

Backyard Boldness: Investigating how Urban Landscape Heterogeneity Affects Mesopredator Risk-Taking Behavior

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ABSTRACT

As urbanization continues, wildlife are increasingly faced with novel challenges imposed by changes to their habitats. Consequently, urban wildlife have been observed as behaviorally different than their non-urban counterparts, often exhibiting greater boldness towards novel or risky situations. However, landscape variation driven by societal inequities (e.g., pollution, infrastructure, vegetation) creates habitat patches of differing quality that could influence an animal's risk perception. Although largely unexplored, the effects of societal inequity may be especially pronounced for generalists who capitalize on anthropogenic resources and are subsequently managed as pest species. In this study, we surveyed the risk-taking behavior of three common North American mesopredators (raccoons- *Procyon lotor*, striped skunks *Mephitis mephitis*, opossums- *Didelphis virginiana*) towards a novel foraging task ("puzzle"). In partnership with urban residents and organizations, we deployed 123 puzzles in yards and public green spaces across California's East Bay to evaluate how interspecies and intraspecies boldness variation relates to (1) habitat quality and (2) human presence. We then assessed whether the occurrence of agonistic interactions among conspecifics could be influenced by urban heterogeneity. Our results suggest that landscape heterogeneity has little to no impact on boldness and agonism; however, we discuss that this conclusion could be premature due to our small sample size. By analyzing our full dataset, our results would give a clear understanding of urban impacts which would contribute to a better understanding of how societal inequities spill over to shape urban wildlife ecology, with applications for improving human-wildlife coexistence and conservation in cities.

KEYWORDS

applied animal behavior, inter/intra species conflict, urban ecology, remote sensing, anthropogenic change

INTRODUCTION

To accommodate our growing population, it is projected that our urban land use will increase by 0.82 million square kilometers to fit the additional 13% of the population expected to live in urban areas by 2050 (Simkin et al. 2022). This expansion reduces habitats and biodiversity, forcing wildlife to either move into cities or retreat farther into more natural areas to make up for the loss of resources and space (Lowry et al. 2013, Russo and Ancillotto 2015). The animals which do move into cities are faced with new challenges, including navigating new resources and predators, overcoming barriers like roads and fences, coping with various types of pollution, and avoiding humans (Barrett et al. 2019, Lowry et al. 2013). The individuals whom overcome these obstacles are often behaviorally different from their rural counterparts, having altered foraging strategies, dispersal patterns, and sociality, as well as increased dietary flexibility, boldness, risk-taking, habituation, tolerance, and aggression (Lowry et al. 2013, Candolin et al. 2023, Farr et al. 2023, Stanton et al. 2021, Allen et al. 2022, Breck et al. 2019). To date, these behavioral shifts are seen in comparative analyses between rural to urban individuals, which characterize cities by decreases in natural resources and increases in anthropogenic disturbances (Stanton et al. 2021, Lowry et al. 2013). However, this depiction of urban spaces is oversimplified because the complexity of cities lies in the dispersal differences of natural and anthropogenic resources (Stanton et al. 2021, Lowry et al. 2013, Magle et al. 2021).

This unequal distribution of resources shapes the ecology of cities and impacts biodiversity, community composition, and species richness (Magle et al. 2021, Estien et al. 2024, Schell et al. 2021, Hassell et al. 2021, Murray et al. 2019). This mosaic distribution of resources typically stems from the social inequalities built into our neighborhoods as exemplified by luxury effects, legacy effects, and prestige ecology (Schell et al. 2020, Estien et al. 2023, Des Roches et al. 2021, Ramalho and Hobbs 2012). As cities become the next frontier for conservation, it is critical to understand how eco-social city dynamics impact wildlife behavior and health so we can create cities which embrace cohabitation and conservation (Uchida et al. 2021, Lowry et al. 2013, Murray et al. 2019). Despite this need, there is a knowledge gap on how our cities' heterogeneity impacts wildlife behavior and health (Uchida et al. 2021).

Prior to understanding effects from city heterogeneity, we must understand the species which live and thrive in cities. While larger species are slowly becoming more frequent sights in cities, they are pushed out of urban spaces first due to their extensive home ranges (Stanton et al. 2021). However, those species which are medium generalist carnivores (i.e., mesopredators) are the most successful in cities (Barrett et al. 2019, Stanton et al. 2021). The most notable of these synanthropic species are raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*; referred to as “skunks”), and Virginia opossums (*Didelphis virginiana*) (Barrett et al. 2019, Stanton et al. 2021). While each species has very different life histories and traits, they all tend to be nocturnal, flexible in diet, and habitat generalists (Allen et al. 2022, Wright et al. 2012, *Raccoon Nation* 2012, Stanton et al. 2021). These traits may have made them inclined to higher levels of boldness, which could be highly rewarding for individuals in the form of increased access to resources, increased territory acquisition, and more offspring (Lowry et al. 2013, Breck et al. 2019, Candolin et al. 2023). Despite their many achievements in urban survival, as non-charismatic species raccoons, skunks, and opossums face much backlash from humans due to their reputation for spreading diseases like rabies, making messes out of trash receptacles, and their unwanted residency in our homes (*Raccoon Nation* 2012, Allen et al. 2022, Wright et al. 2012). They are considered “nuisance” or “pest” species and face increased risk of lethal encounters with humans and heavier wildlife management (Schell et al. 2021, Barrett et al. 2019, Stanton et al. 2021). So while boldness may prove advantageous to urban species, it can also backfire and result in increased injury or death of individuals, making finding how the intricacies of our cities impact boldness critical to coexistence so we can create better management practices (Lowry et al. 2013, Schell et al. 2021).

