REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.12.e1850
ISSN 2007-3380

Original article / Artículo original

Absence of Top Predators: No sign of Jaguars (*Panthera onca*) or Pumas (*Puma concolor*) in Punta de Mita, Nayarit, Mexico

Ausencia de depredadores tope: sin pistas de jaguares (*Panthera onca*) ni pumas (*Puma concolor*) en Punta de Mita, Nayarit, México

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Please cite this article as/Como citar este artículo: Guzmán-Báez, D. J., Luja, V.H. (2025). Absence of Top Predators: no sign of Jaguars (*Panthera onca*) or Pumas (*Puma concolor*) in Punta de Mita, Nayarit, Mexico. *Revista Bio Ciencias*, 12, e1850. https://doi.org/10.15741/revbio.12.e1850

Article Info/Información del artículo
Received/Recibido: December 11th 2024.
Accepted/Aceptado: May 20th 2025.
Available on line/Publicado: May 08th 2025.

ABSTRACT

Top predators are essential for ecosystems, and their absence can trigger trophic cascades. In Punta de Mita, Nayarit, Mexico, tourist urbanization, livestock farming, and road infrastructure threaten the presence of the jaguar (*Panthera onca*) and the puma (*Puma concolor*). From April to June 2023, 36 camera traps were set up to determine the presence and relative abundance of jaguar and puma in an area surrounded by roads and subject to intense human disturbance. With a sampling effort of 1,291 trap-days, 1,975 independent photographic records were obtained. A total of 11 species of medium and large wild mammals were recorded. The most abundant species were the coati (*Nasua narica*) and the white-tailed deer (*Odocoileus virginianus*). No records of jaguars or pumas were obtained, which could be related to habitat loss and fragmentation due to livestock activities and road presence, as well as detection difficulties caused by their low densities. The coati, collared peccary, and raccoon were more abundant than in other studies where large predators were recorded. This highlights the need for long-term studies to understand the effects of the absence of large predators in fragmented ecosystems.

KEY WORDS: Apex predators, camera traps, diversity, habitat fragmentation, mammals, Nayarit, prey, relative abundance.

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ISSN 2007-3380

RESUMEN

Los depredadores tope son esenciales en los ecosistemas y su ausencia puede desencadenar cascadas tróficas. En Punta de Mita, Nayarit, México, la urbanización turística, ganadería e infraestructura vial amenazan la presencia del jaguar (*Panthera onca*) y el puma (*Puma concolor*). De abril a junio de 2023, se colocaron 36 cámaras trampa para determinar la presencia y abundancia relativa de jaguar y puma en un área rodeada por carreteras y sometida a intensa perturbación humana. Con un esfuerzo de muestreo de 1,291 días-trampa, se obtuvieron 1,975 registros fotográficos independientes. Se registraron un total de 11 especies de mamíferos silvestres medianos y grandes. Las especies más abundantes fueron el coatí (*Nasua narica*) y el venado cola blanca (*Odocoileus virginianus*). No se obtuvieron registros de jaguar ni de puma, lo cual podría estar relacionado con la pérdida y fragmentación del hábitat, como consecuencia de las actividades ganaderas y la presencia de carreteras, así como con la dificultad de detección por sus bajas densidades. El coatí, el pecarí de collar y el mapache fueron más abundantes que en otras investigaciones donde se registraron grandes depredadores. Se resalta la necesidad de estudios a largo plazo para comprender los efectos de la falta de grandes depredadores en ecosistemas fragmentados.

PALABRAS CLAVE: Abundancia relativa, cámaras trampa, depredadores tope, diversidad, fragmentación del hábitat, mamíferos, Nayarit, presas.

