The transhemispherical role of an apex predator: Foraging, energetic and behavioral ecology of the puma

by

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Lo dedico

Desde la raíz que yace bajo tierra, marrón y semi olvidada

al más verde retoño que se alza atrapando la luz.

Células entrelazadas que enduran tempestades

sostenidas por el sustrato enriquecido con frutos del pasado

aguardan el tiempo calmo en donde florecerán nuevas memorias.

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Dissertation abstract

The role of predators has lured attention due to their disproportional effect given their biomass. Through top-down effects, predators not only affect the numbers or behavioral responses of their prey but ultimately can cascade down to the rest of the trophic web. However, in a rapidly changing world, increased human land use change, invasion of exotic species combined with a partial rebound of carnivores can converge in the rise of novel landscapes with novel community interactions. In this dissertation, I explored the ecology of the puma (*Puma concolor*), an apex predator, focusing on its role across different ecosystems within a gradient of human disturbance. This work explores various facets of puma ecology—ranging from their foraging habits, movement patterns and energetics to their facilitating role to the ecological community. Hence, I sought to elucidate the broader ecological implications of this predator.

Chapter 1 explored how a direct outcome of puma predation, killed carcasses, can serve as a complex food resource. This microbe-rich resource can bridge macro- and microbiomes and be an important component of scavenger dynamics, specifically Andean condors (*Vultur gryphus*). Through isotopic analysis, this chapter reveals the trophic identities of an Andean food web and how condors exhibit trophic omnivory by consuming and assimilating plant biomass, the meat of herbivores, and the microbes embedded in it. These results show that carcasses represent trophically heterogeneous resources that can be relevant pieces of the ecosystem functioning. Hence, I underscore the importance of microbes in ecological studies to understand their role in food webs.

Chapter 2 analyzed the importance of energy budget estimations on free-range animals and evaluated the daily energy expenditure of wintering pumas in Colorado. I compared the doubly labeled water method with different movement model estimations. The results indicate

that the energetic expenditure is influenced by distance traveled and temperature and emphasize the role of thermogenesis for winter estimations. This chapter emphasizes the importance of incorporating intrinsic factors that can affect the metabolism of animals for a comprehensive understanding of carnivore energetic strategies.

Chapter 3 assessed how human disturbance influences the diet of pumas. Humans can significantly alter the communities around them by their association with domesticated animals and the facilitation of resources to synanthropic species. I evaluated seven puma populations within a human disturbance gradient across the Americas using stable isotopes and GPS collars. This study found that individual diet specialization decreases with increasing human disturbance, leading to a more generalized diet as pumas incorporate human-associated prey. A change of diet associated with human landscapes can have implications for pumas' functional roles in novel ecosystems, potentially leading to novel ecological relationships.

Chapter 4, explored how the imposed barriers of human development can affect puma movement patterns and activity across five different populations. This chapter revealed that pumas adjust their spatial and temporal behaviors based on the level of human disturbance. Pumas tend to have smaller home ranges and exhibit more nocturnal movement patterns in highly disturbed areas, suggesting a strategy to avoid human interaction. In less disturbed populations, though, human elements in the landscape can even represent attractiveness during the night. I highlight the puma behavioral plasticity in navigating human-altered landscapes, which is crucial for their survival in the face of increasing human expansion.

This dissertation contributes to understanding the role of an apex predator within different ecosystems, representing a variety of human disturbances. I provide insights into how human-induced changes in the landscape affect foraging behaviors and movement patterns. The findings shown here underscore the importance of conserving puma interactions, not only for their intrinsic value but also for their impact on the ecological communities and the maintenance of ecosystemic functions.

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CHAPTER ONE

More than just meat: Carcass decomposition shapes trophic identities in a terrestrial vertebrate.

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Abstract

1. Most food web models fail to account for the full complexity of interactions within a community, particularly where microbes are involved. Carcasses are microbe-rich resources and may represent a common nexus for the macro- and microbiome, effectively uniting autotrophs, consumers, predators, and microbiota.
2. We evaluated the role of carcasses as multitrophic resources and explored dietary partitioning for a sexually dimorphic obligate scavenger known for its hierarchical social system. This study was set in a well-studied community of camelids (*Vicugna vicugna, Lama guanicoe*), pumas (*Puma concolor*), and Andean condors (*Vultur gryphus*) in the Andes. We hypothesized that condors, by feeding on trophically distinct dietary substrates within any given carcass, would have highly variable trophic position (TP) values. Further, we expected that the microbial consumers within the carcass would inflate TP values in both, the carrion and the condors. Thus, we expected that the trophic heterogeneity within a carcass could facilitate sex-based dietary partitioning in condors.
3. We used a multifaceted approach to assess the foraging of Andean condors, using regurgitated pellet and bulk isotopic analyses, and also quantified the TP of the entire community of graminoids, camelids, camelid carrion, pumas, and female and male condors employing compound-specific stable isotopes analysis of amino-acids.
4. Our analysis of condor pellets and bulk isotopes revealed non-trivial plant consumption, close to 10% of condor diet. Isotope analysis of amino-acids revealed that condors had highly variable TPs (2.9±0.3) compared to pumas (3.0±0.0) and camelids (2.0±0.1), likely representing “trophic omnivory”, wherein the condors

consume plants (TP=1.0±0.1) and microbe-colonized carrion (2.3±0.1). Female condors exhibited a TP (2.8±0.2) lower than strict carnivory, suggesting that they consume more plant biomass in a carcass, while males (TP=3.1±0.3) are likely consuming more of the microbe-rich animal tissue.

5. Our study highlights that carcasses represent a trophically heterogeneous resource and that vertebrate scavengers can feed across trophic groups within the carcass, from autotrophs to secondary consumers, and from both the macro- and microbiome. Thus, integration of microbes in macroecological contexts can help to resolve trophic identity, and better characterize the importance of microbes in detritivorous and omnivorous species.

Keywords:

carrion – detritivory – food webs – guanaco – microbe – necrobiome – omnivore – vulture.

Introduction

Predation is one of the most studied and captivating trophic interactions (Sergio et al., 2006; Wilson & Wolkovich, 2011). Apex carnivores exert disproportionate effects on prey both directly ("consumptive") and indirectly ("non-consumptive"), sometimes with cascading consequences for lower trophic levels (Estes et al., 2011). Consequently, the regulation of food webs by apex carnivore has been widely explored not only in an ecological context, but also in terms of animal conservation and ecosystem restoration (Ritchie et al., 2012; Wallach et al., 2015). There has been, however, a growing interest in actors beyond the classic "green food web" (i.e., living primary producers-consumers-predators) to include those in the "brown food web" (i.e., detritus-microbiota-scavengers).

It is becoming increasingly recognized that this traditional view of neatly compartmentalized green or brown food webs is not broadly representative of most food webs—indeed green and brown food-chains are usually so intertwined that it becomes trivial to characterize a higher-order consumer as either ‘green’ or ‘brown’ (Steffan & Dharampal, 2019; Wilson & Wolkovich, 2011).

Carcasses are one example blurring the lines between green and brown food webs and uniting the macro- and micro-biome (Barry et al., 2019; Burkepile et al., 2006; Shukla et al., 2018). Although vertebrate carcasses comprise a small fraction of total detritus in an ecosystem, they have a disproportionate local impact in nutrient deposition and cycling, especially of nitrogen, phosphorous and calcium, and represent nutritionally rich, yet ephemeral, resources (Carter et al., 2007; Parmenter & Macmahon, 2009). Consequently, carcasses are crucial for a diversity of scavengers and decomposers that compete for access to them (Burkepile et al., 2006). Although generally considered as a discrete and homogeneous dietary item, carcasses are a composite of organisms: animal tissue, plant matter and digesta held in the digestive tract, invertebrate scavengers and microbes that are consuming necrotic tissue. A single carcass can hold a diversity of organisms that span multiple trophic levels, from autotrophic biomass to secondary and tertiary consumers, including both macro- and microbiota. This so-called “necrobiome” provides a framework to explore cross-kingdom interactions, where microbes can impact entire ecosystems (Benbow et al., 2019). Scavengers are likely consuming all components of a carcass (Pechal et al., 2019; Steffan & Dharampal, 2019) – not only the carrion (i.e. tissue of the dead animal) but also the innumerable microscopic consumers embedded within the carrion (Steffan et al. 2017, Steffan & Dharampal 2019).

Obligate scavengers have evolved a suite of behavioral, physiological, and anatomical adaptations to exploit ephemeral carrion resources (Ruxton & Houston, 2004).

Predators, by hunting prey regularly and often in a spatially predictable fashion, can provide some spatiotemporal consistency in carcass availability (Wilmers et al., 2003). Vultures occupy an important place in the food web by recycling nutrients (Moleón & Sánchez-Zapata, 2015), regulating disease outbreaks (Wilson & Wolkovich, 2011) and modulating predation rates due to competition-facilitation interactions with predators (Elbroch & Wittmer, 2013; Moleón et al., 2014). In the last century, the global abundance and distribution of vultures have declined in part due to decreased availability of safe carrion (Buechley & Şekercioğlu, 2016; Pauli et al., 2018). These declines are likely disrupting important community interactions involving carcasses and the necrobiome (Benbow et al., 2019).

The Andean condor (*Vultur gryphus*) – the largest vulture (1.3 m height and 3 m wingspan) and one of the heaviest flying birds (13 kg body mass, Ferguson-Lees & Christie, 2001) – is considered carnivorous. Condors primarily consume carrion of large herbivores, generally provisioned by pumas (*Puma concolor*) in areas where native communities have been preserved, but they also can consume smaller-bodied vertebrates including exotic lagomorphs (Ballejo et al., 2018; Duclos et al., 2020; Perrig et al., 2017). Researchers have observed plant matter in the pellets of condors previously (Duclos et al., 2020; Pavez et al., 2019), although it is often considered to be incidental ingestion. Plant consumption among condors is a potential mechanism by which condors acquire pigments, notably carotenoids, which are associated with social dominance (Blanco et al., 2013; Marinero et al., 2018). Although male condors are up to 50% larger than females (Alarcón et al., 2017) and assume priority at carcasses (Alarcón et al., 2017; Donazar et al., 1999;

Wallace & Temple, 1987), xanthophyll carotenoid concentrations in blood are highest in immature and female condors (Blanco et al., 2013). Possibly, then, dominant males are consuming the protein-rich components of the carcass and relegating subordinate individuals to plant material.

Research to-date has found little evidence of sex-based partitioning of diet (Perrig et al., 2021), although this could be overlooking fine-scale diet partitioning that happens within the carcass. Microbial or detrital impact on large vertebrate systems has been documented extensively (Hyodo et al., 2015; Stevens & Hume, 1998); however, the quantification of such microbivory has only recently been documented through trophic inflation of detrital complexes in invertebrates and fish (Dharampal et al., 2019; Steffan et al., 2017). Given that carcasses are a heterogeneous pool of resources, it is possible that sexes partition dietary resource at a carcass, possibly resulting in different trophic positions (TP). To-date, the relative importance of carcass components to Andean condors has not been quantified.

To explore resource allocation from the carcass and to examine trophic identities of scavengers, we studied a camelid-puma-condor community in San Guillermo National Park (hereafter, San Guillermo NP; Fig. 1). Located in northwestern Argentina, San Guillermo NP is one of the few places that maintained trophic interactions among native camelids (vicuñas [*Vicugna vicugna*] and guanacos [*Lama guanicoe*]), pumas and Andean condors (Donadio et al., 2010; Donadio, Buskirk, & Novaro, 2012; Perrig et al., 2017). Previous work in San Guillermo NP identified that puma predation is the primary cause of adult camelid mortality (accounting for >90% of carcasses; Donadio et al., 2012). Given that pumas are solitary hunters and leave large quantities of carcass materials behind (Elbroch et al., 2014), the majority (88%) of the condor diet in San Guillermo NP consists of camelids, 85% of which resulted from puma provisioning (Perrig et al., 2017).

We hypothesized that the trophic identity of a carcass would be an assemblage of multiple trophic positions – plant digesta (TP ~ 1), camelid biomass (TP = 2) and microbes (TP ~ 3) – and that obligate scavengers, by consuming them all, would have intermediate non-integer ($2.5 < x < 3.5$) trophic positions. Specifically, we predicted that camelids and pumas would have integer TPs corresponding to strict herbivory and carnivory (TP = 2 and TP = 3, respectively), and exhibit little variance. However, we predicted that camelid necrotic tissue, i.e., carrion, would be trophically inflated due to microbial colonization (Steffan et al., 2017). Consequently, the Andean condors consuming the microbe-colonized carrion would develop a trophic position above pumas consuming only the camelid meat. In effect, microbes within a carcass are trophically analogous to pumas, thus when a condor consumes both the camelid and the embedded microbes, the condor feeds at a higher trophic position. We also predicted that condors were consuming non-trivial amounts of plant material and hence, the condor population would exhibit higher intra-populational variation in TP. Finally, we predicted that condors would exhibit sex-based dietary partitioning, in which dominant males would consume more carcass meat and females would consume more plant material. To test our hypothesis, we quantified the TP of the entire community using compound-specific stable isotopes analysis of amino acids (CSIA-AA). Additionally, we documented the relative contribution of individual food sources to scavengers via regurgitated pellets and the analysis of bulk isotopes (^{13}C and ^{15}N).

Methods

San Guillermo NP (29°12’S – 69°20’W) extends over 1660 km^2 in the Andean plateau in the province of San Juan, Argentina. This high-altitude region is characterized by a semiarid climate (100 – 500 mm/year) with temperatures ranging between 27 °C and - 23 °C (Salvioli, 2007), where vegetation is dominated by grass and shrub steppes. We reanalyzed 177 regurgitated pellets from active Andean condors roosting sites collected by Perrig et al. (2017) in summer 2013 in the park to estimate the amount of plants consumed (as plants were not accounted for in the original analysis). Pellets were oven-dried (55°C for 48 h) and then microscopically identified dietary items to estimate, 1) occurrence, as the percentage of times an item occurred in the total number of pellets, and 2) the relative volumetric content, in relation to the total volume of pellets, for each category of dietary items, grouped as camelids (vicuña and guanaco), plants, livestock (cow [*Bos taurus*], horse [*Equus ferus*], goat [*Capra aegagrus*] and sheep [*Ovis aries*]) and others (hares [*Lepus europeus*] and southern mountain viscachas [*Lagidium viscacia*]).

We also used samples collected in summer 2013 for a complementary analysis of ^{13}C and ^{15}N bulk isotopes (Perrig et al. 2017), which estimate assimilated diet components avoiding biases associated with differential digestibility, as well as ^{15}N CSIA-AA, to estimate the trophic position of all members of the food web. Past analyses have shown that the enrichment in ^{15}N between the diet and the consumer is uneven across AAs but is highly consistent for particular AAs (Chikaraishi et al., 2009). Thus, by quantifying the ratio of ^{15}N between an AA that reflects the source (e.g., phenylalanine), and one that reflects the fractionation by the consumer (e.g., glutamic acid), one can robustly predict the TP of a consumer (Steffan et al., 2015).

For the ^{13}C and ^{15}N stable isotope analysis of bulk tissue we used samples of condor feathers (*n* = 47, adult males = 14, adult females = 23, immature males = 2, immature females = 8) from feeding and roosting sites, hair from camelids (vicuña *n* = 7, guanaco *n* = 7) found on carcasses and hair from cows (*n* = 10) and goats (*n* = 6) (Perrig et al., 2017). We additionally collected the dominant autotrophs and main dietary items of vicuñas (Cajal, 1989) in the park from living plants: rushes (*Juncus* sp.; *n* = 3) and fescues (*Festuca* spp.; *n* = 3). We molecularly identified the sex and individual for each condor feather and evaluated age class based on their color (Perrig et al., 2019). We prepared hair and feather samples for bulk isotope analysis by washing and rinsing 3 times with a solution 2:1 trichloromethane-methanol to remove dirt and surface oil, then all samples were homogenized in small parts (< 1mm) with surgical scissors and dried for 72 hours at 60°C. Values for δ^{13}C and δ^{15}N in bulk tissue were calculated by weighing the samples (0.6 mg ± 0.005) and placing them in tin capsules, then measured with a Costech 4010 elemental analyzer attached to a Thermo Finnigan DeltaPLUS XP or V Flow Isotope Ratio Mass Spectrometer (University of Wyoming, Laramie, USA). Results are provided in per mil (‰) notation relative to the international standards of Peedee Belemnite (PDB) and atmospheric nitrogen (AIR) for carbon and nitrogen, respectively calibrated against internal laboratory standards.

We estimated the proportional assimilation of isotopically distinct food sources by Andean condors using a Bayesian-based mixing model in MixSIAR (Stock & Semmens, 2016) with Markov chain Monte Carlo (MCMC; chain length = 300,000; burn = 200,000; thin = 100; chains = 3). Based on the pellet analysis, we identified five isotopically distinct and biologically meaningful dietary sources: large livestock (cows), small livestock (goats), camelids (vicuña and guanaco), fescues and rushes. We used raw isotopic compositions values of the dietary source and average and standard deviation for their concentration dependence (i.e. relative weight of N and C; Table S1). We used trophic discrimination factors of 3.1‰ ± 0.1 and 0.4‰ ± 0.4‰ for δ^{15}N and δ^{13}C, respectively, previously observed for Californian condors on a controlled feeding trial (Kurle et al., 2013). We ran four MixSIAR model sets with age-sex class as a fixed effect, using either informed priors based on pellet content or a uniform prior and using both the whole condor data set (*n* = 47, adult males = 14, adult females = 23 and immatures =10) and the subset utilized in CSIA-AA (adults only, *n* = 15, males = 7, females = 8). To make diet estimates from pellets and bulk stable isotopes comparable, we merged a posteriori large and small livestock into "livestock" and fescues and rushes into "plants"; for each category, we added individual estimations from MCMC chains and derived the mean and Bayesian credible interval from the aggregated chain (Phillips et al., 2014; Stock et al., 2018).

We examined the values of δ^{15}N in glutamic acid and phenylalanine for all community members in San Guillermo NP. We used a subset of samples mentioned above, rushes (*n* = 2), fescues (*n* = 2), vicuñas (*n* = 3), guanacos (*n* = 3), adult female (*n* = 3) and male condors (*n* = 2), and we added puma hair (*n* = 4) from individuals captured using foot snares (Smith et al., 2019), and decomposing subcutaneous muscle intermingled with skin from camelid carrion (*n* = 4). Camelid carrion was collected after condors abandoned the carcasses (>3 days after death) from the hind limbs simultaneously with camelid hair. The carrion was analyzed to account for the microbial activity in the necrotic tissue as a contrast to camelid hair that does not change their isotopic signature after growth. Previous work has shown that skin, muscle and keratin are reliable proxies for whole body glutamic acid and phenylalanine δ^{15}N measurements (Dharampal et al. unpublished data). All these samples were analyzed following Chikaraishi et al. (2007; 2009). Our sample sizes exceed those of previous work addressing trophic ecology with CSIA which have repeatedly demonstrated sufficient precision to identify trophic position (Blanke et al., 2017; Pauli et al., 2019; Steffan et al., 2015; Takizawa et al., 2020). This is because of the lower variance of the intra-trophic δ^{15}N difference value between glutamic acid and phenylalanine and the greater magnitude of effect in glutamic acid fractionation compared to bulk isotopes. We nevertheless increased our sample size with additional adult female (n = 5) and male condor (n = 5) samples using the N-acetyl methyl esters (NACME) derivatization technique (Corr et al., 2007; Yarnes & Herszage, 2017; University of California, Davis, USA). Given that we detected no differences between these two approaches in either δ^{15}N in glutamic acid and phenylalanine (multivariate analysis of variance [MANOVA] $F_{2,13} = 1.54$ p = 0.25) or estimated TP values (Welch's $t_{4.5} = 1.85$, $p = 0.13$), we pooled condor CSIA data in subsequent analyses.

