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# MS Environmental Biology Capstone Project

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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

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CAPSTONE PROJECT

by

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# CHAPTER 1. LITERATURE REVIEW: AMENDING THE KNOWLEDGE GAP OF THE MESOPELAGIC ZONE

Currently there are 242,574 oceanic species accepted in the World Register of Marine Species (WoRMS Editorial Board, 2016), with study estimates ranging between 540,000 and 2,210,000 species (Mora et al., 2011; Appeltans et al., 2012). Hiding beneath the ocean waves is a world that holds 99% of the livable space (Costanza, 1999), as well as the highest density of biomass and the most biodiversity on the planet (Nelson, 2006; Irigoien et al., 2014). Below the first 200 meters begins the mesopelagic zone, where the largest migration occurs every night, bringing the greatest abundance of vertebrates in the world (Berge et al., 2009; Haddock et al., 2010). From the deep waters where the pressure is high, and the temperature is low, they come to the surface to feed in a drastically different environment before descending back down as the sun threatens to expose them (Nelson, 2006; Berge et al., 2009). The mesopelagic zone is an ecological machine with biotic and abiotic gears that play crucial roles in the movement of nutrients, as well as repackaging and repurposing of carbon-based material. Survival in this intense ecosystem requires countless evolutionary adaptations. One such adaptation is bioluminescence, a phenomenon that allows an organism to produce light, and it is far more diverse and abundant in the ocean than what is found terrestrially or in freshwater systems (Haddock et al., 2010). Bioluminescent organisms can produce their own light through the breakdown of enzymes, or through symbiotic relationships with bacteria (Haddock et al., 2010; Davis et al., 2016). There are more than 550 genera of marine organisms that are known to be bioluminescent, but many have not been very well described or studied (Haddock et al., 2010). The west Pacific luminous roughy (*Aulotrachichthys prosthemius*) is a ray-finned fish with a bioluminescent organ housing a bacterial symbiont used for counter-illumination. It was originally discovered in 1902, and except for two studies in the 1950's, little research has been done on *A. prosthemius* to assess the specialization of its bioluminescent organ. The mesopelagic zone is one of the least investigated, and most poorly understood ecosystems in the world (Irigoien et al., 2014). A detailed study of *A. prosthemius* and its bioluminescent adaptations will ease the knowledge gap of this enigmatic ecosystem.

The mesopelagic zone begins at the depth where light is still visible, but too weak for photosynthesis to occur, and proceeds down until the light is too dim to effectively see and hunt prey (Robinson et al., 2010). At 200m where the mesopelagic zone begins, the pressure is multiplied from 1 atmosphere at the surface, up to 21 atmospheres (CalcTool: Pressure at depth calculator). At the bottom of the mesopelagic zone, at approximately 1000m, the pressure is nearly 100 atmospheres (CalcTool: Pressure at depth calculator). In addition to the drastic pressure gradient, the mesopelagic zone is where the ocean's major thermocline takes place. At 200m, the temperature averages around 23°C and radically gets colder as it gets deeper, until it evens out at 5°C near 1000m (Figure 1, (Castro et al. 2008)) (Karspeck et al., 2013). From the bottom of the thermocline, all the way down to the deepest depths of the hadopelagic zone (>6000m) the temperature will reach 0°C but will stay liquid due to the high concentrations of dissolved salts and extreme pressure (Karspeck et al., 2013).

Considering the severe living conditions within the mesopelagic zone, it was thought to be a liquid desert, devoid of life except for the occasional transient whale, or the sparse, hyper-adapted permanent resident. While transients are common, the mesopelagic zone is teeming with life and it is estimated that there is over 1.1 billion tons of biomass hiding within the mesopelagic zone (Lam & Pauly, 2005). Sonar data collected while mapping the sea floor consistently gave readings of a "false bottom" hundreds or thousands of meters above the true sea floor. The acoustic frequencies were found to create an anomaly described as the deep scattering layer (DSL) (Eyring et al., 1948). With the examination of over 100 mesopelagic trawls between 275m and 320m, it was determined that the densities and depth of krill and other organisms strongly correlated with the acoustic data regarding the location of the DSL (Boden, 1950). Additionally, the DSL was analyzed over 24-hour periods and the trawl densities continued to match the acoustic depth of the DSL, which was deepest during the mid-day hours, and shallowest during the mid-night hours (Belman, 1978). This led researchers to confirm that the DSL is a phenomenon of acoustic waves reverberating off massive biotic structures made up of thousands of organisms within the mesopelagic zone (Boden, 1950).