Introduction

Apex predators play a crucial role in shaping the dynamics of most ecosystems by regulating prey populations, balancing the food chain, and preventing cascading ecological effects (Di Bitetti, 2008; Ordiz *et al*., 2013; Burgos *et al*., 2023). They hold a fundamental place in conservation efforts, with many species being used as “flagship” species to secure funding for biodiversity and key habitat conservation (Rumiz, 2010; Macdonald *et al*., 2017), as well as “umbrella” species to help preserve entire ecosystems (Rumiz, 2010; Di Minin *et al*., 2016). Despite their importance, apex predators face significant threats, including habitat loss, prey decline, and direct hunting, leading to population declines (Ripple *et al*., 2014; Di Minin *et al*., 2016). Large carnivores require extensive territories and rely on robust prey populations for sustenance (Di Bitetti, 2008; Ripple *et al*., 2014; Jędrzejewski *et al*., 2017), making them more vulnerable to habitat degradation and fragmentation (Di Minin *et al*., 2016). Additionally, they are recognized as indicator species of habitat quality (Crooks, 2002; Benchimol, 2016; Botero-Cañola *et al*., 2018); their absence can lead to uncontrolled growth of herbivore populations, increasing herbivory pressure on vegetation.

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This overexploitation of vegetation alters the habitat and reduces resource availability for other species, triggering a series of landscape-level impacts, including changes in habitat structure and use, as well as a decline in biodiversity (Di Bitetti, 2008; Beschta & Ripple, 2009; 2010; Estes *et al*., 2011; Ripple & Beschta, 2012; Ripple *et al*., 2014; Wallach *et al*., 2015).

The loss of apex predators can trigger complex effects known as trophic cascades (Di Bitetti, 2008; Rumiz, 2010; Ordiz *et al*., 2013; Ripple *et al*., 2014). On one hand, mesopredators (mid-sized predators that prey on small herbivores; Di Bitetti, 2008) may increase in number due to the disappearance of the indirect control that apex predators exert over them, leading to greater pressure on their own prey and potentially causing their decline or local extinction (Prugh *et al*., 2009). On the other hand, the direct prey of apex predators tends to experience population growth due to reduced predation pressure (Pérez-Irineo & Santos-Moreno, 2015; Amaya, 2020; Burgos *et al*., 2023); both processes significantly impact ecosystem balance. This phenomenon has been observed in regions of Africa, Australia, Western Europe, and North America following the disappearance of lions (*Panthera leo*), leopards (*Panthera pardus*), dingoes (*Canis dingo*), Eurasian lynxes (*Lynx lynx*), gray wolves (*Canis lupus*), grizzly bears (*Ursus arctos*), and pumas (*Puma concolor*) (Estes *et al*., 2011; Ripple *et al*., 2014).

The jaguar in Mexico is found in part of its historical range, from Sonora and Tamaulipas to Chiapas and the Yucatán Peninsula, covering the coastal plains of the Gulf of Mexico and the Pacific (Ceballos *et al*., 2021). In western Mexico, the jaguar is best represented in the forests of Nayarit and Jalisco (Núñez, 2007). In the state of Nayarit, the presence of the jaguar (*Panthera onca*) and puma (*Puma concolor*) has been well documented (Ramírez-Silva *et al*., 2015; CONANP, 2016; Figel *et al*., 2016; Illescas, 2019; Medina-Gutiérrez & Ramírez-Silva, 2019; Guzmán-Báez, 2021; Núñez, 2021; Tejeda, 2021; Cortés, 2022; Luja *et al*., 2022). Camera trap studies conducted in Cerro de San Juan (Tejeda, 2021), Sierra de Vallejo (Cortés, 2022), and the Marismas-San Juan corridor (Guzmán-Báez, 2021), using the standard methodology of the National Jaguar Census (CENJAGUAR) (Chávez *et al*., 2013), have recorded that both felines are widely distributed in the state. This is attributed to the availability of sufficient habitat and a stable prey base, including the collared peccary (*Dycotiles tajacu*), coati (*Nasua narica*), and raccoon (*Procyon lotor*) (Guzmán-Báez, 2021; Tejeda, 2021; Cortés, 2022). One area that had not been previously studied is Punta de Mita, in the southern part of the state, near the border between Nayarit and Jalisco. This region is experiencing rapid urbanization due to tourism development, livestock production (which generates conflicts with large predators), and infrastructure expansion. In this regard, the recently opened Las Varas–Puerto Vallarta highway, running parallel to Federal Highway 200 at distances ranging from 500 meters to 3 kilometers in certain sections, adds to these pressures. Given this context, it is hypothesized that cumulative pressures from human activities, including hunting, livestock farming, and tourism, are altering the diversity and abundance of medium and large mammals previously recorded north of the study area by Illescas (2019) and Cortés *et al*. (2022). The objective of this study was to determine the presence and relative abundance of the jaguar (*Panthera onca*) and puma (*Puma concolor*) in the Punta de Mita region, Nayarit, Mexico, using camera trap methods. The study aimed to characterize their spatial distribution, activity patterns, and density within the study area.

