Expanding the Definition of an Urban Ecosystem through Stable Isotope Analysis

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Abstract

The question of defining ecosystem boundaries is important in creating management policies, conservation guidelines, and continuing accurate research. In the case of urban ecosystems, boundaries cannot be defined by strictly cultural or physical methods. Approaching boundary definitions in this manner ignores the importance of energy movement through an ecosystem. Part of the energy budget of an urban ecosystem is the anthropogenic food input, which can serve to attract wildlife to the city. It is proposed that this attraction to urban centers due to food resources influences surrounding classically defined natural ecosystems to such an extent that the urban ecosystem should be redefined to include the environment surrounding cities. The research technique that is the most time and cost effective, least invasive, and allows multiple species to be compared at once is stable isotope analysis. Isotope compositions have been commonly used for decades, and among the most important isotopes for deciphering ecosystem functions are carbon and nitrogen. Through the use of stable isotope analysis, urban wildlife can be differentiated from wildlife that is not influenced by human inputs to the ecosystem by the carbon value. The isotope ratio of anthropogenic foods is unique enough from natural sources of food that it can be used as a marker for human influence on the food chain. Previous work supports that species and individuals within a species can be differentiated using stable isotope analysis. It is proposed that stable isotope analysis be used to analyze samples of wildlife for carbon and nitrogen values in an attempt to measure the impact that people are having on urban and non-urban species.

Introduction

The ability that humans have to influence their environment has shaped perceptions of the modern world, encouraging a separation between modernized human culture and conservation, which promotes restoration of the environment through the minimization of human impact. This separation has been extensive enough that even under an urban ecology symposium in 1980, editors Bornkamm, Lee, and Seaward prefaced their notes with the statement that, "It is not clear how extensive human interference needs to be before the term ecosystem ceases to be applicable"[1]. Artificially determining the boundaries of an ecosystem disrupts potential research opportunities and complicates management practices. Redefining a new approach to urban ecology, specifically in how the boundaries of an ecosystem are determined, could greatly improve management efforts.

Recent ecologists have endorsed the concept of an urban ecosystem where the anthropogenic influences on the environment are incorporated instead of excluded. This led to the creation of the multidisciplinary field of urban ecology, which works to describe the processes occurring in the complex urban environment. Urban ecology joins a multitude of fields, including ecologists, anthropologists, geographers, conservationists, and engineers, to create a better understanding of ecosystems that are dominated by humans (Newsome et al., 2010).

[1] Bornkamm, R., J. A. Lee, and M. R. D. Seaward. "Preface." Preface. *Urban Ecology: The Second European Ecological Symposium*. N.p.: Blackwell Scientific, 1982: pp. xiii.

The first definition of an ecosystem was created by Tansley in 1935, in which the ecosystem was called the "basic unit of nature"[2]. This original ecosystem concept incorporated biotic and abiotic factors, suggesting that there was a constant exchange between these components. The idea of an urban environment and urban ecosystem – an environment in which humans are not excluded – builds on the basic concepts laid out by Tansley (1935). Relying on the abiotic components, an urban ecosystem could be spatially bounded by the urban geographical setting. Defining an urban ecosystem in this way relies exclusively on the human perception of boundaries and ignores territorial components of wildlife that may be considered part of the urban environment. This physical restriction ignores the biota, and therefore the interactive ecosystem components of the definition created by Tansley (1935). Constraining this boundary in a more holistic and quantitative manner is part of the problem of urban ecology.

Building on the groundwork that humans cannot be excluded from an ecosystem, this paper explores the possibility that the human-influenced urban ecosystem significantly impacts wildlife outside of the urban setting. Urban centers are places where food and water resources can be dense and diverse, and are more easily accessed than in natural environmental settings. Due to these factors, urban areas can attract wildlife (Newsome et al., 2010). This paper will look at the application of stable isotope analysis to track the influence of the urban environment on non-urban food webs at a radius spreading from the city center. This is done in an effort to answer the

[2] Tansley, A. G. "The Use and Abuse of Vegetational Concepts and Terms." *Ecology* 16.3 (1935): pp. 299.

question: Should the conceptual understanding of urban ecology be expanded to include areas outside of the city where the concentration of human-influenced food resources inside the city behaves as an attractant to outside wildlife?

Urban Ecology

Urban ecology focuses on the diversity and population of animals inside and near cities, as well as their movement and nutrient and energy budgets (Pickett et al., 2011). The dominant pressure of any urban ecosystem is the presence of humans. Humans alter the physical and energetic components of the urban ecosystem through management of the biota and the abiotic environment. This creates an artificial equilibrium to which urban plants and wildlife adapt (Tansley, 1935). While urban ecosystem is commonly accepted as the proper term today, Tansley (1935) described a human-dominated system as an anthropogenic ecosystem. While an urban ecosystem would always fit the definition of an anthropogenic ecosystem, all anthropogenic ecosystems are not urban ecosystems.

According to the original definition of an ecosystem created by Tansley (1935), and his explanation of the anthropogenic ecosystem, the urban environment should be considered an ecosystem. However, more modern scientists have introduced stricter definitions and new terms in an effort to further define the properties of ecosystems and the urban environment. As a result, the understanding of the urban environment as an ecosystem has been needlessly complicated. Natural ecosystems, unlike urban

ecosystems, are reliant on solar energy, have natural food chains, and have methods of nutrient recycling (Adams, 1994). More recently, urban ecology has expanded to include the social and economic impacts of humans, identifying the urban environment and its components as "heterogeneous ecological systems".[3] This expansion has taken the urban ecosystem back towards the original definition of an ecosystem as laid out by Tansley (1935), incorporating humans as a biotic factor.

Urban Ecosystem Boundaries

Many researchers have posed terms that artificially restrict the ecosystem concept in an attempt to place spatial boundaries on ecosystems. This has also generated uncertainty relating to how urban environments function as ecosystems. Defining the boundaries of a research experiment is an important part of the research process, allowing scientists to tailor experiments to their hypotheses. However, broadly applying definitions of an ecosystem, or the boundaries of an ecosystem, to all ecosystems can be too restrictive. It is proposed that stable isotope analysis be used as a technique to quantitatively define the boundaries of a wide range of urban ecosystems to avoid qualitative definitions. The following section explores a few qualitative definitions that have been broadly applied to ecosystems and their boundaries in an illustrative manner. The goal is to persuade the reader that, while

[3] Pickett, S.T.A., M.L. Cadenasso, J.M. Grove, C. G. Boone, P. M. Groffman, E. Irwin, S. S. Kaushal, V. Marshall, B. P. McGrath, C.H. Nilon, R.V. Pouyat, K. Szlavecz, A. Troy, and P. Warren. "Urban Ecological Systems: Scientific Foundations and a Decade of Progress." *Journal of Environmental Management* 92 (2011): pp. 333.

these terms may be applicable to limited studies, they cannot be broadly applied. For this reason, the use of stable isotope analysis is supported over other methods of defining ecosystem boundaries.

Kondrat'ev et al. (2001) defined an ecosystem as a unit composed of "smaller elementary substructures"[4], which they called biogeocenoses. Biogeocenoses is a method that relies on vegetation type to define the boundaries of an ecosystem. Vegetation is grouped together into units called facies. If biogeocenosis is applied to the urban landscape, the resulting facies of the urban ecosystem would be drastically different than those of a natural environment. If impermeable surface cover is used as a vegetation type in the facies definition, variable percentages of pavement could be a first cut at breaking the urban ecosystem into various parts based on landscape. The advantage of defining the urban ecosystem by its parts and not as a whole is to better understand the role that wildlife, plants, and people play in different windows of the ecosystem. Additionally, percentage of pavement compared to true vegetation cover could more quantitatively define an urban ecosystem's boundaries (Kondrat'ev et al., 2001).

The edges of biogeocenoses, however, are more specifically bounded by the interactions between species and their environment. The presence of high levels of human activity in an urban environment, which violate energy regulation due to massive food inputs and severely reduced decomposition and nutrient recycling, degrade the

[4] Kondrat'ev, K. Y., K. S. Losev, M. D. Ananicheva, and I. V. Chesnokova. "Elementary Structural Units of the Biosphere and Landscapes." *General Biology* 380.1 (2001): pp. 136.

concept of biogeocenoses (Kondrat'ev et al., 2001). This degradation can "[reduce] the biological potential of natural ecosystems," [5] therefore causing an unstable environment that may be unsuited for many species. The biogeocenosis approach is best used in low diversity environments that have visually clear boundaries. While a facies composed of paved surfaces could be used to define the physical urban ecosystem boundaries, relying on a strictly physical boundary ignores the potential for an urban ecosystem to influence wildlife outside of the human-observed city borders.

In contrast to the biogeocenosis concept, Post et al. (2007) recommended abandoning a purely physical boundary definition in favor of an ecosystem boundary that was more functionally appropriate. Using a functional boundary instead of a physical boundary takes into consideration energy movement, which is of interest when analyzing the potential influence of an urban ecosystem on the so-called natural ecosystems surrounding it. A functional boundary can be defined by a sharp decline in species interactions. This boundary, therefore, permits the definition of the urban ecosystem to include the surrounding areas if the energy exchange between the natural and urban environments warrants this expansion (Post et al., 2007). A functional and not strictly physical boundary better encompasses the control that anthropogenic inputs to the food web have over wildlife. If the definition of the urban ecosystem is to be expanded based on the ability of urban centers to attract wildlife due to their resources, the expansion should be based on energy movement and not simply on physical

[5] *Ibid.* 4, pp. 137.

characteristics of the environment. This functional boundary, which is the energetic boundary of an ecosystem, could be measured through stable isotope analysis.

The classic understanding of an urban ecosystem uses the concepts of gradients to differentiate between the urban, suburban, rural, and natural environments. The area of highest urbanization, typically corresponding to the city center, is considered to be the urban environment. The farther one moves from the city center, the less urbanization is present, and the environment grades into suburban and finally rural settings. The distinction between these settings is based on the assumption that the ecosystem function changes in a gradient respective to landscape zones or linear distance from the city center moving into the rural environment (Figure 1). This gradation is therefore an example of facies. Gradient concepts of an ecosystem can be defined through quantitative methods using energetic budgets through stable isotope analysis. In this way, an urban ecosystem could be better defined by its specific characteristics as opposed to the physical characteristics of the landscape.