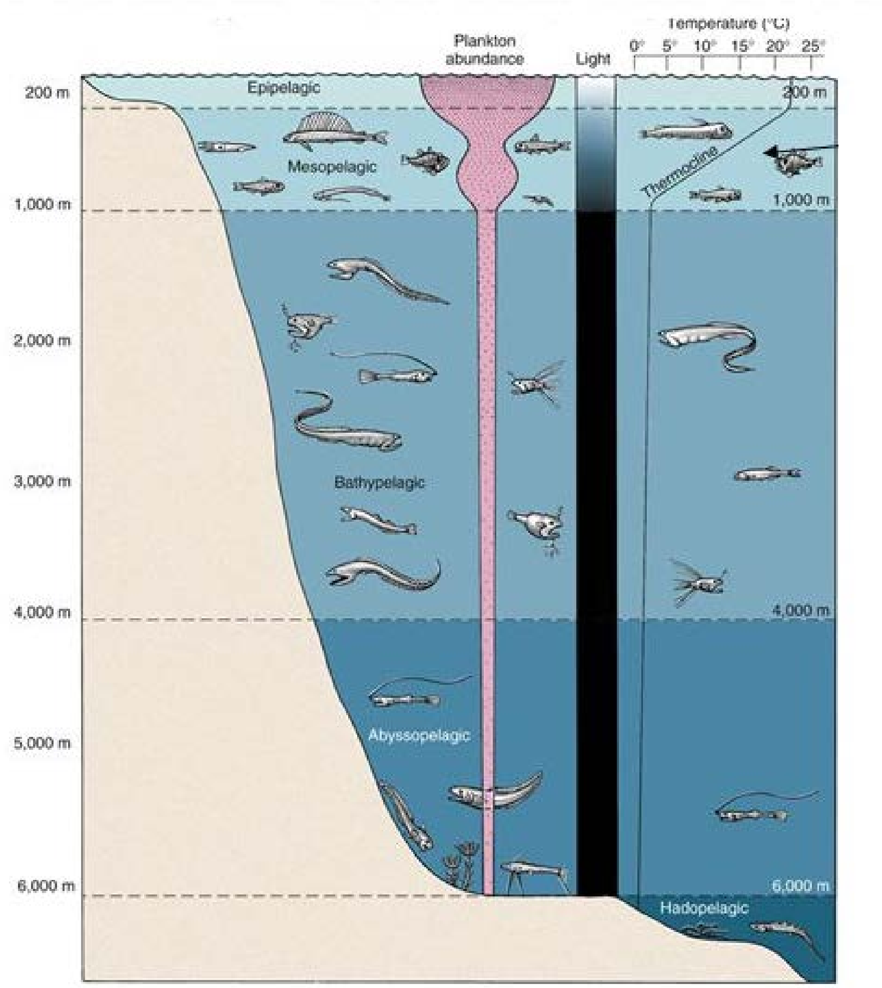



*Figure 1: Diagram of the different ocean zones with depths, plankton abundance, light penetration, and temperature. Note the major thermocline in the mesopelagic zone. (Castro et al. 2008)*

Dual-frequency acoustic and optical probes, combined with trawl nets, were used to gain a better understanding of the functions and structure of mesopelagic biota (Kloser et al., 2016).

The acoustic frequencies were used to target mid-trophic groups of macrozooplanton, and micronekton, which are operationally defined as organisms too agile to be caught by plankton nets, and too small to be retained by the larger mesh trawl nets (Brodeur et al., 2005). The masses of organisms that have swim bladders resonate and scatter the acoustic waves in a conspicuous way. Furthermore, the frequency and scatter of the sound allow for a general estimation of the size of the swim bladder, which can then be used to infer the size of the fish as well as the density of the organisms (Kloser et al., 2016). While collecting these data, trawl nets were deployed to collect organisms from the DSL, then the nets were brought aboard to clean, sort, photograph, categorize, and freeze. The sampled animals were sorted into four categories based on their acoustic resonance: cephalopods, crustaceans, fish, and gelatinous organisms like siphonophores. The fish were split into smaller groups by weight, and the density of fish with swim bladders was determined by the number of fish in each weight class, divided by the volume of water pulled through the net. When possible, the fish were identified down to the species level, and the lengths and widths of the individual organisms was obtained. It was found that 95% of the fish caught that were 100mm or larger were lantern fishes, a family of fish well known to exhibit bioluminescence (Irigoien et al., 2014; Davis et al., 2016), and 35 species in that length class had swim bladders. The researchers recognized that these methods have biases when the selectivity of the nets and catchability of the organisms are scrutinized. Regardless of that, they attributed 88% of the acoustic scattering to organisms with swim bladders and most of those organisms were bioluminescent lantern fishes (Kloser et al., 2016).