We estimated the trophic position for plants, vicuñas, guanacos, pumas, condor and camelid carrion based on the δ^{15}N values of glutamic acid ($\delta^{15}N_{glu}$) and phenylalanine ($\delta^{15}N_{phe}$) using the equation: $\mathrm{TP} = \frac{\delta^{15}\mathrm{N}_{\mathrm{glu}} - \delta^{15}\mathrm{N}_{\mathrm{phe}} + \beta}{\Delta_{\mathrm{glu-phe}}} + \lambda$ (Chikaraishi et al., 2009), where β corrects for the difference in δ^{15}N values between glutamic acid and phenylalanine in C_3 plants; $\Delta_{glu\text{-}phe}$ represents the net trophic discrimination between glutamic acid and phenylalanine (7.2; Steffan et al., 2015, 2017); and λ represents the basal trophic level (1). A TP estimate can be improved by using a β value that has been empirically derived from the study system (Steffan et al., 2013). While most terrestrial C3 plant β are assumed to be near 8.4‰ (Chikaraishi et al., 2014), we measured our system-specific β to be 8.35‰ (± SD = 0.80). We tested for normality in our TP estimates using the Shapiro–Wilk test and for homoscedasticity with a Bartlett test (Bartlett's K^2) with subsequent pairwise comparisons. To compare TP among all trophic entities we used Welch's ANOVA. We then conducted pairwise comparisons between camelid carrion and living camelids, and between pumas and male and female condors using Welch’s *t*-test. We compared observed TP values to expected TP categories with a one sample *t*-test.

Research permits were issued by the Argentine National Park Administration (#DRC265 and DCM255 and subsequent renewals) and the Argentine Ministry of Environmental and Sustainable Development under CITES permit No. 15US94907A/9.

Results

Camelids were the most common dietary item found in Andean condor pellets, constituting 98.3% of occurrence and 86.9% (mean ± 21.9 SD) of volumetric content (Table 1). Plant matter was the second most important dietary item, found in 57.6% of the pellets analyzed, which on average constituted 9.9% (± 15.8) of the pellet volumetric content. Other sources of food, such as livestock, hares and mountain viscachas were present only in 12.9% of the pellets and accounted for 3.1% (± 4.2) of volumetric content.

Our dietary mixing model involving bulk $\delta^{13}C$ and $\delta^{15}N$ for the subset of condors that we also analyzed TP (Fig. 2), suggested that the diet of male condors was 65.2% (median, 95% credible interval [35-89]) camelids, whereas the diet of females was 53.1% [26-75] camelids. Additionally, livestock and plants represented 24.3% [3-58] and 7.8% [1-28] of male diet, respectively, while the diet of females were 32.6% [7-65] livestock and 13.0% [2-33] plants (Fig. 2b). When we analyzed the entire dataset of condors, mixing models revealed the same ranked importance in diet items at the population level (Table 1): camelids, followed by livestock and then plant matter, with sex and age-based differences in proportional diets overlapping (Table S2).

Our TP estimates for discrete trophic entities differed predictably ($F_{5,11} = 284.9$, $p < 0.001$), with plants (TP = 1.01 ± 0.11 [SD]) at the base of the food web, camelids (TP =

2.02 ± 0.05) registering as primary consumers, and pumas (TP = 3.00 ± 0.04) as strict consumers of herbivores. Camelid carrion was significantly elevated relative to living camelids (TP = 2.26 ± 0.1; $t_{3.8} = -3.91$, $p = 0.02$). TP estimates for male (TP = 3.09 ± 0.34) and female condors (TP = 2.83 ± 0.15) did not differ ($t_{8.1} = -1.70$, $p = 0.13$; condor population TP = 2.9 ± 0.29). However, the TP of pumas was significantly higher than the TP for female condors ($t_9 = 2.67$, $p = 0.027$), and marginally lower than male condors ($t_6 = -0.64$, $p = 0.054$). Indeed, TP of female condors represent a non-integer value ($t_7 = -2.85$, $p = 0.025$), lower than strict carnivory (3.0), while male condor TP was indistinguishable from strict carnivory ($t_6 = 0.64$, $p = 0.549$). Among all the trophic groups in this Andean food-web, Andean condors exhibited the widest range of TP values, from 2.6 to 3.8 (Fig. 3), and had a significantly greater variance compared to that found in pumas ($K^2 = 7.70$, $p = 0.006$). TP variance was marginally higher in male than female condors ($K^2 = 3.63$, $p = 0.057$).

Discussion

Our results reveal that carcasses are not just meat to obligate scavengers but represent a heterogeneous pool of resources that span taxa and trophic positions. In the process, the consumption of a carcass and the attendant necrobiome effectively integrates the macro- and microbiome within scavenger populations. Indeed, we found that plant material was common within regurgitated pellets of condors and assimilated in relatively large amounts by these scavengers. Our estimates of TP suggested that carcasses also have non-trivial amounts of microbial matter and that there was a detectable, and important, consumption of microbes by condors. Finally, our non-integer estimates of TP for condors, as well as the high level of inter-individual variation in TP estimates, demonstrates that this highly specialized scavenger is, indeed, a "trophic omnivore" (Steffan & Dharampal, 2019) and consumes multiple trophic levels.

Andean condor diet partitioning among sexes and age classes has been widely debated. Strong sexual dimorphism and hierarchical dominance structures have been suggested as potential mechanisms that drive partitioning of space and time (Alarcón et al., 2017; Donazar et al., 1999), with eventual implications for the diet. However, evidence documenting sex-based dietary partitioning in condors is inconsistent between sites (Perrig et al., 2021). Our results show some support for dietary partitioning between the sexes. Similar to Perrig et al. (2017), but with the inclusion of plants, we did not detect difference in the assimilated diet between age-sex classes, nor did we detect TP differences between the sexes. On the other hand, adult male condors possessed TP estimates indistinguishable from strict carnivory (TP - 3.0), while females had lower TP estimates attributable to trophic omnivory. This aligns with studies showing that adult males can exert control and gain priority over high quality sites (Alarcón et al., 2017; Marinero et al., 2018; Wallace & Temple, 1987) and, therefore the highest-quality food, as well as with the observation that males have less pigments derived from plant material in the blood (Blanco et al., 2013). The lack of consistent evidence of partitioning between sexes may be due to high unpredictability in resource availability in the landscape (Mancini et al., 2013). That is to say, the camelid carcasses regularly provisioned by pumas contain varying amounts of residual meat (Elbroch et al., 2014), which could modulate the potential dietary differentiation. Hence, future research should evaluate the impact of carcass quality (i.e. amount of remaining meat) at a finer scale.

Diet estimates obtained from bulk isotopes and mixing model differed from those derived from pellets. Notably, bulk isotopes estimated greater reliance on livestock and less consumption of camelids. However, camelids remained the most important diet item regardless of the approach. This difference in point estimates is likely a result of the different integration times that these methods reflect: isotopes from feathers reveal assimilated diet over a wider spatio-temporal frame (~3 months), while the pellets reflect more immediate foraging events. Additionally, estimates of the mixing model could be affected by the wide range ^{15}N found in the plant material, inflating error estimates. However, a previous study that did not include plants as a potential diet item, found similar results in the relative contribution of camelids and livestock (Perrig et al., 2017).

We found that condors consumed a considerable amount of plant biomass. The ingestion of plant material may be incidental (Duclos et al., 2020) and associated with the consumption of the guts of the carcass. However, its consistent presence in the diet of condors could link this feeding habit to other functions. It is possible that plant consumption allows the assimilation of carotenoids (Blanco et al., 2013), which can be important in social hierarchies for Andean condors (Marinero et al., 2018) and associated with their immune response (Plaza et al., 2020). Also, plant digesta and associated volatile fatty acids produced in the rumen of camelids may provide nutritional inputs that would otherwise be unavailable and limiting to condors, given that vultures do not possess the capacity for fermentation and the cecum is vestigial or absent (Clench & Mathias, 1995). Additionally, plant digesta can be a source of water, as moisture is retained for longer periods in the internal tissues of the carcass (Carter et al., 2007; Schotsmans et al., 2011), which might be a relevant factor in arid and semiarid environments. It is also possible that plant material is ingested not only for nutrition or digestion but also as an emetic, as suggested for other vultures (Thomson et al. 2013). Regardless of the ultimate function of plant ingesta, it showed as a non-trivial and consistent item across the condor population.

As predicted, camelid carrion exhibited an inflated TP of 2.26, most likely produced by microbial consumption of the carrion (Steffan et al., 2017). As microbes consume carrion and propagate through the carcass, they stockpile ^{15}N within certain amino acid pools, including glutamic acid (Steffan et al. 2013, 2015). This compound-specific enrichment is how microbes cause trophic inflation within a detrital mass (Steffan et al. 2017; Steffan & Dharampal 2019). Autolytic processes during decomposition produce a general isotopic enrichment of bulk ^{15}N in carrion as volatilized compounds such as ammonia are lost (Keenan & DeBruyn, 2019), although the role in ^{15}N enrichment of glutamic acid over phenylalanine should be minor. Glutamic acid newly synthesized, and enriched in ^{15}N, is unexpected to happen by itself in a dying tissue. Indeed, enrichment of bulk ^{15}N is only perceptible in tissues with a prolonged decomposition stage (Keenan & DeBruyn, 2019; Yurkowski et al., 2017) when microbes are dominant. Intra-trophic enrichment of glutamic acid ^{15}N and elevation of TP by catabolic processes has been documented in living organisms, sometimes generating *de novo* tissues, that sustain these processes for prolonged periods like hibernation, starvation, and egg production in animals (Whiteman et al., 2021) or flowers and bud development in plants (Takizawa et al., 2017). Because the primary cause of mortality of camelids in San Guillermo NP is puma predation, and all carrion we sampled were the result of predation, trophic elevation of camelid carrion due to prior starvation is highly unlikely; rather it is far more plausible that the trophic elevation of carrion was driven by microbial decomposition. While trophic inflation of decomposed tissues colonized by microbes has been described uniquely in detritus of vegetation and invertebrates (Steffan et al., 2017; Steffan & Dharampal, 2019) and bulk ^{15}N enrichment has been reported in decaying vertebrate tissue (Keenan &

DeBruyn, 2019); this study provides the first evidence for trophic position inflation of vertebrate tissue using CSIA.

Even though condors consumed and assimilated measurable amounts of plant material, their average TP was close to that of pumas (3.0), suggesting that condors are also eating non-trivial quantities of secondary consumer biomass (i.e., the microbes embedded in carcasses that that are trophically analogous to pumas). Thus, it appears the consumption of the microbes likely elevates the condor TP, effectively neutralizing the depressive effects of the plant digesta. Given that condors unlikely scavenge on pumas due to their lower population density in relation to camelids, and that camelid carrion has an already inflated TP of 2.26, it is almost certain that condors are eating the heterotrophic microbes of the carrion as secondary consumers. This is one of the few studies to report trophic inflation (i.e. higher TP due to consumption of detritivores organisms) in a terrestrial vertebrate consumer (Pauli et al., 2019) and the first suggesting microbivory for a large vertebrate. These findings highlight the importance of CSIA in trophic positions and necrobiomes where regular bulk isotopes analysis have shown variable differences (Burrows et al., 2014; Keenan & DeBruyn, 2019; Payo-Payo at al., 2013; Yurkowski et al., 2017). It is notable that estimated TPs of pumas showed little variability around 3.0, suggesting little-to-no ingestion of microbial-colonized tissues. Pumas in our study site abandon carcasses shortly after killing (on average 34 hours), which may be insufficient for microbial trophic inflation. Andean condors, in contrast, consume carcasses after pumas have given up and up to 3-7 days after death. It is possible that other organisms, notably scavenging insects, could have been also consumed by condors; however, the complete absence of insect remains or chitin in pellets, their infrequent occurrence on puma-killed carcasses and the fact that carrion is trophically inflated suggests that the TP of condors came primarily from microbes on the carrion.

Puma predation provides obligate scavengers not only with a regular supply of carrion (Barry et al., 2019; Elbroch & Wittmer, 2013) but also with other dietary components as plant digesta and high concentration of microbes. This is especially important given that vulture reliance on landfills or other artificial food sources is increasingly common (Duclos et al., 2020; Moreno-Opo et al., 2015; Plaza & Lambertucci, 2017) and these sources do not always provide all the components of naturally occurring carcasses. The existence of microbivory in vultures requires further study, as it could represent a link between scavenger gut microbiota and the microbiome of the carrion, as shown for other taxa (Weatherbee et al., 2017). Vulture gut microbiota similarly to other scavenger, the American alligator, is composed mainly of Clostridia and Fusobacteria (Keenan & Elsey, 2015; Roggenbuck et al., 2014), generally considered pathogenic for other vertebrates and associated with flesh decomposition (Zepeda Mendoza et al., 2018). It could be that condors and other scavengers exploit microbes from carrion to inoculate gut flora and increase digestion efficiency, which is critical for birds that feed on patchy and ephemeral food resources (Grémillet et al., 2012). Our study represents an initial contribution to the understanding of the trophic heterogeneity found within a carcass and the role of scavengers in uniting the macro- and micro-biome, emphasizing the utility of multifaceted approaches in reconstructing the diets of free-ranging animals within their natural habitat. Our work also highlights how the integration of microbes into food webs can help resolve the trophic identities and dietary differences for large vertebrates and that resolving interkingdom interactions can shed light on the necrobiome functioning.

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Author Contributions

GB, JNP and SAS designed the study. PLP and ED collected field data. GB and PD analyzed the data. GB and JNP lead the writing and all authors contributed critically and approved the final manuscript.

Data availability statement

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.vhhmgqnrj (Barceló et al., 2022).

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Tables

Table 1: Mean diet estimates of Andean condors based on isotopic mixing models (n = 47; ±95% credible interval) and the volumetric content in the pellets (n = 177; ±95% confidence interval) from San Guillermo National Park, Argentina, 2013. Diet components consisted of camelids (*Vicugna vicugna*, *Lama guanicoe*), plants (*Juncus* sp., *Festuca* spp.), livestock (*Bos taurus, Capra aegagrus, Ovis aries*, *Equus ferus*) and others (*Lagidium viscacia, Lepus europeus*).

Diet items	Bulk stable isotope (% diet)	Pellets (% volume)
Camelids	68.8 (44.5 – 84.8)	86.9 (83.6 – 90.1)
Plants	8.3 (1.5 – 22.8)	9.9 (7.6 – 12.2)
Livestock	22.1 (3.9 – 47.3)	2.5 (1.1 – 03.9)
Others	-	0.7 (0.0 - 1.3)

Figures

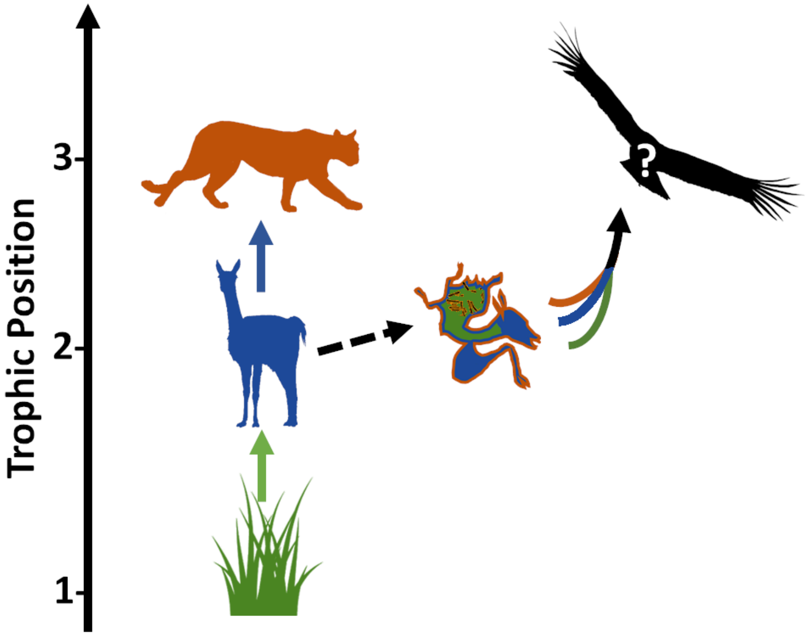

Fig. 1 The trophic relationships and positions of food web members in the high Andes of Argentina: primary producers (TP = 1: rushes [*Juncus* sp.] and fescues [*Festuca* spp.]; green), primary consumers (TP = 2: vicuñas [*Vicugna vicugna*] and guanacos [*Lama guanicoe*]; blue), secondary consumer (TP = 3: pumas [*Puma concolor*] and microbes; orange). Dashed line represents predator provisioning camelids for scavengers and decomposers. The carcass is a conglomerate of three trophic positions (TP): plant digesta (TP ~ 1), camelid meat (TP = 2) and microbes (TP ~ 3) of which we predict Andean condors (*Vultur gryphus)* is consuming across.

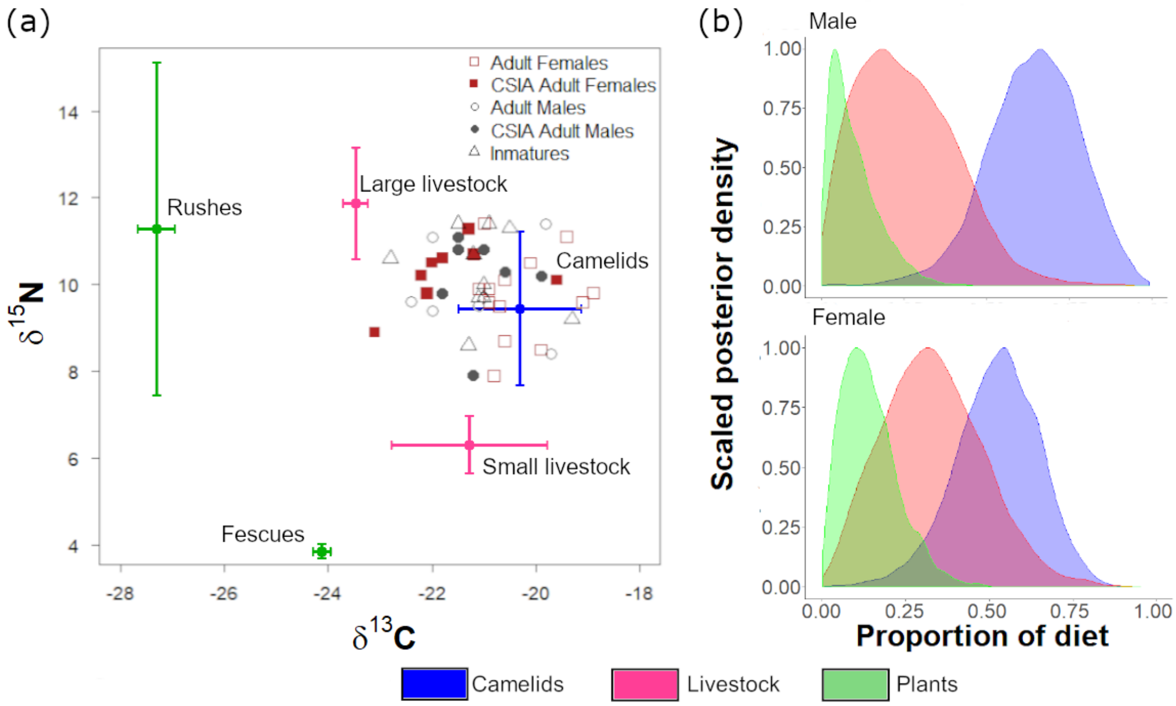

Fig. 2 (a) Stable isotope signatures (δ^{13}C and δ^{15}N) of Andean condors (*Vultur gryphus*; adult males (circles), adult females (squares), and immature males and females (triangles). Individuals analyzed also for bulk only (open), and for bulk and CSIA-AA (filled). Also shown are the main food sources for Andean condors in our study system (mean ± SD, corrected by trophic discrimination factor): plants (in green; fescues, *Festuca* sp.; and rushes *Juncus* sp.), large livestock (in red; *Bos taurus*), small livestock (in red; *Capra aegagrus*) and camelids (in blue; *Vicugna vicugna* and *Lama guanicoe*). (b) Proportion of camelids, livestock, and plants in the diet of male and female condors, estimated from Bayesian mixing model based on a uniform prior from individuals analyzed for CSIA-AA.