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Material and Methods

Study area

This study was conducted in Punta de Mita, located in the southwestern portion of the Sierra de Vallejo, covering an area of approximately 52.8 km², outside the Sierra de Vallejo protected natural area. It is situated between the towns of Sayulita, Higuera Blanca, Cruz de Huanacaxtle, and El Guamúchil, within the municipality of Bahía de Banderas, on the southern coast of the state of Nayarit (Figure 1). The climate is warm subhumid with summer rainfall, with an average annual temperature of 28°C (Arriaga *et al*., 2000; CONANP, 2012). The predominant vegetation consists of secondary vegetation of medium sub-deciduous tropical forest, along with other vegetation types such as low deciduous tropical forest and medium deciduous tropical forest (Arriaga *et al*., 2000; Rzedowski, 2006; Martínez & Ceballos, 2010). Human activities in the region include extensive cattle ranching, agriculture, fishing, and forestry; the rest of the economically active population is engaged in secondary and tertiary sectors, such as construction, commerce, services, and tourism (CONANP, 2012).

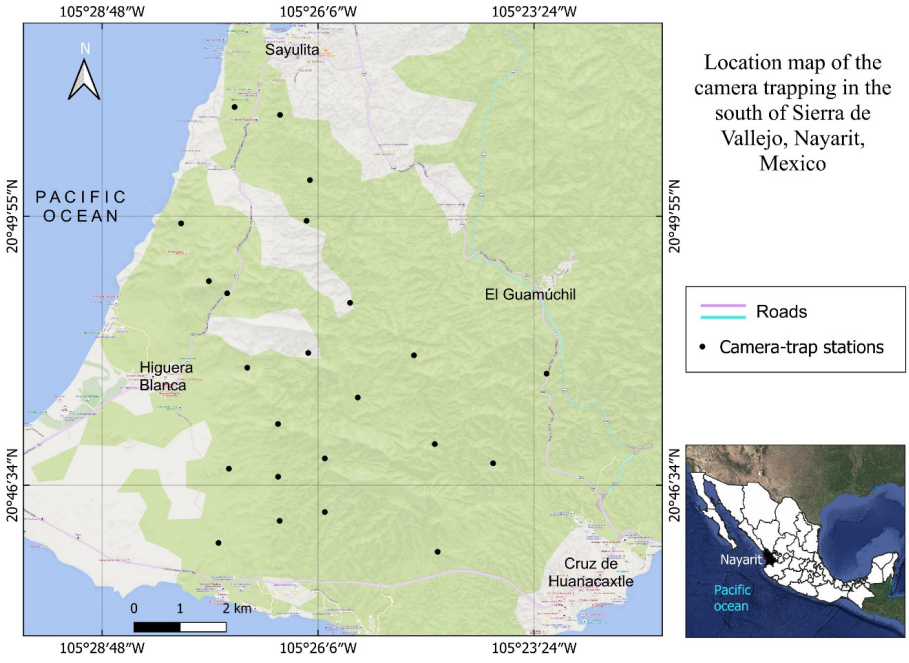

Figure 1. Sampling area and location of camera trap stations located south of Sierra de Vallejo, Bahia de Banderas, Nayarit, Mexico.