Acoustic data, combined with a sensitivity analysis, suggests that the previous estimate of mesopelagic biomass should be increased by, at the bare minimum, an order of magnitude, from 1.1 billion tons to over 11 billion tons of biomass (Irigoien et al., 2014). Additionally, several characteristics were described that highlight the contribution of the mesopelagic biota to our ecosphere. The most apparent component is the vertical migration by the micronektons, as well as larger mesopelagic fishes, to the upper layers of the epipelagic zone to feed on mesozooplanton and descending back to the darkness during the daylight hours (Irigoien et al., 2014). The vertical migration contributes to a biological pump to transfer carbon and other organic debris, or marine snow, from the upper layers of the ocean, down towards the layers beyond the mesopelagic zone at a faster rate compared to the natural downward drift (Irigoien et al., 2014; Isla et al., 2015). This flux is accomplished by the consumption of biotic material by the micronektons in the shallow waters at night, and as they descend the carbon and other nutrients are physically moved through the water column. Throughout the day, they respire and excrete at a depth of 500m – 700m, bypassing a significant part of the water column, to allow the material to reach the lower layers of the ocean (Irigoien et al., 2014). This is a vital resource for the bathypelagic zone beneath mesopelagic zone, since most of the organic carbon is lost in the sediment of the upper layers (Irigoien et al., 2014; Isla et al., 2015). The active relocation of carbon by the largest abundance of vertebrates in the world (Nelson, 2006), has a profound effect on the oceanic carbon cycle. Including the suggestion that the density of those vertebrates could possibly be underestimated by an order of magnitude, the implications could have significant revelations about the global biogeochemical cycle (Irigoien et al., 2014; Isla et al., 2015).

The mesopelagic fishes that contribute to the carbon flux have many specialized adaptations to allow them to thrive while migrating through the varying environmental factors within the mesopelagic zone. A fundamental biological adaptation in the darkness of the mesopelagic zone is the production of light via bioluminescence as well as the diversity in which it is presented among biotic organisms. The evolution of bioluminescence among marine fishes has 27 separate evolutionary events across 14 lineages of Actinopterygii, or ray-finned fishes (Davis et al., 2016). Using 301 taxa, 10 nuclear gene fragments, and 1 mitochondrial gene fragment, the phylogenetic relationships between fishes and their bioluminescence, was examined (Davis et al., 2016). After reconstructing ancestral nodes using previously documented sources (Figure 2) (Widder, 2010; Haddock et al., 2016), the singular gain of bioluminescence in some fishes was revealed, while other clades experienced several loss-then-gain iterations, where each is considered a separate evolutionary event (Davis et al., 2016).