Ramalho and Hobbs point out one concern with this broad-brushed approach to break-up the definition of an urban ecosystem – it "does not fit with the non-linear and complex growth of contemporary cities"[6]. Their proposed alternative, called the dynamic urban framework, relies on social and economic contexts to better plan, manage, and restore urban ecosystems (Ramalho and Hobbs, 2012). This approach

[6] Ramalho, C. E., and R. J. Hobbs. "Time for a Change: Dynamic Urban Ecology." *Trends in Ecology and Evolution* 27.3 (2012): pp. 179.

incorporates humans as part of the environment while simultaneously removing the misleading tags that describe only the physical characteristics of the landscape as perceived by people. While the percentage of urbanization as presented in the dynamic urban framework is a better approximation of the urban ecosystem, it still ignores the movement of energy from the physically-defined urban environment into the surrounding landscape.

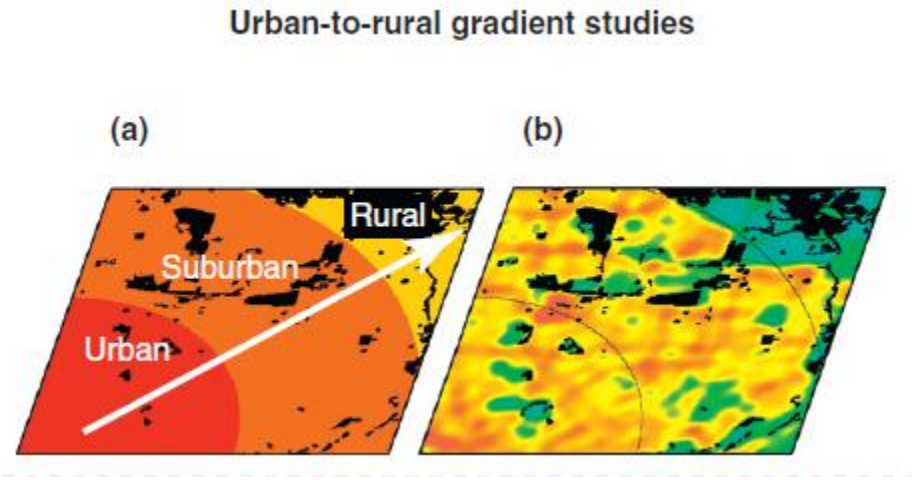

Figure 1: "The urban-to-rural gradient approach classifies the degree of urbanization of remnant ecosystems using either categorical classes or quantitative measures of linear distance between the city center and the rural matrix (a) (remnant vegetation in black); or a combination of those with socio-economic, land cover, land use, or built infrastructure metrics (b) (road density depicted here). Data analysis usually focuses on the comparison between ecological responses across different urban classes or on the single effects of a simplified set of explanatory variables."[7] From Ramalho and Hobbs (2012).

There is another aspect of an ecosystem which the surrounding natural environments could use to describe the urban ecosystem and how it influences their ecosystems. A spatial subsidy occurs when the physical edge of an ecosystem does not correspond with the edge of the community and ecosystem processes. While the

[7] *Ibid.* 6, pp. 184.

expanded definition of an urban ecosystem proposed above is an open ecosystem framework, the relationship between the urban and natural ecosystems could be considered a spatial subsidy. An urban ecosystem has a boundary that is clear to humans in the form of city limits, but these limits are not adhered to by plants and animals. The energy flow of an urban ecosystem, primarily through the fluctuation of food resources, also ignores city limits (Post et al., 2007). While an urban ecosystem performs a limited level of ecosystem processes, it does foster interactions between people and wildlife that do not occur, or occur in limited fashion, in other environments. These new interactions help to define the urban environment as an ecosystem and a spatial subsidy. If a human city is subsidizing the food chain outside of the physical urban environment, the localized food chain of these surrounding natural environments should be heavily influenced, expanding the spatial scale of the urban ecosystem.

The physical, energetic, and dynamic urban framework approaches to boundaries in urban ecosystems do not address the potential for the urban environment to influence control over energy flow in surrounding natural environments. The concept of a spatial concept created by Post et al. (2007) allows the urban ecosystem to be physically and energetically defined with separate boundaries. Despite this, direct measurement of the exchanges between the urban ecosystem and adjacent environments through animal movement and the food web would create a custom ecosystem boundary for each city. Using stable isotope analysis instead of relying on

previously defined terms would yield a quantitative answer to how to best define the boundaries of an urban ecosystem.

Urban Landscape

The term urbanization is used to describe the process through which the natural landscape is altered by urban development. Urbanization, and the urban environment, is a recent landscape type that appeared in the last 100 to 200 years. The resulting anthropogenic pressures are the greatest of any other environment, but these pressures create new ecological niches for wildlife (Luniak, 2004). Urban environments are dominated by a high density of developed land, including manmade structures with impervious surfaces and a decline in the percentage of vegetation compared to natural ecosystems. Cities have higher levels of pollution and noise from car exhaust, industry, and day-to-day human life. Pollution inside cities can also take the form of heat, which increases water and air temperatures compared to surrounding, natural environments (Adams, 1994). Because urban settings vary in key physical aspects from the classic views of an ecosystem, urban ecosystems can be characterized by that which separates them from surrounding, natural ecosystems.

The urban ecosystem revolves around two primary landscape types: patches, and developed areas. Patches are unpaved areas that have either had little modification by humans or have been restored. The interactions of wildlife with and within patches most closely resemble a natural ecosystem. Examples of patches include ponds, parks,

and even cemeteries (Adams, 1994). In contrast, developed areas are not designed to serve an ecological purpose, but may offer critical components to survival unique to the urban ecosystem. Parking lots, backyards, and canals are pieces of developed landscape in an urban ecosystem. While the process of urbanization threatens native habitats, it can also create new spaces for wildlife to inhabit.

Urban Wildlife

The urban environment is one that wildlife must adapt to in order to survive. This creates a subset of plants and animals residing in urban ecosystems that are either from adjacent environments or exotic species that are common in cities. Nondomestic species that can tolerate the differences between urban environments and more natural settings are termed urban wildlife (Adams, 1994). The process by which wildlife adapt to urban environments has been coined synurbanization (Luniak, 2004).

Synurbanization outlines the changes that wildlife undergo to be considered urban species. While urbanization reduces ecological diversity across urban landscapes, some populations of wildlife are able to adapt their behavior to take advantage of the niches that urbanization creates. These adaptations include greater population densities and reduced territory sizes, reduced or absent migratory behavior, prolonged breeding season which can include winter breeding cycles, increased lifespan due to increases in food availability and survival rates in winter, alterations to or extensions of circadian activity to accommodate artificial lighting and movement during the lowest

points of human activity, adaptations to nesting and feeding behavior to take advantage of anthropogenic resources, and adjustments to stress levels and escape distances around humans. In some cases, synurbanization is successful enough that populations living in the urban environment are more successful than native populations living in their natural habitats. A number of species in Europe, for example, have exhibited such successes, including omnivorous mammals like the red fox (*Vulpes vulpes*), predatory falcons like the kestrel (*Falco tinnunculus*), and small herbivorous mammals like the striped field mouse (*Apodemus agrarius*) and the red squirrel (*Sciurus vulgaris*). While genetic independence between urban and wild populations has not been proven, the adaptations that urban populations exhibit are clearly distinct from the behavior of native populations (Luniak, 2004).

Another example of a synurbic species is the common raven (*Corvus corax*) of the Mojave Desert. A native species, the population of the common raven has increased alongside the increase in human population of the desert. Naturally predators and scavengers, the ravens' scavenging success is greatly amplified by the presence of humans. Opportunistic generalists, ravens have taken advantage of the food and habitat resources of the urban environment. One study revealed that 24.2% of all material in raven pellets consisted of human trash such as paper, plastic, aluminum foil, wood, and styrofoam. The stable, anthropogenic food sources have increased fledging success near urban areas, and also increased their population size (Kristan, Boarman, and Crayon, 2004).

Urban animals are most often generalists, a term describing species that have the ability to adapt to a wide range of habitat types through evolution (Adams, 1994). This plasticity in behavior and habitat requirements allows populations to undergo the process of synurbanization and readily adapt to the urban environment (Luniak, 2004). Furthermore, generalists are more common in urban environments due to their naturally widespread distribution, high rate of dispersal, ability to successfully assume risks, high reproductive rate, and fast-paced life history (Pickett et al., 2011; Moller, 2009). Generalist bird species are often found at points where multiple types of habitat meet, which are common in urban environments where the landscape is a patchwork of habitats. Exotic species flourish in the urban environment due to the tendency of these species to be generalists (Adams, 1994). As urbanization increases, which can be extrapolated to be an increase in impervious surface area, generalist species increase in frequency while native species that exist in specialized niches decline. Generalists can more readily adapt to urbanized, and therefore disturbed, environments, while other environmental and climate factors are maintained by humans (Pickett et al., 2011).

In addition to being dominated by habitat generalists, wildlife found in urban ecosystems are structurally and compositionally similar despite geographical differences. This tendency towards similar community structure and high numbers of exotic species in urban ecosystems is called biotic homogenization. This homogenization is encouraged in part by the stress of the urban environment as well as the maintenance of the environment by humans (Pickett et al., 2011). Urban

ecosystems include reliably watered lawns, consistent non-native food sources, and climate conditions with fewer extremes compared to the natural habitat (Pickett et al., 2011; Adams, 1994). Lastly, the movement of goods and people from city to city promotes similar exotic species to transition from one urban ecosystem into another (Pickett et al., 2011). By manipulating resources, humans eliminate and create niches for wildlife, exerting indirect control over the species present in the urban environment.

Exotic plant species, which are not often recognized as food sources by native wildlife, are more common inside cities. The presence of exotic plants can limit indigenous species' access to native sustenance. While natural housing and native sources of food for wildlife are reduced, unnatural resources are available. Urban ecosystems have massive food inputs from people, which can be distributed to wildlife through intentional and unintentional feeding. The most common intentional source of food for urban wildlife is the backyard birdfeeder. Unintentional feeding primarily comes from access to trash cans and dumpsters. These energy inputs into the system in the form of food results in an ecosystem that is unbalanced. High concentrations of wildlife in small areas cause the natural carrying capacity of the landscape to be quickly outpaced, forcing animals to become reliant on people for their survival. This can be a strong enough influence to disrupt the natural migratory patterns of birds living in urban ecosystems (Adams, 1994). Urban wildlife that become reliant on people develop altered behavioral patterns, leading to human inputs to the food chain becoming the oasis of the otherwise resource-limited urban environment.