To understand the impact our cities have on mesopredators like raccoons, skunks, and opossums, it is necessary to see which factors influence boldness, including natural and anthropogenic elements. Our objective was to understand how the boldness of mesopredators varies as a function of urban landscape heterogeneity. In our approach, we looked at how boldness is predicted by 1) habitat quality, 2) human presence, and 3) species as well as how the 4) frequency of agonistic encounters between conspecifics varies in response to landscape heterogeneity. We measured the proximity of individuals and their behavior towards a novel foraging puzzle to estimate the boldness of an individual towards novel anthropogenic objects with a possibility of a

food reward. To evaluate what has the highest effect on boldness, we examined the influence of seven landscape variables, which were grouped into indicators of habitat quality or human presence, and species on an individual's behavior towards the puzzle. Regarding agonism, we compared the occurrence of agonistic behavior to the spatial distribution of our landscape variables. We expected that mesopredators occupying areas with higher habitat quality (i.e., source habitats) would show decreased boldness relative to conspecifics. Similarly, we expected mesopredator boldness to be higher in areas with increased human presence. Regarding species effects on boldness, we predicted that raccoons will demonstrate higher boldness compared to other mesopredators (i.e., striped skunks and Virginia opossums). Finally, we expected to see higher occurrences of agonism in areas of lower habitat quality.

METHODS

Study sites

The Bay Area is a rapidly evolving urban landscape growing in population and urban sprawl located in Northern California containing the cities of San Francisco, Berkeley, and Oakland. The Bay Area has a Mediterranean climate defined by cool, wet winters and warm, dry summers (Ackelrly et al. 2018). With a variety of governments implementing different ordinances on land-use management in combination with preexisting legacy effects, the East Bay's urban areas have developed differently, resulting in differences in socio-environmental factors.

Within the East Bay, we selected puzzle setup sites based on transect sampling and permission availability of sites. In conjunction with the Urban Wildlife Information Network (UWIN) and one other project examining differences in biodiversity and cognitive behavior of mesopredators, we set up a total of 123 unique sites in a mix of residential, park, and industrial areas (Figure 1). Within each site, we identified a wildlife corridor/wildlife-frequented area and set up a Bushnell Trophy Cam HD E3 trail camera facing the puzzle (Figure 2).

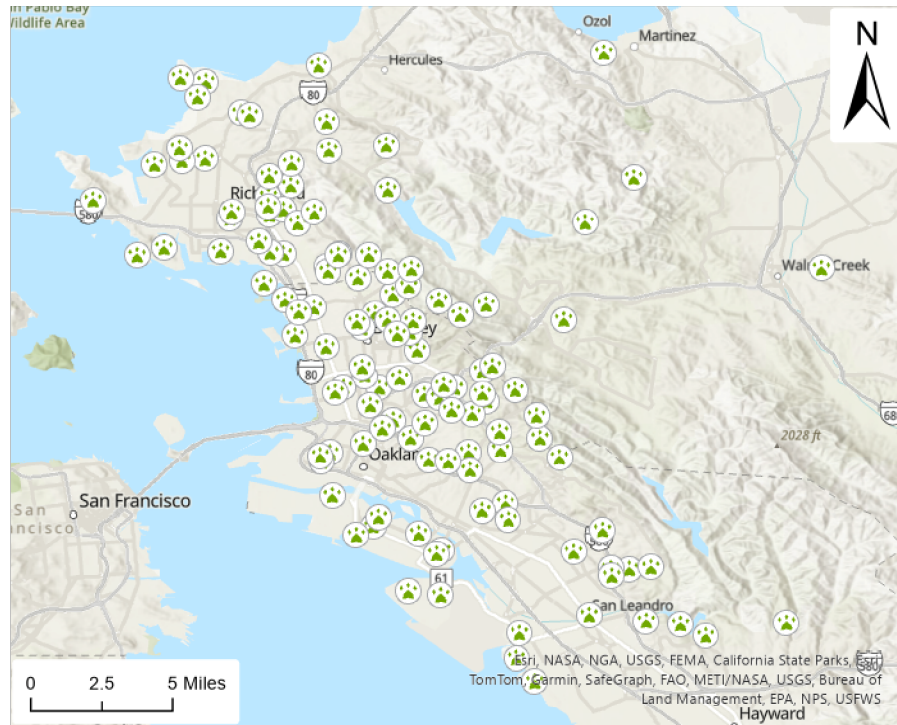


Figure 1. Map of study sites across East Bay, California. Each green paw print represents a single test site, with a total of 123 unique sites. Some sites were retested due to camera errors, human interference, and other miscellaneous disturbances, resulting in 132 total trials.

Puzzle construction

The puzzle consists of a 3.5 inch ABS plastic tube with two caps on either end of it, one of which has a ground anchoring system and the other which is attached via garden sisal rope. When subjects pull the rope or the cap off the tube, they are awarded a food reward. We placed the puzzle on the pathway in view of the camera, baited it, and secured it to the ground while allowing some movement. Once deployed, we recorded the time and date of setup, latitude and longitude, and environmental details of each site. We left puzzle setups to run for 2 weeks with weekly check-ins and removed them once the puzzle was solved or the trial time expired.



Figure 2. Camera and Puzzle Set Up. a) We set up cameras to capture 30-second videos of the puzzle from the clearest angle, with a 1-second interval between detections at normal trigger sensitivity. The camera is secured in a lockbox with a keyed lock, and strapped to a vertical structure (in this case, a tree) to capture movement in the cone of detection. We also placed placards near set-ups to deter human interference. b) Puzzle designed by L Stanton. The puzzle was secured to the ground allowing for slight movement and baited using 2 Tbsp of Friskies Seafood Sensations Dry Cat Food and a Sheba Salmon Flavored Meaty Tender Stick Cat Treat, which animals could scent through holes drilled into the tube. c) In order to be solved, animals had to either pull on the rope or cap of the puzzle, which was secured evenly across sites.

Measuring boldness

To measure the boldness of urban mesopredators we caught interacting with the puzzle, we created an ethogram outlining the different behaviors observed (Table 1). The ethogram helps quantify boldness by including the different “levels” of boldness (e.g., vigilant, curious, interactive, inattentive), social/conflict, and proximity to the puzzle which can be recorded using software called Behavioral Observation Research Interactive Software (BORIS) (Friard and Gamba 2016). Behavioral data was coded by three individuals, all of whom had coding agreeability scores greater than 0.8 for 10% of our sites to ensure no/limited coder bias. Protocols and independent variables used are included in Appendices A and B respectively. Sites were divided among coders, which included reliability coding which was done at the beginning, middle, and end of all assigned sites so that coder agreeability was consistent throughout all the videos.

Table 1. Boldness Ethogram. Behaviors are grouped according to the different metrics of boldness that they indicate (e.g., proximity, vigilance).