In blue, the Las Varas-Puerto Vallarta Highway is shown, and in purple, the Federal Highway 200 (Tepic-Puerto Vallarta).

Source: own elaboration based on base maps [Bing Maps], created in QGIS (QGIS, 2020).

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Sampling design and fieldwork

Camera trapping was conducted from April to June 2023 using camera traps to monitor wildlife. The protocol of the National Jaguar Census (CENJAGUAR) (Chávez *et al*., 2013) was adapted for this study. A total of 23 stations with 36 camera traps were deployed, using devices from the following brands and models: Cuddeback (Cuddeback X-Change Color, Cuddeback IR Plus, Cuddeback H20 MP IR; company: Cuddeback; city: Green Bay), Browning (Dark Ops Full HD; company: Prometheus Group; city: Birmingham), and Wosoda (G300; company: Haofan Technology Co., Limited; city: Hong Kong). The cameras were programmed to operate continuously 24 hours a day, capturing only photographs with the shortest possible interval between shots according to the technical specifications of each model. The camera traps remained active for 68 days. The study area was divided into 12 sampling quadrants, each covering 9 km². Within each quadrant, sites showing signs of wildlife activity, such as trails, tracks, and scrapes, were selected for camera placement. Each quadrant aimed to include two single-camera stations and one double-camera station. The stations were placed at a minimum distance of 1 km from each other under the assumption that all individuals had an equal probability of being photographed (Karanth & Nichols, 1998; de la Torre, 2009). Cameras were mounted on trees at a height of 40–50 cm above ground level, positioned perpendicularly to the trails (Chávez *et al*., 2013). For image organization and analysis, the protocol of Sanderson & Harris (2013) was followed. All spatial analyses were conducted using QGIS Desktop version 3.4.4 (QGIS, 2020) and Google Earth.

Species Richness

Alpha diversity was estimated as the number of species or site-specific species' richness (Moreno, 2001; Halffter & Moreno, 2005; García-Morales *et al*., 2011), calculated by counting the species present in the study area. Following the classification by Ripple *et al*. (2014), large mammals were defined as those with a body mass of ≥ 15 kg. All recorded data were organized into a database, from which species accumulation curves were generated using the program EstimateS (Colwell, 2013); a presence-absence matrix was used along with the ACE and CHAO 1 estimators, the latter applied for estimates with fewer than 10 individuals (Moreno, 2001; Jiménez-Valverde & Hortal, 2003). The conservation status of the recorded species was determined based on the Official Mexican Standard NOM-059-SEMARNAT-2010, updated in 2019 (SEMARNAT, 2019).

Species Abundance

Relative abundance, defined as the "number of individuals of a species in relation to the total number of individuals of all species" (Morláns, 2004), was estimated using the Relative Abundance Index (RAI). This index is interpreted as the average number of independent photographs per 100 trap-days (Pérez-Solano *et al*., 2018). The calculation followed the formula proposed by Maffei *et al*. (2004) and Jenks *et al*. (2011): RAI= (C / SE) x 100 where C represents the number of independent photographic records, and SE is the sampling effort, multiplied by 100 days (standard correction factor). To avoid overestimation of species abundance, only independent records were

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considered. A record was deemed independent when photographs of individuals of the same species at the same station were separated by at least 60 minutes (Di Bitetti *et al*., 2014).

Results and Discussion

With a sampling effort of 1,291 trap days, a total of 12,839 photographic records were obtained, of which 1,975 were independent. No records of jaguar or puma were obtained. Instead, 11 species of medium and large wild mammals were recorded, classified into four orders, eight families, and ten genera. Additionally, humans and domestic animals such as cows, dogs, and cats were recorded (Table 1). Among the wild mammals, the most represented order was Carnivora, with four families and seven species. The sampling efficiency using the ACE estimator was 96%, and with CHAO1, it was 100%, indicating that the sampling effort was sufficient to capture the species richness in the study area (Figure 2).