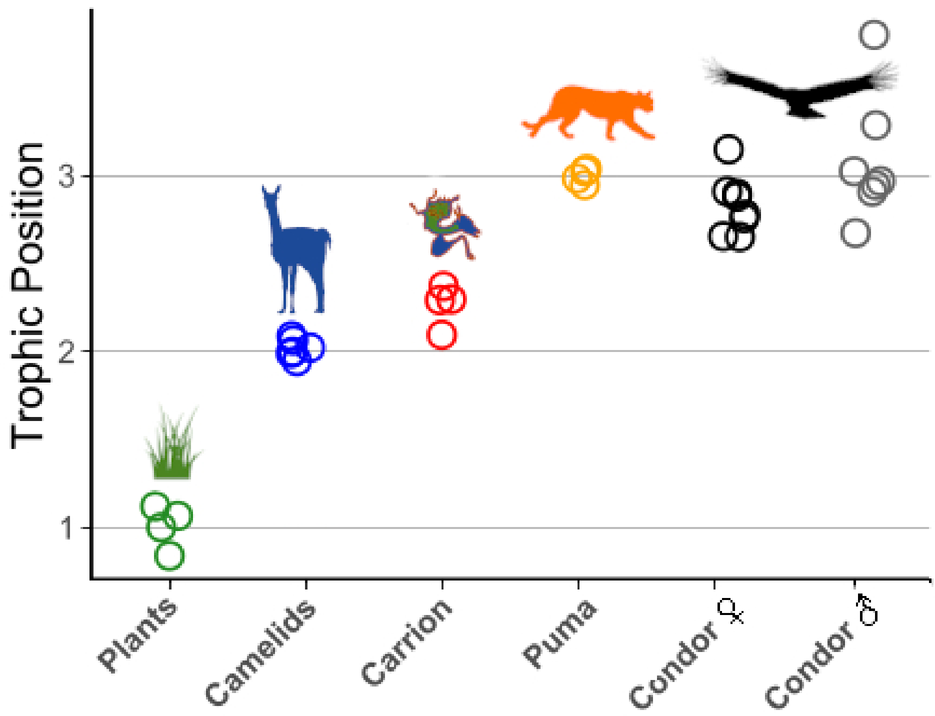

Fig. 3 Trophic positions estimated through the analysis of $\delta^{15}N$ in glutamic acid and phenylalanine of each component of the Andean food web: plants (*Festuca* sp. and *Juncus* sp.; green), camelids (*Vicugna vicugna* and *Lama guanicoe*; blue), camelid carrion (red), Andean condor (*Vultur gryphus*; females in black, males in gray) and puma (*Puma concolor*; orange).

Supplementary Materials

Methodical details for compound-specific stable isotopes analysis

We examined the values of $\delta^{15}N$ in glutamic acid and phenylalanine within a subset of samples of rushes (n = 2), fescues (n = 2), vicuñas (n = 3), guanacos (n = 3), carrion (n = 4), pumas (n = 4), male adults condors (n = 2) and female adult condors (n = 3) following Chikaraishi et al. (2007, 2009); and a second subset of adult female (*n* = 5) and male condors (*n* = 5) using the N-acetyl methyl esters (NACME) derivatization technique (Corr et al., 2007; Yarnes & Herszage, 2017).

Samples of the first subset were analyzed in the Hokkaido University (Sapporo, Japan), where they were hydrolyzed, and the hydrolysate was washed with n-hexane/dichloromethane. Derivatization of amino acids was performed sequentially with thionyl chloride/2-propanol (1/4, v/v) and pivaloyl chloride/dichloromethane (1/4, v/v). Amino acid abundance was determined using a 6890N Gas Chromatography (GC) connected to a flame ionization detector and nitrogen–phosphorus detector. The Pv/iPr derivatives of amino acids were injected using a vaporizing injector (Gerstel) into a HP-5 ms capillary column (Agilent Technologies). Stable nitrogen isotopic composition of amino acids was determined by GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS) using a 6890N GC (Agilent Technologies) instrument coupled to a DeltaplusXP IRMS instrument through combustion (950 °C) and reduction (550 °C) furnaces, countercurrent dryer (Permeable membrane, Nafion™), and liquid nitrogen CO_2 trap via a GC-C/TC III interface (Thermo Fisher Scientific).

The second subset, adult male condors (n = 5) and adult female condors (n = 5), was analyzed in the University of California, Davis, USA. We used the N-acetyl methyl esters (NACME) derivatization technique (Corr et al. 2007; Yarnes and Herszage 2017). Before derivatization, amino acids were liberated from sample material proteins by acid hydrolysis (6 M HCl, 70 min, 150 ºC under N2 headspace). NACME amino acid derivatives were injected at 260 ºC (splitless, 1 min) and separated on an Agilent DB-35 column (60 m x 0.32 mm ID x 1.5 µm film thickness) at a constant flow rate of 2 mL/min under the following temperature program: 70 °C (hold 2 min); 140 °C (15 °C/min, hold 4 min); 240 °C (12 °C/min, hold 5 min); and 255 °C (8 °C/min, hold 35 min). GC-C-IRMS was performed on a Thermo Trace GC 1310 gas chromatograph coupled to a Thermo Scientific Delta V Advantage isotope-ratio mass spectrometer via a GC IsoLink II combustion interface. The combustion reactor was a NiO tube containing CuO and NiO wires maintained at 1000 °C. Water was subsequently removed through a Nafion dryer before the analyte gases were transferred to the IRMS. During ^{15}N analysis, CO_2 was removed from the post-combustion carrier stream through the use of a liquid nitrogen trap to prevent isobaric interferences within the ion source. All samples were analyzed in duplicate.

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Table S1. Dietary sources items used in the mixing model, their mean values and standard deviation (SD) of δ ^{13}C and δ ^{15}N and their concentration of carbon and nitrogen, as weight percentage. Dietary items correspond to camelids (*Vicugna vicugna and Lama guanicoe*), the plants fescues (*Festuca* sp.) and rushes (*Juncus* sp.), large livestock (*Bos taurus*) and small livestock (*Capra aegagrus*).

Sources	Mean δ ^{15}N (‰)	SD δ ^{15}N (‰)	Mean δ ^{13}C (‰)	SD δ ^{13}C (‰)	Conc. N (%)	Conc. C (%)	n
Rushes	8.190	3.848	-27.695	0.364	0.012	0.440	3
Fescues	0.759	0.147	-24.520	0.176	0.017	0.459	3
Camelids	6.347	1.779	-20.711	1.183	0.155	0.472	14
Small Livestock	3.204	0.664	-21.679	1.500	0.150	0.450	4
Large Livestock	8.770	1.272	-23.884	0.231	0.150	0.450	10

Table S2. Estimated proportional contribution of different items to Andean condor (*Vultur gryphus*) diet in San Guillermo National Park (Argentina) as calculated by isotopic mixing models in MixSIAR using uniform or informed prior based on pellets data (see Figure 2). Dietary items correspond to camelids (*Vicugna vicugna and Lama guanicoe*), the plants fescues (*Festuca* sp.) and rushes (*Juncus* sp.), large livestock (*Bos taurus*) and small livestock (*Capra aegagrus*).

Whole data set *n* = 47 - Uniformed prior

	Adult male condors		Adult female condors		Immature condors	
Diet items	Median	95% CI	Median	95% CI	Median	95% CI
Camelids	0.767	(0.56 - 0.93)	0.815	(0.62 - 0.95)	0.772	(0.56 - 0.94)
Fescues	0.035	(0.00 - 0.21)	0.028	(0.00 - 0.17)	0.030	(0.00 - 0.19)
Rushes	0.022	(0.00 - 0.13)	0.017	(0.00 - 0.10)	0.019	(0.00 - 0.13)
Large livestock	0.084	(0.00 - 0.31)	0.067	(0.00 - 0.26)	0.106	(0.00 - 0.34)
Small livestock	0.025	(0.00 - 0.16)	0.021	(0.00 - 0.15)	0.020	(0.00 - 0.13)

Whole data set *n* = 47 - Informed prior

	Adult male condors		Adult female condors		Immature condors	
Diet items	Median	95% CI	Median	95% CI	Median	95% CI
Camelids	0.847	(0.69 - 0.97)	0.891	(0.74 - 0.98)	0.863	(0.69 - 0.97)
Fescues	0.038	(0.00 - 0.17)	0.026	(0.00 - 0.14)	0.030	(0.00 - 0.15)
Rushes	0.034	(0.00 - 0.13)	0.023	(0.02 - 0.10)	0.029	(0.00 - 0.12)
Large livestock	0.025	(0.00 - 0.17)	0.018	(0.00 - 0.13)	0.026	(0.00 - 0.19)
Small livestock	0.012	(0.00 - 0.10)	0.009	(0.00 - 0.08)	0.010	(0.00 - 0.07)

TP subset *n* = 16 – Uninformed prior

	Male Condors		Female Condors	
Diet Items	Median	95% CI	Median	95% CI
Camelids	0.652	(0.35 - 0.89)	0.531	(0.26 - 0.75)
Fescues	0.040	(0.00 - 0.25)	0.075	(0.00 - 0.29)
Rushes	0.019	(0.00 - 0.13)	0.037	(0.00 - 0.16)
Large Livestock	0.186	(0.01 - 0.47)	0.258	(0.03 - 0.50)
Small Livestock	0.036	(0.00 - 0.22)	0.060	(0.01 - 0.23)

TP subset *n* = 16 - Informed prior

	Male Condors		Female Condors	
Diet Items	Median	95% CI	Median	95% CI
Camelids	0.855	(0.65 - 0.97)	0.840	(0.76 - 0.90)
Fescues	0.035	(0.00 - 0.18)	0.051	(0.02 - 0.11)
Rushes	0.033	(0.00 - 0.13)	0.053	(0.02 - 0.11)
Large Livestock	0.023	(0.00 - 0.18)	0.031	(0.01 - 0.08)
Small Livestock	0.010	(0.00 - 0.10)	0.015	(0.00 - 0.05)

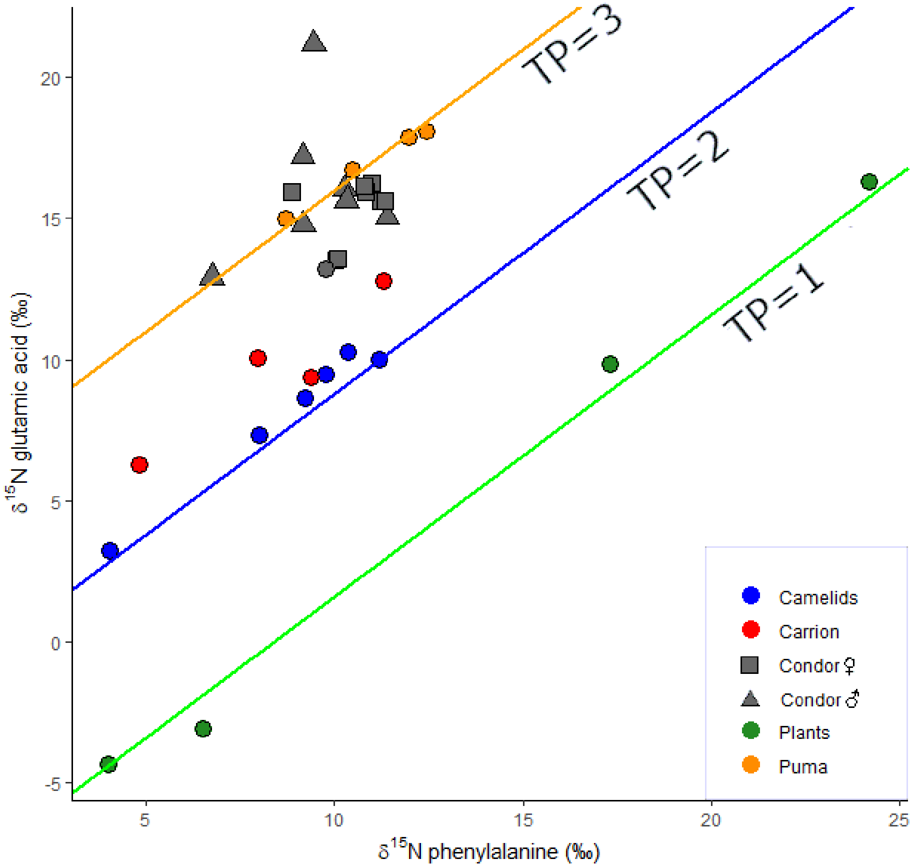

Figure S1: δ^{15}N ratios in phenylalanine and glutamic acid of plants (green, *Festuca* sp. and *Juncus* sp.), camelids (blue, *Lama guanicoe* and *Vicugna vicugna*), carrion (red), male condors (gray triangles, *Vultur gryphus*), female condors (gray squares, *Vultur gryphus*) and pumas (orange, *Puma concolor*). Diagonal lines represent trophic isoclines corresponding to integer trophic positions.

CHAPTER TWO

Assessment of behavioral energetics model on *Puma concolor* using doubly labeled water.

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Abstract

The estimation of energy expenditure is key to resolve ecological questions and conservation strategies. The puma (*Puma concolor* (Linnaeus, 1771)) has been the subject of several efforts to measure the daily energy expenditure (DEE). Most of the estimations have been made using movement or activity models that have been questioned because of discrepancies with kill rates. This study looks at one movement model estimation and validates it using doubly labeled water (DLW), which can also account for energy expenditures beyond those associated with activity. We captured six pumas (51.5 ± 9.9 [s.d.] kg) during the winter in Colorado that were GPS collared and injected with DLW for an approximately three-week trial. We found that DEE obtained from the DLW (14.5 ± 6.1 MJ/d) did not differ from that estimated using the movement model or from the predicted allometric equation based on DLW studies on other mammals. Decreasing air temperature and increasing daily distance movement were correlated with increasing DEE for monitored pumas. Both DLW and movement models provide a reasonable proxy to estimate DEE at the population level but exhibit low precision in estimating individual values for free-ranging puma.

Keywords: Doubly labelled water - Energetic expenditure - Felids - Field metabolic rate - Mountain lion - *Puma concolor* - Thermogenesis.

Introduction

Quantifying energetics provides a portal to understand how physiological, morphological and behavioral traits of organisms optimize energy budgets on ontogenetic or evolutionary scales (Hedenström 2008). Evaluation of the daily energy expenditure (DEE) of free-living animals is considered a challenging task, although body size has shown to be a reliable predictor (Nagy 2005). However some ecological features may have a strong and variable effect on the energy expenditure, such as the activity of the animals (Scantlebury et al. 2014; Pagano et al. 2018), reproductive status, the heat increment of feeding (i.e. the energy required in digesting and assimilating food, Secor 2009) and thermoregulation costs (Song and Beissinger 2020).

Consideration of energy expenditure of species can support management and conservation strategies to evaluate habitat quality and carrying capacity (Bernhardt et al. 2018). Pumas (*Puma concolor* (Linnaeus, 1771)) present a broad distribution in the Americas, where they exhibit an opportunistic predatory behavior relying on a variety of prey (Iriarte et al. 1990). The size and availability of prey can influence kill rates, and the estimation of energy expenditure can help assess kill-rates in the context of game species management or livestock conflict. For these reasons, the puma has been the object of many efforts to estimate their energy budget (Laundré 2005; Elbroch et al. 2014; Williams et al. 2014; Wang et al. 2017).

First attempts to quantify the energy expenditure of pumas were based on basal metabolic rate and a multiplier for different activities individuals undergo while free-living (Ackerman et al. 1986). Activity level is considered one of the important factors modifying the overall DEE (Garland Jr. 1983; Scantlebury et al. 2014). Later, Laundré (2005) revisited these energy estimations by using standardized mammalian metabolic rates while in movement (Taylor et al. 1982) and more effectively measuring movement distance. However, Laundré (2005) estimated lower kill rates than registered by kill site investigations (Anderson and Lindzey 2003; Allen et al. 2014). The lower estimates could be explained by several factors, such as (i) the use of energy allometric equations generated for mammals, even though carnivores have higher basal metabolic rates and locomotion costs than the average mammal (Garland Jr. 1983; Muñoz-

Garcia and Williams 2005); (ii) use of a proportionally low locomotive cost component in the model (Girard 2001) and (iii) because the consumption of meat per kill was overestimated (Elbroch et al. 2014). More recent research has improved estimations of the locomotion cost based on higher precision and frequency of the spatial data using GPS (Dunford et al. 2020) or acceleration loggers (Williams et al. 2014). Nevertheless, none of the previously developed methods to estimate DEE based on activity has been compared with a respirometry-based method simultaneously on free-living pumas.

More importantly, an accurate estimation of the metabolic rate including features beyond locomotion might be even more relevant to the overall DEE. The doubly labeled water (DLW) method, which measures CO_2 production by the differential turnover rates of ^{18}O and ^{2}H administered to the animal, arguably has an advantage of capturing all components of metabolism, including those not directly related to the activity in the individual, e.g. expenditures associated with thermoregulation and diseases. Thermoregulation costs are typically not included in movement energy models, especially for large animals, because it is assumed that their generally low lower critical temperature (LCT, the ambient temperature at which thermogenesis starts) and low whole body thermal conductance, plus the heat increments of feeding and locomotion, and the use of thermal refugees, all mitigate thermoregulatory costs. However, temperature could be a relevant factor in determining DEE even on large mammals (Fristoe et al. 2015), particularly on pumas, where air temperatures around -10°C could double the resting metabolic rate (McNab 2000) .

In this sense, evaluating different methods of estimation of DEE could shed light about what are the most relevant factors on it. We applied the DLW method to estimate the DEE of free-living pumas along with a model relying on distance traveled using GPS to cross-validate these energy models. We tested the *a priori* predictions that the DLW method would provide estimations of DEE similar to movement models based on daily distance traveled (DDT), that DEE increases with DDT, and that low daily air temperature could also have a positive impact on the DEE.

Methods

Six *Puma concolor* adult individuals (3 males – 3 females) were captured using cage traps in the Southern Front Range of the Rocky Mountains (Fig. 1) by Colorado Parks & Wildlife (Animal Care and Use Committee [ACUC] # 09-2018) during the winter of 2019 and 2020. Captures were done during January-February when sites are mostly covered by snow and average minimum and maximum temperatures were -15°C and 3°C (Westcliff station, Custer County, Colorado, 2357 m.). Over the course of ~45 minutes, the animals were anesthetized (medetomidine/ketamine hydrochloride; reversed at release with atipamezole), weighed using a calibrated hanging scale, and a blood sample was immediately collected in a vacutainer lined with dry EDTA to obtain baseline level of ^{18}O and ^{2}H. Then a premixed isotonic dose of doubly-labeled water was injected intravenously (0.68 g/kg body mass), approximately two parts by mass of 96% $H_2{}^{18}O$ (Sigma Aldrich) and one part of 99% $^{2}H_2O$ (Cambridge Isotope Laboratories, MA, USA) made isotonic mixing it with hypertonic serum 7.2% NaCl at a 1:7 dilution ratio (see Table 1 for exact dosing). Before the trials, we calculated the desired initial excess enrichment of ^{18}O (equation 12.1, Speakman 1997) to be over 320 ppm, required to go through 4 half-lives in pumas for the 3 weeks and get a final excess enrichment over 20 ppm (the minimum to resolve differences from the background; Speakman, 1997). A GPS collar (Lotek Wireless, ON, Canada) was fitted to collect spatial data. A post-injection blood draw was taken at minute 45, maximum time allowable to have the animal sedated accordingly to our agency Animal Care and Use Committee (ACUC), but it was not used in the calculation because the equilibrium was not reached at that time. A final blood sample was collected 24.8 ± 3.2 (S.D.) days after release to determine the isotope turnover rate. Pumas were recaptured tracking GPS information with hounds and immobilizing them with anesthetic darting.