Behavior	Behavior Type	Description	Proximity
<i>Close</i>	State event	Focal animal is within one body length of puzzle	≤ 1 body length
<i>Far</i>	State event	Focal animal is greater than one body length of puzzle	> 1 body length

<i>Vigilant</i>	State event	Focal animal is alert and demonstrates hesitancy towards puzzle.	> 1 body length (generally)
<i>Curious</i>	State event	Focal animal is alert and demonstrates interest towards puzzle.	Any
<i>Interactive</i>	State event	Focal animal is alert and engaged with puzzle.	< 1 body length (generally)
<i>Inattentive</i>	State event	Focal animal demonstrates a lack of awareness, interest, or fear towards puzzle.	Any

<i>Social</i>	State event	Focal animal is not the only animal in frame	Any
<i>Conflict</i>	Point event	Focal animal aggresses another animal.	Any

<i>Look at camera</i>	Point event	Focal animal's eyes are directly facing camera.	Any
<i>Urinate</i>	Point event	Focal animal releases urine onto the ground near, or directly onto, puzzle while squatting, spraying, and/or lifting its leg.	< 1 body length (generally)
<i>Defecate</i>	Point event	Focal animal releases feces onto the ground near, or directly onto, puzzle while in a squatting position.	< 1 body length (generally)
<i>Muzzle</i>	Point event	Focal animal places muzzle in close proximity to the puzzle.	< 1 body length (generally)
<i>Solve</i>	Point event	Focal animal removes cap from end of tube.	Any
<i>Already solved</i>	Point event	Puzzle is open at the start of the video.	Any
<i>Other</i>	Point event	Other behavior of significance that is not included in ethogram.	Any
<i>Exit</i>	Point event	Focal animal exits frame for > 5 seconds	Any
<i>Video ends</i>	Point event	Video has stopped. (Not necessarily end of observation)	Any

Note: Additional row containing clarifying notes for video coders on description not included due to length. Full details can be found in Appendix C.

Measuring agonism

To measure agonistic behavior between conspecifics, we coded for when multiple individuals were captured by the camera and labeled this as “social” (Table 1). Within “social” durations, anytime an individual aggressed the other, “conflict” was recorded as a binary, point event. While these behaviors were recorded for intra and interspecies, they are most applicable for raccoons, as they tended to be more social of the three focal species.

Isolating environmental predictors

Datasets & geospatial processing

In order to find correlations between boldness behaviors and environmental heterogeneity, we retrieved environmental data for each puzzle set-up location. By inputting the coordinates of all the sites into ArcGIS Pro, we isolated the environmental data by rasterizing the raw variable data, creating a 500-meter buffer around each site, and extracting the mean and standard deviation of each variable using data zonal statistics. The environmental variables included as predictors were Normalized Difference Vegetation Index (NDVI) (USGS 2023), pollution burden (California Environmental Protection Agency 2022), Artificial Light At Night (ALAN) (Elvidge et al. 2017), noise pollution (U.S. Department of Transportation 2020), land surface temperature (USGS 2023), population density (ESRI Demographics 2023), and percentage of impervious surfaces (Dewitz et al. 2023).

For the Landsat 8 satellite imagery which was sourced from USGS, we selected for the year 2023 to match the years of the study, cloud cover of <10%, and selected for November to January to get the times with the highest vegetation. We downloaded Band 4 and 5 in order to calculate NDVI and Band 10 to calculate the land surface temperature.

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)} \text{ or } \frac{(Band 5 - Band 4)}{(Band 5 + Band 4)}$$

$$TOA = M_L \times Q_{cal} + A_L$$

M_L = Band — specific multiplicative rescaling factor

$$Q_{cal} = \text{Band 10}$$

A_L = Band — specific additive rescaling factor

$$LST = \left(\frac{K_1}{\left(\ln\left(\frac{K_2}{TOA}\right) + 1 \right)} \right) - 273.15$$

K_1 = Band — specific thermal conversion constant from the metadata

K_2 = Band — specific thermal conversion constant from the metadata

CalEnviroScreen 4.0 utilizes many environmental effects and exposures including air quality (ozone), particulate matter (PM2.5), children’s lead risk from housing, diesel particulate matter, drinking water contaminants, pesticide use, toxic releases from facilities, traffic impacts, cleanup sites, groundwater threats, hazardous waste generators and facilities, impaired water bodies, and solid waste sites and facilities to generate the pollution burden of a given area (California Environmental Protection Agency 2022). However, this metric compares rural and urban locations using percentiles, so the percentile scores require rebinning to ensure comparability across an urban gradient. To establish a new baseline for the pollution burden data, we calculated the mean raw pollution scores of each buffer zone and then generated new percentiles to make the burden scores relative to each other.

Predictor PCAs

To reduce the dimensionality of the dataset, as these landscape variables are often found heavily correlated with each other, we separated the variables into two groups habitat quality (NDVI, pollution burden, ALAN, noise pollution, and surface temperature) and human presence (population density and percentage of impervious surfaces) and isolated the environmental predictor variables for each site (Liu et al. 2022). Subsequently, we conducted a PCA for habitat quality and human presence in R and plotted it using the *ggplot2* package (R Core Team 2021, Wickham 2016). This process aided in identifying priority sites and outliers that could be utilized

for preliminary data or excluded from the dataset. Habitat quality PC1 and human presence PC1 were subsequently employed as the predictor variables for all further analyses.

Creating predictor models

To assess boldness in response to the predictors, we used the first observation of each species and created generalized linear mixed models (GLMMs) which plotted the binomial distribution of whether an individual made contact with the puzzle (yes or no) relative to chosen predictors (habitat quality or human presence) with species as the random factor using the ‘*nlme*’ packages (Pinheiro et al. 2023). Then we confirmed no assumptions of the models were violated. To test our models, we used the traditional significance level of a p-value greater than 0.05 along with ensured our confidence intervals did not overlap with zero. Finally, we assessed how well our model fit our data by calculating the R^2 value, which revealed whether boldness was due to the fixed effects or the random effect of species.

To assess boldness in response to species, we created a bar plot to which demonstrated the probability of an individual within species likelihood of muzzling with the puzzle for all species encountered. Furthermore, we ran a chi-square test to see if there were any significant differences between our three species, raccoons, striped skunks, and Virginia opossums. We then ran a post hoc analysis using Tukey’s HSD test to tell which species combinations were significantly different.

