Stress in the city: investigating the effect of urbanization on coyote oxidative stress and diet

Background: As the world urbanizes, animals are adapting to novel environmental disturbances in human-dominated landscapes. The type and magnitude of these stressors vary considerably with human activity, culture, and *socioeconomic status (SES*), and each can influence the amount of biodiversity within a city[1,2]. Wealthier neighborhoods (higher SES areas) generally exhibit higher biodiversity and greater food availability, collectively known as the *luxury effect*[1,2]. Paradoxically, some low SES areas exhibit high biodiversity by providing greater refugia (e.g., abandoned buildings) for prey[2]. Overall, the luxury effect has repeatedly been shown to affect ecological dynamics at the community level, principally shaping species assemblages and interactions, which ultimately affect population and organismal ecology[1,2]. **Few studies, however, have investigated how the luxury effect and urban stressors (e.g., light pollution) interact to affect the ecology and physiology of urban wildlife.**

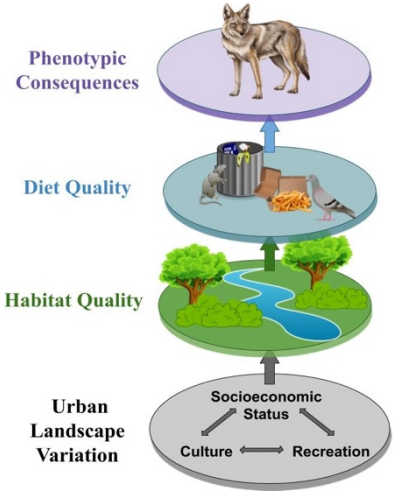

Figure 1. Here, I depict the cascading effect of urbanization. Urbanization directly influences habitats which can alter food availability. These factors can vary within cities, changing the phenotypes of urban wildlife (e.g., physiology).

Wildlife in urban and low SES environments face distinct environmental pressures relative to their conspecifics in rural and high SES areas[1]. However, how within-city differences in disturbance (e.g., noise pollution) affect stress levels and fitness outcomes is largely unexplored. Given that stressors in low SES neighborhoods are magnified[1], organisms in these areas may experience greater oxidative stress, an imbalance between free radicals and antioxidants in the body[4,5]. Biological responses to oxidative stress can vary with environment, genotype, and activity levels[5]. Further, antioxidants gained through food can provide a defense against oxidative stress[6]. Anthropogenic food, however, is often protein-poor and low in antioxidants[5]. Hence, wildlife that exploits such resources are likely to develop health risks[7] (e.g., hyperglycemia, which is correlated with low immunity[8]) and are at higher risk of the deleterious effects of free radicals[4], including apoptosis[5]. Because these effects can reduce fitness[4], **it is imperative to uncover how human-driven impacts on habitat and diet shape an individual's ability to cope metabolically with anthropogenic stressors (Fig. 1).**

My research will investigate the effects of within-city and among-city variation on the physiology of coyotes (*Canis latrans*). Specifically, **I will test how oxidative stress and diet vary along an urban-socioeconomic gradient.** Urban coyotes are well-suited model organisms to address the phenotypic consequences of variation in human disturbances within urban systems. Coyotes are ubiquitous across North America and have assumed the apex predator role in urban areas following the local extirpation of tertiary carnivores (e.g., wolves, *Canis lupus*)[3]. Moreover, coyotes often consume anthropogenic food[9], but exhibit variation in diet[9,10]. As apex predators, stressors that affect coyote behavior or physiology will have top-down effects in urban ecosystems[3].

I predict low SES areas will represent poor habitat and diet quality via a reduction in prey diversity[1]. Therefore, coyotes will supplement their diets with a greater proportion of anthropogenic food subsidies relative to conspecifics in rural and high SES areas. I will trap coyotes (n=60) at 15 predetermined locations along an urban-socioeconomic gradient based on land cover, household density, and median household income[11] across the Seattle-Tacoma, WA metropolitan region over three years during winter and summer. Coyotes show more restricted home ranges in urbanized areas and are unlikely to forage in non-adjacent territories[12]. I will collect blood and hair samples from captured animals and scat samples from within and around trapping sites. I will deploy GPS collars and use spatial data to determine the mean habitat type and SES area used by each individual, and relate these habitat measures to physiological data.

Aim 1: Quantify oxidative stress variation in coyotes along an urban-socioeconomic gradient.
H1: Coyotes in low SES areas are exposed to more stressors, leading to greater oxidative damage and hyperglycemia relative to rural and high SES coyotes. Alternatively, coyotes may cope with urban stress via access to higher prey diversity in low SES areas, where refugia for prey is more common. **To quantify oxidative damage**, I will analyze lipid erythrocyte proteins from blood samples for peroxidation[4]. Additionally, I will test for hyperglycemia by examining glycated serum protein levels[8].