Doubly labeled water and DEE

Water was microdistilled from blood samples and isotopic enrichments were measured using cavity ring-down spectroscopy (Picarro model L2120-I, Santa Clara, California) at the Isotope Ratio Mass Spectrometry laboratory at the University of Wisconsin-Madison. Rates of CO_2 production (rCO_2, in mol/d) were calculated using the double-pool equation (Speakman 1997; section 17.1.7[h], p. 315) and the single-sample approach, which does not require knowledge of the enrichment at the equilibrium but instead relies on the ratio $^{18}O_{inj}/^{2}H_{inj}$ of the injected solution (Speakman 1997; Bourne et al. 2019):

$$rCO_2 = (N/2.078) \bullet (k_o\text{-}k_d)\text{-}0.0115 \bullet k_d \bullet N \qquad \text{(eq. 1)}$$

where body pool size (N, in mol.) was estimated by the body water in felids (65% ± 4.0% of body mass; Clarke and Berry 1992; Speakman 1997), $k_o\text{-}k_d = \ln(^{18}O_{inj}/^{2}H_{inj} \bullet {}^{2}H_f/^{18}O_f)/t$ (Speakman 1997; Bourne et al. 2019) and $k_d = (\ln[^{2}H_i]-\ln[^{2}H_f])/t$. $^{18}O_{inj}/^{2}H_{inj}$ is the ratio of isotopes in the injection, $^{2}H_f$ and $^{18}O_f$ are final enrichments background-corrected, and *t* is the trial time in days. As we used the single-sample approach, the initial $^{2}H_i$ enrichment was based on the pool size, the background enrichment and the enrichment and quantity of the injected solution (Speakman 1997; eq. 17.1). CO_2 production rate was converted to L/day using a standard conversion factor of 22.4 L/mol, and transformed to kJ/d using an RQ of 0.8 and the conversion factor of 20.08 kJ/L O_2 (Schmidt-Nielsen 1997). As body pool size was estimated on bibliographical references, a sensitivity analysis indicates that variation in 5% of body water will reflect in a variation of 7.8% of DEE. To minimize discrepancies in the body water percentages due to fat content, we incorporate body size measurements of the pumas. We calculated the residuals of body size principal component analysis (body, skull and hind foot length, chest and neck girth; Supplementary Table 1) against body mass, obtained a transformation factor and then calculated body water percentage as 0.65-0.012**Residuals* (Supplementary Table 2).

We transformed DEE values to estimated consumption of meat, to compare our results to more commonly reported values of kill rates and meat consumption in pumas:

$$\text{Meat consumption} = \text{DEE}/(7.9 \bullet 0.86) \qquad \text{(eq. 2)}$$

where 7.9 is the energy value of deer meat in kJ/g wet mass, and 0.86 is the assimilation efficiency for meat consumed by pumas (Elbroch et al. 2014).

As trials were done in winter, we evaluated the contribution of thermogenesis to the DLW DEE:

$$E = C \bullet (M_b)^{0.794} \bullet \Delta T^\circ \qquad \text{(eq. 3)}$$

where E is the energy cost of thermogenesis in kJ/day, C is the conductance reported for felids, including pumas ($8.62\ \text{kJ} \bullet \text{kg}^{-0.794} \bullet \text{day}^{-1} \bullet {}^\circ\text{C}^{-1}$; McNab 2000), M_b is body mass in kilograms, ΔT° is the difference between the LCT (10°C, McNab 2000) and the average air temperature recorded in Westcliff station during the trial.

Distance movement and activity quantification

GPS collars collected at least 6 GPS locations per day during the DLW trial times, which are listed in Table 1 and Fig. 1. This sampling frequency was used to save battery charge over 2 years in the frame of another broader long-term study. We eliminated GPS points fixed with less than 3 satellites or with a dilution of precision higher than 10 (Frair et al. 2010), less than 10% of the total. The minimum daily distance traveled (DDT) was estimated by the sum of the distance between locations during the length of the DLW trial and divided by the number of days using the *geosphere* package (Hijmans et al. 2017) in R software. The movement model for DEE of non-reproductive adult pumas is calculated using Laundré (2005) equation:

$$\text{DEE (kJ d}^{-1}\text{)} = 580 \bullet M_b^{0.75} + 10.9 \bullet \text{DDT} \bullet M_b^{0.60} \qquad \text{(eq. 4)}$$

where DDT is in km, and M_b is the body mass in kg.

Additionally, we included 3-axial accelerometers (Lotek Wireless, ON, Canada) in the collars of two individuals. Acceleration data were collected continuously at 6 Hz and summed up into overall dynamic body acceleration (ODBA). We used the equation developed by Williams et al. (2014) for pumas, to transform our ODBA data to oxygen consumption (VO_2) and subsequently to DEE assuming 20.08 kJ/L O_2 (Schmidt-Nielsen 1997), and presented as a daily average.

$$VO_2 = 3.52 + 58.42 \bullet (\text{ODBA}_{avg}) \qquad \text{(eq. 5)}$$

Statistical analysis

Results are expressed as the mean ± standard deviation (S.D.), and because sample size was relatively small we favored nonparametric tests. We used two-tailed Mann Whitney U test to evaluate differences between sexes. To test for possible animal distress following initial handling, we used Wilcoxon matched pairs test between the 3 initial and 3 finals days of the trial of each individual. We also used Wilcoxon matched test to compare if DEE measured with DLW (eq. 1) differed from the DDT model (eq. 4) and to compare our DLW results with predictions from Nagy (2005) allometric equation (DEE [kJ•day^{-1}] = 4.82•M_b[g]$^{0.734}$) based on measurements of metabolic rate in 79 free-range mammals using DLW. Additionally, to explore the effect of winter temperatures, we ran a linear multiple regression with DEE as the dependent variable and DDT, body mass and mean daily temperature as the independent variables. In this single instance we relied on this parametric test, lacking an equivalent nonparametric test. Variance inflation factor of 1.01 discarded collinearity between independent variables. We selected the model with the lowest AIC as the best fitting.

Results

Body mass was higher in males (57.1 ± S.D. 8.3 kg) than in in females (41.6 ± 3.6 kg; Z = -1.96, p = 0.049). DDT in males (3.7 ± 1.1 km/d) did not differ from that in females (2.5 ± 0.9; Z = -1.09, p = 0.27). There was no difference in distance moved when comparing the first 3 days after capture and release with the last 3 days prior to recapture (Z = 0.94, p = 0.35). Mean DEE obtained from DLW was 14,482 ± 6,102 kJ/day (n=6) and showed no differences between sexes (Z = 0.44, p = 0.66; Table 1).

Among the multiple regression models evaluated (Table 2), the two top ranked models included air temperature and DDT as predictors, as well as body mass for one of them. The model with DDT and T° showed that the regression coefficient was significant for daily mean temperature (-2,426 ± 56 kJ d^{-1} ^{o}C^{-1}, t_3= -4.29, p=0.023) and for DDT (3,712 ± 940 kJ d^{-1} km^{-1}, t_3= 3.24, p=0.029). Temperature correlated negatively with DEE. Indeed, the cost of thermoregulation (eq. 3), assuming a LCT of 10°C, constituted 17.6% ± 4.2 of DEE. The cost of locomotion (eq. 4) constituted 2.4% ± 1.0 of DEE. As predicted, DEE measured with DLW (eq. 1) did not differ from either the estimated values from the model based on DDT (eq. 4; t_5 Z =

1.871.57, $p = 0.1311$) or the DEE predicted by the allometric equation (Nagy 2005; DEE = $4.82 \cdot M_b^{0.734}$; t_5 $Z = -0.9331$, $p = 0.3975$; Fig. 2). For those individuals with ODBA, we obtained values of 755 and 654 kJ•day^{-1}•kg$^{-0.75}$, which represent 91% and 120% of the values obtained from DLW (Table 1).

Discussion

The estimated DEE for our individuals (15.6 ± 5.6 MJ/day) falls within the range of those estimated by Ackerman et al. (1986; 12.6 – 23.1 MJ/day), and the estimated meat consumption derived from our values (2.3 ± 0.8 kg/day)– is also within the range estimated by Elbroch et al. (2014) in Colorado (3.3 ± 1.8 kg/day). Importantly, the handling and sedation of the animals had no apparent significant effect on the daily distance traveled by the animals. As predicted, DEE was correlated with DDT when multifactorial analysis accounted for thermoregulation. Most past models used a slope that links DDT to energetics, known as the incremental cost of locomotion (ICL), close to 10.7 kJ•kg$^{-0.684}$•km^{-1} (Taylor et al. 1982) or 10.9 kJ•kg$^{-0.60}$ •km^{-1} (Laundré 2005). However, our low sample size and high variance of the data did not allow us to do a direct evaluation of the ICL slope.

Movement and activity energy models, in general, underestimate consumption and killing rates (Elbroch et al. 2014). Girard (2001) considers that costs of transportation have been underestimated in relation to the total energy expenditure when calculated allometrically from ICL equations, as conditions of the field can represent additional challenges over those estimated in treadmill on laboratories, or because of the low estimation of the distance traveled by each animal. Low estimation of distance traveled has been greatly overcome with high precision and frequency of GPS data recently, but ICL in models has not been updated since. Steep terrains can increase ICL up to 42% when associated to 20° slopes (Dunford et al. 2020).

DEE in our results showed lower values for the movement model, although non-significantly different. This could be explained because DDT is likely underestimated in our results by at least a factor of two because of low frequency of GPS sampling. Other studies have shown that pumas can move around 4.4-9 km/day (Allen et al. 2014; Elbroch et al. 2014). We can confirm with our own data that two individuals with higher GPS sampling frequency have twice the distance traveled when DDT was calculated on a 30 minute basis instead of 4-hour intervals. Lower values of DEE coming from the movement model can be associated to the lower energy attributable to locomotion accordingly to the model, which corresponds to a 2.4% (eq. 4) of the DEE. Even accounting for the underestimated DDT and doubling it, the locomotion cost seems low in relation to the DEE and may be indicative of the underestimation that Elbroch et al. (2014) and Girard (2001) accuse in movement models. Another possibility is that in fact locomotion cost in pumas, as ambusher predators, are indeed low, where more active hunters as wild dogs can have locomotion cost that can surpass the 30% of the DEE (Gorman et al. 1998; Hubel et al. 2016).

On the other hand, activity models usually do not consider thermoregulatory costs, as it is often assumed that these costs are marginal for large mammals because of thermal inertia and low conductance of larger bodies (Fristoe et al. 2015). However, we showed that thermogenesis could plausibly constitute up to 17.6% of the DEE in our site of study during winter, where temperatures had a daily average of -3.5°C and a daily minimum average of -15°C. These calculations are a conservative approach as we used the LCT that is calculated on resting individuals (McNab 2000). The effective LCT could be lower due to thermogenic contributions from the heat increment of feeding and moving (Hindle et al. 2003). Nonetheless, the best fitting model based on DDT and daily mean temperature suggests that temperature may be important to include when studying free-range puma during winter or when they are thermally challenged.

The high variability in DLW energy estimates could be explained, first by our low sample size, but also by activities beyond locomotive and thermoregulatory costs. In this sense, the ODBA model represents a better fit for two individuals where acceleration can reflect a broader range of activities than GPS data and the DDT model. Other studies that evaluate DLW as a method to link DEE with activity or heart rate have also shown great individual variability (Speakman 1997; Martin et al. 2020). Although the increasing use of acceleration loggers and ODBA has improved the strength of the correlation between activity and DLW (Sutton et al. 2021), they are more useful to understand general trends in the population than individual adjustment. This work suggests that thermoregulation can be a relevant predictor of the DEE beyond locomotion or activity, even though DLW and movement models estimates of DEE represent adequate population estimates for pumas. Ecological studies quantifying the DEE of free-living large carnivores should consider the variance of results using this DLW approach and also account for other metabolic variables, as thermoregulation, when using behavioral or mechanics models. Indeed, by not accounting for these other variables, research could underestimate energy costs during the winter, or more generally, introduce greater uncertainty when animals face particular ecological challenges. Understanding the seasonality and variance of energetic expenditure and the ecological factors connected with DEE can improve the knowledge of food, energy, and space requirements associated with managing and conserving large carnivores.

Contributors’ statement

All authors designed the study. G.B. and M.A. collaborated on fieldwork. G.B and W.H.K analyzed the data and G.B., J.N.P and W.H.K. wrote the manuscript with advice from M.A.

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Tables

Table 1. Data used in the estimation of daily energy expenditures (DEE) for the double labeled water (DLW) and behavioral methods in pumas (*Puma concolor*) during winter of 2019 and 2020 in the Southern Rocky Mountains of Colorado.

ID	Sex	Mass (kg)	Time (days)	Dose (g)	^{2}H final* (ppm)	^{18}O final* (ppm)	k_o- k_d	mol CO_2/day	DLW DEE kJ/day	DDT km/day
AF151	F	38.6	22.2	21.6(1)	51.0	21.3	$2.21 \bullet 10^{-03}$	37.465	21190	3.26
AF118	F	45.6	26.0	31.7(1)	82.0	56.6	$1.09 \bullet 10^{-03}$	18.925	10702	1.49
AF194	F	40.5	24.0	30.9(1)	55.6†	34.8†	$1.35 \bullet 10^{-03}$	23.647	13373	2.83
AM313	M	47.7	24.1	35.9(2)	77.3	66.6	$9.23 \bullet 10^{-04}$	17.516	9908	2.50
AM172	M	63.6	31.1	47.3(3)	47.7	19.8	$1.69 \bullet 10^{-03}$	44.260	25034	4.27
AM177	M	60	21.1	45.3(3)	114.4†	104.5†	$9.38 \bullet 10^{-04}$	23.684	13394	4.46
Mean		51.5	25.3	38.2	71.3	50.6	$1.20 \bullet 10^{-03}$	25.606	14482	3.11
SD		9.9	3.7	7.6	25.4	32.4	$3.2 \bullet 10^{-04}$	10.789	6102	1.2

* *Values above isotopic background.* † *Background values were estimated based on the average value of the rest of the individuals. Enrichment of the injected dose varied among different mixtures: (1): 362447 ppm $^{2}H/^{1}H$, 493069 ppm $^{18}O/^{16}O$; (2):347679 ppm $^{2}H/^{1}H$, 511542 ppm $^{18}O/^{16}O$; (3): 359491 ppm $^{2}H/^{1}H$, 528531 ppm$^{18}O/^{16}O$.*

Table 2: Model selection for estimation of daily energetic expenditure (kJ/d) of free-ranging pumas (*Puma concolor*) in Colorado during the winter of 2019 and 2020 in response to body mass (M_b) daily distance traveled (DDT) and mean daily temperature (T°).

Model	AIC	Δ AIC	Likelihood	Weight AIC	Mb coef.	DDT coef.	T° coef.
DDT + Mb + T°	102.2	0.0	1	0.56	159 (-476, 794)	2767 (-2695; 8229)	-2690 (-5284;-95)
DDT + T°	102.9	0.7	0.689572	0.39	-	3712 (719; 6706)	-2426 (-4226; -626)
Mb + T°	107.5	5.3	0.070692	0.04			
T°	111.9	9.7	0.007895	0.00			
DDT	112.7	10.5	0.005155	0.00			
DDT + Mb	114.6	12.4	0.002069	0.00			
Mb	114.9	12.7	0.001755	0.00			

Figures

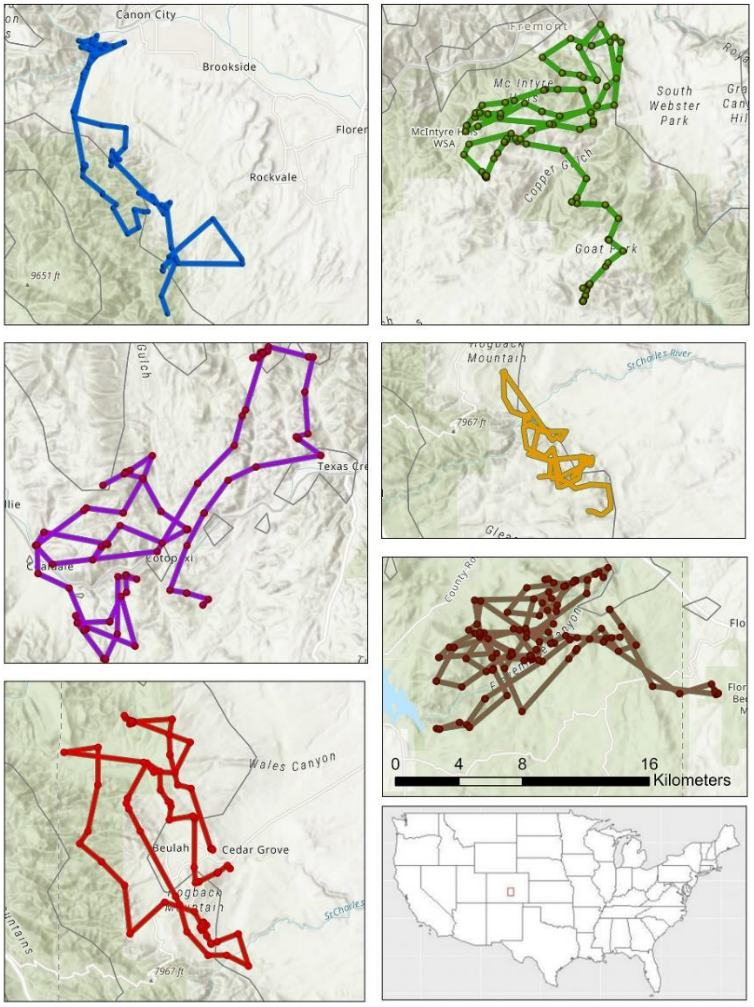

Figure 1. Map of the study site (red rectangle) showing the locations and distance traveled by each puma (*Puma concolor*) during the DLW trials (AF151: green, January 3-25, 2019. AF118: orange, February 12 – March 3, 2019. AF194: red: February 19 – March 15, 2019. AM313: blue, February 23 – March 19, 2019. AM172: brown, February 5 – March 7, 2020. AM177: purple, February 19 – March 11, 2020) (Esri, 2017. "Topographic" [basemap], "World Topographic Map". WGS 84 Web Mercator. Attributes: Esri, HERE, Garmin, FAO, NOAA, USGS, © OpenStreetMap contributors, and the GIS User Community).

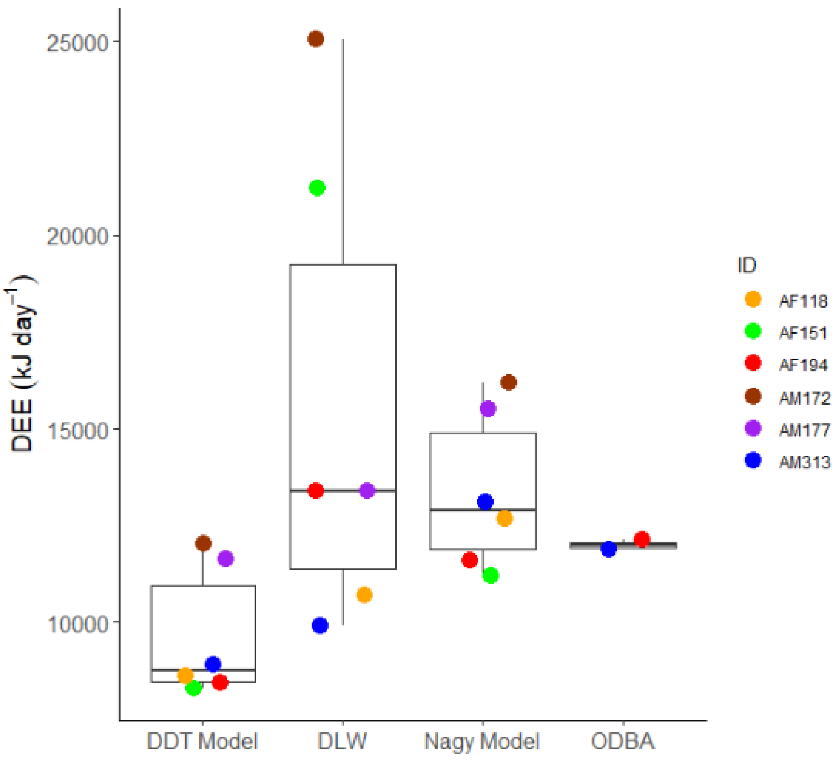

Figure 2: Comparison of the values of daily energetic expenditure (DEE) of pumas (*Puma concolor*) calculated under four different methods: The daily distance traveled (DDT) model (Laundré, 2005), doubly labeled water (DLW) method, the Nagy (2005) allometric model, and the overall dynamic body acceleration (ODBA) method.

Supplementary Materials

Supplementary Table 1. Measurements of body size in pumas (*Puma concolor*) in the Southern Rocky Mountains of Colorado.