Urban wildlife was, at one point, almost exclusively small- to medium-sized mammals and songbirds. Larger species such as white-tailed deer and coyotes, which were once thought to be easily disturbed by people, have more recently become incorporated into the urban ecosystem (Ditchkoff et al., 2006). This movement into cities implies that these animals are either adapting out of necessity, or because there is some advantage to moving into the urban environment. Regardless of the cause, if animals are moving between what is traditionally considered the urban environment and the natural environment, this movement may indicate a need to expand the definition of an urban ecosystem to accommodate the increased human impact outside of the physical bounds of the city.

Much like watering holes in the African savannah, the plentiful food resources in the urban environment attract wildlife. This attraction outlines the need to better define the boundaries of an urban ecosystem through energetic and not purely physical methods. Examples of food sources include human trash, backyard birdfeeders, and non-native plant species maintained in urban environments. Animals living near waste disposal areas in cities are observed to have a diet that includes higher proportions of human foods (Ditchkoff et al., 2006). It has been recorded that urban coyotes are not only reliant on, but attracted to human food resources. Coyotes, however, are not the only species to ingest food that is unique to the urban environment (Adams, 1994). Raccoons, opossums, and red foxes have all been observed to ingest significant amounts of human garbage, dog food, and cat food (Hoffmann and Gottschange, 1977; Manski

and Hadidian, 1987; Adams, 1994). Human activity in many urban centers is greatest during the day, encouraging animals that are "easily disturbed by human activity"[8] to alter their feeding habits (Ditchkoff et al., 2006). Ditchkoff et al. (2006) proposed that this behavioral change resulted in predators being unable to hunt as effectively as their counterparts in natural environments. By reducing the ability for predators to feed on natural prey items, it increases the amount of scavenging, and therefore encourages animals to use human trash (Ditchkoff et al., 2006). While human food resources are plentiful in cities, the urban environment may also be influencing certain species towards a diet more similar to people. The variety of omnivores and carnivores that ingest processed human foods suggests that part of this food source could be used as the marker for urban compared to truly wild animals. Details of how to approach this marker are provided in successive sections of this paper.

Attraction to human food also causes animals to cluster in higher densities in a given territory than would naturally be seen (Adams, 1994). Squirrels in one urban setting were recorded at up to five times higher densities than in rural areas, the increased density attributed to feeding by people (Hathaway, 1973; Flyger 1959; Manski, VanDruff, and Flyger, 1981; Adams, 1994). Another study in Tucson, Arizona by Emlen in 1974 showed that the biomass and density of birds was twenty-six times greater in the city than the surrounding desert. Even larger, ubiquitous omnivores, like

[8] Ditchkoff, S. S., S. T. Saalfeld, and C. J. Gibson. "Animal Behavior in Urban Ecosystems: Modifications Due to Human-induced Stress." *Urban Ecosystems* 9.1 (2006): pp. 6.

the red fox, have been reported in densities of eighteen times higher in towns in England compared to rural settings in North Dakota (Harris and Rayner, 1986; Sergeant, Allen, and Hastings, 1984; Adams, 1994). Density of urban wildlife is also influenced by the decline in natural predation. While humans remove large carnivores, such as mountain lions and polar bears, from the urban environment, other carnivores are deterred from hunting by human presence. This causes them to instead focusing on scavenging (Ditchkoff et al., 2006; Adams, 1994). In some areas, this has led to increases in white-tailed deer densities in cities. For these deer, the urban environment offers protection from predators, readily-available food resources, and enough cover for their survival (Adams, 1994). These factors influence species other than deer, and the increase in population density seen in the urban environment may be an indication that these areas are attracting wildlife from natural environments.

One example of a more transitory species to the urban ecosystem is the mule deer. These deer enter the relatively sheltered environment of the city during severe winters, and return to more natural environments come spring (Adams, 1994). Individuals belonging to common urban species may also be transitory like the mule deer as a whole, and the anthropogenic impact to these individuals could represent a strong influence over environments surrounding urban cities. Through the use of stable isotope analysis, it may be possible to track this influence in an effort to redefine the ecological boundaries of an urban ecosystem.

Beyond reducing the presence of high trophic level predators and directly influencing resource availability, humans can also alter the food web by influencing the biodiversity, favoring generalists (Warren et al., 2006). The dominance of habitat generalists in urban ecosystems means that omnivores and herbivores are favored over predators. Additionally, humans tend to forcibly remove or strongly deter high trophic level predators from their cities, further disrupting the food chain (Adams, 1994). These disruptions result in a much more simplified food chain, where humans can exert both top-down and bottom-up controls (Figure 2; Adams, 1994; Warren et al., 2006). These alterations to the food web, which decline with distance from the city center, should have a greater effect outside of physical boundaries to the ecosystem. This effect could cause nearby natural ecosystems to be impacted by the urban center strong enough to be considered part of the urban ecosystem.

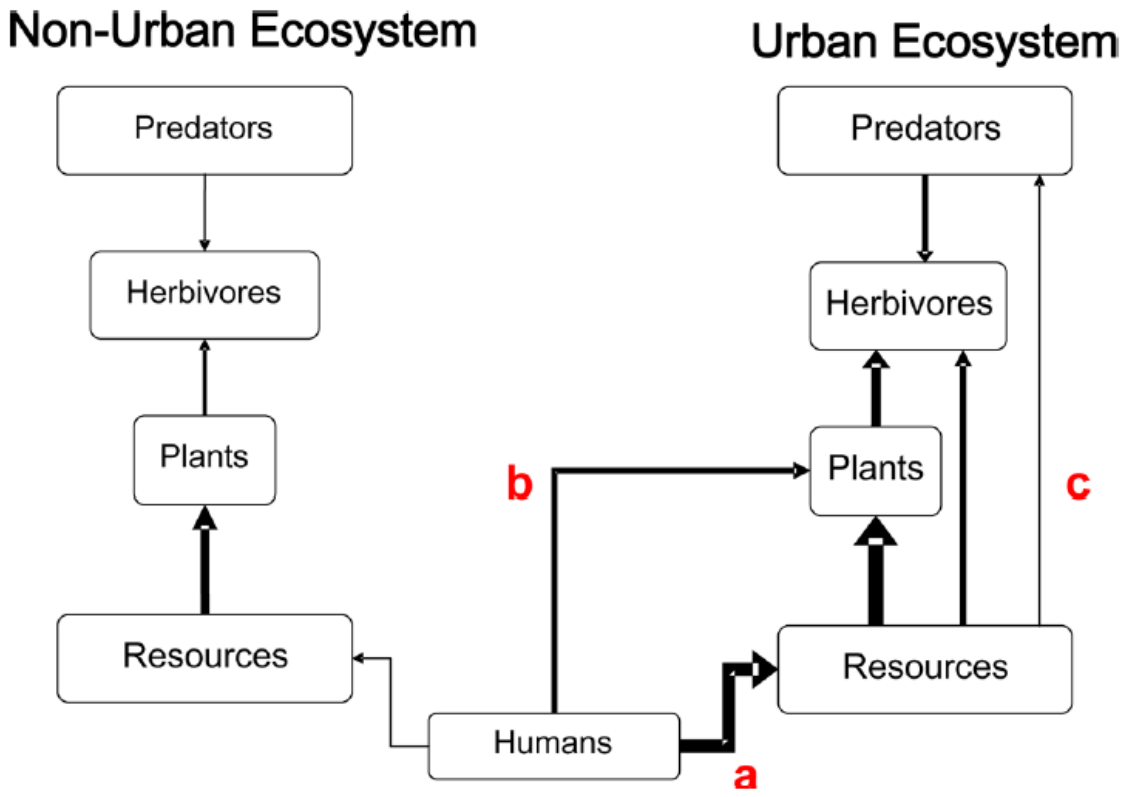

Figure 2: A generalized comparison of the urban and natural food web systems, where arrow size represents strength of influence. Human influences to the urban ecosystem include: (a) increased resources vital to plant growth and survival such as water and fertilizer, (b) inclusion of exotic plant species and exclusion of native plant species from the urban environment, and (c) increased sources of intentional and unintentional food. Originally from Warren et al. (2006).

Stable Isotope Analysis

Isotopes are radioactive and stable forms of elements with the same number of protons and electrons and the same chemical properties, but that differ in their neutron count. Stable isotopes, unlike radioactive isotopes, do not decay. Almost all elements required for sustaining life have stable isotopes, and most often one is more abundant than the other. These different stable isotopes are preferred in various biogeochemical

cycles (Ehleringer et al., 1986). Using stable isotopes, scientists can track the progression of material – like food and water – through the environment using relatively small sample sizes (Weathers et al., 2013). Stable isotope analysis is the preferred experimental approach to redefine boundaries of urban ecosystems.

Measurement of Isotope Ratios

Generally, isotopes are divided into light and heavy elements. This labeling differentiates the process through which a sample acquires its isotopic signature. The light elements, which include C, Ca, O, N, H, and S, vary based on isotopic fractionation during geologic and ecologic processes (Wassenaar, 2008; Smith, 1988). Heavier elements, including Sr, Nd, Ur, Os, Hf, and Ce, are generated when parent isotopes radioactively decay (Smith, 1988). Stable isotopes do not decay, and the lighter elements are the five building blocks of life, making these five vital to the study of ecology. These elements are the bulk majority of all animal tissues, with C comprising roughly 50% of the total weight (Wassenaar, 2008).

There are many common elements and their isotopes measured in stable isotope analysis, however some of the most common for ecology applications include: ^{13}C and ^{12}C, ^{15}N and ^{14}N, ^{34}S and ^{32}S, ^{18}O and ^{16}O, as well as ^{2}H and ^{1}H. Samples are considered to be enriched if there is more of the heavier isotope compared to the lighter isotope, while depleted samples are the reverse (Lajtha and Michener, 1994). The lighter isotope (i.e. ^{12}C) is typically more abundant than their heavier counterpart (i.e. ^{13}C). The

ratio between these two stable isotopes varies due to natural processes, and it is this ratio that is reported (Wassenaar, 2008).