To assess agnostic responses in response to landscape heterogeneity, we ran a GLMM plotting “conflict” frequency within raccoons to PC1 habitat quality and PC1 human presence. Since our response variable of conflict is a binary response, we created a binomial distribution. We then assessed multicollinearity and made sure we didn't over fit the model with the steps described above. For any instances of multicollinearity, we ran individual binomial distributions.

Finally, to see what was driving any trends found within the three boldness models using PCA data, whether they were due to a combined effect of landscape variables or if they were individual effects, we ran an akaike information criterion (AIC) using the ‘*MuMIn*’ package to compare individual landscape variables to see which best fit the model and has the highest impact on boldness (Bartoń 2023).

RESULTS

Puzzle interaction summary

Of our 132 sites, we were able to analyze 41 of them in depth, which resulted in 60 observations for our three focal species first encounter with the puzzle. Our preliminary data found that 83 out of 132 of them were interacted with and solved. Within our observations, we had 70% of raccoons muzzle the puzzle on their first observation at each site, 75% of opossums, and 26% of skunks (Figure 3). Of those who muzzled the puzzle, 95% of raccoons, 92% of opossums, and 100% of skunks continued to engage with the puzzle and interacted with it. In contrast with the 30% of raccoons, 25% of opossums, and 74% of skunks who did not interact with the puzzle at all (Figure 3).

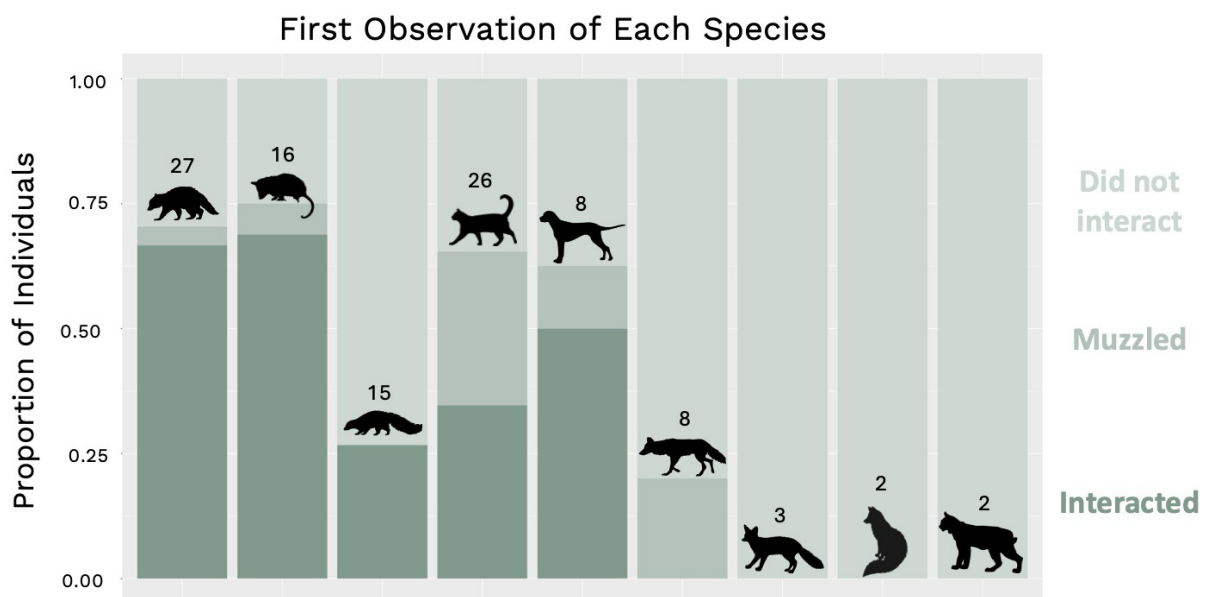


Figure 3. Behavioral State of Each Species During First Observation. Bar plot indicates the proportional breakdown of species' behavioral states (inattentive/vigilant, curious, or interactive) for the first observation of each species at each site. More vigilant or inattentive individuals who neglected to interact with the puzzle are indicated by light green. The slightly darker green represents individuals who got close and muzzled the puzzle, but didn't move it. While bolder individuals who interacted and moved the puzzle are denoted by the darker green. Column labels from left to right are raccoons, Virginia opossums, skunks, domestic cats, domestic dogs, coyotes, gray foxes, red foxes, and bobcats.

With a visual analysis, we found that the environmental variables used to measure urbanization were decently covered by our 41 sites, as displayed in Figure 4. Furthermore, our variables were found to be heavily correlated with each other (Appendix D). The strongest positive correlations were between

population density and impervious surface percentage (0.77), land surface temperature and ALAN (0.60), noise and ALAN (0.45), and impervious surface percentage and pollution burden (0.41). Those with the strongest negative correlations were between NDVI and ALAN (-0.76), land surface temperature and NDVI (-0.42), and noise pollution and NDVI (-0.37).