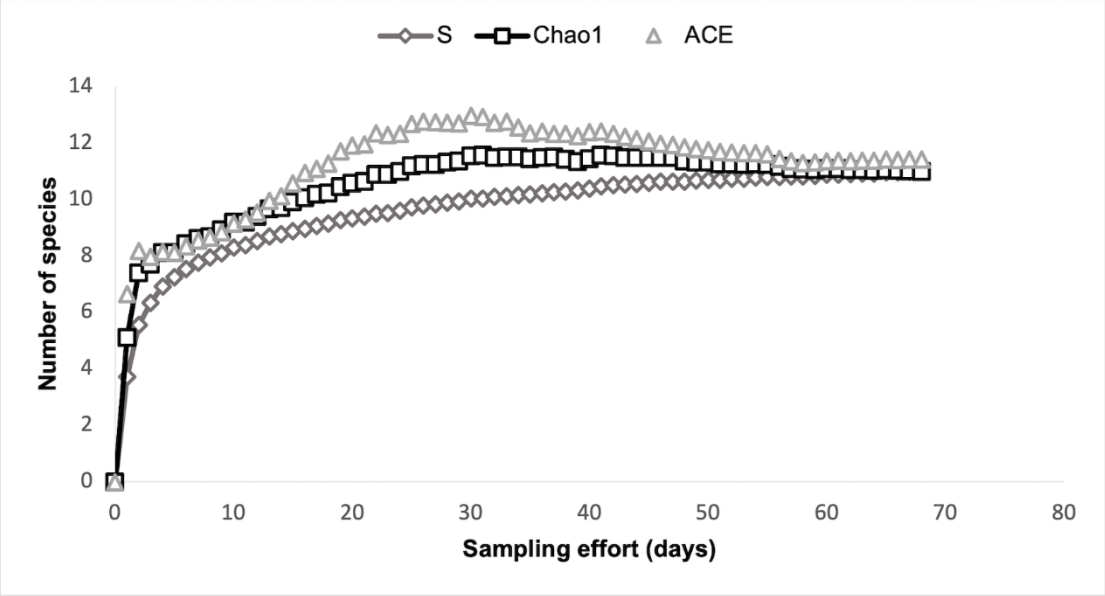

Figure 2. Species accumulation curve (S), with Chao 1 and ACE estimators for medium and large mammals in the southern Sierra de Vallejo, Nayarit, Mexico.

Cattle accounted for the majority of the records (*n* = 850, RAI = 43), followed by humans (*n* = 493, RAI = 24.9). Among wild mammals, the most abundant species was the coati (*Nasua narica*) (*n* = 252, RAI = 12.75), followed by white-tailed deer (*Odocoileus virginianus)* (*n* = 85, RAI = 4.30), and collared peccary (*Dicotyles tajacu*) (*n* = 53, RAI = 2.68). Additionally, two medium-

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sized felid species were recorded: the ocelot (*Leopardus pardalis*) (*n* = 47, RAI = 2.3) and the margay (*Leopardus wiedii*) (*n* = 3, RAI = 0.14), both classified as endangered according to the Mexican Official Standard (NOM-059-SEMARNAT-2010; Table 1). Regarding the percentage of human detections in the area, 465 independent photos were analyzed, showing that 49.67% were tourists, 31.61% were livestock farmers, 14.83% were individuals engaged in other activities, and 3.87% were hunters.

Table 1. Relative abundance indices of medium and large mammals reported in the mangroves of the central coast of Nayarit (Guzmán-Báez, 2021), on the western face of Sierra San Juan (Tejeda, 2021), in the Sierra de Vallejo Protected Natural Area (Cortés, 2022), and in the southwestern end of Sierra de Vallejo (outside the protected area).