ID	Body Length (cm)	Tail Length (cm)	Skull Length (cm)	Hind Foot Length (cm)	Chest Girth (cm)	Neck Girth (cm)	Body Mass (Kg)
AF151	120	71	25	25	71	38.5	38.6
AF118	135	73	24	37	65	38	45.64
AF194	129	73.5	24	26	73	38.5	40.5
AM313	131	79	24	29	76	44	47.7
AM172	137	74	26	31	76	41	63.6
AM177	154	57	29	28	74	44	60

Supplementary Table 2. Residuals of body mass based on the body size principal component analysis and the resulting body water percentage and body water in volume (L) for each individual puma (*Puma concolor*).

ID	Body Water %	Body Mass Residuals	Body Water (L)
AF151	0.65	-0.30	25.23
AF118	0.66	-1.03	30.23
AF194	0.66	-0.71	26.67
AM313	0.66	-1.23	31.71
AM172	0.56	7.50	35.62
AM177	0.70	-4.23	42.04
Mean	0.65		31.92
SD	0.05		6.19

CHAPTER THREE

An analysis of puma foraging across a gradient of human disturbance in South and North America.

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Abstract

Carnivore recovery is often promoted as an approach to restore ecosystem functioning. However, the trophic role of returning carnivores may differ from the past, especially in human-disturbed landscapes that feature novel ecological communities. To test if foraging strategies differed in human-disturbed landscapes and followed the niche variation hypothesis, we studied seven populations of pumas (*Puma concolor*) along a gradient of human disturbance in the temperate latitudes of North and South America (Colorado, USA and Argentina). We deployed GPS-collars to identify landscape features in puma home ranges and, using stable isotopes, quantified individual diet, specialization, and population dietary niche width. Prey composition varied across populations, and individual diet specialization decreased with increasing human disturbance, as pumas incorporated human-associated prey. Nevertheless, pumas exhibited high diet similarity within each location, contrary to our prediction within the niche variation hypothesis. Our results indicate that human-disturbed landscapes can alter the diet and specialization of pumas in both temperate North and South America with likely effects on their functional role. Given these changes in puma foraging within human-disturbed landscapes, the evaluation of the degree of human presence would help to determine whether carnivore repatriation will bring back historical ecological relationships or display novel interactions.

Introduction

Conservation biologists have advocated for the recovery of carnivores, given their global decline and the disproportionate effect that these species can have on ecosystems [1]. The functional role of carnivores –regulating prey populations through consumptive [2] and non-consumptive mechanisms [3], which can generate cascading effects [4] – is thought to be key in restoring ecosystem functioning [5]. However, rapid land-use change in the Anthropocene is restructuring both the stage and actors involved in the ecological play, even to the point of creating novel ecosystems [6]. Human-disturbed landscapes often feature environments with altered prey species [7], and interactions [8], as well as allochthonous subsidies from humans [9]. Consequently, the occurrence of carnivores in novel ecosystems [10] may result in very different functional roles [11–13]. Indeed, these novel landscapes likely provide opportunities for less specialized foraging strategies among individuals [9], and potentially novel roles for returning carnivores [14,15]. Such important changes to biotic interactions highlight the importance of conservation planning to not focus only on the taxa to be recovered but the ecological context in which recovery is planned.

The puma (*Puma concolor*) is the terrestrial predator with the widest distribution in the western hemisphere (Figure 1), able to persist in a variety of environments with varying conditions. Pumas are considered generalist and opportunistic hunters, and utilize a diversity of prey across their distribution [16]. Pumas inhabiting temperate latitudes generally feed upon large ungulates: in North America, they primarily prey upon mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus canadensis*); in southern South America, pumas mostly hunt wild camelids, the guanaco (*Lama guanicoe*) and vicuña (*Vicugna vicugna*). Pumas are increasingly rebounding to human-disturbed landscapes, both rural and urban, across their distributional range [17,18]. In these human-disturbed landscapes, the prey base of pumas has been modified, and they are increasingly consuming livestock and small synanthropic and exotic prey [19,20]. However, an analysis quantifying puma diet as a function of human disturbance across their distribution range and utilizing common measures to estimate their functional role has not yet been conducted (but see [16,21] for reviews). Following the niche variation hypothesis [22], pumas inhabiting novel ecosystems, where prey subsidies could expand their diet and niche width [19], should show less similarity within populations. Consequently, elucidating the trophic relations of pumas in varying ecological context can help to identify their functional role and the scenarios in which recovery may bring the ecosystem services sought or potentially increase conflict with human interests.

Given the increasing occurrence of pumas in human-disturbed landscapes [19,23] due to recolonization of former ranges [24,25] or human expansion, there is uncertainty around the functional consequence of puma on these novel landscapes. To test how pumas respond to human disturbance, we quantified the individual diets of marked pumas and calculated diet specialization and similarity within seven populations inhabiting temperate zones of North and South America with varying degrees of human disturbance. Our study sites in Colorado and Argentina ranged from mostly pristine areas to rangelands and urban-wildland interfaces. We collected hair samples from 83 pumas and used GPS data to account for landscape metrics within the home range for each puma, including fine-scale tracking for five of those populations, involving 57 individuals. We hypothesized that pumas would follow the niche variation hypothesis and individuals would exhibit dietary dissimilarity within each population as they incorporate more prey, and populations would exhibit dietary plasticity associated with prey availability from different sites. Specifically, we predicted that puma populations inhabiting more disturbed landscapes will show wider trophic niches and shift from native ungulates to domestic and small-sized exotic and synanthropic species in these anthropogenic environments, exhibiting a more generalist diet.

Methods

Study Sites

Pumas were sampled in seven different sites distributed across the temperate latitudes of the Americas (Figure 1): (1) Northern Front Range, CO (40° 0′ N, 106° 2’ W) is an urban-wildland interface featuring high human density and development. (2) Southern Front Range, CO (38° 26 N, 105° 13′ W); corresponds to a less populated urban-wildland interface. (3) Uncompahgre Plateau, CO (38º 20’ N, 108º 05’ W) corresponds primarily to undeveloped land, with few developments only around the perimeter of the study site. (4) San Guillermo National Park, Argentina (29° 13’ S, 69°31’ W) is in the Andean high plateau exhibiting virtually no human presence and near-pristine landscape. (5) Laguna Blanca National Park, Argentina (39° 02’ S, 70° 21’ W) in the Patagonian steppe is protected but also actively used as grazing land for livestock. (6) Patagonia Park, Argentina (46° 36′ S, 71° 24′ W) on the plateau of the Patagonian steppe has little human development and past land use was primarily for rangelands. (7) Monte León National Park, Argentina (50° 06’ S, 68° 54’ W) is located on the Atlantic coast of the Patagonian steppe, formerly used as rangeland but since 2006 part of a National Park.

Study Animals

Pumas were captured as part of associated studies using snares or cage traps and anesthetized to collect hair samples (n = 83) and fit GPS collars (n = 57) following the protocols of Colorado Parks and Wildlife (ACUC # 09-2018, 16-2008 and 08-2004), Argentine National Park Administration (permits DRC265, DRPA162 and DRPN1678), and Consejo Agrario Provincial, Santa Cruz (dispositions 19/2018, 09/2019, 37/2019, and 4/2020). Pumas were captured within three years for each site (See Supplementary Data for details). Hair and feathers from prey were collected opportunistically in each site from carrion and road kills, except for domestic animals in the Colorado analysis that were collected only from the Northern Front Range. Prey included in the analysis for each study site was based on kill-site investigations, accounting for at least 10% of the population kills or 20% of individual kills (see Supplementary Materials, Appendix S1 for details).

Isotopic Analysis of Diet

We used bulk ^{13}C and ^{15}N isotopes analysis to determine the diet and the niche width of pumas. Hair and feather samples were prepared following standard procedures [26] and analyzed with a Costech 4010 Elemental Combustion System attached to a Thermo Finnigan DeltaPLUS XP Continuous Flow Isotope Ratio Mass Spectrometer. Results are provided as per mil (‰) ratios relative to the international standards of Pee Dee Belemnite (VPDB; $\delta^{13}C$) and atmospheric nitrogen (AIR; $\delta^{15}N$) with calibrated internal laboratory standards. Firstly, to compare the isotopic niche breadth between populations and their respective prey sources, we used ^{13}C and ^{15}N values to calculate the area (‰^{2}) of Bayesian standard ellipses (SEA_B) and standard ellipses corrected by small sample size (SEA_C) using the *SIBER* package in R software[27].

Secondly, to estimate the relative contribution of prey to the diet of pumas, we used *MixSiar* package [28] for a Bayesian stable isotopes mixing model with Markov chain Monte Carlo (MCMC; chain length = 300,000; burn = 200,000; thin = 100; chains = 3), including the isotopic discrimination factor ($\delta^{13}C$ = + 2.6‰; $\delta\ ^{15}N$ = + 3.4‰, obtained for other carnivore, *Vulpes vulpes,* but proven effective on puma and other carnivore analysis [19,29,30]). We ran the model independently for each site using individuals as a fixed effect to estimate the individual diet and as a random effect to estimate the population diet. We used diet estimates obtained from kill sites and scats [31] as informative priors to the model. We also ran these models with uniform priors but did not detect substantial differences in diet estimation between the two (Supplementary Materials, Table S1). We categorized prey as domestic animals, large native animals (>20 kg, [32]) or small wild animals (<20 kg, including native, synanthropic and invasive species). We combined prey that were in the same category but that were isotopically different a posteriori [28,33]. Finally, we calculated similarity and specialization indices (ranging 0-1, where 1 represents high similarity and specialization, respectively) using the values of the Bayesian diet estimates for each individual [34].

Landscape Analysis

To quantify the influence of landscape variables on the diet, we used the Human Footprint Index (HFI, [35]) and the cumulative gross primary productivity (GPP) of the year [36], as primary productivity can inform the prey abundance [37]. We separated the HFI into agricultural footprint (i.e., cropland and pasturelands) and urban footprint (human population density, roads, railways, night-time lights and built environments). We used GPS locations obtained every 3 or 4 hours for one year to calculate the home range using the Local Convex Hull method with a 95% isopleth and adaptative radius in the package *adehabitat* [38] within the program R. The home range obtained from each individual was used to extract the mean value for each landscape variable. We could not obtain annual GPS data for pumas on two sites (Laguna Blanca and Uncompahgre Plateau), for which we used a 8 km radius as a circular buffer around the sample collection point, based on the average area of the other collared individuals.

We examined whether a series of a priori covariates influenced diet specialization. Covariates examined were cumulative GPP, total HFI, urban HFI and agricultural HFI, age-class (adult – subadult), sex and subcontinent (North or South America) as fixed effects. We used linear beta regression with a logit link function for all models. We selected the best model to predict diet specialization using Akaike's information criterion, corrected for small sample sizes (AIC_C) and using maximum likelihood in the selection process. All variables we used have a variance inflation factor <2, discarding multicollinearity. To account for the effect of site we tested our top-ranked model with an ANCOVA using site as the covariate.

Results

Trophic niche width was similar between sites (SEA_B range = 0.64 to 1.45 %^{2}), except for one site in South America which exhibited a niche width at least >4 times larger than the rest which was principally driven by consumption of marine resources (Figure 2a). We also found a positive correlation between the predator ellipse area with the corresponding base-prey analyzed at the population level (R^2=0.54, p=0.034, n = 7, Table S2). Diet among individuals within each population showed high similarity, except for the costal site of Monte León ($F_{6,76}$=9.8, p < 0.001), where around half of the population preyed on Magellanic penguins (*Spheniscus magellanicus*), when penguins were available from September to April. Pumas exhibited great variability in their diet depending on the site, consuming mainly native ungulates (0 to >95%), and in most cases supplementing the diet with domestic animals (0 to 90%), and small wildlife (0 to 40%, Figure 1). According with our top model (Table 1), puma diet specialization decreased with increasing human footprint index overall (R^2 = 0.32, β = -0.140, 95%CI [-0.19, -0.09], Figure 2b), which was a better predictor than urban footprint (β = -0.114, 95%CI [-0.16, -0.07]) or agricultural footprint (β = -0.043, 95%CI [-0.24, 0.15]) alone. Other variables, such as GPP (β = -0.021, 95%CI [-0.62, 0.20]) and subcontinent (β = 0.19, 95%CI [-0.23, 0.62]) did not affect diet specialization. When top ranked variable, HFI, accounted for the site effect as a covariate ($F_{7,66}$=223.2, $p < 0.001$, $R^2 = 0.95$; Figure S1) we found that specialization still decreased with HFI but at a lower degree (β = -0.0052, 95%CI [-0.23, -0.62], while the study site has a strong predictor effect.

Discussion

As expected, pumas exhibited great variability in their diet across their range in both South and North America. Large ungulates generally predominated as their prey, but we found that pumas incorporated a variety of prey types depending on location. These results reinforce the concept that pumas are, indeed, generalist and opportunistic specialists depending on prey availability [16,39]. Notably, we found that the human footprint index composite, is an important predictor of the degree of dietary specialization, with pumas in more human-disturbed landscapes exhibiting greater dietary generalization regardless of agricultural or urban change. Other studies investigating pumas in human-disturbed landscapes have also found plasticity in the foraging of pumas, and an expansion of the niche to include domestic and synanthropic prey [19]. Our work, however, reveals that this trophic plasticity is at some extent driven by human disturbance, regardless of continent. Indeed, pumas inhabiting more pristine areas were more specialized predators of native ungulates, whereas pumas in landscapes with higher human footprint consume a panoply of prey from domestic livestock and pets to native and exotic synanthropic animals and even penguins. This trophic plasticity of pumas likely modulates the strength of their top-down forces in the ecosystem they inhabit. Indeed, we suggest that the functional role of pumas, and potentially other carnivores, could be altered in human-disturbed landscapes, as they reduce their interaction with large ungulates [13].

Accounting for human disturbance with global products like HFI could be useful in conservation planning to predict what the role of the repatriating carnivores will be. However, there should be consideration of the local aspects of the terrain as within the sites there were high similarities in diet independently of the HFI index, and the site was also a factor affecting specialization. We found that pumas with home ranges encompassing HFI values >7.0 became generalists (specialization indices <0.5). Thus, a starting point in planning future puma recovery initiatives should determine the degree of human development to assess the specialization and functional role of pumas. Nevertheless, the local understanding of the environmental conditions is also necessary to predict the effect of returning carnivores. For example, pumas inhabiting Laguna Blanca, a site featuring low HFI, exhibited high specialization. However, what is unique to Laguna Blanca is that the native prey base has been completely replaced by exotic livestock. Even though specialization values were high in Laguna Blanca, the functional role of pumas has dramatically changed, even to the point of creating conflict with some local ranchers [31,40]. Thus, while a useful starting point, HFI values may not always capture the full extent of ecological change that has already occurred. Notably, HFI is weighted to urban components and, consequently, the effects of rural effects may be underestimated. Further, HFI does not explicitly account for differences or changes in prey availability and hence the particularities of the local prey base independent of the human impact may also have a big involvement in explaining the diet of puma.

While foraging differed between sites, the diets of individual pumas were highly similar within the same population, regardless of the degree of human disturbance or the diversity of the prey base. The lack of niche variation among individuals within a population suggests that individuals opportunistically hunt whatever prey is most available, likely due to no major trade-off associated with the different diet items [41]. While our results do not follow the niche variation hypothesis, they are consistent with optimal foraging, which predicts that diets should be similar due to site specific prey availability [42]. In general, our results align with previous analysis on puma kill sites, in which the diet of individuals within each population tended to be similar [43]. Notably, the only site where pumas exhibited dietary dissimilarity, in coastal South America, was due to the presence of a penguin colony [44]. This unusual dissimilarity within a population could be related to the particularities of this coastal site. Here, penguins are clustered in a colony where just some individual pumas have access. Overall, these results suggest that pumas are good candidates for recovery, given their adaptability to consume whatever prey is present. This adaptability, however, can present its own conservation dilemmas as alternative prey maybe not be well adjusted to puma predation.

Human-disturbed landscapes tend to reinforce generalist foraging strategies in carnivores, as well as in other species [46], via two non-mutually exclusive forces. First, human-disturbed landscapes typically feature abundant prey and food subsidies that are taxonomically and functionally (e.g., body size) diverse [47]. Both prey availability and direct human subsidies have been previously shown to greatly alter puma diet [19,48] and the diet of a diverse guild of carnivores across a range of landscape conditions [9]. Second, the presence of humans themselves, as an ultimate predator, can greatly alter foraging decisions of pumas, and their prey, based on perceived risk [49]. Indeed, pumas feeding in proximity to humans exhibit less feeding time on their prey, and higher rate of carcass abandonment [15,50], which likely reduces the benefits of hunting large prey. Previous studies in urban landscapes have shown pumas indeed consume smaller prey [19]. This apparent subordinate role of pumas within human landscapes appears similar to their role in the tropic where they are sympatric with jaguars (*Panthera onca*)

[16]. In that region, pumas are subordinate to jaguars, which primarily depredate large (>15kg) mammals [51,52]), and pumas consume a wider subset of prey, skewed to smaller species [39]. Thus, the more generalized diet of pumas in human landscapes or tropical latitudes could be explained in part by their subordinate role and interference competition [53].

Carnivores are being reintroduced to restore ecosystems, and this is increasingly occurring in human-disturbed landscape [54,55]. However, there is a dearth of evidence as to whether the recovery of these species restores the original ecological interactions [11,56]. Our results reveal that the degree of specialization, and consequently the functional role of a large carnivore is influenced by the presence of humans and their associated changes to the landscape in addition to other site-specific particularities. Notably, generalist foraging strategies should reduce top-down forces and the role of top predators in regulating prey populations. Thus, the return of carnivores to increasingly disturbed landscapes may not always return the exact ecological relationships sought to pre-disturbed states. Instead, we should aim to understand the role carnivores can play on a case-by-case basis and their contribution to ecosystems in a gradient of human disturbance.

Conservation practitioners can measure human disturbance in anticipation of what carnivore recovery will functionally mean. Such measures can be accomplished with readily accessible products. By quantifying the ecological context in advance of recovery, we can be more prepared to advocate and prioritize restoration areas where the original role is maintained. Such information can also help predict where new ecological roles are likely and help to avoid or prepare for potential conflict with humans or with other conservation efforts.

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Tables

Table 1: Results of model selection to predict the diet specialization index of pumas (*Puma concolor)* across seven sites in the Americas.