Isotope ratios are always recorded in comparison to a set international standard, and are expressed as delta (δ). The unit used to describe an isotope ratio is per mil (‰), which is an arbitrary standard. The value for δ is calculated using the formula below, where R represents the isotope ratio (Ehleringer et al., 1986):

$$\delta = \frac{R_{sample} - R_{sample}}{R_{standard}} \times 1000$$

Determining absolute concentrations of an isotope is difficult, which is why stable isotope ratios are compared against an international standard (Wassenaar, 2008). This measurement is done through the use of an isotope ratio mass spectrometer, which compares the isotope ratio of the sample to the international standard (Lajtha and Michener, 1994). An ultrapure gaseous analyte from the sample material is required for processing by the mass spectrometer (Wassenaar, 2008). This system also has the benefit of the ability to test a wide range of sample types, including tissue, fur, and feathers. Due to the range of samples that can be analyzed, both terrestrial and marine organisms can be analyzed (Michener and Schell, 1994). While the actual cost of stable isotope analysis will depend on the type of sample and the isotope being used, it is a relatively economic research approach (Lajtha and Michener, 1994). The low cost allows many samples to be run on a smaller budget, increasing the range of use for stable isotopes.

The primary advantage of using naturally abundant stable isotopes over other forms of tracking is the lack of health risk associated with their handling to both the environment and people (Weathers et al., 2013). Isotopes also have a wide range of applications in the study of ecosystems, as they are viable in determining food webs, differentiating mechanisms of nutrient movement in a single organism, and tracking the uptake and release of elements on an ecosystem scale. Additionally, isotope analyses can be run from a wide range of sample materials, making it an exceptionally versatile approach (Lajtha and Michener, 1994). Isotopes are an intrinsic marker, allowing quantitative measurements of the boundaries of an ecosystem to be determined without continual recapture of the same subject. Noninvasive sampling methods – such as using fur or feathers – also mean that the animal is unharmed and its behavior pattern is not altered by a physical marker such as a radio collar (Hobson and Norris, 2008). This paper proposes that isotopes are used to analyze samples from wildlife caught in transects from city centers into what is classically considered the natural habitat in an effort to determine if bordering ecosystems are supplemented by urban ecosystems and ultimately humans.

The main drawback of isotope analysis is dependent on the samples taken. As only a portion of each sample is dried, ground, and combusted, it is necessary to assume that the sample combusted is homogenous. Homogeneity insures that the combusted sample represents the original organism. If there is heterogeneity within the sample, grinding a portion and not the entirety of the sample will skew the results. One of the

concerns in working with the terrestrial food web is that fur and feathers grow over time, and the isotopic ratios will change depending on where the animal was consuming resources at the time that millimeter or centimeter grew. Additionally, while instrumentation can generate errors, utilizing proper collection and preparation techniques will reduce the likelihood of error and sample heterogeneity.

Isotope Ratios and Diet Analysis

Stable isotope analysis can be used to determine the diets of terrestrial and marine animals. This is because the isotope ratios of tissues from the organism are similar to the isotope ratios of the items they have consumed (Ehleringer et al., 1986). As long as the various resources available to the species analyzed have distinct, predictable isotopic ratios, stable isotope analysis can be used to determine which resource, or resources, the target species is ingesting (Figure 3; Lajtha and Michener, 1994). These ratios will vary depending on the type of sample taken from the animal, as assimilation rates vary dependent on tissue type. Tissues that are not involved in active metabolic processes, such as feathers, fur, and nails, provide a record of stable isotope ratios from when they were formed. In contrast, tissue like blood and muscle will reflect the most recent diet of the animal (Miller et al., 2008). Stable isotope analysis can therefore be used to map the dietary history of an animal over the course of the growth period for metabolically inactive samples. Comparing fur, feather, or nail samples collected from urban and natural ecosystems should show if wildlife caught in

natural ecosystems are moving between that ecosystem and the urban environment to feed.

	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Fox		
Urban	9.43±0.26	–20.65±0.85
Rural	10.56±0.17	–14.37±0.23
South Farms	9.82	–21.96
Rabbit		
Urban	3.85±0.60	–22.67±1.30
Rural	3.27±0.67	–22.38±1.49
South Farms	5.24±0.19	–23.11±0.95
Soybean		
Rural	0.42±0.36	–26.29±0.53
Corn		
Rural	1.66±1.44	–11.62±0.13

Figure 3: A list of stable isotope measurements from red fox and rabbit fur samples taken from urban and rural environments, as well as stable isotope values for two common rural crops. South Farms is a rural environment that is devoid of another predator, the coyote. Taken from Lavin et al. (2003).

The application of stable isotopes to diet analysis involves three assumptions. Stable isotope analysis relies on proportionality to be successful when determining food sources. This means that, in order for stable isotope analysis to be successful, available food sources and their isotope compositions must be known. Without knowledge of the isotopic compositions of what is being consumed, assumptions on which food sources are influencing the isotopic composition of a sample cannot be made. As stable isotope analyses reflect the relative contributions of different sources, not only must all sources be known, but their isotopic values must also be known. It is assumed in this experiment that the isotope ratios of anthropogenic food sources are substantially different than those of other, natural sources of food. If these food sources contain

similar isotope ratios, few conclusions can be drawn from the data. Finally, a thorough understanding of isotope fractionation across trophic levels is required to determine what level of enrichment indicates a new food source compared to a natural concentration of isotopes as seen in predators (Merkle, Derbridge, and Krausman, 2011).

The two most common stable isotopes in food web and diet analysis are $\delta^{13}C$ and $\delta^{15}N$ (Ehleringer et al., 1986). Carbon isotopes in animals are similar to the isotope compositions of their food, usually enriched by only about 1‰ (DeNiro and Epstein, 1978). In comparison, $\delta^{15}N$ ratios are typically enriched in the animal by 3-5‰ at the bottom trophic level (Ambrose and DeNiro, 1986). With each successive trophic level, the $\delta^{15}N$ values tend to be 2-4‰ greater. In other words, the rabbit eating the grass has a $\delta^{15}N$ value 3-5‰ greater than the grass, while the fox eating the rabbit has a 2-4‰ increase in their $\delta^{15}N$ value compared to the rabbit. This change is thought to be a result of catabolic metabolism, which fractionates the nitrogen isotopes as they pass through the food chain (Ehleringer et al., 1986). The turnover rate of the tissue sampled will alter how quickly the stable isotope ratio influenced by diet will be recorded inside the tissue. In combination, $\delta^{13}C$ and $\delta^{15}N$ together can be used to differentiate between species that would have overlapping isotopic signatures for one of the two elements (Figure 4; Ehleringer et al., 1986).

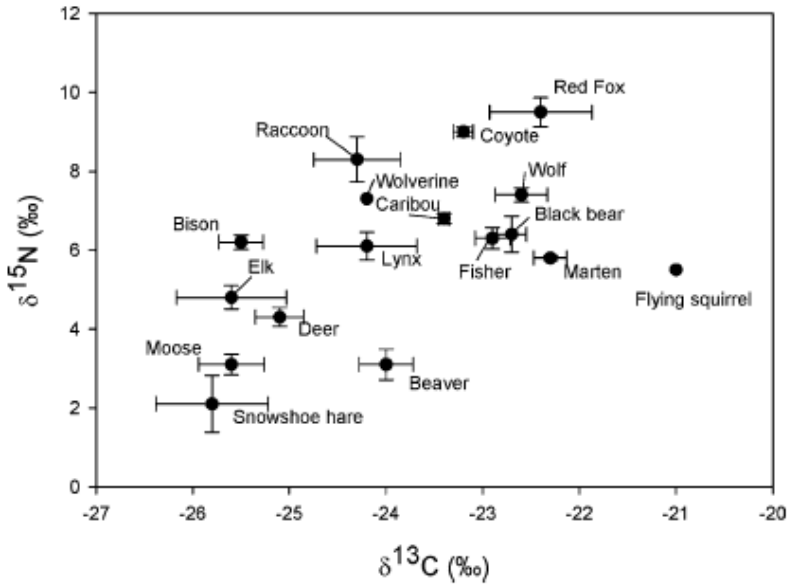

Figure 4: An example of carbon and nitrogen isotope ratios for various mammals. From Urton and Hobson (2005).

Challenges of Using Stable Isotopes in Dietary Analysis

One potentially complicating factor related to turnover time is that of digestibility. The time period before the expected change in isotope signature following a dietary shift occurred varied in goat feces and blood samples (Codron et al., 2011). It was believed that this change was dependent on how readily the animal was able to digest and therefore assimilate the carbon isotopes from its food. Their results indicated that, while the isotopic signatures began to shift within a few days, the animals were not at equilibrium with the dietary change until almost three months later. It was speculated that the reason for this was because the C_3 plant used in the dietary switch was not naturally foraged by the goats (Codron et al., 2011). It is possible that urban wildlife, especially wildlife moving between and ingesting food in the urban

environment and surrounding natural environments, would not show a strong relationship to the $\delta^{13}C$ and $\delta^{15}N$ values expected from a corn-dominated diet (Newsome et al., 2010). However, while tissue may not be in equilibrium with the diet change, the stable isotope ratio for the sample should still indicate a difference between wildlife that is feeding at least partially in urban environments and wildlife that is feeding exclusively on native food sources.

Differentiating individuals within the same species based on the influence that their diet has on carbon and nitrogen stable isotope ratios poses a challenge - understanding the differences between the stable isotope ratios of urban and wild food resources. Wildlife influenced by the urban environment, which is dominated by human inputs to the food chain, should have stable isotope ratios of carbon and nitrogen similar to human food. Anthropogenic food resources have high corn stable isotope ratios, present in meat products, sweets, and other processed food items. It is proposed that noninvasive tissue samples, primarily fur, be used from wildlife to check for the isotopic signature of corn in their diet.

Corn as a Marker for Urban Food Sources

In North America, food commonly consumed by people has an enriched $\delta^{13}C$ value indicative of the widespread use of corn and corn products in processed foods. Corn is a C_4 plant, and therefore has a high $\delta^{13}C$ value that ranges from -12‰ to -14‰. Sources of corn are lacking in the natural environment, making this crop an almost

exclusively human-grown food source. The distinct signature can theoretically be used as a marker for animals feeding on anthropogenic food (Newsome et al., 2010). Carbon isotope ratios vary between C_3 and C_4 plants because of the different photosynthetic pathways used by each plant group. Plants that have a C_4 photosynthetic pathway use phosphoenolpyruvate (PEP) carboxylase as their primary enzyme. PEP carboxylase is a different photosynthetic enzyme than Rubisco, which is the enzyme found in C_3 plants. Photosynthesis performed by C_3 plants encourages isotopic fractionation both when the carbon dioxide enters the plant through the stomata and by the enzyme Rubisco. In comparison, C_4 plants have an apparatus called a bundle sheath cell which limits the amount of carbon dioxide that is not processed. This means that the bulk of carbon metabolized by the plant, and therefore incorporated into its tissues, is processed by PEP carboxylase. These differences result in different stable isotope ratios for the plant, which are proliferated through the food chain (Farquhar, Ehleringer, and Hubick, 1989).