	Guzmán-Báez (2021)	Tejeda (2021)	Cortés (2022)	This study
Taxa				
ORDER DIDELPHIMORPHIA				
Family Didelphidae				
Didelphis virginiana	1	0.8	0	0.7
ORDER CINGULATA				
Family Dasypodidae				
Dasypus novemcinctus	0.5	1.2	0.2	0.2
ORDER LAGOMORPHA				
Family Leporidae				
Silvilagus cunicularius	12	0	0	0
ORDER CARNIVORA				
Family Felidae				
Herpailurus yaguarundi	0.1	0	0	0
Leopardus pardalis	3.9	1.3	3.15	2.3
Leopardus wiedii	0	0.08	0.1	0.1
Lynx rufus	1.3	0	0	0
Panthera onca	5.2	0.5	3.6	**0**
Puma concolor	0	0.1	0.1	**0**
Family Canidae				
Canis latrans	3.3	2.6	0.5	0.05
Urocyon cinereoargenteus	0.04	2.5	0.8	2.27
Family Mephitidae				
Conepatus leuconotus	0	0	0.1	0.1

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Continuation

Table 1. Relative abundance indices of medium and large mammals reported in the mangroves of the central coast of Nayarit (Guzmán-Báez, 2021), on the western face of Sierra San Juan (Tejeda, 2021), in the Sierra de Vallejo Protected Natural Area (Cortés, 2022), and in the southwestern end of Sierra de Vallejo (outside the protected area).

	Guzmán-Báez (2021)	Tejeda (2021)	Cortés (2022)	This study
Taxa				
Family Procyonidae				
Nasua narica	2.1	3.7	2.43	**12.7**
Procyon lotor	0.6	0.6	0.2	**2.4**
ORDER ARTIODACTYLA				
Family Tayassuidae				
Dicotyles tajacu	0.4	0.2	0.7	**2.6**
Family Cervidae				
Odocoileus virginianus	4.5	3.1	6.2	4.3
Total mammal species	13	12	12	11
Bos taurus	20.1	11.8	42.6	43
Homo sapiens	40.9	70.7	35.1	24.9
Canis familiaris	2.3	14	3.54	2.4
Felis catus	0	0	0	0.1
Sampling effort	2740	2581	2045	1291

The taxonomic list of wild mammals is presented, including values for humans and domestic species (livestock, dogs, and cats). Sampling effort = camera days.

The species diversity of medium and large mammals recorded in this study (11 species) is similar to that reported in other studies (12–13 species) that have used camera trapping to assess the diversity of this group in the state (Guzmán-Báez, 2021; Tejeda, 2021; Cortés, 2022; Luja *et al*., 2022). Our results indicate that, despite previous studies being conducted in environments highly modified by human activities, a baseline community of medium and large mammals can still be found. However, the RAI values obtained in this study for the collared peccary (*Dicotyles tajacu*), coati (*Nasua narica*), and raccoon (*Procyon lotor*), as well as the absence of jaguar (*Panthera onca*) and puma (*Puma concolor*) records, are noteworthy.

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In other sites where camera trapping has been used to assess the diversity of medium and large mammals in Nayarit, jaguars, pumas, or both have been recorded, depending on the type of vegetation (Illescas, 2019; Medina-Gutiérrez & Ramírez-Silva, 2019; Guzmán-Báez, 2021; Hernández-Cortés, 2021; Cortés, 2022). Although the presence of these felids is well documented in Sierra de Vallejo, just 20 kilometers north of our camera traps (Núñez & Saracho, 2018; Illescas, 2019; Cortés, 2022), no records of jaguar or puma were obtained in our study area.

During a camera trap survey, there are two possible scenarios: capturing images that confirm the presence of a species or the lack of images, which could be mistakenly interpreted as absence. However, this zero can result from either imperfect detection or the actual absence of the species (Martin *et al*., 2005). The lack of records for these species does not necessarily imply their absence in the area but may instead be related to a low detection probability (Díaz-Pulido & Payán, 2012; Chávez *et al*., 2013; Andrade-Ponce *et al*., 2021).