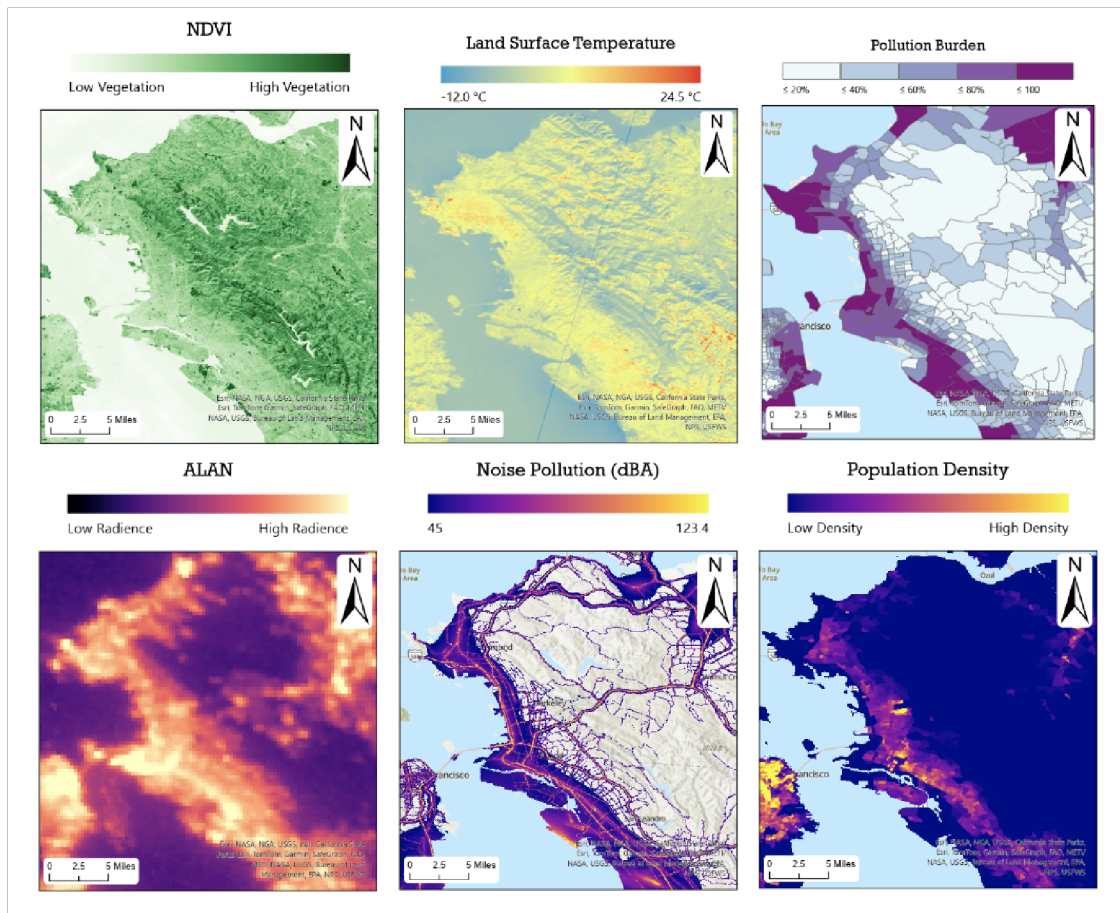


Figure 4. Urban Landscape Heterogeneity of East Bay, California. The figure includes multiple maps displaying the distribution of a different environmental predictor variable across the East Bay.

Predicting boldness

Habitat quality as a predictor of boldness

We found habitat quality was a weak predictor of boldness, since only the model predicting whether an individual would interact with the puzzle. We found that the landscape variables were highly correlated with each other (Appendix D). This violated the assumption of the GLMM regarding multicollinearity so used the PCA groups we ran which revealed that the [x%] of the

habitat quality predictors were correlated and that [x%] of the human presence predictors were correlated so the PC1 for each group contain [x amount and x amount] of the dataset, respectively. We found that the p-value of Model 1 was [x.xxx] which suggests that habitat quality has a significant effect on whether an individual would touch (muzzle) the puzzle. The confidence intervals didn't overlap with 0 supporting the p-value. The effect size was [x] which revealed habitat quality to be a strong predictor of muzzle probability. Finally, our model's R^2 was [x > 0.3] which indicates the model is a good fit for the data and that individuals interacting with the puzzle can be explained more by the effects of habitat quality than the random effects of species (Figure 5).

Human presence as a predictor of boldness

Since the predictor variables of human presence were collinear, we used the PC1 human presence as a predictor rather than independent variables. Similar to habitat quality, we found that PC1 human presence was a poor predictor of whether an individual would touch/muzzle the puzzle due to the p-value well over the desired significance value, the confidence intervals overlapping with 0, all of which indicate that human presence is an insignificant and poor predictor of whether an individual will muzzle the puzzle. The R^2 value was [x > 0.3] indicating that the model is a poor fit to the binomial distribution and that the effects seen are due more to the effects of species than human presence. However, in comparison to PC1 habitat quality, the habitat quality is a worse predictor of the likelihood of touch since the R^2 value is greater than that of the human presence model, [R^2 habitat quality > R^2 human presence] (Figure 5).

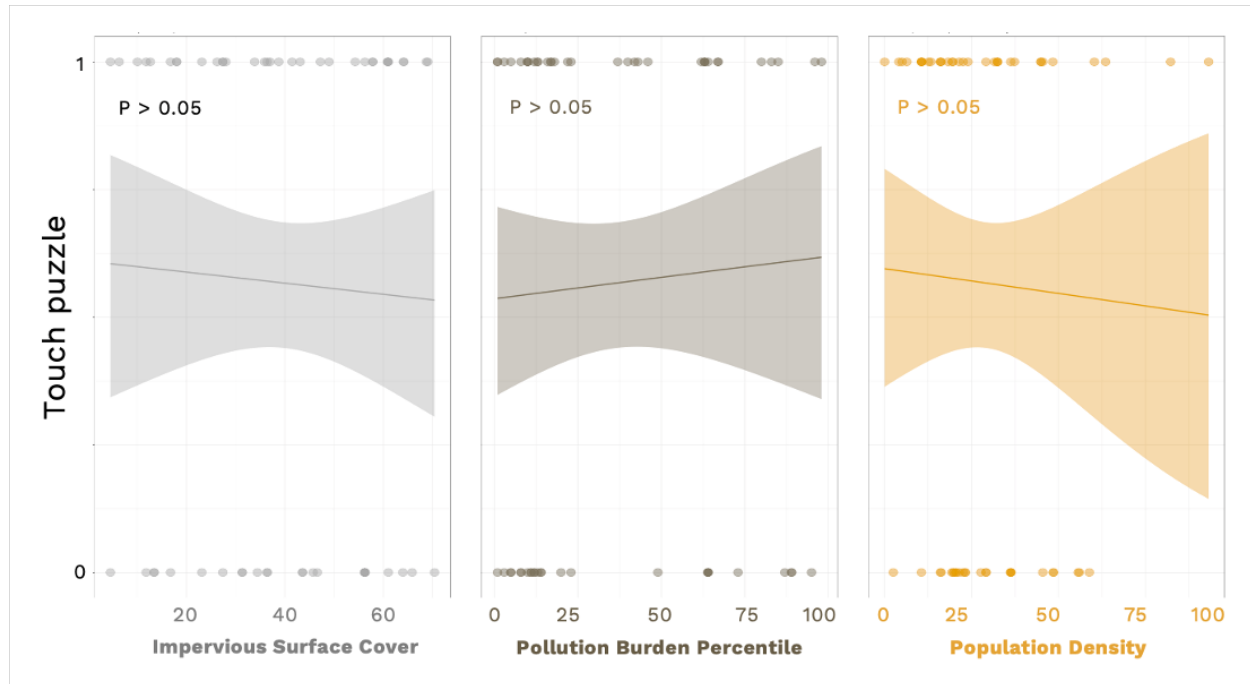


Figure 5. Boldness Predictor Models. a) PC1 Habitat Quality on Probability of Touch. b) PC1 Human Presence on Probability of Touch.