Utilizing carbon and nitrogen stable isotope ratios to compare urban trash with fur samples of wildlife in transects from the urban center should result in a $\delta^{13}C$ and $\delta^{15}N$ spread that differentiates wildlife impacted by anthropogenic inputs to the food chain and those predominantly relying on natural sources of food. In 2008, Jahren and Kraft performed a $\delta^{13}C$ and $\delta^{15}N$ study using popular food components from fast food restaurants across the United States (Figure 5). Their experiment showed that even meat products in urban environments contained strong signatures for corn, suggesting that urban omnivores and carnivores could be differentiated from non-urban species

based on their stable isotope values. While variability existed between chains and regional location, a clear difference between corn-fed and grass-fed meat was determined. The $\delta^{13}C$ values that are less than -21‰ may indicate a source of carbon that is not corn. The closer to -21‰ the value, the less corn is present in an animal's diet. Low $\delta^{15}N$ values indicate prevalent fertilizer, like with corn and other cultivated crops. In comparison, natural fertilizers such as animal waste have a higher $\delta^{15}N$ value. Due to these differences, $\delta^{15}N$ values can be used as a marker for anthropogenic food sources (Jahren and Kraft, 2008).

Summary of $\delta^{13}C$ and $\delta^{15}N$ values of fastfood sampled

	Mean, ‰	Range, ‰	Minimum, ‰	Maximum, ‰
All samples				
Beef ($n = 162$)				
$\delta^{13}C$	−18.0	11.8	−25.5	−13.7
$\delta^{15}N$	6.1	2.1	5.3	7.4
Chicken ($n = 161$)				
$\delta^{13}C$	−17.5	2.7	−19.3	−16.6
$\delta^{15}N$	2.3	1.3	1.5	2.8
Fries ($n = 161$)				
$\delta^{13}C$	−26.9	4.9	−28.8	−23.9
$\delta^{15}N$				

Figure 5: Even anthropogenic meat products contain a strong corn signature. This chart illustrates the $\delta^{13}C$ and $\delta^{15}N$ values determined from food sampled across the United States in three fast food chains. Averages show that fries are the least enriched in corn-derived carbon. All beef and chicken indicate the use of fertilizer through the $\delta^{15}N$ values. After Jahren and Kraft (2008).

Using stable isotope analysis to determine the boundaries of an ecosystem through the isotope signature of anthropogenic food has many advantages, including its low cost. Measurements can be taken through noninvasive means, allowing researchers to sample threatened and endangered wildlife without negative population impacts. The same technique can be applied to multiple mammal species, and avian species can

be tested utilizing different sample preparation methods. Extensive research has also been done on sources and assimilation of $\delta^{13}C$ and $\delta^{15}N$, providing the groundwork for applying these techniques to the understanding of urban ecosystems.

Alternative Experiment Options

While stable isotope analysis appears to be the best choice for determining ecosystem boundaries, it is still important to analyze the effectiveness of alternative methods. Stomach content analysis, trace element analysis, and home range analysis are the three techniques presented and compared to the use of stable isotope analysis.

The classical approaches to ecology involve long-term observation of individuals and often require sedation or euthanization for blood, tissue, or stomach content analysis. Scat analysis is another technique commonly used to supplement observations, but determining anthropogenic food in the diet of an animal this way has proved ineffective. Most processed foods, which are predominantly what people would be eating in an urban environment, contain no indigestible material that could be easily identified (Newsome et al., 2010). In place of direct observations and GPS or radio tracking, many techniques have been developed to track movements of animals and define territory sizes. Observational techniques require manpower to track multiple individuals or lengthy field work performed by a single individual. An additional drawback to using observational techniques is that they rely on the ability of observers to continually identify a single individual from the rest of its species. Other alternatives

and supplements to the traditional observation approach are outlined and then compared to the use of stable isotope ratios in the following sections.

Stomach Content Analysis

Stomach content analysis involves the trapping and dissection of individuals within a species. It allows researchers to construct a detailed account of the indigestible and recently consumed material, but has additional drawbacks beyond its destructive approach. While specialized equipment is not required for stomach content analysis, the researcher does need to have an expansive knowledge base. The resulting data only provides a limited snapshot of diet, which is dependent on how quickly matter consumed by the animal is digested. Differentiating between natural and anthropogenic sources of food using stomach content analysis is also complicated by the potential variety of protein sources found in human refuse. Without bones or other distinct morphological components of the protein, differentiating between natural predation and scavenging on human resources may be difficult (Michener and Schell, 1994). Stable isotope analysis is non-invasive and non-destructive, allowing it to be utilized without influencing population. A thorough background in taxonomy is not required, and stable isotope analysis offers the benefit of showing long-term and short-term consumption patterns based on the sample.

Trace Elements

Trace elements are, like stable isotopes, a type of intrinsic marker. Using as little as 3 mg of tissue sample, an inductively coupled plasma mass spectrometer can process the material to generate a unique chemical profile. This profile should match the geographic location of the animal when the tissue was grown (Hobson and Norris, 2008). While specificity of location is a concern when attempting to see a gradational change between populations, differences in elemental profiles for sandpiper feathers were seen at just 3 km apart (Norris et al., 2007). This detail suggests that trace elements could potentially be a better testing method than stable isotope analysis. However, while trace elements can differentiate populations based on chemical signatures across relatively small spatial scales, some elements may be regulated within members of the same species. Trace elements essential to marine animals, such as Cu, Mo, Se, and Zn, displayed no significant spatial differences between twenty-nine fish species caught off the coast of China in eight locations. The other possible explanation for the lack of differentiation is that there was no source of metal input to the diets of the fish. This possibility indicates that a thorough understanding of the trace elements and their sources in a given environment are required to ensure the proper trace elements are analyzed (Zhang and Wang, 2012).

The main drawbacks of using trace elements are cost and the lack of relative abundance maps for the elements. The lack of knowledge of variability in elemental abundances across terrain requires the initial step of a trace element analysis in ecology

to begin with analyzing different pieces of the study area in detail. Additionally, running trace element analysis is an expensive process compared to stable isotope analysis. While trace elements are a viable alternative, and they do provide additional data on anthropogenic contaminants that could also be used as markers for the urban environment, the cost and need for abundance maps seems to be more limiting than stable isotope analysis (Hobson and Norris, 2008).

Home Range Analysis

The home range of an animal, also called a territory, is defined as "where [the animal] enact[s] their day-to-day activities"[9] (Powell, 2000). In this way, territory can be defined by the movement of an animal among resources (Hobson and Norris, 2008). It comprises areas the animal frequents with food and water resources, access to potential mates, and locations to raise young (Powell, 2000). Understanding the territory of an animal, and if that territory changes through time, would be another potential method for identifying species that are reliant on anthropogenic inputs to the urban ecosystem, even if they are not necessarily sleeping in the urban environment.

As a home range is frequented by the same individual over a given time period, it is feasible to monitor urban wildlife and construct home range maps. Part of the

[9] Powell, R. A. "Animal Home Ranges and Territories and Home Range Estimators." *Research Techniques in Animal Ecology: Controversies and Consequences*. Ed. L. Boitani and T. K. Fuller. New York: Columbia UP, 2000. Pp. 65.

definition of a home range implies "predictability"[10] related to the likelihood of the animal being at any given place in time (Powell, 2000). All types of home range maps are created using point data based off of direct observation or satellite tracking of the location of individuals. These maps could be used to analyze the percentage of urban and non-urban terrain the animal occupies in place of creating isotope ratio transects from urban centers to the natural environment. The percentage of territory or time spent in the urban landscape could then be expanded to draw conclusions on the relative importance of each area to an individual (Powell, 2000).

Many techniques have been designed for estimating home range size and are most frequently accomplished through the use of spatial analysis tools in geographic information system (GIS) programs like ArcMap (Figure 6). This section will briefly compare the advantages and disadvantages of stable isotope analysis to three types of home range estimators: utility distributions, kernel estimators, and home range core.

Utility Distribution

Utility distribution maps are created using known sightings of the individual to generate point locations. These maps represent the intensity with which an animal uses a particular piece of its territory, which is generated from the point locations using a utility function. The map is dependent on the territory probability chosen by the designer, as the goal is to "define […] the smallest area that accounts for a specified

[10] *Ibid*. 9, pp. 74.

proportion of total use"[11] (Powell, 2000). Generating a utility distribution map requires extensive point data as well as knowledge about the individual animal gathered from observation. It is unlikely feasible to create these maps for multiple species and individuals without extensive resource expenditures. For this reason, using utility distribution to define territory is less suited for this study compared to stable isotope analysis.

Kernel Estimators

Using the kernel estimator method, also called the kernel density estimator, a territory map can be created based entirely on data points. The advantage of this technique over other home range estimation techniques is that it does not require the creation of a somewhat-arbitrary grid system, and the kernel size can incorporate measurement error. Kernel size, called band width, can be constant in the case of a fixed kernel or vary, such as with adaptive kernels. Based on the average value at each kernel, a distribution is created and then projected as a surface using the size of the kernel (Figure 6). Varying the kernel size alters the detail of the resulting surface and the visibility of sampling error (Powell, 2000). According to Powell (2000), fixed kernel density is the most accurate and best of the home range estimators.

[11] *Ibid*. 9, pp. 75.

