532. https://ijcs.ro/public/IJCS-19-48

Lafferty, D. J., Gillman, S. J., Jeakle, L. K., Roell, B. J., & McKenney, E. A. (2022). Mink (Neovison vison) fecal microbiomes are influenced by sex, temperature, and time postdefecation. *Journal of Mammalogy*, *103*(2), 316-327.

https://doi.org/10.1093/jmammal/gyab140

Lasch, P., Haensch, W., Naumann, D., & Diem, M. (2004). Imaging of colorectal adenocarcinoma using FT-IR microspectroscopy and cluster analysis. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1688*(2), 176-186. https://doi.org/10.1016/j.bbadis.2003.12.006

Lee Foote, A., & Rice Hornung, C. L. (2005). Odonates as biological indicators of grazing effects on Canadian prairie wetlands. *Ecological Entomology*, *30*(3), 273-283. https://doi.org/10.1111/j.0307-6946.2005.00701.x

Leopold, B. D., & Krausman, P. R. (1991). Factors influencing desert mule deer distribution and productivity in southwestern Texas. *The Southwestern Naturalist*, 67-74. https://doi.org/10.2307/3672118

MacCracken, J. G., & Van Ballenberge, V. (1987). Age-and sex-related differences in fecal pellet dimensions of moose. *The Journal of wildlife management*, 360-364. https://doi.org/10.2307/3801017

Manley, F. (1994). *Multivariate statistical methods: a primer.* Chapman and Hall. https://doi.org/10.1201/9781315382135

Maurer, M., Peralta Martínez, K., Trevelline, B. K., Tripoli, D., Dearing, M. D., Derting, T., Martinez Mota, R., Pasch, B., & Kohl, K. D. (2021). Diet alters rodent fecal pellet size: implications for paleoecological and demographic studies using fecal dimensions. *Journal of Mammalogy*, *102*(6), 1619-1626. https://doi.org/10.1093/jmammal/gyab098

Morden, C. J. C., Weladji, R. B., Ropstad, E., Dahl, E., & Holand, Ø. (2011). Use of faecal pellet size to differentiate age classes in female Svalbard reindeer Rangifer tarandus platyrhynchus. *Wildlife Biology*, *17*(4), 441-448. https://doi.org/10.2981/10-023

Nawaz, A., Mirza, Z. B., Zakaria, V., Rafique, M., Shah, M., Malik, R., Khan, N. K., & Younas, M. (2007). *Himalayan Wildlife Foundation.* Margalla Hills National Park Ecological Baseline; Himalayan Wildlife Foundation: Islamabad, Pakistan.

Neff, D. J. (1968). The pellet-group count technique for big game trend, census, and distribution: a review. *The Journal of wildlife management*, 597-614. https://doi.org/10.2307/3798941

Niknam, T., & Amiri, B. (2010). An efficient hybrid approach based on PSO, ACO and k-means for cluster analysis. *Applied soft computing*, *10*(1), 183-197. https://doi.org/10.1016/j.asoc.2009.07.001

Origin. (2010). *Origin In (Version 8.5) Origin Labs Corp.* https://www.originlab.com/index.aspx?go=Company/NewsAndEvents/PressRoom&pid=1720

Page, B. D., & Underwood, H. B. (2006). Comparing protein and energy status of winter‐fed white‐tailed deer. *Wildlife Society Bulletin*, *34*(3), 716-724.

https://doi.org/10.2193/0091-7648(2006)34[716:CPAESO]2.0.CO;2

Popp, J. N., Priadka, P., Young, M., Koch, K., & Morgan, J. (2020). Indigenous guardianship and moose monitoring: Weaving indigenous and Western ways of knowing. *Human–Wildlife Interactions*, *14*(2), 17. https://doi.org/10.26077/67f5-d36b

Prosekov, A., Kuznetsov, A., Rada, A., & Ivanova, S. (2020). Methods for monitoring large terrestrial animals in the wild. *Forests*, *11*(8), 808. https://doi.org/10.3390/f11080808

Pukazhenthi, B. S., & Wildt, D. E. (2003). Which reproductive technologies are most relevant to studying, managing and conserving wildlife? *Reproduction, Fertility and Development*, *16*(2), 33-46. https://doi.org/10.1071/RD03076

Putman, R. J. (1984). Facts from feces. *Mammal Review*, *14*(2), 79–97. https://doi.org/10.1111/j.1365-2907.1984.tb00341.x

Roberts, T. J. (1997). *Mammals of Pakistan.* (Revised ed.). Oxford University Press.