Model	AIC_C	Δ AIC_C	AIC_C weight
Human Footprint (HFI)	-27.12	0	0.3
HFI + Continent	-26.29	0.83	0.21
HFI + Cumulative GPP	-25.03	2.1	0.1
HFI + Sex	-24.62	2.5	0.09
Urban Footprint	-21.39	5.73	0.02
Null	-2.16	24.96	0
Agricultural Footprint	0.34	27.46	0

Figures

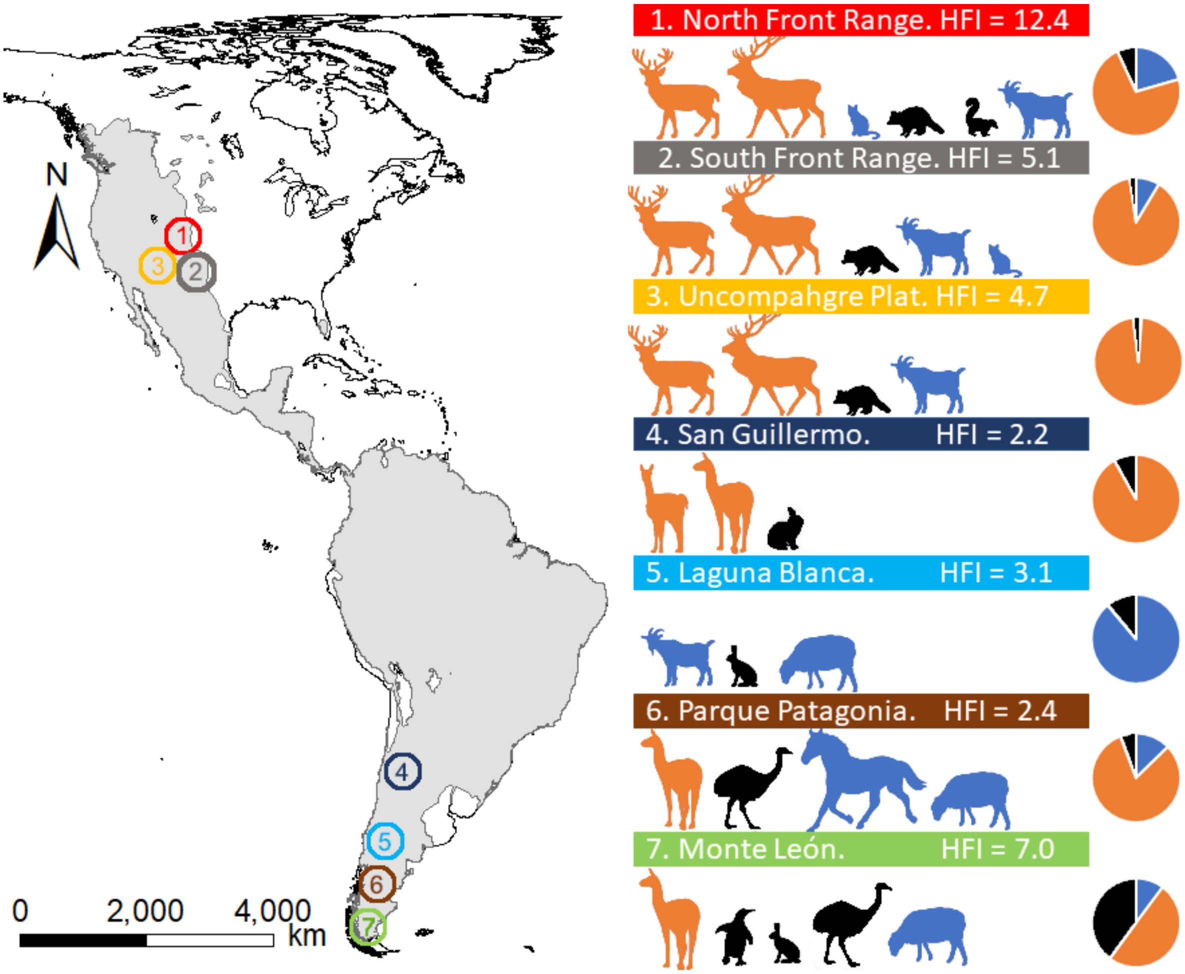

Figure 1: Puma (*Puma concolor*) current distribution in the Americas (light grey, [57]) showing the seven study sites with the corresponding prey species found in kill-site investigations and the average human footprint index (HFI) for the overlapping home range of the population in each site. Pie charts indicate the assimilated prey using Bayesian mixing models medians and categorized as domestic, including livestock and pets (blue; goat, cat, sheep, horse); large wild animals, corresponding to native ungulates (orange; mule deer, elk, vicuña, guanaco); and small wild prey, which include wild prey weighting less than 20 kg (black; racoon, skunk, viscacha, European hare, rhea, Magellanic penguin).

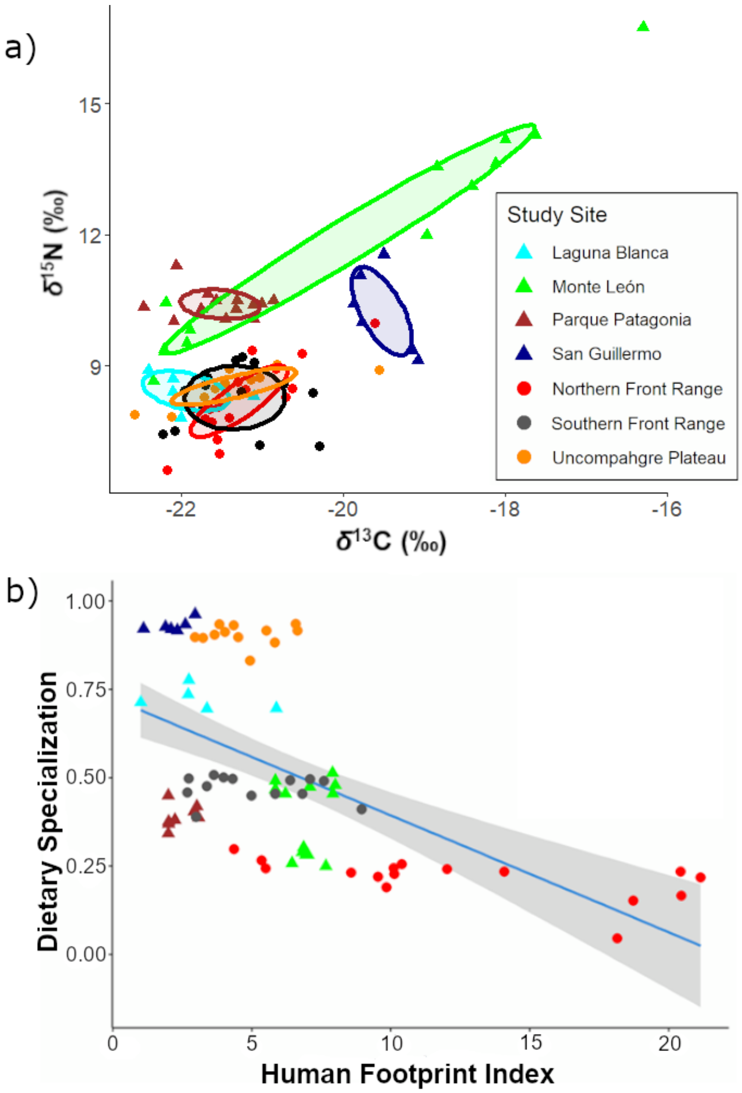

Figure 2: (a) Standard Ellipse Area corrected for sample size (SEAc) for each population of pumas (*Puma concolor)* on the seven different study sites using the ^{13}C and ^{15}N isotopic space. **(b)** Specialization index for each puma in response to the human footprint index [35] encompassing seven different populations in North (circles) and South America (triangles). The relationship between dietary specialization and the human footprint index is shown as a trend line (blue line) ± 95% CI (gray shaded area), R^2=0.32, p<0.001.

Supplementary Material

Results

Given the high HFI in the North Front Range site, we also performed a regression analysis excluding that site. We found that diet specialization was still negatively correlated to the HFI ($R^2 = 0.11$, $\beta = -0.152$, 95%CI [-0.26, -0.04], Figure S2).

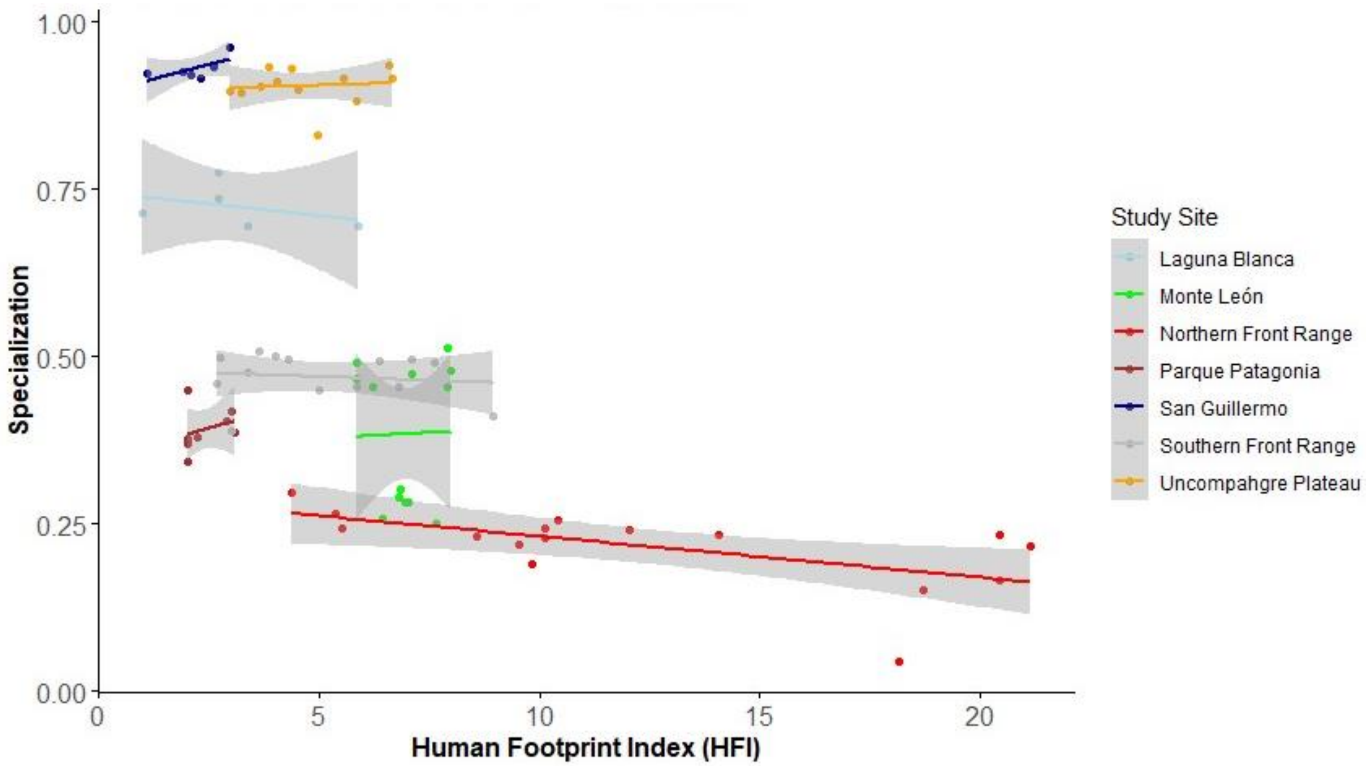

Figure S1: Diet specialization index for each puma in response to the human footprint index (Venter et al. 2006) encompassing seven different populations in North and South America . The relationship between dietary specialization and the human footprint index is shown as a trend line (solid line) ± SE (gray shaded area) per site.

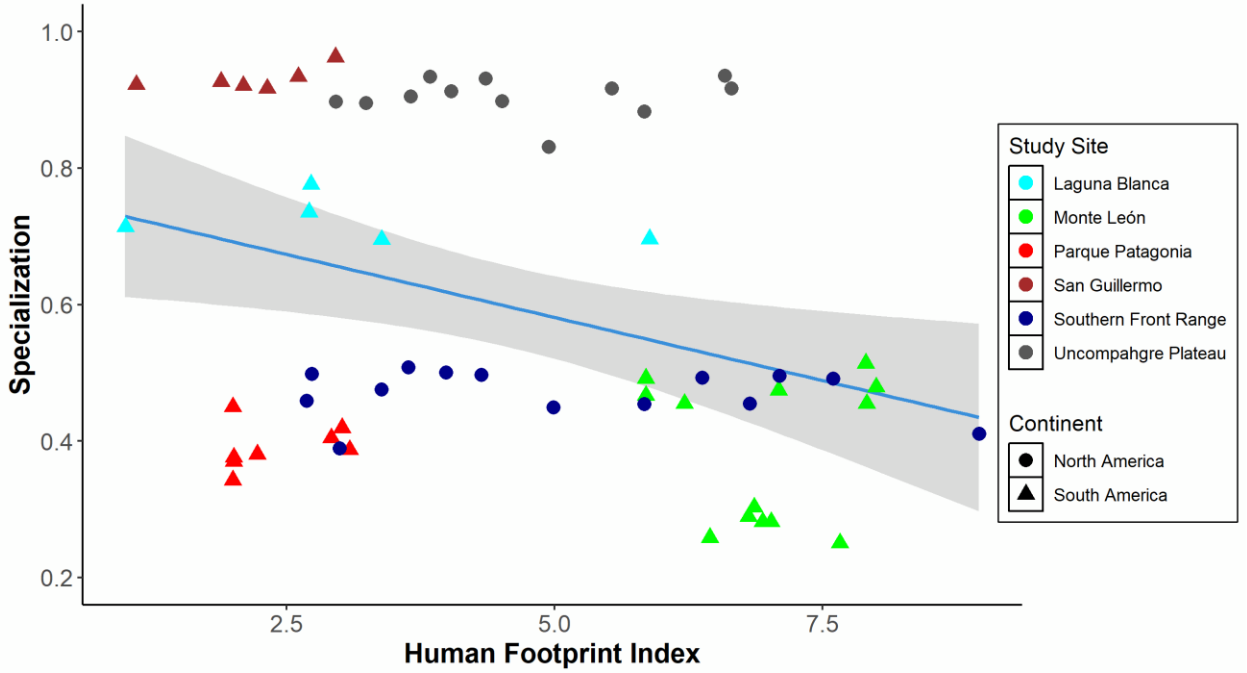

Figure S2: Diet specialization index for each puma in response to the human footprint index (Venter et al. 2006) encompassing six different populations in North (circles) and South America (triangles). The relationship between dietary specialization and the human footprint index is shown as a trend line (blue line) ± SE (gray shaded area), $R^2=0.11$, $p<0.01$.

Table S1: Diet composition of pumas *(Puma concolor*) at the population level on seven different sites as the mean and 95% credible intervals. Prey is categorized as domestic, including livestock and pets; large wild animals, corresponding to native ungulates; and small wild prey, which include wild prey weighting less than 20 kg.

Study Site	HFI	**Informed Prior** **Domestic Prey**	**Large Wild Prey**	**Small Wild Prey**	**Uninformed Prior** **Domestic Prey**	**Large Wild Prey**	**Small Wild Prey**
		%	%	%	%	%	%
San Guillermo	2.2	0	91.9 [29-100]	8.1 [0-70]	0	41.4 [13-86]	58.6 [12-86]
Laguna Blanca	3.1	88.9 [36-100]	0	11.1 [0-64]	76.7 [25-99]	0	23.3 [1-75]
Parque Patagonia	2.4	12.7 [0-57]	81.6 [37-100]	5.7 [0-38]	36.9 [12-66]	43.1 [16-71]	18.0 [2-42]
Monte León	7.0	10.3 [0-41]	50.4 [5-85]	40.3 [12-90]	28.3 [3-65]	21.0 [2-61]	44.6 [19-81]
North Front Range	12.4	20.0 [3-43]	71.2 [57-85]	6.8 [0-18]	22.9 [6-39]	70.2 [57-85]	6.1 [0-18]
South Front Range	5.1	8.6 [1-22]	89.0 [76-97]	2.3 [0-8]	21.9 [6-43]	70.3 [53-83]	6.3 [0-20]
Uncompahgre Plateau	4.7	1.2 [0-7]	96.9 [88-99]	1.9 [0-9]	15.2 [3-36]	80 [60-94]	3.5 [0-15]

Table S2. Isotopic standard ellipses area (SEAc) for each puma population and their corresponding prey base.

Population (n size)	**S**EAc Pop Area (‰^{2})	**S**EAc Prey Area (‰^{2})	**Proportion**
San Guillermo (6)	1.005	11.47	8.8
Laguna Blanca (5)	0.798	3.91	20.4
Parque Patagonia (13)	0.521	3.05	17.1
Monte León (13)	4.184	28.32	14.8
Northern Front Range (20)	0.866	17.46	5.0
Southern Front Range (14)	1.44	16.83	8.6
Uncompahgre Plateau (12)	0.64	16.19	4.0

CHAPTER FOUR

A large carnivore shows a gradual spatial-temporal movement response to human disturbance.

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Abstract

Large carnivore populations are increasingly occurring in human-disturbed landscapes. Carnivores in these landscapes typically avoid humans or human development by limiting movement rates and distances and shifting their diel activity. However, there is evidence that some carnivores can select human landscapes that provide cover or increase their movement rate near urban areas, among other contrasting responses of carnivores to different types of human disturbance. Here we evaluated the behavior of a large carnivore and explored how space and time interact with human-disturbed landscapes across different sites in North and South America. We equipped 58 pumas (*Puma concolor*) with GPS collars and evaluated the impact of human disturbance, using the Human Footprint Index (HFI), on the home range, step length, diel activity, and step selection of pumas. We hypothesized that the movement and activity of pumas would respond to the degree of human disturbance, independent of the type of disturbance. We predicted less movement for pumas occupying high HFI areas and greater avoidance of heavily impacted areas. We also predicted that to compensate for the restriction on movement, pumas would be more active at night in areas with higher human disturbance. Pumas decreased their step length with increasing human disturbance which was associated with a slight reduction of their home ranges. Surprisingly, puma diel activity was not affected by HFI – regardless of human disturbance pumas were generally mostly active at night than during the day. However, the puma population within the higher disturbance site traveled less at night. Nevertheless, when exploring the interaction of spatial and temporal variables, we found that puma step length increased at night, especially in areas with higher HFI, suggesting a finer scale behavioral response to human disturbance. Moreover, pumas avoided high human disturbance areas during the day, but at night ventured into them more. This interaction of time of day and avoidance of

HFI was especially pronounced in sites that featured overall lower HFI, while avoidance was sustained regardless of time of day in sites with overall higher HFI. Our research highlights that the plasticity in the behavioral response of pumas to human development exhibits a strong spatial-temporal response to human disturbance and that this response was consistent across regions regardless of the human type of disturbance. Moreover, this plastic response to the amount of human disturbance likely reflects a perceived landscape of varying risks and rewards associated with humans that pumas navigate.

Keywords: Human disturbance – Mountain lion - Movement patterns – Nocturnality – Step selection.

Introduction

Carnivores are increasingly inhabiting human-disturbed environments (Athreya et al. 2013, Chapron et al. 2014, Pauli et al. 2018). These novel landscapes (Radeloff et al. 2015) feature altered habitats, prey bases, and interactions, all of which have important consequences on carnivores (Wang et al. 2017, Sévêque et al. 2020). Notably, the construction of roads and conversion of landscapes to agriculture or urban areas has not only resulted in habitat loss and fragmentation but also imposed physical barriers to the movement of animals (Crooks et al. 2011, Tucker et al. 2018). Due to the large space used by carnivores, the likelihood of encountering human landscapes is higher for any given individual (Di Minin et al. 2016, Carter and Linnell 2016). While carnivores generally avoid human-disturbed areas or travel quickly through them (Whittington et al. 2022), the degree of human disturbance is inversely related to the distance traveled (Tucker et al. 2018); in other words, carnivores in human-disturbed landscapes often exhibit smaller and more confined home ranges (Šálek et al. 2015) selecting for small patches of natural areas in and around human landscapes (Bateman and Fleming 2012). Human development is also associated with important human food subsidies (Manlick et al., 2020, Penteriani et al., 2021) in the form of food waste, intentional bait or supplemental feed, agricultural crops as well as domestic and synanthropic species (Shochat et al. 2010, West et al. 2016, Kirby et al. 2017). This abundance of food in human-disturbed landscapes also reinforces predators to spend less time searching and moving shorter distances to forage (Parsons et al. 2022).

However, other studies have reported an increase in the movement of large carnivores in human-disturbed landscapes (Wang et al. 2017, Dickie et al. 2017, Thorsen et al. 2022). Some carnivores select agricultural features that possess sufficient low horizontal cover, despite the presence of humans (Naha et al. 2021). In such systems, large carnivores like tigers (*Panthera tigris*) have been shown to increase both their movement rate and home range (Habib et al. 2021). Additionally, the total distance traveled by carnivores has also been documented to be higher in landscapes with high housing densities (Wang et al., 2017). Additionally, large carnivores will utilize roads and other human linear features (such as pipelines, railways, or transmission lines) as movement corridors, increasing the daily distance traveled while searching for prey (Dickie et al. 2017). Finally, increases in distance traveled and speed of movement in human-disturbed landscapes have also been attributed to avoidance of human activity rather than the direct impact of human features (Whittington et al. 2022). Indeed, increased movement within developed areas has been attributed to risk-induced effects of humans on large carnivores; specifically, carnivores prematurely abandoning carcasses due to human activity (Smith et al. 2015).

Human activities also alter the temporal activity of animals (Frey et al. 2020), with carnivores generally becoming increasingly nocturnal to avoid encounters with humans (Gaynor et al. 2018, Suraci et al. 2019). In many cases, carnivores increase the total distance traveled during the night, as well as movement becomes more direct and less sinuous (Barocas et al., 2018). This increased nocturnality, however, appears to be dependent on the intensity of human exploitation rather than the mere presence of human activity (Mills et al. 2023). This shift towards nocturnality appears to be an important adjustment to predict the persistence of carnivores within disturbed landscapes as it enables individuals to navigate through the landscape, maintaining access to prey while minimizing lethal encounters (Mills et al. 2023; Gaynor et al., 2018). However, this temporal shift and contraction also increases their overall interactions with other species, potentially reshaping predator-prey interactions (Gilbert et al.