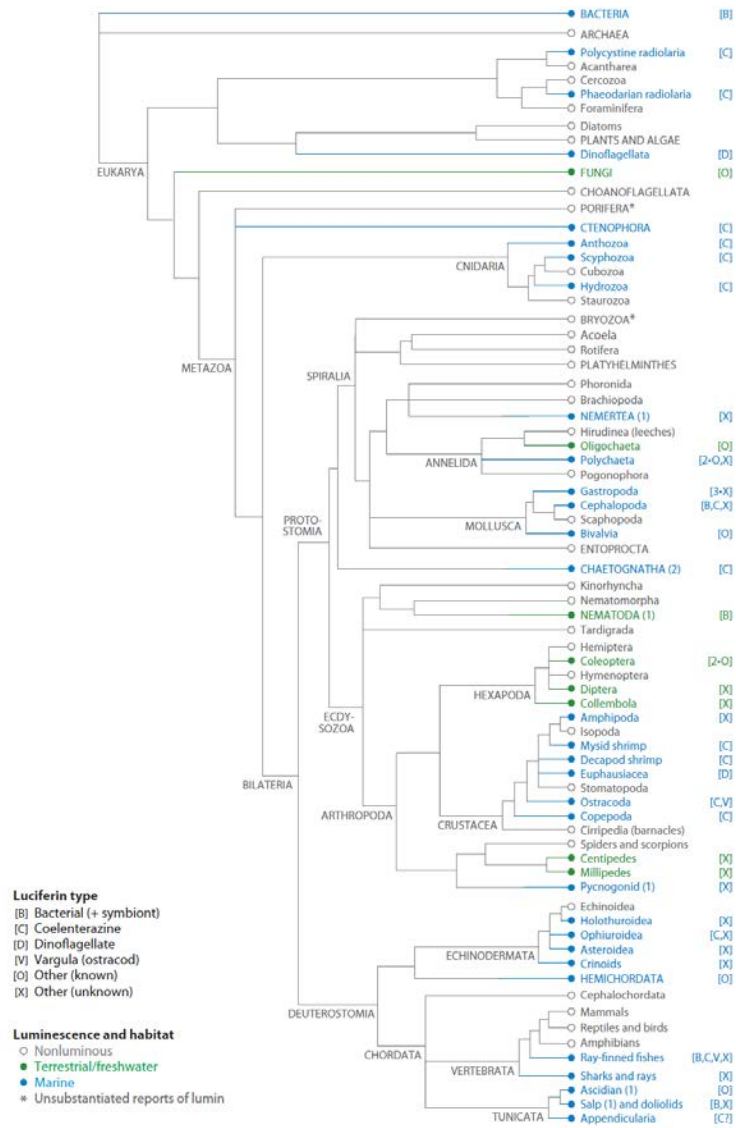



*Figure 2: The phylogenetic tree detailing the evolution of bioluminescence in eukaryotes. Note the color of the phylum names as an indication of the method of luminescence. (Haddock et al., 2016)*

More than half of the marine fishes known to be bioluminescent intrinsically produce light. This indicates that the fish has evolved a self-contained system to produce and emit light, typically by producing luciferin. The process of breaking the enzyme down with luciferase releases the photons. This greatly contrasts with the other common method where the fishes form a symbiotic relationship with a light producing bacteria as seen with *A. prosthemius* (Davis et al., 2016). Regardless of the method of light production, most mesopelagic bioluminescence is blue in color, matching the hue of the down-welling light (Haddock et al., 2016).

Due to the many functions of bioluminescence, it plays a critical role in the fitness of an individual organism, and for the entire ecological community (Haddock et al., 2016). Bioluminescence aids in survival through predator evasion, prey attraction, reproduction, and several other mechanisms (Haddock et al., 2016). Offensively, many species use bioluminescent lures to attract prey as seen in anglerfishes or illuminate prey in the case of lantern fishes. Defensively, an organism could startle potential predators, or counter-illuminate themselves. This specialized type of camouflage uses bioluminescent organs typically found on the underside of the fish (Haddock et al., 2016). The fish will break up its silhouette by blending into the ambient down-welling light by producing light of similar color, intensity, and distribution (Widder, 2010). Many predatory fish have developed eyes that are oriented upward to see the silhouettes of potential prey, making counter-illumination an effective method to evade predators (Poulsen et al., 2016).

The west Pacific luminous roughy (*Aulotrachichthys prosthemius*) is a small mesopelagic fish with a ventral bacterial bioluminescent organ used for counter-illumination. It was initially identified by D.S. Jordan and H.W. Fowler in 1902, and since then, very little research has been done on this fish. One study of the external anatomy of the fish and histology of the bioluminescent organ included hand-drawn histological figures that showed the luminous organ encircles the anus and is covered by a muscular hyaline lens (Kuwabara, 1955). The hyaline lens, a structure that is found in many counter-illuminating fishes to diffuse light more evenly, lies beneath the luminous organ (Poulsen et al., 2016). Another study described the luminous organ and noted that it was a capsule-like structure with inner and outer layers (Haneda, 1957). While many counter-shading fish can block the light being produced by contracting muscular shutters (Dunlap et al., 2012; Davis et al., 2016), *A. prosthemius* has chromatophores distributed through the inner layer of the luminous capsule (Haneda, 1957). These chromatophores could possibly be expanded and contracted to control the output of light, although it is not certain. Additionally, there is a structure situated on top of the luminous gland that seems to have an important role in *A. prosthemius'* bioluminescence. It is unknown what tissues it is made up of, or what function it serves (Kuwabara, 1955; Haneda, 1957). Perhaps the most unique aspect of *A. prosthemius'* bioluminescent organ is the duct system, which may be similar in structure and function to the bioluminescent organ found in *Siphamia versicolor* (sea urchin cardinalfish) (Dunlap et al., 2012). *Siphamia versicolor* will release excess bacteria in the bioluminescent organ through the duct system and into the intestinal tract to be excreted from the body. Alternatively, the duct system of *A. prosthemius* could be unique and new to science.