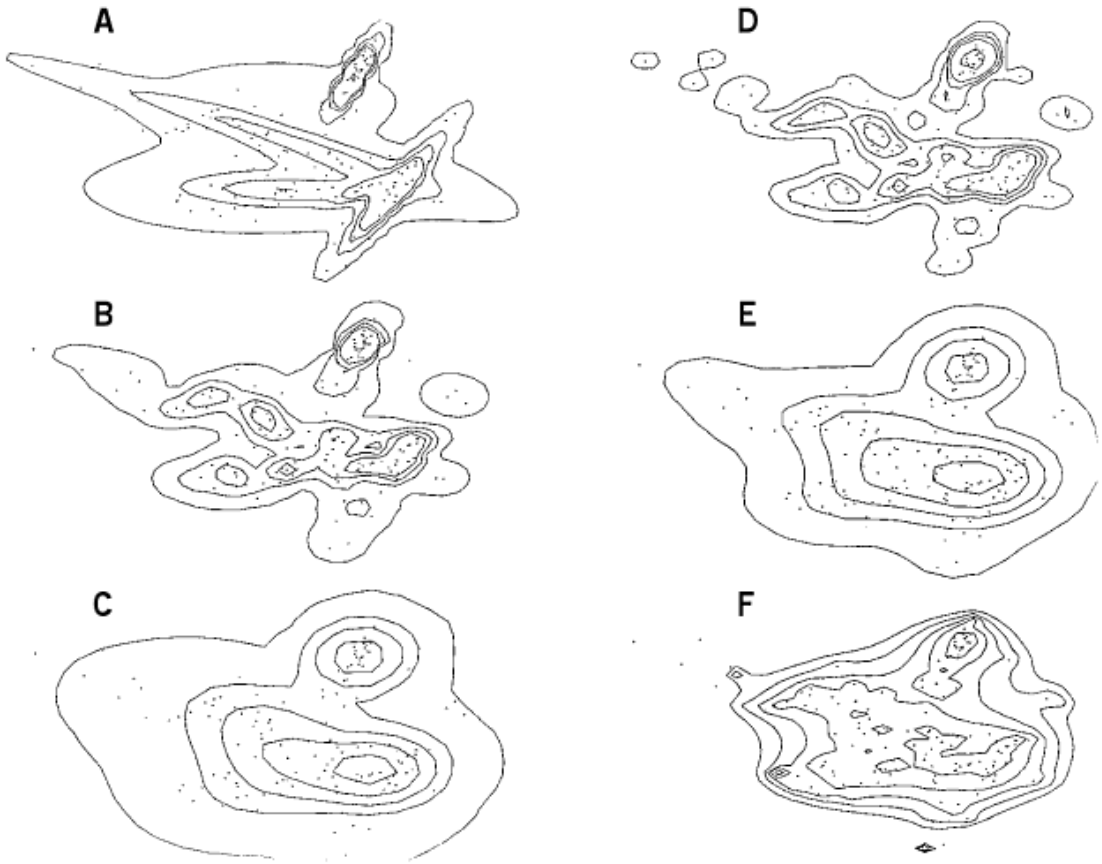

Figure 6: Examples of home ranges simulated by Powell et al. (1997) and modified by Powell (2000). "(A) True density contours. (B) Fixed kernel density estimate with cross-validated band width choice. (C) Fixed kernel density estimate with ad hoc band width choice. (E) Adaptive kernel density estimate with ad hoc band width choice. (F) Harmonic mean estimate"[12] Taken from Powell (2000).

Kernel estimators do have known drawbacks that include a loss of time sequence information that may have been collected during observations, territory maps produced rely on probability and can therefore have disconnected sections, and the map produced lacks a weight to how important each area of a territory are to the animal. Kernel estimators create territory outlines that are a snapshot in time restricted to the collection period of the data set, and therefore rely heavily on the amount of data collected for each individual (Powell, 2000).

[12] *Ibid*. 9, pp. 88.

Using isotope ratios to compare individuals allows for a much smaller sampling period, and the relative importance of food sources would be incorporated into the carbon and nitrogen isotope values. Defining home territory using kernel estimators would generate the probability that an animal incorporated urban terrain and possibly anthropogenic food sources in its daily routine, but this probability would be limited to the study time, and would lack the relative importance of these resources to daily use. For these reasons, stable isotope ratios more strongly address the research question and are therefore better suited to answering this hypothesis.

Home Range Core

The home range core is an attempt to weigh different sections of a territory by importance. Generating a home range core map requires the assumption that areas of the territory that are important to an animal are used more frequently than would be caused from random use. Cores incorporate known behavioral patterns of the species, including territoriality and foraging methods. An objective method for defining a core involved the simple definition of a core as any part of the territory used more frequently than would be expected by evenly distributed use. The primary drawback is that this method is arbitrary. The non-arbitrary method for defining a territory core was developed by Powell (2000), and plots percentage of home range against probability of use to generate a slope. This slope can then be used to map intensity of use and delineate which areas of territory are actually peripheral to the animal (Powell, 2000).

While the home range core solves the problem of determining frequency of use and outlines areas of relative importance to the animal, extensive field work is required to build the data set necessary to generate a home range core. Field assessments of home range cores would also be needed to determine what the animals are getting from these areas in order to determine if anthropogenic food inputs were included. In contrast, stable isotope ratios would not require constant field time, and the data generated in the lab would be indicative of food sources. For these reasons, it is again apparent that stable isotope ratios best address the research question and are the best method.

While alternative methods for research exist, they fall short of the ability of stable isotope analysis to analyze the anthropogenic impact to the food chain. Home range analysis requires extensive field work and data collection on individual animals, while stomach content analysis requires thorough knowledge of taxonomy and is a destructive approach. Using trace elements is cost-prohibitive, and there is also a lack of elemental abundance maps required for analysis. Despite the availability of alternatives to using stable isotope ratios, the goal of this research question to address multiple individuals from multiple species makes stable isotope ratios the best approach.

Case Studies

Next, specific instances of stable isotope analysis being applied to ecosystem and urban ecosystem research will be explored. These case studies serve as examples of experiments that do and do not support the feasibility of using C and N stable isotope analysis to analyze the anthropogenic impact on the traditionally-defined natural ecosystems bordering physical urban environments through noninvasive sampling of omnivorous urban wildlife.

Non-Supportive Case Studies

Isotopes and Anthropogenic Foraging in Bears

In 2011, Merkle, Derbridge, and Krausman released the results of their study on black bear foraging habits. Their experiment was designed to use stable isotope analysis to compare fur samples from urban and rural caught individuals in an attempt to assess the contribution of anthropogenic foods to black bear diets (Merkle, Derbridge, and Krausman, 2011). Merkle, Derbridge, and Krausman (2011) focused on comparing the ratio of ^{13}C and ^{14}C isotopes in their study. The source of ^{14}C for black bears was the native, terrestrial vegetation, derived from the C_3 photosynthetic pathways. The ^{13}C isotopes in black bears were related to the proportion of human food sources the bears ingested (Merkle, Derbridge, and Krausman, 2011).

Merkle, Derbridge, and Krausman (2011), similar to what has been proposed as a research technique for defining urban ecosystem boundaries, relied on the influence of

corn found in human foods to enrich ^{13}C isotope. In this manner, black bears that were reliant on humans, and could therefore be considered a wildlife management and human safety problem, could more reliably be identified than bears that were simply moving through urban environments. The noninvasive collection of fur samples from these bears also supports the same procedure as viable for an expansive study of urban wildlife (Merkle, Derbridge, and Krausman, 2011).

The 2011 experiment performed by Merkle, Derbridge, and Krausman revealed that urban and wild bears in their sample period did not significantly differ in isotope values, implying that the bears were not consuming garbage in large enough proportion to the rest of their diet. This suggests that, despite using the urban environment as part of their territory, these bears could not be identified as urban wildlife through the use of stable isotope analysis. While possible that sampled bears had not consumed large amounts of human trash, or that the wild bears had consumed some portion of trash, Merkle, Derbridge, and Krausman (2011) also proposed that it was possible the diet assumptions they had made concerning black bears were incorrect. While a large percentage of processed foods and packages are created from corn, garbage also consists of fruit and vegetable matter that could be more selectively ingested by foraging bears. Merkle, Derbridge, and Krausman (2011) stated that they were confident with their assumption that bears consumed corn-based products, however, and proposed another alternative for the reason that wild bears foraged inside the urban environment – non-native vegetation.

If, instead of primarily feeding on garbage, the bears were drawn to the urban environment by exotic fruit trees, the assumption that the $\delta^{13}C$ values would reflect a human influence would be incorrect. Exotic fruit trees use the same C_3 photosynthetic pathway as native vegetation, making it impossible to differentiate the native and non-native proportion of the bear's diet using stable carbon isotope ratios. Additionally, the majority of conflicts between bears and humans occur during the fruit-bearing season for apple trees (Merkle, Derbridge, and Krausman, 2011). While the likelihood that this particular set of circumstances is true for all urban wildlife, it does suggest that the seasonality of movement between the city environment and the more natural environments is vital to making assumptions about expanding the boundaries of an urban ecosystem. This also illustrates the need to fully understand the natural behaviors of the animals being studied.

Isotopes in Urban and Rural Red Foxes

Lavin et al. (2003) released the results of their carbon and nitrogen isotope study on red foxes in urban and rural environments of Illinois. They compared both fox groups against coyotes in an effort to compare the diets of the two specie and determine if competition existed for food resources. They theorized that competition in the rural environment would be reduced due to the bottom-up control on the food chain exhibited by agriculture. In addition to running blood, fur, and serum samples from the predators, prey remains were opportunistically collected from den sites to use as

isotopic markers for food sources. All of the collected samples were analyzed for their $\delta^{15}N$ and $\delta^{13}C$ values. After the captured predators were released, they were then tracked using radiotelemetry and then by spotlight during the animals' nocturnal routines. The tracking allowed Lavin et al. to estimate home ranges using an adaptive-kernel analysis (Levin et al., 2003).

The analysis of fur samples run by Levin et al. (2003) also concluded that the isotope ratios they displayed were signatures of when the hair was growing. This supports the hypothesis that fur of varying lengths and growth periods could be tested to show if the animal had been moving between urban and wild environments for different food sources.

The experiment performed by Levin et al. (2003) showed urban foxes to have a higher $\delta^{13}C$ ratio, while their $\delta^{15}N$ values were lower than rural foxes (Figure 3). While Levin et al. (2003) did not test non-natural food sources in the urban environment for red foxes, their rural counterparts had a stronger carbon isotope ratio signature for corn. If the classic delineations of urban and non-urban ecosystems are applied, the human impact on the diets of animals in the rural environment should be less than that of the urban environment. In contrast, the bottom-up controls on the ecosystem that humans exert over agricultural areas has altered the feeding habits of prey items, which has altered the isotopic composition of animals farther up the food chain (Levin et al., 2003). These results show a strong anthropogenic impact to the food chain outside of

the classic urban environment, and this alteration should be considered when defining ecosystem boundaries.