Rouco, C., Starkloff, A., Delibes-Mateos, M., Schubert, M., & Rödel, H. G. (2012). Differentiation of animals from different age classes by means of pellet size: assessment of a field method in European rabbits. *Mammalian Biology*, *77*, 451-454. https://doi.org/10.1016/j.mambio.2012.03.009

Rowland, M., White, G., & Karlen, E. (1984). Use of pellet-group plots to measure trends in deer and elk populations. *Wildl Soc Bul (1973-2006)*, *12*(2), 147-155. https://www.jstor.org/stable/3781605

Sánchez-Rojas, G., & Gallina, S. (2000a). Factors affecting habitat use by mule deer (Odocoileus hemionus) in the central part of the Chihuahuan Desert, Mexico: an assessment with univariate and multivariate methods. *Ethology Ecology & Evolution*, *12*(4), 405-417. https://doi.org/10.1080/08927014.2000.9522795

Sanchez-Rojas, G., Gallina, S., & Equihua, M. (2004b). Mule deer (Odocoileus hemionus) density in a landscape elements of the Chihuahuan desert, Mexico. *Journal of Arid Environment*, *44*, 357-368. https://doi.org/10.1080/08927014.2000.9522795

Sánchez‐Rojas, G., Gallina, S., & Equihua, M. (2004). Pellet morphometry as a tool to distinguish age and sex in the mule deer. *Zoo Biol: Published in Affiliation with the American Zoo and Aquarium Association*, *23*(2), 139-146. https://doi.org/10.1002/zoo.10119

Shaw, C. N., Wilson, P. J., & White, B. N. (2003). A reliable molecular method of gender determination for mammals. *Journal of Mammalogy*, *84*(1), 123-128. https://doi.org/10.1644/1545-1542(2003)084<0123:ARMMOG>2.0.CO;2

Singh, B., Kumar, A., Uniyal, V. P., & Gupta, S. K. (2019). Complete mitochondrial genome of northern Indian red muntjac (Muntiacus vaginalis) and its phylogenetic analysis. *Molecular Biology Reports*, *46*(1), 1327-1333. https://doi.org/10.1007/s11033-018-4486-z

Tahir, Z., & Asim, M. (2018). Surface measured solar radiation data and solar energy resource assessment of Pakistan: A review. *Renewable and Sustainable Energy Reviews*, *81*, 2839-2861. https://doi.org/10.1016/j.rser.2017.06.090

Thomas, J. W., & Toweill, a. D. (1982). *Elk of North America: Ecology and Management.* Stackpole, Harrisburg, Pennsylvania.

Turek, K., KAMLER, J., & PROCHÁZKA, L. (2016). The Impact of Thinning Type on Bark Stripping Damage Intensity Caused by Red deer (Cervus elaphus L.). *Baltic Forestry*, *22*(2), 246-250.

Valdez, R., & Ortega-S, J. A. (2014). *Ecología y manejo de fauna silvestre en México.* Editorial del Colegio de Postgraduados.

Webbon, C. C., Baker, P. J., & Harris, S. (2004). Faecal density counts for monitoring changes in red fox numbers in rural Britain. *Journal of Applied Ecology*, *41*(4), 768-779. https://doi.org/10.1111/j.0021-8901.2004.00930.x

Whisson, D. A., Engeman, R. M., & Collins, K. (2005). Developing relative abundance techniques (RATs) for monitoring rodent populations. *Wildlife Research*, *32*(3), 239-244. https://doi.org/10.1071/WR03128

Wilson, G. J., & Delahay, R. J. (2001). A review of methods to estimate the abundance of terrestrial carnivores using field signs and observation. *Wildlife Research*, *28*(2), 151-164. https://doi.org/10.1071/WR00033

Woodruff, S. P., Johnson, T. R., & Waits, L. P. (2016). Examining the use of fecal pellet morphometry to differentiate age classes in Sonoran pronghorn. *Wildlife Biology*, *22*(5), 217-227. https://onlinelibrary.wiley.com/doi/pdf/10.2981/wlb.00209

Zemanova, M. A. (2020). Towards more compassionate wildlife research through the 3Rs principles: moving from invasive to non‐invasive methods. *Wildlife Biology*, *2020*(1), 1-17. https://doi.org/10.2981/wlb.00607