*Aulotrachichthys prosthemius* is an exceptional example of how little is truly understood about the mesopelagic zone. Despite being identified over a century ago, only a small handful of research has been conducted that sincerely appreciates this organism. The method of light production and the benefits to overall fitness may be typical, but the structures with which *A. prosthemius* emits light is peculiar. The investigation of the bioluminescence of *A. prosthemius* is a vital part of filling the intellectual void surrounding the mesopelagic zone.

# References

Appeltans, W., Ahyong, S. T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., … Costello, M. J. (2012). The magnitude of global marine species diversity. *Current Biology*, *22*(23), 2189–2202. http://doi.org/10.1016/j.cub.2012.09.036

Belman, B. W. (1978). Respiration and the effects of pressure on the mesopelagic vertically migrating squid Histioteuthis heteropsis. *Limnology and Oceanography*, *23*(4), 735–739.

Berge, J., Cottier, F., Last, K. S., Varpe, Ø., Leu, E., Søreide, J., … Brierley, A. S. (2009). Diel vertical migration of Arctic zooplankton during the polar night. *Biology Letters*, *5*(1), 69–72.

Boden, B. P. (1950). *Plankton organisms in the deep scattering layer*. San Diego, CA: U.S. Navy Electronics Laboratory.

Brodeur, R. D., Seki, M. P., Pakhomov, E. A., & Suntsov, A. V. (2005, January). Micronekton - What are they and why are they important? *PICES Press*, *13*, 7-11.

CalcTool: Pressure at depth calculator. (n.d.). Retrieved November 28, 2016, from http://www.calctool.org/CALC/other/games/depth_press

Castro, P., Huber, M. E., Ober, W. C., & Garrison, C. W. (2008). *Marine biology*. Dubuque, IA: McGraw-Hill.

Costanza, R. (1999). The ecological, economic, and social importance of the oceans. *Ecological Economics*, *31*(2), 199–213.

Davis, M. P., Sparks, J. S., & Smith, W. L. (2016). Repeated and widespread evolution of bioluminescence in marine fishes. *PLOS ONE, 11*(6).

Dunlap, P. V., Gould, A. L., Wittenrich, M. L., & Nakamura, M. (2012). Symbiosis initiation in the bacterially luminous sea urchin cardinalfish Siphamia versicolor. *Journal of Fish Biology*, *81*(4), 1340–1356.

Eyring, C. F., Christensen, R. J., & Raitt, R. W. (1948). Reverberation in the sea. *The Journal of the Acoustical Society of America*, *20*(4), 462-475.

Haddock, S. H. D., Moline, M. A., & Case, J. F. (2010). Bioluminescence in the sea. *Annual Review of Marine Science*. (2), 443-493.

Haneda, Y. (1957). Observations on luminescence in the deep sea fish, *Paratachichthys prosthemius*. *Science Report of the Yokosuka City Museum*, *2*, 15–23.

Irigoien, X., Klevjer, T. A., Røstad, A., Martinez, U., Boyra, G., Acuña, J. L., . . . Kaartvedt, S. (2014). Large mesopelagic fishes biomass and trophic efficiency in the open ocean. *Nature Communications, 5*.

Isla, A., Scharek, R., & Latasa, M. (2015). Zooplankton diel vertical migration and contribution to deep active carbon flux in the NW Mediterranean. *Journal of Marine Systems, 143*, 86-97.

Karspeck, A. R., Yeager, S., Danabasoglu, G., Hoar, T., Collins, N., Raeder, K., … Tribbia, J. (2013). An ensemble adjustment kalman filter for the CCSM4 ocean component. *Journal of Climate*, *26*(19), 7392–7413.

Kloser, R. J., Ryan, T. E., Keith, G., & Gershwin, L. (2016). Deep-scattering layer, gas-bladder density, and size estimates using a two-frequency acoustic and optical probe. *ICES Journal of Marine Science*, *73*(8), 2037–2048.

Kuwabara, S. (1955). Some observations on the luminous organ of the fish *Paratrachichthys prosthemius*. *Journal of the Shimonoseki College of Fisheries*, *4*(2), 197–202.