The strength of their conclusion varied with the different types of tissues analyzed, but Lavin et al. (2003) believed that the presence of coyotes did alter the prey selection of red foxes in rural environments. Rural foxes had an increased trophic level that ranged from 4.21-7.54% due to coyote presence, which forced the foxes to feed more on prey that consumed larger proportions of C_4 plants. However, they also observed a 4.14-10.96% increase in trophic level based solely on habitat differences of rural to urban foxes, which was the likely cause for variations in the nitrogen isotope ratios between the two populations (Levin et al., 2003). The differences seen due to feeding patterns in different environments and not the pressures of other predators is an indicator that populations of urban and non-urban wildlife could be differentiated from one another using stable isotope analysis.

Supportive Case Studies

Isotope Variation in Gray Wolf Individuals

In 2005, Urton and Hobson published a paper titled "Intrapopulation Variation in Gray Wolf Isotope ($\delta^{15}N$ and $\delta^{13}C$) Profiles: Implications for the Ecology of Individuals", which detailed their research on the relationship between gray wolves and their prey items using stable isotope analysis. Their research used stable isotope analysis of fur from gray wolves (*Canis lupus*) and seventeen other species in a natural forest habitat

near Saskatchewan, Canada to determine the trophic relationships between the animals. Guard hairs were collected from animals of all eighteen species over the course of two winters, as well as from road kills that occurred in the same time period (Urton and Hobson, 2005). In order to determine the trophic relationships between the species, Urton and Hobson used a dietary mixing model called isosource (2005). Isosource used "the isotopic values of consumer tissues, isotope values of the dietary inputs, and the diet-tissue discrimination factor relevant to the tissue being analyzed"[13] (Urton and Hobson, 2005). By incorporating the feeding habits of the wolves into the isosource computer model, Urton and Hobson (2003) hoped to increase the accuracy of the estimations made by the model. The feeding habits were reflected in the fur samples taken from the animals, and it was theorized that they would be isotopically distinct from other samples (Urton and Hobson, 2003).

Wolves tested matched the expected $\delta^{13}C$ values associated with an ecosystem that was dominated by C_3 plants. What was not expected were the broad ranges of isotope values for the wolves; $\delta^{15}N$ values ranged from 5.4‰ to 11.2‰, while $\delta^{13}C$ values ranged from -19.7‰ to -24.3‰ (Figure 7). These ranges reflected individual diets of the gray wolves, which sometimes correlated on the population level with different territory locations. The tested stable isotope values for elk and deer showed no differences, indicating that the species browsed similar if not identical vegetation.

[13] Urton, E. J. M., and K. A. Hobson. "Intrapopulation Variation in Gray Wolf Isotope ($\delta^{15}N$ and $\delta^{13}C$) Profiles: Implications for the Ecology of Individuals." *Oecologia* 145 (2005). Pp. 319.

The isosource model, however, indicated that wolves primarily preyed on elk and white-tailed deer (Urton and Hobson, 2003).

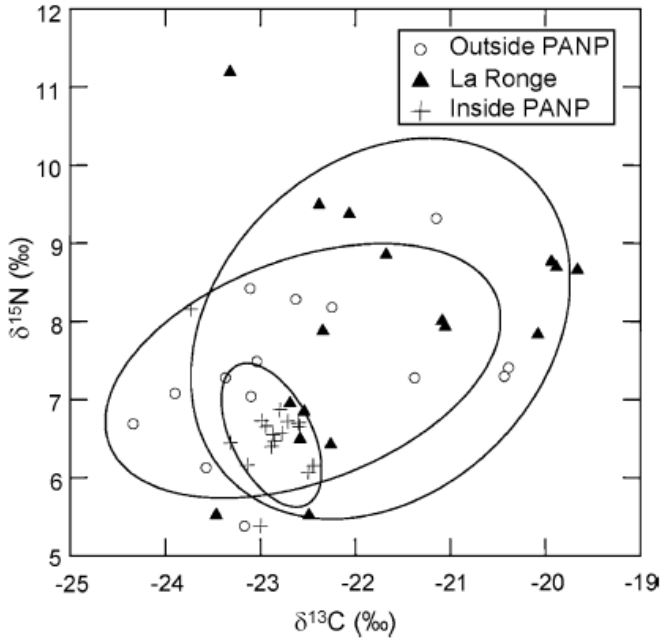

Figure 7: This depicts $\delta^{13}C$ and $\delta^{15}N$ values from fur samples of gray wolves across three sample groups, underlying the ability of stable isotope analysis to differentiate individuals. The ellipses represent the standard deviation of each group. Taken from Urton and Hobson (2003).

Urton and Hobson (2003) theorized that the variation in stable isotope values for gray wolves were due to diet differences based on prey distribution and abundance. Additionally, two of the three populations of gray wolves tested were from human-exploited areas. The least variance was found in the pack of wolves from the natural, protected environment. These results support other theories that exploited gray wolf populations are more likely to have a wider range of prey sources. This is because exploited populations have more unstable packs, leading to individuals that roam farther from a central territory, and are therefore exposed to more prey items than

wolves that maintain strict territorial boundaries. Further support that the stable isotope variability was due to diet is in the assimilation time of fur. The stable isotope signature of prey items are incorporated into fur over the course of months, which is likely too fast of a time period to reflect changes based solely on location (Urton and Hobson, 2003).

The experiment performed by Urton and Hobson (2003) on gray wolves supports that fur samples for multiple species can be used to compare stable isotope values. Additionally, the variation that individuals of the same species displayed also supported that stable isotopes can be used to determine different food resources and location. With the isotope compositions of food resources being assimilated into fur isotope values in a timespan of months, the viability of using fur to show short-term or seasonal use of anthropogenic resources is also supported (Urton and Hobson, 2006).

Illustrating Anthropogenic Feeding in Silver Gulls

In 2011 Auman et al. released a study on the impacts of human-derived food on silver gulls entitled "Urbanization of the Silver Gull: Evidence of Anthropogenic Feeding Regimes from Stable Isotope Analyses." Their hypothesis focused on there being a clear difference between the isotope ratios of silver gulls feeding on anthropogenic compared to natural food sources. In order to test their hypothesis, Auman et al. (2011) collected blood samples from silver gulls nesting in the urbanized environment of Hobart and on a remote island near Tasmania. Samples were taken before and after the breeding

season in an effort to determine if there were differences in isotope compositions of birds dependent on the time of year. The $\delta^{13}C$ and $\delta^{15}N$ values were compared between the two populations. Additionally, regurgitated food samples from both populations were used to supplement knowledge of food resources (Auman et al., 2011).

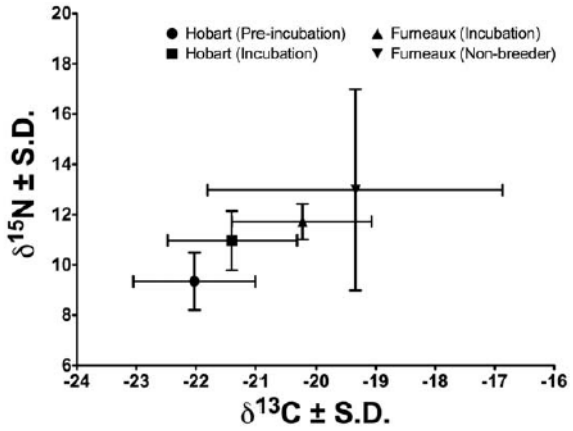

Figure 8: This shows the stable isotope values for carbon and nitrogen in silver gull populations in an urban (Hobart) and natural (Furneaux) environmental setting. Taken from Auman et al. (2011).

The results of their experiment showed that Hobart silver gulls had a higher average $\delta^{13}C$ value compared to the non-urban island-dwelling population (Figure 8). The $\delta^{13}C$ values suggested that the urbanized silver gulls were ingesting higher amounts of freshwater and terrestrial foods, which was supported by the regurgitation data. Analysis of regurgitated foods from the two gull populations revealed a higher percentage of garbage in the diet of the urban silver gulls, including foods such as dog and cat food, potatoes, and pasta and non-food items such as paper and cigarette butts (Auman et al., 2011).

In this experiment, the combination of stable isotope analysis and direct observation of regurgitated food was used to support the hypothesis of Auman et al.

(2011) that dietary differences in silver gulls could be identified. The dietary differences shown in this experiment support that stable isotope analysis could be used to quantitatively identify human impact to the food chain. This can then be used to outline boundaries for an urban ecosystem environment.

Relating Isotopes in Kit Foxes to Humans

In 2010, Newsome et al. published a paper that focused on the anthropogenic impact to the food resources of the endangered kit fox population. Their study focused on three populations of San Joaquin kit foxes residing near the city of Bakersfield, Kern National Wildlife Refuge, and Lokern - an area described as a natural environment. Fur and vibrissae, also known as keratin, samples were collected from each population of foxes. Samples were also taken from natural prey species of the kit foxes in both of the natural environments, and hair samples from the residents of Bakersfield. Scat analysis was performed to ensure that proper prey species were analyzed for the two wild populations. The kit fox vibrissae samples were continually collected to establish a record that would exhibit temporal shifts in diet if any existed (Newsome et al., 2010).

The study resulted in no differences between the mean $\delta^{13}C$ of humans and the Bakersfield kit foxes. The fur samples collected from the natural environments showed lower mean $\delta^{13}C$ amounts compared to their urban counterparts, and higher mean $\delta^{15}N$ values. Despite the mean differences between populations, there was an approximate 8% overlap in both carbon and nitrogen isotope ratios between the populations. Even

after correcting for "tissue-specific trophic discrimination"[14] in the kit fox fur samples, the differences between the two populations was apparent (Newsome et al., 2010). The correction that Newsome et al. (2010) applied to $\delta^{15}N$ values was 3‰, which is similar to that proposed by Ehleringer et al. in 1986. The $\delta^{13}C$ correction used by Newsome et al. (2010) was 1‰. Additionally, the Bakersfield human hair samples revealed a slightly higher $\delta^{15}N$ mean value compared to the Bakersfield kit foxes. The continuous analysis of vibrissae from wild foxes also showed that their $\delta^{13}C$ and $\delta^{15}N$ values differed significantly from year-to-year (Newsome et al., 2010).