2022) or carnivore intraguild dynamics (Rodríguez Curras et al. 2022). Nevertheless, in some cases, carnivores can maintain their general temporal activity even on human landscapes by being more selective of the space used when temporal overlap with humans exists (Van Cleave et al. 2018).

Overall, then, there are multiple and sometimes contrasting responses of carnivores to disturbed landscapes. Some of these differences can be attributed to different species or sites under study (Lowry et al. 2013), but also the form of human disturbance appears to play an important role in carnivore space and temporal activity (Sévêque et al., 2020). Urban and exurban environments can have marked effects compared to agricultural ones, as large predators may avoid human activity rather than the human-disturbed area (Nickel et al., 2020). Near urban areas, high human density and traffic on roads reinforce carnivore avoidance of human disturbance (Kautz et al. 2021), while providing an abundance of wild synanthropic and small domestic prey (Moss et al. 2016a; Barceló et al. 2024). In rural areas, while human-carnivore conflict exists due to the presence of livestock (Ohrens et al. 2016), carnivore spatial avoidance appears to be lower due to reduced human activity (Naha et al., 2021). Previous studies, though, have not disentangled the potential differing roles of human disturbance types or investigated such responses simultaneously in different populations of the same species. Furthermore, there has been a general bias towards research in North America in relation to other parts of the globe, particularly more than tripling the research in human-carnivores relations done in South America (Lozano et al. 2019). Whether carnivores respond similarly across different parts of their range and the universality of these responses has just started to be explored (Guerisoli et al. 2021).

The puma (*Puma concolor*) possesses one of the widest distributions of a wild terrestrial mammal in the Americas, ranging from the Yukon in Canada to the southernmost extremes of continental South America. The puma, a generalist predator, exhibits great behavioral plasticity and can occupy human-disturbed landscapes when hunting pressure is reduced, occupying exurban areas (Moss et al. 2016a) and agroecosystems (Azevedo et al. 2018). Consequently, the puma has recovered some parts of their historic range (Mazzolli 2012, O′Neil et al. 2014); however, it is still unclear what are the human landscape variables that strongly interact with puma habitat use, and the universality of them across the Americas. In general, though, rural areas are perceived by pumas as less hostile compared to exurban and urban areas, which are avoided more frequently (Burdett et al. 2010). This trend, however, can be attenuated or even reversed by the composition of the wildland-urban interface, especially the degree to which natural areas are interspersed and juxtaposed with human disturbance (Lewis et al. 2015). Pumas can habituate to human landscapes by incorporating non-native prey in their diet on both continents (Novaro et al. 2000, Moss et al. 2016a); in North America, there are more frequent reports of individuals selecting urban-wildlands interfaces (Moss et al. 2016b, Wang et al. 2017); while in South America, human-carnivore conflict is associated with more reliance of pumas on agricultural lands and livestock (Guerisoli et al., 2021). Thus, the spatiotemporal dynamics of how pumas navigate a range of human disturbances have been understudied or restricted to only a few sites.

To better understand the spatiotemporal behavior of a large carnivore across a gradient of human disturbance, we studied pumas across five populations inhabiting a range of human disturbance in both North and South America. We hypothesized that the intensity of human disturbance would modulate the movement pattern and activity of pumas and that this carnivore responds differently in exurban and rural environments. Specifically, we predicted that the pumas inhabiting sites with higher overall human-disturbed landscapes will exhibit smaller home ranges and will move on shorter and less straight steps. We also predicted that pumas will avoid more consistently developed areas when the overall human disturbance is higher and that higher activity during the night, as distance traveled, will be associated with higher disturbance.

Methods

Study Sites

We studied pumas at five sites in North and South America (Figure 1): (1) Northern Front Range, Colorado, USA (40° 0′ N, 106° 2’ W), an urban-wildland interface featuring high human density and development (n = 13). (2) Southern Front Range, Colorado, USA (38° 26 N, 105° 13′ W); corresponds to a less populated urban-wildland interface (n = 13). (3) San Guillermo National Park, Argentina (29° 13’ S, 69°31’ W) is in the Andean high plateau exhibiting virtually no human presence and near-pristine landscape (n = 8). (4) Patagonia National Park, Argentina (46° 36′ S, 71° 24′ W) on the plateau of the Patagonian steppe has little human development and past land use was primarily for rangelands (n = 11). (5) Monte León National Park, Argentina (50° 06’ S, 68° 54’ W) is located on the Atlantic coast of the Patagonian steppe, formerly used as rangeland but, since 2006, part of rewilding and restoration efforts (n = 12).

Pumas were captured within three years for each site as part of associated studies using snares or cage traps and anesthetized to fit GPS collars (n = 58) following the protocols of Colorado Parks and Wildlife (ACUC # 09-2018, 16-2008 and 08-2004), Argentine National Park Administration (permits DRC265, DRPA162 and DRPN1678), and Consejo Agrario Provincial, Santa Cruz (dispositions 19/2018, 09/2019, 37/2019, and 4/2020). GPS collars collected at least 8 locations per day over one year. GPS points fixed with less than three satellites or a dilution of precision higher than six were eliminated (Frair et al. 2010).

Home range, Movement and Daily activity estimations.

To estimate the home ranges of each individual, we used the Local Convex Hull method with a 95% isopleth and adaptative radius in the package adehabitat (Calenge 2006) within the program R using our GPS dataset. The home range obtained from each individual was used to extract the mean value of the Human Footprint Index (HFI; Venter et al. 2016) as a global scale index to compare human disturbances in Argentina and the USA.

We evaluated the step length and the turning angles of each real step as a measure of the activity of pumas where larger steps and angles closer to zero indicate more direct traveling and higher activity, while shorter steps and more pronounced angles are evidence of lower activity. We evaluated these variables in response to the time of the day and HFI using general linear mixed model accounting site and ID as random effects, as well as evaluating the direct relation of HFI with the step length and turning angle using a generalized additive mixed model with site and ID as random effects.

To quantify diel activity patterns for pumas, we calculated the mean movement rate of individuals between successive points as the mean distance traveled per hour. We examined diel movement rates for all five sites by fitting generalized additive mixed models (GAMM) with a cyclic spline and a random intercept for individuals using the mgcv package in R (Kohl et al. 2018). We also categorized the diel period as day (1 hour after sunrise and one before sunset), night (1 hour after sunset and 1 hour before sunrise), or dusk/dawn (intermediate times), ran a mixed-effects analysis of variance (ANOVA) tests using R package nlme for pumas with the diel period as the predictor, distance traveled as the response variable, and a random intercept for individual and site. Finally, we calculated the nocturnality value for each individual (the proportion of distance traveled during the night). Then, we ran a linear mixed-effects analysis with nocturnality as the response variable, the HFI of the home range as the predictor, and a random intercept for the site.

Step Selection

To account for the environmental variables and the human influence over the puma movement pattern, we used an integrative Step-Selection Analysis (iSSA, Avgar et al. 2016). iSSA can estimate resource selection parameters concurrently with the movement pattern of the animal, allowing joint inference on both processes. As iSSA requires a constant step duration, we fractioned the whole individual puma trajectory into several bursts with constant intervals between fixed locations. For each real step between two successive locations, we generated ten random steps with the *amt* package in R (Signer et al. 2019), using a gamma distribution for the step lengths and von Mises distribution for the turning angles (Lund et al., 2017). We also categorized each step as day or night based on the sunset and sunrise for each given location and time of the year. As environmental covariates for the iSSA, we used the components of the Human Footprint Index (Venter et al., 2016), specifically: road proximity, human density, agricultural intensity, the extent of built human infrastructure and the total HFI. To account for natural variables, we considered elevation, roughness and slope (USGS, 2018), distance to water (Kummu et al. 2011), and cumulative, minimum and variability of the gross primary productivity (GPP; Radeloff et al. 2019). We scaled all variables and discarded those with high collinearity (Pearson's $R > 0.5$), namely slope, minimum GPP and GPP variability. We extracted the landscape covariates value at the end of each step and proceeded to create a conditional logistic regression model for each individual using the two-stage approach (Fieberg et al. 2010) to escalate the model to the population level accounting for individual and site effects. Briefly, this approach tests for the best-fitting model among a series of a priori models using the Akaike information criterion (AIC) for each individual and then selects the model with the lowest AIC average among the population. Then, for the best competing model, we obtained the selection coefficients for each landscape variable and each individual in the five sites for day and night and their variance-covariance matrix. Then we used parametric bootstrapping (Fieberg et al. 2020) to resample each coefficient from a multivariate normal distribution with the original fitted coefficient and their variance-covariance matrix and got individual estimates for the population-level coefficient as the mean of each resampled coefficient across 2000 bootstrap iterations.

Results

A total of 58 pumas were used in our analyses, which had > 6 months of monitoring with a total of 179,372 high precision locations, leading to 104,523 true steps within constant track bursts. The home range size of pumas declined with increasing HFI (β = -10.3 km^2 95%CI [-24.9-3.4]). The home range for males in the most pristine areas was nearly twice (β_0= 395 km^2, [302, 488]) compared to females in the most pristine areas (β_0 = 233 km^2; [105,351], Figure 2). Additionally, step length diminished with the increasing HFI ($F_{8.1,8.7}$= 7.40, $p < 0.01$, Figure 3) independently of site, while the turning angle did not change in function of HFI ($F_{2.3,2.9}$= 1.31, $p = 0.22$), but the variance of it increased in sites with higher HFI (Figure 3b).

We did not detect an association between nocturnality, the proportion of distance traveled during the night, and the HFI (β = 0.00, [-0.1, 0]), although there was an effect of the site showing that at our most developed site in the northern Front Range proportion of distance traveled between sunset and sunrise was just 45% [39, 51], while for all the other sites the nocturnality was around 60% [56, 64]. ($F_{4,53}$ = 7.5, $p < 0.01$, Fig 4). While the pumas appeared to not associate their turning angle to HFI or the time of day (β = 0.01 rads, [-0.12, 0.034]), the time of day affected the step length. Specifically, the log step lengths during the night were significantly longer (β = 0.483 95%CI [0.435, 0.532]) compared to the daytime. While the longer steps on low HFI sites were not significant (β = 0.09, [-0.203 to 0.387], there was an interaction of time and HFI, indicating that at high HFI sites there is an increased step length during the night (β = 0.1709, [0.108, 0.233] Figure S1).

The iSSA model that best explained the true movement steps included Elevation, Cumulative GPP, Distance to Water, Roughness, HFI, and the interaction of HFI and the time of day (Table 1). In decomposing the effects of specific components of the HFI index, we found that the total HFI was a better predictor to inform iSSA rather than any component by itself (Table 2). In general, pumas selected for roughness and distance to water, but against elevation and HFI. However, these values varied when analyzed by site. Nevertheless, pumas generally avoided areas with higher HFI, but that avoidance of HFI was attenuated during the night (Figure 5a). When considering the effect of the different sites and the time of day, we found that pumas regardless of the sex of individual (β = 0.27, [-5.5, 6.1]), were slightly more likely to move through developed areas during the night in regions characterized by overall low HFI sites but generally avoided developed areas regardless of the time of day in regions featuring overall high HFI (β = 0.44, -0.34, 1.2], Figure 5b).

Discussion

Our findings highlight the behavioral plasticity of pumas in response to human disturbance. Indeed, we observed that the rate of movement of pumas was decreased with human disturbance, while their space selection was affected by human disturbance depending on the time of the day.

Specifically, we observed reduced home ranges and movement rates in areas featuring higher human disturbance. However, contrary to what we expected, puma populations inhabiting higher human-disturbed sites did not shift their diel activity to becoming more nocturnal. When considering movement as a function of both space and time, pumas exhibited reduced movement during the day, especially in areas with higher human disturbance. Furthermore, the space selection by pumas was a function of both the time of the day and the level of human disturbance. Specifically, pumas inhabiting areas with higher HFI consistently avoided human disturbance regardless of the time of day. In contrast, pumas inhabiting areas with lower HFI showed less avoidance and even selection of areas associated with human disturbance during the night.

The reduction in the movement rate with increasing human disturbance aligns with previous studies (e.g., Tucker et al. 2018) that have shown that pumas modify their paths to navigate smaller and more fragmented habitats. Similarly, the reduced home ranges in pumas have been widely documented in response to human disturbance (Nickel et al. 2021). Similar to our findings, pumas have been shown to reduce their home ranges by >50% in places featuring high housing density (Nickel et al. 2021). We did not detect an effect of human disturbance on the sinuosity of path movements for pumas, regardless of sex. This finding contrasts with other studies that have found that pumas exhibit more tortuous travel paths in human landscapes, which is associated with elevated perceived risk due to higher traffic or activity in areas with high human density (Nickel et al. 2021). Our results suggest that while pumas alter how far they move, the characteristics of their paths are relatively unchanged. In general, these adjustments in reduced space use by pumas indicate that they consistently minimize exposure to human presence on the landscapes across sites.

Surprisingly, we did not find a relationship between nocturnality and the HFI value within individual puma home ranges. Nocturnality has been widely documented as a strategy to avoid human encounters (Gaynor et al. 2018; Suraci et al. 2019); nevertheless, community dynamics play an important role in whether temporal shifts occur in carnivores (Frey et al. 2020). If pumas can avoid spatially humans, the need to change the temporal activity is less likely (Van Cleave et al. 2018). Interestingly, though, the time of the day had an important effect over other variables, namely step length and step selection in an interaction with HFI. Step length increased at night only in areas featuring high HFI. This might suggest that nocturnality could be observed at a finer scale, where each puma travels longer distances during the night when confronting areas of higher disturbance within their home range (Barocas et al. 2018). Similarly, this finer-scale association was observed when pumas selected human disturbance features on the landscape differently depending on the time of day.

Our finding that pumas avoided human disturbance more during the day than the night suggests that pumas have the capability to avoid human landscapes when the probability of encounter with human activity is higher. However, pumas may adjust their movement patterns not only to avoid human disturbance directly but also to align with the activity patterns of prey species, which themselves may shift in response to human activity (Crawford et al. 2022), which would imply an indirect impact of humans on large carnivores as well. Attenuated avoidance, and even selection for higher HFI areas at night in sites with overall lower HFI could suggest that pumas select these areas for subsidies associated with livestock ranches (Kuiper et al. 2024) or use of human linear features for facilitated travel (Dickie et al. 2017). Maintaining the use of these human-associated areas in overall low HFI sites may indicate a tolerated risk that diminishes in sites with higher human activity (Moss et al. 2016b). The intensity of human disturbance plays a key role in shaping puma behavior and movement, as interactions between the time of the day and HFI have a significant impact on the step length and the step selection. Hence, we suggest that pumas adjust their spatiotemporal behavior based on the degree of human modification, which is consistent with a higher landscape resistance in human landscapes (Gilbert et al., 2022; Suraci et al., 2019; Tucker et al., 2018).

Our study shows that pumas across different populations and geographical areas respond in a similar trend towards human disturbance, regardless of the component associated with human disturbance. This response, however, was expressed to different extents depending on the intensity of the human disturbance and includes an interplay between the spatial and temporal activity of the pumas. Thus, the inconsistencies that have previously been observed in the behavioral response of large carnivores to human disturbance may reflect different degrees of development featuring varying risks and rewards associated with human landscapes (Oriol-Cotterill et al. 2015, Carter and Linnell 2016). Ultimately, the responses that we have observed among pumas, as well as those documented among other carnivores, to human disturbance likely reverberate throughout the vertebrate community (Suraci et al. 2019). For example, avoidance of the top predator of human-disturbed landscapes has contributed to mesopredators expanding into new areas (Frey et al. 2020), increased activity of small mammals (Suraci et al. 2019), and the increased reliance of large carnivores on small prey (Moss et al. 2016a). These changes can disrupt local food webs and can result in altered ecosystem functions, broadening the implications of puma responses beyond immediate predator-prey interactions (Wirsing et al. 2021). To that end, the spatial-temporal dynamics of predators in human-disturbed ecosystems will be important in determining their functional role in these emerging ecosystems.

We highlight that pumas will utilize different parts of the landscape during different times of the day to access resources while avoiding humans, suggesting a perception of risk in both space and time. This spatiotemporal partitioning from humans allows carnivores to persist in novel landscapes despite the challenges posed by human expansion. This “landscape of coexistence” can be strategically managed to enhance the adaptive behaviors of carnivores, ensuring their survival and functional role within ecosystems and limiting human-carnivore conflict (Oriol-Cotterill et al. 2015). To that end, carnivore conservation efforts in developed areas can focus on mitigating impacts on the landscape, generating restoration measures and corridors that allow free passage of carnivores and the maintenance of their community interactions and foraging activity.

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Tables

Table 1: Structure of the integrative step selection models tested using a conditional logistic approach for puma (Puma concolor) movement in five sites across the Americas. All models included the movement variables: step length + log (step length) + cos(turning angle). Time-of-day refers to day or night calculated for each specific end of the step. The response variable (Case) indicates whether a step was observed or available. For each observed step, ten available steps were randomly generated. AIC values show the average from all the individual puma models.

Model	AIC	ΔAIC	AIC_{weight}
Elevation + Cumulative GPP + Distance to Water + Roughness + HFI + HFI:Time-of-day	7327.0	0.0	1.0
Elevation + Cumulative GPP + Distance to Water + Roughness + HFI	7343.1	16.1	0.0
Elevation + Cumulative GPP + Distance to Water + Slope + HFI	7344.2	17.3	0.0
Elevation + Cumulative GPP + Distance to Water + Roughness	7348.6	21.6	0.0
Elevation + Cumulative GPP + Distance to Water + HFI + HFI:Time-of-day	7352.0	25.0	0.0
Elevation + Cumulative GPP + Distance to Water + HFI	7368.5	41.5	0.0
Elevation + GPP Variability + Distance to Water	7374.2	47.2	0.0

Table 2: Model selection from the components of the Human Footprint Index for puma (Puma concolor) integrative step selection analysis in five sites across the Americas using the structure defined in the previous model selection Case ~ Elevation + Cumulative GPP + Distance to Water + Roughness + Human Variable + Human Variable:Time-of-day + step length + log (step length) + cos(turning angle). AIC values show the average from all the individual puma models.

Human Variable in Model	AIC	ΔAIC	AIC_{weight}
Total HFI	7327.0	0.0	0.9
Road Proximity	7330.6	3.6	0.1
Human Density	7347.6	20.6	0.0
Building Distance	7347.9	20.9	0.0
Agriculture Intensity	7348.7	21.7	0.0

Table 3: Selection coefficients (log-Resource Selection Strength) ± standard error showing habitat selection of pumas (Puma concolor) during day and night. Positive values represent preference, while negative values represent avoidance, in bold significant values.