Lam, V. W., & Pauly, D. (2005, July). Mapping the global biomass of mesopelagic fishes. *Sea Around Us Project*, *30*, 4.

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., & Worm, B. (2011). How many species are there on earth and in the ocean? *PLoS Biology*, *9*(8), 1–8.

Nelson, J. S. (2006). *Fishes of the world*. Hoboken, NJ: John Wiley.

Poulsen, J. Y., Sado, T., Hahn, C., Byrkjedal, I., Moku, M., & Miya, M. (2016). Preservation obscures pelagic deep-sea fish diversity: doubling the number of sole-bearing Opisthoproctids and resurrection of the genus Monacoa (Opisthoproctidae, Argentiniformes). *PLOS ONE, 11*(8).

Robinson, C., Steinberg, D. K., Anderson, T. R., Arístegui, J., Carlson, C. A., Frost, J. R., . . . Zhang, J. (2010). Mesopelagic zone ecology and biogeochemistry – a synthesis. *Deep Sea Research Part II: Topical Studies in Oceanography, 57*(16), 1504-1518.

Silas, E. G. (1972). Investigations on the deep scattering layers in the Laccadive Sea.

Widder, E. A. (2010). Bioluminescence in the Ocean. *Science*, *704*(5979), 704–708.

WoRMS Editorial Board (2016). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2016-11-28.

# CHAPTER 2. GRANT PROPOSAL: EXAMINATION OF BIOLUMINESCENCE IN THE WEST PACIFIC LUMINOUS ROUGHY (*AULOTRACHICHTHYS PROSTHEMIUS*)

## Abstract

The mesopelagic zone is a vast portion of the ocean that light barely penetrates. There is not enough light for photosynthesis to occur in the shallowest parts, and at the deepest depths of the mesopelagic zone there is not enough light to effectively hunt prey. Many of the fishes living here have adapted to break the perpetual darkness by producing their own light through bioluminescence. This versatile evolutionary trait allows these fish to communicate, lure prey, and defend themselves. Counter-illumination is a specialized form of camouflage, typically associated with bioluminescent organs found on the underside of deep sea fishes. The hazy blue light will disrupt their silhouette and allow them to hide from potential predators that may be lurking below. The west Pacific luminous roughy (*Aulotrachichthys prosthemius*) is a mesopelagic fish with a small bioluminescent organ on its abdomen. It was initially discovered in 1902, but little research has been done since then. I will examine the external and internal anatomy of *A. prosthemius*, as well as conduct a histological examination of the bioluminescent organ. The histological study will identify what types of cells make up the organ, and how it is related to other organs within the fish. Alongside the histology, the external and internal anatomical studies will give insight on how the light produced within relates to other structures to effectively counter-illuminate. This will provide a more accurate representation of the functions of bioluminescence, as well as better understanding of the development of bioluminescence within this fish species.

# Background, Rationale, and Significance

Currently there are 242,574 oceanic species accepted in the World Register of Marine Species (WoRMS Editorial Board, 2016), with study estimates ranging between 540,000 and 2,210,000 species (Mora et al.,2011; Appeltans et al., 2012). Hiding underneath the ocean waves is a world that holds 99% of the livable space found on Earth (Costanza, 1999), as well as the largest amount of biomass, the highest concentration of vertebrates, and the most biodiversity on planet Earth (Nelson, 2006; Irigoien et al., 2014). Below the first 200 meters begins the mesopelagic zone, where animals that live in near darkness have evolved to produce their own light (Haddock et al., 2010). The world's greatest migration happens within the mesopelagic zone every night, bringing the largest abundance of vertebrates on the planet from the deep waters where the pressure is high, and the temperature is low, to the surface to feed in a drastically different environment before descending back down as the sun threatens to expose them (Nelson, 2006; Berge, 2009). The mesopelagic ecosystem is important for the distribution of life in the ocean via movement of nutrients, repackaging and repurposing of carbon-based material, and deep-sea current systems. Despite this, it remains one of the least investigated, and most poorly understood ecosystems in the world (Irigoien et al., 2014).