Species as a predictor of boldness

We found that opossums were the most likely to muzzle and interact with the puzzle, with raccoons coming in a close second. Domestic cats, domestic dogs, and skunks were also more likely to muzzle the puzzle compared to coyotes, gray foxes, red foxes, and bobcats (Figure 3). When we ran our chi-squared test on our three focal species, we found that there are significant differences among species ($p = 0.004314$). Our post hoc analysis revealed this trend to be driven mainly by skunks, which had a pvalue of 0.027 between opossums vs. skunks and a value of 0.040 between raccoons vs. skunks (Figure 3).

Predicting agonism

Regarding predicting agonistic behavior between individuals, we found that out of all our sites, there were only 13 which had social interactions between raccoons. Within this dataset, 38% of social interactions had agonistic interactions occur between raccoons.

Since the predictor variables were proved to be collinear with each other, we opted to run each landscape variable individually rather than a PCA due to our small sample size. Each of our models were found to be insignificant; however, visual analysis suggests beginning trends forming between higher raccoon agonism and human presence over habitat quality. Within habitat quality, higher aggression was found to increase with noise pollution, light pollution, and pollution burden. From visual analysis, NDVI had no impact on raccoon conflict.

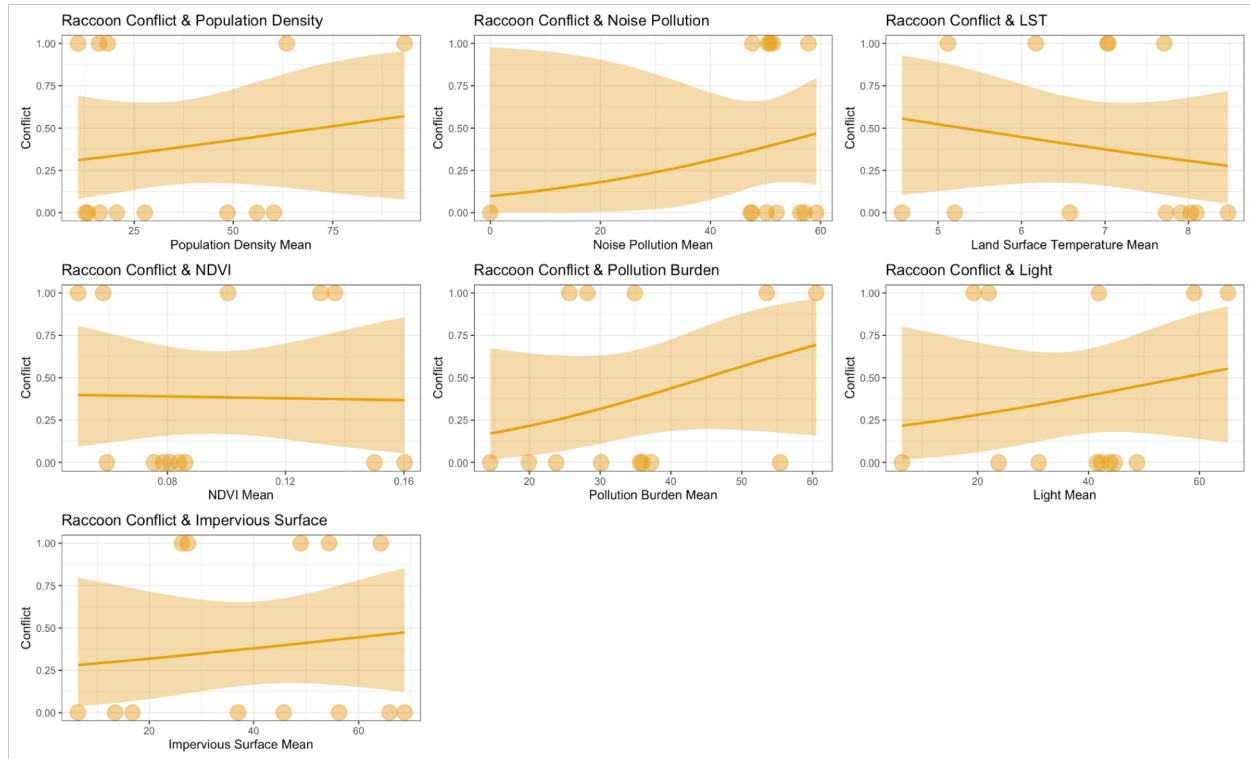


Figure 6. Agonism Probability Models. Due to a sample size of 13, all analyses were insignificant and trends were determined visually.

DISCUSSION

Boldness is established as an advantageous trait for urban survival, but carries just as many dangers due to the increased risk of injury and death (Lowry et al. 2013, Candolin et al. 2023). We compared how variations in urban landscapes and species impact boldness of three synanthropic mesopredators. While all landscape variables had insignificant effects on boldness, prior research shows significant impacts of landscape heterogeneity on the ecology in cities (Schell et al. 2020, Estien et al. 2023, Mangle et al. 2021). We attribute this result to a low sample size rather than a lack of an effect of landscape on boldness. Furthermore, we are able to validate our hypothesis that

species is a good predictor of boldness, based on a visual analysis of the species boldness variation and our post hoc analysis. This revealed that among species, skunks exhibit different responses, most likely driven by differences in life history and habituation (Allen et al. 2022, Uchida et al. 2022, Stanton et al. 2021). More data will help us determine whether there is a potential positive trend occurring between agonist frequency and variation in the urban landscape. The developing patterns in our data could provide key insights into which aspects of our cities we should enhance or reduce to manage wildlife, as well as show how the bias we build into our neighborhoods affect the wildlife whom are learning to live with us. Despite only having a third of our total data processed, we are seeing trends start to show a connection between increased boldness and higher light pollution, and we expect that with more observations these trends will strengthen. Regardless, the continuation and need for more research on how our cities' affect wildlife behavior and health will allow us to make more informed decisions of wildlife management and elevate our cities as areas of biodiversity conversation.