Imperfect detection occurs when a species present in a survey area is not detected, leading to the erroneous conclusion of its absence (false negative, according to MacKenzie *et al*., 2002). In camera trapping, imperfect detection can occur at two levels: in relation to the species' area of use and in relation to the camera trap station (Burton *et al*., 2015; Findlay *et al*., 2020). The first level is influenced by the species' spatial distribution and abundance and is linked to the probability that an individual moves within its territory in the area where the camera traps are placed. The second level refers to the effective detection area, defined by the radial distance (r) and angle (θ) at which a species triggers the sensor and is photographed (Rowcliffe *et al*., 2008). In this regard, the research team has deployed 350 camera trap stations using the same camera models as in this study, and pumas and/or jaguars have been recorded in all of them (Guzmán-Báez, 2021; Tejeda, 2021; Cortés, 2022; Luja *et al*., 2022).

Therefore, although imperfect detection is a possibility, the absence of jaguars and pumas in Punta de Mita could be due to the actual absence of both species in the study area. Anthropogenic factors such as high human presence and livestock activities, as well as habitat fragmentation caused by road construction disrupting connectivity with Sierra de Vallejo, may be limiting habitat use by these large felids.

The study area is located within a territory shaped like a "peninsula," bordered to the north, west, and south by the Pacific Ocean, and connected only at its northeastern portion to the Sierra de Vallejo (Figure 1). However, this connection is physically interrupted by two roads: Federal Highway 200 (a two-lane road without a central divider) and the new Las Varas–Puerto Vallarta highway (Duarte *et al*., 2017).

While roads fragment ecosystems by dividing habitats into smaller, isolated patches, the Barrier Effect arises because roads act as physical obstacles, preventing or limiting the movement of organisms and hindering the free movement of wildlife. This disruption affects critical ecological processes such as reproduction, foraging, and sheltering, and may restrict gene flow between populations, potentially leading to inbreeding and a reduction in genetic diversity (Sanz *et al*., 2001; Arroyave *et al*., 2006; Benítez & Escalona-Segura, 2021).

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The lack of jaguar and puma records in our study area, despite their reports in the past six years north of the Las Varas–Puerto Vallarta highway (Illescas, 2019; Cortés, 2022), could explain the high relative abundance of their main prey species (collared peccary, coati, and raccoon). This finding aligns with previous studies suggesting that the absence of apex predators can lead to an increase in prey populations (Estes *et al*., 2011; Ripple *et al*., 2014).

The RAI of the collared peccary (*Dicotyles tajacu*) recorded in this study was 3.7, 6.5, and 13 times higher than the values reported by Cortés (2022), Guzmán-Báez (2021), and Tejeda (2021), respectively. Similar patterns emerged for the RAI of the raccoon (*Procyon lotor*), which was 12, 4, and 4 times higher, and for the coati (*Nasua narica*), which was 5.2, 6, and 3.7 times higher, respectively. In the aforementioned studies, jaguar, puma, or both are present, and these species are recognized as predators of the mentioned mammals (Ávila-Nájera *et al*., 2018; Luja *et al*., 2020). These patterns could be explained by the release from predation pressure in the absence of large predators or in areas where their densities are very low. With reduced energy investment in defensive or vigilance behaviors, prey species can allocate more resources to feeding and reproduction, leading to higher population sizes compared to areas where predators are present (Ordiz *et al*., 2013; Pérez-Irineo & Santos-Moreno, 2015; Spindler & González, 2022; Burgos *et al*., 2023). However, this increased abundance of these species may have significant ecological consequences, such as the overexploitation of plant resources due to higher herbivory levels (Ripple & Beschta, 2012; Pérez-Irineo & Santos-Moreno, 2015; Ripple *et al*., 2016), potentially leading to drastic changes in the structure and composition of local vegetation. Additionally, heightened intraspecific competition among prey species could result in decreased physical condition, lower reproductive success, and increased disease transmission (Levi *et al*., 2012; Ripple *et al*., 2014; Wallach *et al*., 2015).