[14] Newsome, S. D., K. Ralls, C. V. H. Job, M. L. Fogel, and B. L. Cypher. "Stable Isotopes Evaluate Exploitation of Anthropogenic Foods by the Endangered San Joaquin Kit Fox (Vulpes Macrotis Mutica)." *Journal of Mammology* 91.6 (2010). Pp. 1317.

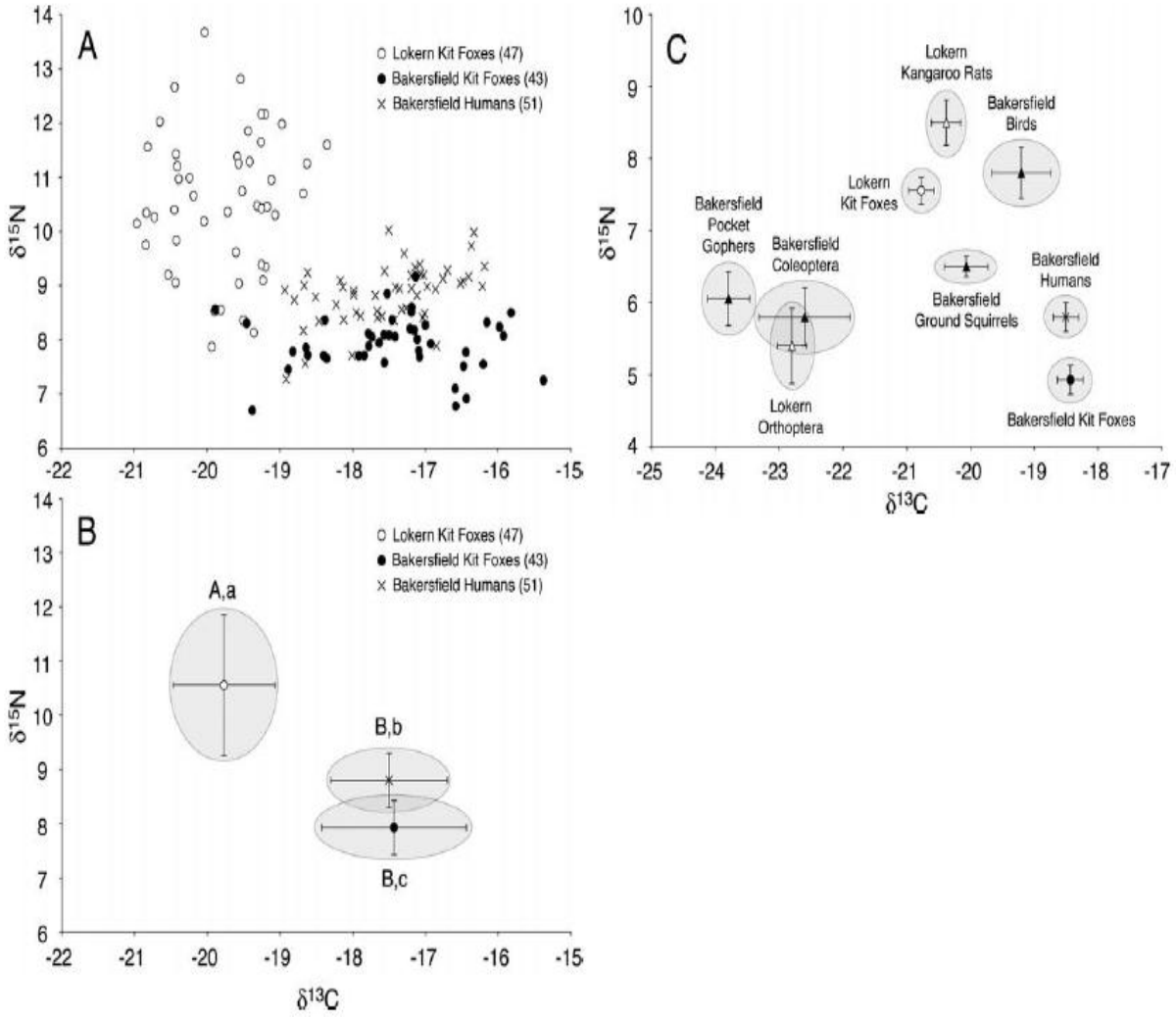

Figure 9: A comparison of Lokern kit foxes, a natural population, with populations of humans and kit foxes living in Bakersfield, California. (A) displays individual samples, (B) depicts mean isotope values, and (C) compares the trophic-corrected mean isotope values of common kit fox prey items, humans, and kit foxes. After Newsome et al. (2010).

The results discovered by Newsome et al. (2010) offer support for the procedures proposed here to use stable isotope analysis in urban and natural wildlife to analyze the impact of anthropogenic food sources. A clear anthropogenic trend was demonstrated in the kit fox population by Newsome et al. (2010), and their analysis negated the need to estimate or test multiple sources of urban food by comparing fur

samples with human hair samples (Figure 9). While overlap exists between the urban and natural populations, the raw individual data is clear enough to display a difference without the mean calculation. The statistically indistinguishable $\delta^{13}C$ values between kit foxes and humans in Bakersfield presents strong support for the use of stable isotope analysis to aid in redefining the boundaries of an urban ecosystem (Newsome et al., 2010).

Conclusions

This research was aimed at analyzing why and how the definition of an urban ecosystem should be expanded to include areas that are impacted by urban centers but are not necessarily inside the human delineation of a city. Understanding the impact humans have on ecosystems is important for wildlife management and conservation. For this understanding to be thorough, it must be taken into consideration where these ecosystem boundaries are, and that they cannot simply be defined with city delineations. In a 2011 paper on urban ecological systems, Pickett et al. states that it is well known that urban ecosystems have exchanges beyond the boundaries often prescribed to them. They go on to state that "an ecological understanding of urban systems also must include less densely populated areas in order to capture the full range of urban effects […]"[15] (Pickett et al., 2011). At first glance, these two points of view are seemingly at odds with one another. However, as has been explored in this

[15] *Ibid.* 3, pp. 333.

paper, defining the boundaries of an ecosystem is a complicated, yet vital, process that requires detailed analysis. Compared to the alternative research techniques investigated, using stable isotope analysis across multiple urban species appears to be the most accurate and least field-intensive approach. Stable isotope analysis is relatively cheap, and requires no invasive or destructive procedures on the study animals. For these reasons, it was determined that the use of stable isotope analysis was the best fit for redefining the boundaries of urban ecosystems.

Not all wildlife living in the urban environment are common species, evidenced by the 2010 research completed by Newsome et al. on urban populations of the endangered kit fox. Endangered and threatened wildlife can be sampled for stable isotope analysis without damaging the population, and with relatively little stress to the animal. The use of noninvasive sampling techniques to collect fur, hair, or feathers allows a wide range of species to be analyzed using similar lab techniques. Most urban species that are mobile enough to cross between the classical boundary of the urban and natural environments are mammals or birds. Every member of these two large groups can be compared with their stable isotopes.

The ability to run variable samples and cost efficiency are some of the reasons that stable isotope analysis is better suited to address this hypothesis. The cost of running samples for stable isotope analysis is relatively low (Lajtha and Michener, 1994). Trace element analysis is more expensive, and the field currently lacks detailed maps to show spatial trends and changes in trace element abundance (Hobson and Norris,

2008). Other techniques analyzed in this paper that could be used to determine where animals were frequenting include the array of home range analyses. While utility distributions, kernel estimators, and home range core analyses were excellent at statistically generating boundaries for the probable territory of an individual, each technique required assumptions about the behavior of the animal. More importantly, all home range analyses investigated required a large amount of data points for each individual. The amount of data required to run the modeling for home range analysis is prohibitive when dealing with multiple species. With less background research required, no trend maps to create, and a more cost-effective way to run samples, stable isotope analysis is the most adaptable of research techniques analyzed.

Stable isotope analysis has been applied to a wide range of experiments, and its versatility is apparent. Most importantly, however, many studies have used isotopes in analyzing diet, which is a major aspect of the ecosystem that humans in urban environments influence. The two most common isotopes for diet analysis, and therefore the two that were focused on as part of this report, were $\delta^{13}C$ and $\delta^{15}N$ (Ehleringer et al., 1986). Stable isotope analysis of food webs and individual diets relies on the fractionation through trophic levels as well as the natural abundances of each stable isotope in different food sources. Plants that undergo C_3 and C_4 photosynthesis create different $\delta^{13}C$ signatures in their tissues, which propagate up the food chain and are strong enough to determine food resources for animals (Farquhar, Ehleringer, and Hubick, 1989; Merkle, Derbridge, and Krausman, 2011).

Urton and Hobson (2003) used stable isotope analysis with fur samples from gray wolves, revealing that individual differences in stable isotope values could be observed. Their research implied that the urban and non-urban wildlife of the same species could then be differentiated based on the stable isotopic signature of their food sources (Urton and Hobson, 2003). Merkle, Derbridge, and Krausman (2011) were able to use isotopes in an effort to determine the anthropogenic impact on black bears. Their results showed that the sampled bears were not foraging primarily from human trash, but they hypothesized that this could be a seasonal variation or that the bears were actually consuming non-native fruit trees that humans were maintaining in the city (Merkle, Derbridge, and Krausman, 2011). This study pointed out the importance of understanding the target species being sampled. Lavin et al. (2003) was able to sample urban and non-urban red foxes in an effort to differentiate between the two populations. Their research also supports that it is possible to use stable isotope analysis to determine food sources, even when dealing with non-native food supplies. Auman et al. (2011) illustrated the use of stable isotope analysis to differentiate diet of urban and natural populations of silver gulls. Finally, Newsome et al. (2010) analyzed urban and wild populations of kit foxes, comparing their urban population to the isotope ratios of humans living in the same city. The research performed by Newsome et al. (2010) is a strong indication that wildlife are relying on the same food sources as humans, and support that stable isotope analysis is the best approach for identifying this relationship.

While Pickett et al. (2011) also propose that distinguishing between the urban environment and the natural environment is less important for science moving forward than the gradational change that occurs between these environments, this is not necessarily the case. From a strictly scientific standpoint, knowing the proportion of the environment at any given point influenced by humans in some manner is important. However, management and conservation efforts and government policy require defined areas. By being able to define the point at which the impact of a city to the surrounding ecosystems is minimal or negligible, better management and policies to support the urban ecosystem can be created.

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