Predicting boldness

Urban landscape heterogeneity as a predictor of boldness

Habitat quality is able to explain boldness a little more than human presence, as indicated by the AIC model fitness test, which listed light pollution as the model with most impact despite insignificance. However, due to the fact that each grouping of variables was arbitrary and all the landscape variables are correlated with each other, we ran additional models which looked at the independent effects of each variable (Appendix D). Once separated, we found light has the most impact on boldness. Due to light pollution increasing visibility in an area, mesopredators like raccoons, opossums, and skunks may be deterred from interacting with the puzzle as the increased light makes it more difficult to hide from predators (Gaynor et al. 2018, Longcore & Rich 2004). While light was the best model for boldness for landscape variables, all the other landscape variables were insignificant. We attribute these insignificant variables mainly due to our low sample sizes; however, if the trend of insignificance continues once all the sample size is fitting to the number of fixed effects in our model, this could be due to our current inability to account for

inattentive behavior. Since we can't make assumptions on animal behavior without additional cues, reasons for inattentiveness are difficult to pinpoint. Whether the lack of interactions can be explained by species simply not detecting the puzzle or out of a lack of motivation, we do not have the current methodology to quantify these differences. If species are monopolizing other more accessible anthropogenic resources like trash cans or pet food dishes, they may simply neglect to engage with the puzzle since it would be an additional energy expenditure. On the other hand, if species are experiencing higher predation pressure, the puzzle may serve as a distraction to prey being vigilant from the rest of their surroundings (Ciuti et al. 2012). With this in mind, we can't be so hasty to dismiss the possibility of these budding trends having significant effects until we finish our dataset and have a larger sample size.

Species as a predictor of boldness

As the most significant predictor of boldness, differences in boldness among species shed light on how habituation and lifestyle histories impact risk taking. From all these videos, we found variation in behavioral responses to the puzzle within and between species. With the species which were more likely to touch and muzzle the puzzle, being those which are known to thrive in urban spaces and experience high levels of habituation (Stanton et al. 2020, Uchida et al. 2019). For our three primary species of interest (raccoons, opossums, skunks) we conducted a chi-square test that showed significant differences among species. Post-hoc analysis confirmed that this difference is driven by skunks, who are exhibiting a different response compared with raccoons and opossums, whereas raccoons and opossums are not significantly different from each other. These differences may be driven by dissimilarities in physiology, foraging methods, and general methods of interaction driven by morphological constraints (Allen et al. 2019, Iwaniuk and Whishaw 1999). When we looked at landscape variables in relation to boldness, we did notice grouping of species in connection with the landscape differences, which may indicate that the distribution of natural and anthropogenic effector may have some effect on species occurrence.

Predicting agonism

With the emergence of trends indicating agonism is increased in areas of higher pollution burden, noise pollution, and light pollution, we suspect that increases in anthropogenic pollutants could be connected with increased aggression which has similarly been mirrored in various other interspecies conflict and human-centric conflict (Krapf et al. 2023, Wilkinson et al. 2023). While some altered skunk interactions were expected due to past literature, none were observed possibly due to the low sample size of species interactions; however, raccoons are known to form loose social groups which could account for the increased amounts of conciliatory behavior towards each other (Allen et al. 2022, *Raccoon Nation* 2012). Regardless of our very limited data regarding agonism occurrence during social encounters, (n=13), visual analysis suggests the beginning of positive trends forming between lower habitat quality and increased agonist occurrence, as well as higher human presence and increased agonist occurrence. These starting trends are as we expected since it's known that as resources become more limited, competition increases, resulting in more conflict (Schell et al. 2021, Allen et al. 2022). Within the habitat quality grouping, the models which best explained these rising trends were those that align with increased pollution burden, noise pollution, and light pollution. The connection between increased pollution and aggression/agonism implies that those species living in more polluted areas are more prone to conflict, which opens more opportunities for injury or death. Overall, we attribute the insignificance of our data to be a result of our current data deficiency, but expect that with more data, stronger conclusions can be drawn.

Limitations and future directions

The largest limitation of our study was the amount of data we were able to process and analyze over a short timespan. While we set up for 123 sites, we were only able to process 41 of them due to the number of videos we caught at each site as well as the sizeable time commitment that coding each site entails. Traditional statistical practices suggested that for each fixed effect one has in a mixed model that there should be 10–20 observations; however, with seven fixed effects and only 60 observations, our study misses that mark. As a result of this, our results lack the sufficient data needed to find correlations. Furthermore, as most behavioral studies, a large

limitation of this project is the inability to measure the interest and motivation of the participants of our study, which was evident in the amount of time we coded for “inattentive” behavior. As such, we are unable to state that they had increased boldness as a result of not being wary of the puzzle or whether they simply failed to detect the puzzle. Additionally, individuals may have neglected to interact with the puzzle not out of caution for anthropogenic items, but for the fact that the puzzle would require them to spend less time being vigilant toward other dangers, especially if there is high predation pressure in the area (Ciuti et al. 2012). Finally, since our study required the training of multiple individuals to be able to recognize certain behaviors with as little bias as possible, our protocol, and ethogram evolved over time, so results should be interpreted with caution.

Nevertheless, as we collect more data and continue to code for these boldness behaviors, we can draw stronger conclusions regarding what increased boldness in cities. By applying a new framework towards measuring boldness, as we did in our study, we may be able to quantify the perceptions of urban wildlife towards novel anthropogenic objects in less invasive ways than measuring flight initiation distance and alert distance (Uchida et al. 2021). In an effort to better understand boldness, studies like this should be conducted in multiple cities of differing size, population, and range as California is known for its many natural wonders including the most national parks of any other state which could suggest higher affinities for the outdoor and wildlife. With increased access and usage of natural spaces, people may be more inclined to feed animals or leave food sources for them, leading to higher habituation levels which can effect boldness heavily (Stevens 2024, Uchida et al. 2021).