This study recorded a community of medium and large-sized mammals similar to those reported in other studies in the state of Nayarit, but it also documented a significant presence of livestock in the study area. Livestock farming is a key sector in Mexico's economy (Rosas-Rosas *et al*., 2015). In Sierra de Vallejo, extensive cattle ranching practices carried out by rural producers allow livestock to roam freely in forests and jungles. This type of livestock management, in an environment where large predators are present and where habitat is relatively fragmented, increases conflicts between jaguars, pumas, and ranchers. Big cat attacks on livestock cause economic losses for ranchers (Thirgood *et al*., 2005; Peña-Mondragón *et al*., 2017; Tortato *et al*., 2017) and often lead to retaliatory lethal actions (Zimmermann *et al*., 2005; Garrote, 2012; Torres-Romero *et al*., 2024). A study conducted in the Chamela-Cuixmala and Sierra de Manantlán regions in Jalisco documented that livestock intrusion into the habitat of big cats, combined with the hunting of their natural prey, contributed to the decline of predator populations (Núñez, 2007).

Globally, the increase in habitat fragmentation and accessibility to natural areas is bringing humans closer to wildlife populations (Preisler *et al*., 2006). This can lead to a series of impacts on the distribution, reproduction, and survival of wildlife (George & Crooks, 2006; Reed & Merenlender, 2008; Otavo & Echeverría, 2017). In our study area, 24% of the photographed records correspond to human presence. Of this percentage, 49.6% were tourists, 31.6% were ranchers, 14.8% were people with other activities, and 3.8% were hunters. It has been observed that human presence

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causes disturbance to wildlife species, triggering costly behavioral responses such as the interruption of feeding (Fernandez-Juricic & Tellería, 2000) or changes in spatial/temporal habitat use (Rogala *et al*., 2011).

Conclusions

Our results suggest that top predators in the region (pumas and jaguars), if still present, are at densities too low to be detected by our cameras during a sampling effort of 1,291 trap-days. In contrast, the relative abundance of their prey, such as coati, collared peccary, and raccoon, is very high compared to other sites where the presence of large predators has been previously confirmed using the same methodology. The absence of jaguar and puma records may be linked to specific anthropogenic factors in the region, such as proximity to livestock activities and habitat fragmentation due to road construction, which interrupts the connection between the northwest part of the study area and the Sierra de Vallejo, potentially limiting the dispersal of these felines. Although this study presents values for the diversity and relative abundance of medium and large mammals, as well as the presence of humans, no conclusions can be drawn about the effects of human presence on wildlife. It is recommended that future studies be conducted to better understand these effects on the spatial and temporal patterns of wildlife. Furthermore, the importance of conducting long-term studies is emphasized in order to analyze more deeply the potential effects of the absence of large predators on prey populations in fragmented ecosystems, as well as to evaluate the impact of human activities on wildlife connectivity and mobility in the region.

Authors contribution

Conceptualization of work, DJG-B y VHL; methodology development, DJG-B; software management, DJG-B; experimental validation, DJG-B y VHL; analysis of results, DJG-B y VHL; data management, DJG-B; manuscript writing and preparation, DJG-B y VHL; writing, revising and editing, DJG-B y VHL; project manager, VHL; fund acquisition, DJG-B y VHL.

All authors of this manuscript have read and accepted the published version of this manuscript.

Financing

This research was funded with personal funds.

Acknowledgments

We thank Vista Paraíso for funding the batteries for the camera traps, providing transportation support, and for their hospitality. We also extend our gratitude to J. Ramírez and Manuel from

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ISSN 2007-3380

Sangre Azul Kennels for their invaluable support and companionship during fieldwork. D. J. Guzmán Báez also acknowledges the Doctoral Program in Biological and Agricultural Sciences at the Universidad Autónoma de Nayarit and the Secretariat of Science, Humanities, Technology, and Innovation (SECIHTI) for the scholarship granted. We also appreciate the reviewers who contributed to improving this manuscript, and to R. Thompson for language review.

Conflict of interest

The authors declare that they have no conflicts of interest.

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