Variable	Day	Night	Total
Elevation	**-0.24 ± 0.03**	0.05 ± 0.03	**-0.10 ± 0.02**
Cumulative GPP	**-0.37 ± 0.04**	0.08 ± 0.05	**-0.20 ± 0.03**
Distance to Water	**0.18 ± 0.06**	0.00 ± 0.06	**0.12 ± 0.04**
Roughness	**0.21 ± 0.10**	**-0.52 ± 0.12**	**1.05 ± 0.07**
HFI	**-0.30 ± 0.04**	**0.69 ± 0.06**	-0.03 ± 0.04
HFI:ToD(Night)	**-**	**-**	**0.40 ± 0.05**

Figures

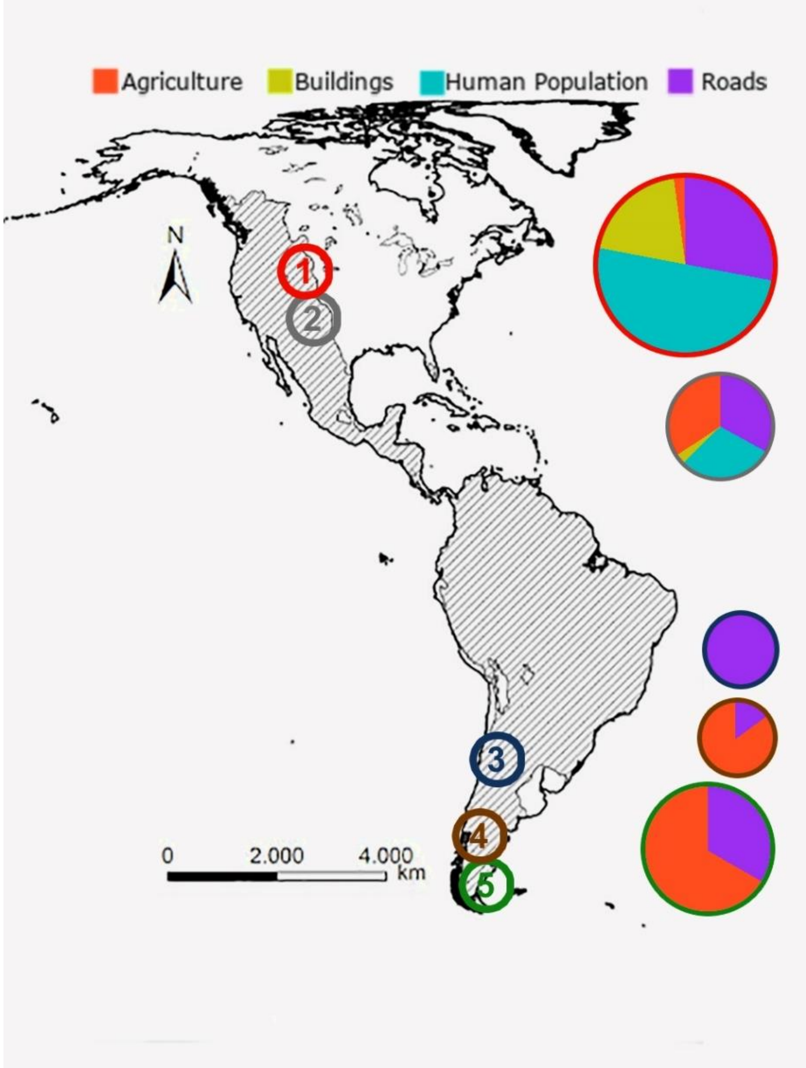

Figure 1: Puma (<u>Puma concolor</u>) study sites in the Americas: 1) Northern Front Range; 2) Southern Front Range; 3) San Guillermo National Park; 4) Parque Patagonia; 5) Monte León. Hatched area reflects the current distribution range of the puma (IUCN). Pie chart area reflects the total human footprint index (HFI) of each study site; the components of the HFI are categorized as agricultural (light red), road proximity (purple), human density (light blue) and built infrastructure (green).

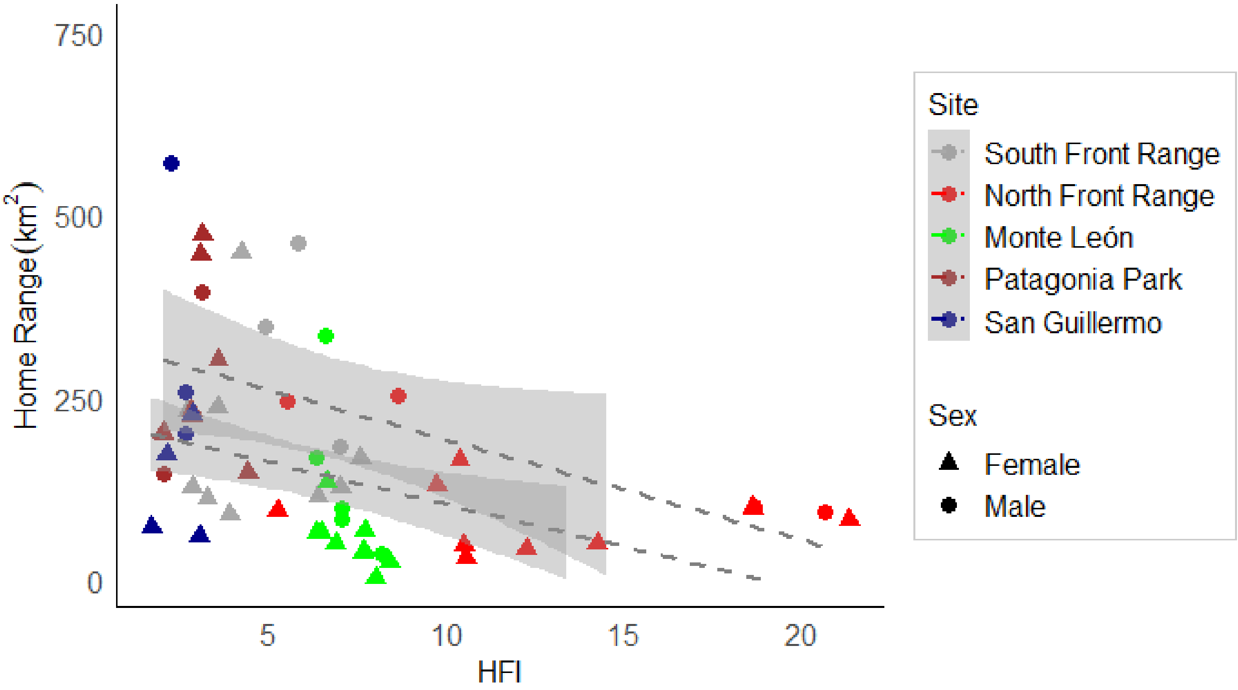

Figure 2: *Association between individual home range (HR) of pumas (Puma concolor) to the mean value of the human footprint index (HFI) within their respective home range in five different locations: South Front Range (grey) and North Front Range (blue) in Colorado, USA; Monte León National Park (green), Patagonia National Park (brown) in Santa Cruz, Argentina; and San Guillermo National Park (blue) in San Juan, Argentina. Trend lines represent the linear regression for males (dashed line and triangles) and females (solid line and circles) ± standard error.*

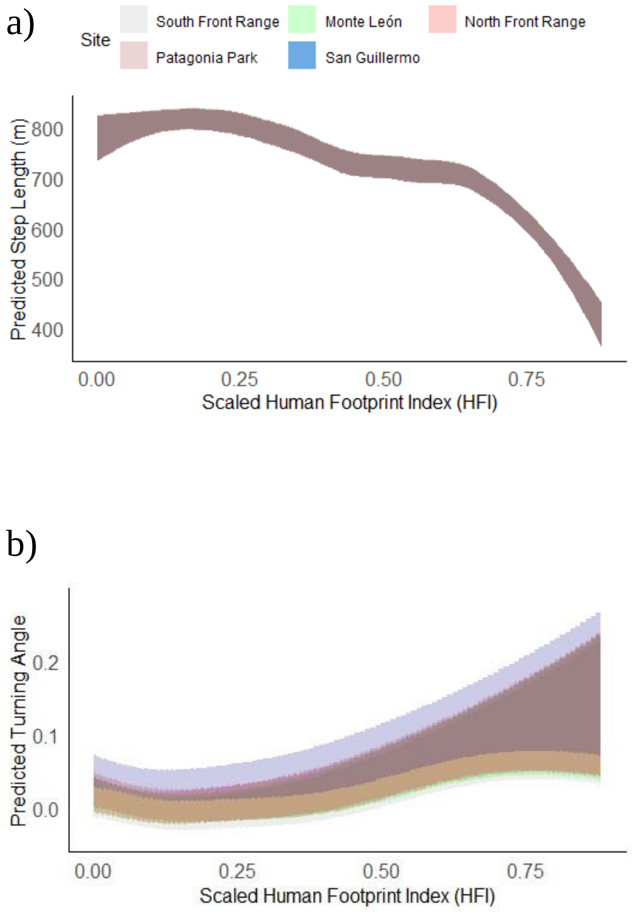

Figure 3: Predicted step length (a) and turning angle (b) of pumas (Puma concolor) from five sites across the Americas in response to the human footprint index (HFI) using a generalized additive model with site and individual ID as random factors ± standard errors. The predicted turning angle represents absolute values.

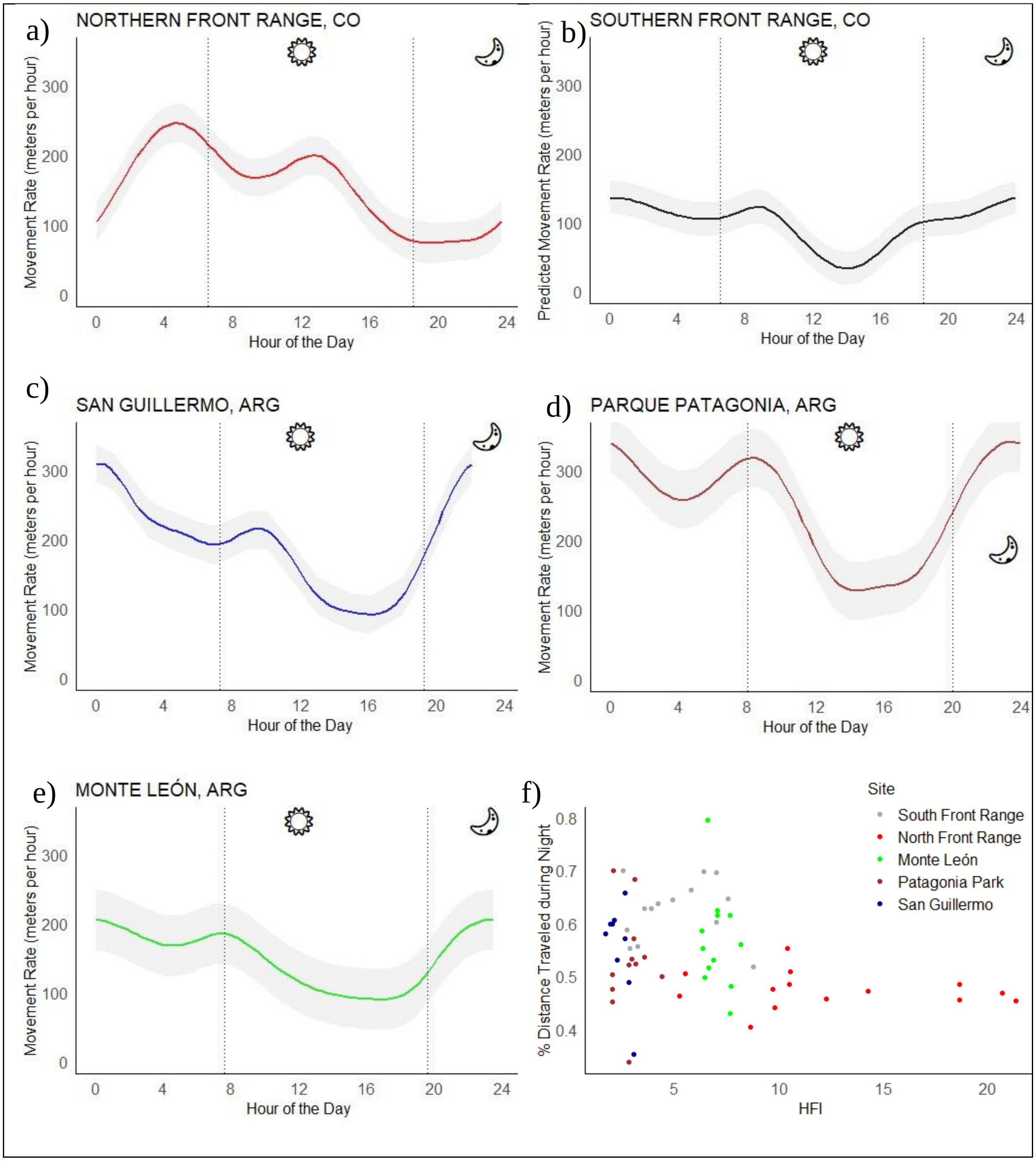

Figure 4: a-e)Predicted daily movement rate (m/h) of the puma (Puma concolor) with standard error from five sites fitted with a generalized additive mixed model. Dotted lines represent the sunrise and sunset times at the equinox. f) Individual values of the proportional distance traveled

during the night (nocturnality) and the mean human footprint index (HFI) value of the home range across the five sites.

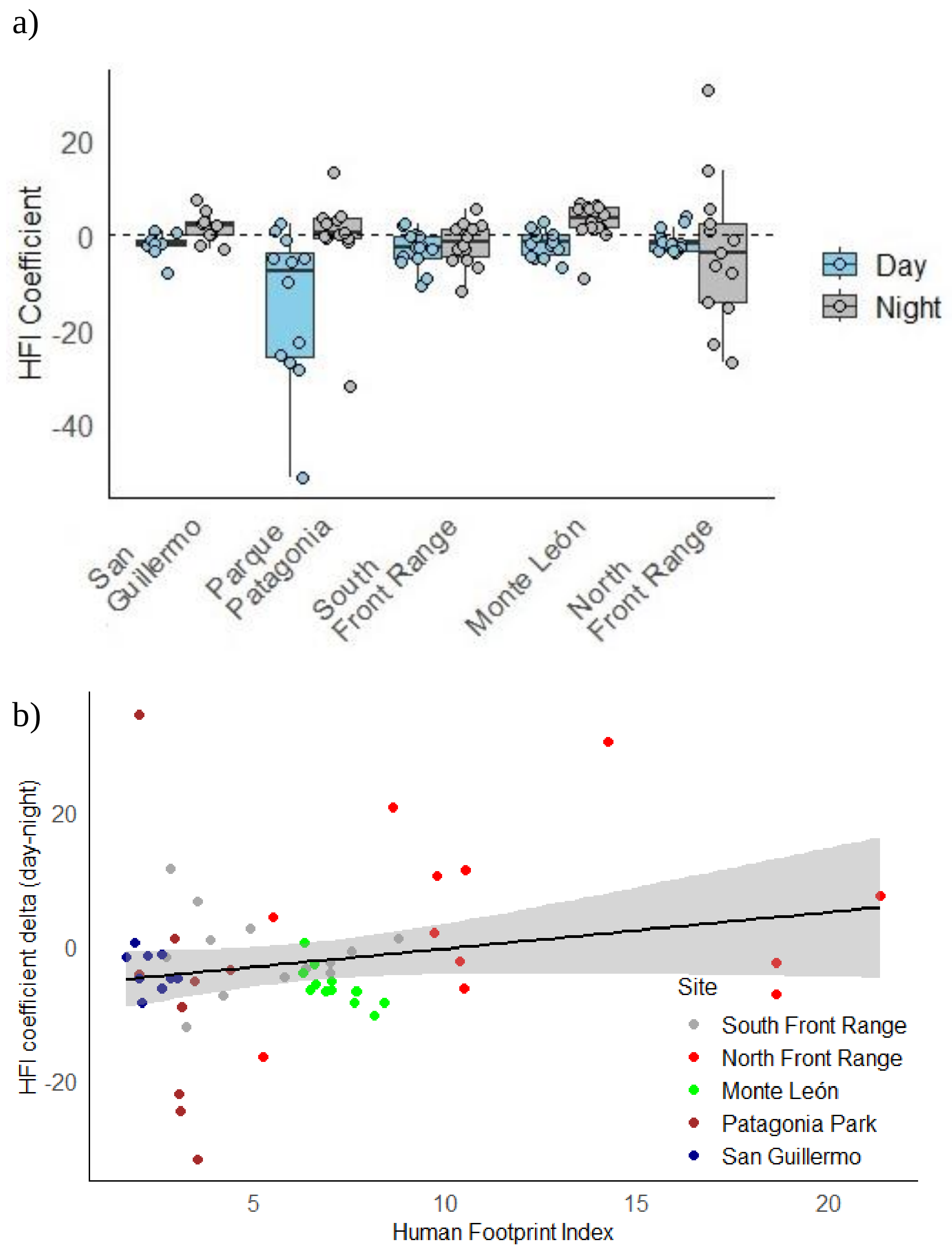

Fig 5: Selection coefficients of the integrative step selection analysis of the puma (Puma concolor) for the variable human footprint index (HFI): a) plotted from day (grey) and night (light blue) models in the five different populations. b) Delta HFI coefficient (as HFI day coefficient minus HFI Night Coefficient for each individual) as a function of the mean HFI of home range for each individual in the five sites.

Supplementary Materials

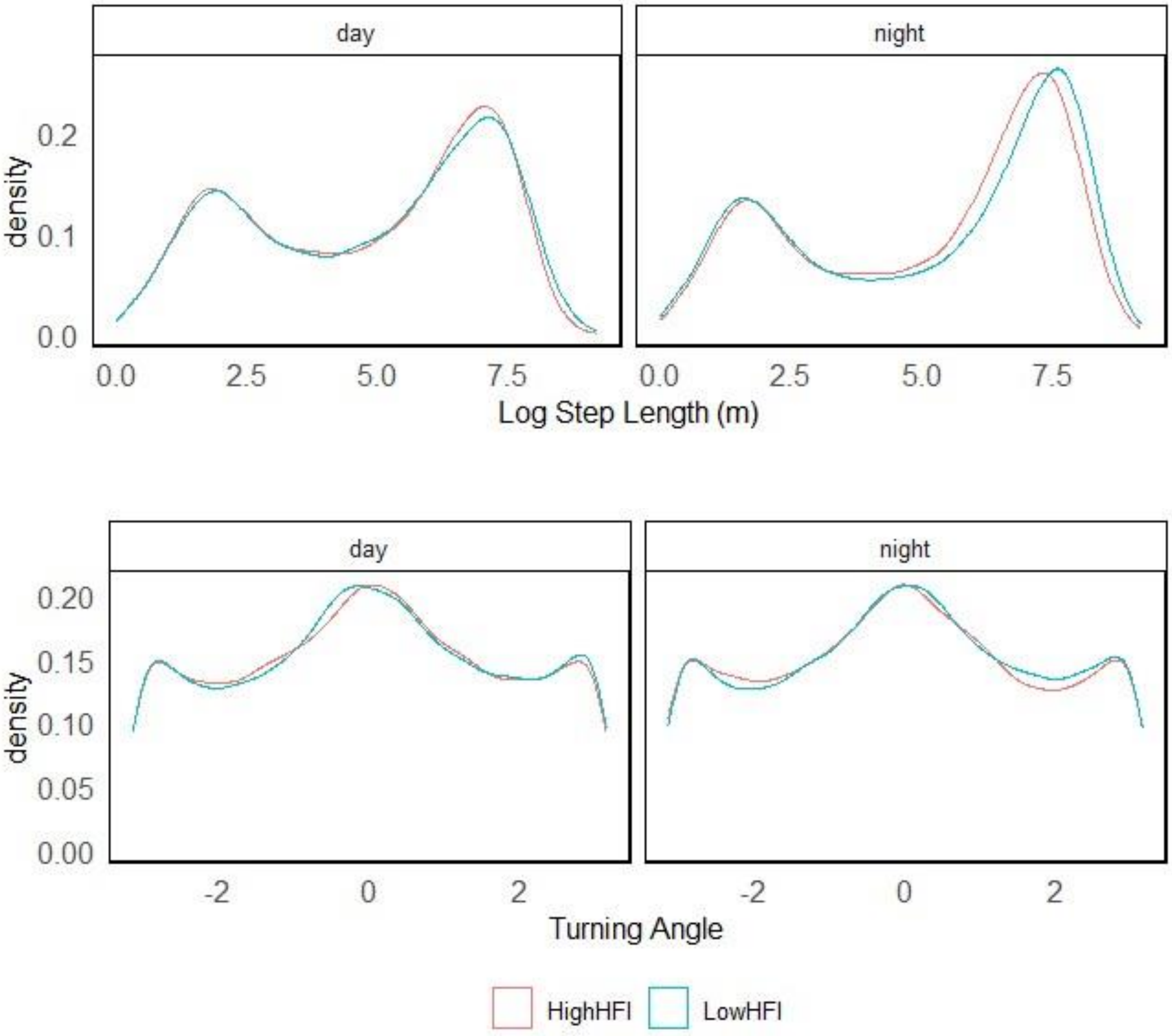

Figure S1: Puma (Puma concolor) step lengths (log, above) and turning angles (below) during day and night in low (in blue; San Guillermo, Parque Patagonia) and high (in red; Monte León, South Front Range, North Front Range) human footprint index (HFI) sites.