Aim 2: Determine the effect of urbanization on coyote diet composition and how diet influences their ability to mitigate oxidative stress. H2.1: Urban coyotes consume less natural food and more anthropogenic food than rural coyotes. Urban coyote fecal and hair samples will have lower nitrogen ($\delta^{15}N$) signatures, demonstrating a protein-poor diet, and higher carbon ($\delta^{13}C$) signatures, reflecting anthropogenic food consumption[13]. H2.2: Urban coyotes increase their antioxidant capacity by up-regulating antioxidant enzymes to cope with urban stress. **To evaluate how coyotes mitigate stress**, I will evaluate their **(a)** total antioxidant capacity, **(b)** activity of antioxidant enzymes[4], and (**c)** diet composition. To evaluate **(a)**, I will use the ferric reducing ability of plasma assay to describe the global antioxidant balance[4]; **(b)**, I will measure glutathione peroxidase and superoxide dismutase activity[4]; **(c),** I will perform stable isotope analysis (using $\delta^{13}C$ and $\delta^{15}N$)[11] on hair and scat samples to determine diet composition (i.e., anthropogenic vs. non-anthropogenic food sources). Using a linear mixed-effects model framework with AIC model selection, I will analyze the effect of site characteristics (rural/low SES/high SES), sex, and reproductive status on oxidative stress and the effects of urbanization on diet and oxidative stress.

Feasibility: My research project will bring a new avenue of research to an established system, the Grit City Carnivore Project (Dr. Christopher Schell, University of Washington). I will collaborate with the Urban Wildlife Information Network to effectively identify coyotes near trapping locations through existing camera trap data. **Field sites and permits have already been approved.** My field experience (trapping/sampling) and computational skills (database management/R), along with access to cutting-edge equipment and collaborators, will lead to the success of this project.

Intellectual Merits: Understanding the consequences of urban systems on wildlife physiology and stress is essential to developing effective wildlife conservation plans. My proposed research will fill knowledge gaps by exploring the luxury effect in coyotes and identifying links between urbanization, SES, and oxidative stress. **These results will bring a novel perspective to an emerging field investigating the influence of urbanization on the life-history of urban wildlife.** This study will form a foundation for future studies on fitness outcomes and adaptation to oxidative damage and could establish coyotes as bioindicators that reflect the health of urban environments (e.g., high oxidative stress may reflect exposure to pollutants[14]). Further, this project will advance our knowledge of the biological processes within cities.

Broader Impacts: ***(1) Community Engagement and Education:*** In addition to disseminating my results throughout the scientific community, I will also present these findings locally (e.g., Tacoma News Tribune, high schools). I will also engage directly with residents and students near urban trapping sites to observe coyotes closely and showcase the vibrancy of the urban biome. **I will partner with Environmentalists of Color and The Nature Conservancy to create community engagement opportunities and accessible education materials in urban ecology, focusing on Black and Brown communities in the Seattle-Tacoma metropolitan area.** I will work with Treehouse, an organization aiming to close the education gap between underrepresented foster youth and their peers, to develop engaging interdisciplinary assignments with real data that links math, science, and urban history. ***(2) Management Implications:*** I will leverage existing connections to work with Point Defiance Zoo and Aquarium and Woodland Park Zoo to develop workshops about urban wildlife natural history and conservation. **My research will reveal how wildlife are modifying their behavior in urban areas and how wealth disparities in humans influence wildlife stress, which will help managers develop natural areas for urban wildlife.** Through my collaborations, I will be able to interact directly with Seattle and Tacoma city officials to help develop environmental justice and urban conservation policies.

References: [**1**] Schell et al. (2020) *Science*. [**2**] Kuras et al. (2020) *Landscape Urban Plan*. [**3**] Prugh et al. (2009) *BioSci.* [**4**] Herrera-Dueñas et al. (2017) *Front. Ecol. & Evol.* [**5**] Isaksson (2015) *Funct. Ecol*. [**6**] Arnold et al. (2010) *Biol. J. Linn. Soc*. [**7**] Strandin et al. (2018) *Phil. Trans. R. Soc. B Biol. Sci.* [**8**] Schulte et al. (2018) *Cons. Physio.* [**9**] Morey et al. (2007) *Am. Midl. Nat.* [**10**] Newsome et al. (2015) *Oecologica.* [**11**] Magle et al. (2015) *Anim. Conserv.* [**12**] Gehrt (2007) *Proc. 12th Wildl. Damage Mgmt. Conf.* [**13**] Windberg et al. (1991) *J. Wildl. Dis*. [**15]** Pérez-Coyotl et al. (2019) *Env. Poll.*