Broader implications

Despite being a new field of research, studies done within urban ecology have drawn clear connections between how as a part of the ecosystem, we humans, have large impacts on the ecology in and of our cities (Pickett et al. 2016, Schell et al. 2021, Murray et al. 2019, Des Roches et al. 2021). With the human population increasing at an exponential rate, figuring out how we can embrace the interconnectedness between environmental health, human health, and wildlife health while acknowledging social justice's necessary role in the policies we make will lead us to create

better cities for humans and wildlife alike (Schell et al. 2020, Murray et al. 2022). Since the human population will undoubtedly continue to increase, designing better cities ensures we can minimize our impact on preexisting biodiversity hotspots as we expand, before conservation becomes a matter of saving a species from extinction (Weller 2018, Seto 2022). Furthermore, as we fortify the ecological relationships within our cities, we create stronger barriers which protect One Health, making all species healthier in the long run (Murray et al. 2022, Pickett et al. 2016).

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APPENDIX A: Video Coding Protocol

Step	Description
1	Once animal is detected, determine the first and last video of an observation. Note that observations are separated by at least 2 minutes and/or by species (e.g., if a skunk is interacting with a puzzle and leaves and a raccoon comes 30 seconds later, that should be scores as two separate observations) and is ended once (1) an individual solves the puzzle by removing the cap or (2) the individual exits and doesn't return for >2 minutes
2	Enter observation ID as date_location_observation# (e.g., 11142023_Ahern_01).
3	Enter date and time of first video for observation (collected from trail camera).
4	Fill out independent variables (second sheet in Workbook).
5	Add media (videos) for the identified observation.
6	Code for behaviors as specified in ethogram (either Boldness or Exploration, depending on your group). Save observations frequently (at least after every video) by hitting save or COMMAND+S.
7	Once coding for an observation has been completed, review next set of videos until the next observation is identified and begin again.
8	Once all observations for a date have been completed, select export events > aggregated events and select all for everything. Save as date, location name, and observer initials (e.g., 111421_Ahern_LAS).

APPENDIX B: Independent Variables Noted in Videos

Variable	Description
Location	Name of study site where the observation occurred (taken from folder name).
Observer	Initials of observer (3 letters)
Observation Number	Specific observation number for a given location; in chronological order
Date	Date (mm/dd/yyyy) that observation occurred; collected from trail camera.
Time	Time (military) that observation occurred; collected from trail camera
Species	Mesopredator species that was observed on camera. Most common include opossum, skunk, raccoon, coyote, and domestic cat. Others may include gray fox, red fox, domestic dog, bobcat, mountain lion. All other species will be ignored. If unsure of species identification, ask Lauren or Gretel. If multiple species in observation include both species names here seperated by a coma (e.g., skunk, raccoon)
# of Individuals	Max number of individuals in a group that appear during the observation. If multiple species in observation include number of individuals seperated by a coma with respect to the order made in species variable (e.g.. 2, 1)
Weather	Windy and/or clear or precip (e.g., clear, clear&windy, windy, windy&precip, precip).
Photoperiod	Day = 7:00am-5:00pm; Night = 5:01pm-6:59am.
Visible Water Sources	Record the number of water sources set out in location (e.g., water bowls, buckets, etc.)
Notes	Include observed state of health (e.g., healthy, missing tail, mange) Any notes regarding what was observed. If puzzle was solved off camera, make a note and include any guess as to which species solved it (if possible).

APPENDIX C: Additional Ethogram Notes

Behavior	Notes
Close	Focal animal in video must always be labeled as either close or far. If animal is on the border of close and far, label as close.
Far	Focal animal in video must always be labeled as either close or far.
Vigilant	The focal animal's head/eyes/nose are focused on the puzzle. They will be walking hesitantly towards or around puzzle, stretching their head and nose towards puzzle while keeping body and limbs away, and generally avoiding making contact with the puzzle. Their ears may be erect or tucked, and their tail may also be either erect (skunk, cats) or tucked (canids). The focal animal may also be flinching, bobbing their head, leaning away from the puzzle, or actively scanning its surroundings.
Curious	The focal animal's head/eyes/nose are focused on the puzzle. They may be walking assuredly towards the puzzle and "muzzle" or sniff the puzzle at least once each time you make curious. Their ears are likely to be erect, the tail may or may not be relaxed (up or down), and they are likely to be actively sniffing. When they make contact with the puzzle, the behavior should switch to interactive.
Interactive	The focal animal's head/eyes/nose are focused on puzzle and they are generally making continuous contact with puzzle. Brief pauses < 5 sec in interactions are permitted. Interactions for canids (foxes, coyotes) include leaning or digging at puzzle.
Inattentive	The focal animal's head/eyes/nose are NOT focused on the puzzle and they are not engaging with the puzzle. E.g., animal walks past puzzle without looking at it, animal is grooming next to puzzle, animal stops and scans its surroundings for > 5 seconds. Please clarify why animal is inattentive in notes by specifying whether they are apathetic (aware but do not seem to care), distracted (aware but something is happening in the environment), or unaware (unclear whether they know the puzzle is there).
Social	Both animals should be present in same observation at least once. Identify other species present in notes.
Conflict	The focal animal may charge, snap/bite, body-block, or vocalize at another individual. The behavior should be scored for the aggressor, not the animal receiving the aggression (unless both participants demonstrate aggression, then both should receive a score).
Look at camera	Distinct bouts of looking at the camera must be separated by the animal looking away before looking back at camera.
Urinate	
Defecate	
Muzzle	Puzzle does not move and should only be scored when there is no other behavior taking place (e.g., paw, push, pull, etc.). Includes hovering over, sniffing, and light touching of any part of the puzzle with the nose and/or mouth (inc. tongue). Does not need to be scored during "interactive" state.
Solve	Once puzzle is solved, the current observation will end and no further observations for the given location are required.
Already solved	Only scored when solving of puzzle is not recorded on video. Animal does not have to be in the video. E.g., if the puzzle is solved at the beginning of the video and you are unsure of who opened it, then select "unknown" as the animal ID and then select "already solved."
Other	Examples: vocalizations, animal carrying prey, etc.
Exit	When this is selected, ongoing recordings of behaviors will end.
Video ends	When this is selected, ongoing recordings of behaviors will end.

APPENDIX D: Variable Correlations