The mesopelagic zone begins at the depth where light is still visible, but too weak for photosynthesis to occur, and proceeds down until there is not enough light to effectively see and hunt prey (Robinson et al., 2010). The differences between the top and the bottom of the mesopelagic layer are dramatic. At 200m where the mesopelagic zone begins, the pressure is multiplied up to 21 atmospheres. At the bottom of the mesopelagic zone, approximately 1000m, the pressure is nearly 100 atmospheres (CalcTool: Pressure at depth calculator). In addition to the drastic pressure gradient, the mesopelagic zone is where the ocean's major thermocline takes place. At 200m, the temperature averages around 23°C and radically gets colder as it gets deeper, until it evens out near 1000m at 5°C (Karspeck et al., 2013). From the bottom of the thermocline, all the way down to the deepest depths of the hadopelagic zone (>6000m) the temperature will reach 0°C but will stay liquid due to the high concentrations of dissolved salts and extreme pressure (Karspeck et al., 2013).

Sonar data collected while mapping the sea floor consistently gave readings of a “false bottom” hundreds or thousands of meters above the true sea floor. The acoustic frequencies were found to create a phenomenon described as the deep scattering layer (DSL) (Eyring et al., 1948). It was then determined that the DSL is a phenomenon of acoustic waves reverberating off massive groups of biotic organisms in the mesopelagic zone (Boden, 1950; Belman, 1978). Recent research suggests that previous estimates of mesopelagic biomass calculated using the DSL should be increased by, at the bare minimum, an order of magnitude. That would increase the current assessment from 1,100 million tons to over 11,000 million tons of biomass (Irigoien et al., 2014). When deep sea ROVs that are deployed to map and explore the mesopelagic zone, new species are discovered on nearly every dive.

There are many mesopelagic fishes that have been identified but are not very well described or studied. More than 550 genera of marine organisms are known to be bioluminescent, producing their own light through symbiotic relationships with bacteria, or the breakdown of enzymes (Haddock et al., 2010; Davis et al., 2016). Mesopelagic fishes commonly use bioluminescence for intraspecific communication, attracting prey, startling predators, as well as counter-illumination. This specialized type of camouflage uses bioluminescent organs found on the underside of the fish (Haddock et al., 2010). The fish will break up its silhouette by blending into the ambient down-welling light by producing light of similar color, intensity, and distribution (Widder, 2010). Many predatory fish have developed eyes that are oriented upward to see the silhouettes of potential prey making counter-illumination an effective method to avoid predators.

The west Pacific luminous roughy (*Aulotrachichthys prosthemius*) is a small mesopelagic fish with a ventral bioluminescent organ used for counter-illumination. It was initially identified by D.S. Jordan and H.W. Fowler in 1902, and since then, very little research has been done on this fish. One study of the external anatomy of the fish and histology of the bioluminescent organ discovered the luminous organ encircles the anus, and is covered by a hyaline lens (Kuwabara, 1955). The hyaline lens, a structure that is found in many counter-illuminating fishes to diffuse light more evenly, lies beneath it (Poulsen et al., 2016). Another study described the organ and noted that the luminous gland was a capsule-like structure with inner and outer layers (Haneda, 1957). While many counter-shading fish can block the light being produced by contracting shutter-like scales (Dunlap et al., 2012; Davis et al., 2016), *A. prosthemius* has chromatophores distributed through the inner layer of the luminous capsule (Haneda, 1957). These chromatophores expand and contract to control the output of light. Additionally, there is a structure situated on top of the luminous gland that seems to have an important role in *A. prosthemius'* bioluminescence. It is unknown what tissues it is made up of, or what function it serves (Kuwabara, 1955; Haneda, 1957). Perhaps the most unique aspect of *A. prosthemius'* bioluminescent organ is the deepest gland and duct system, which may be similar in structure and function to the bioluminescent organ found in *Siphamia versicolor* (sea urchin cardinalfish) (Dunlap et al., 2012). *Siphamia versicolor* will release excess bacteria in the bioluminescent organ into the intestinal tract to be excreted from the body.

With so little research in the last 100 years, I will examine *A. prosthemius* for a precise external anatomical study using high definition imaging of external and internal structures. Additionally, I will examine the bioluminescent organ using histological slides to identify the different types of cells found in the light organ and help determine how the organ is connected to the rest of the organism's biological systems. *Aulotrachichthys prosthemius* is an exceptional example of how little is truly understood about the mesopelagic zone. Despite being identified over a century ago, only a small handful of research has been conducted that directly examine this organism. The method of light production and the benefits to overall fitness may be typical, but the structures with which *A. prosthemius* emits light is peculiar. The investigation of the bioluminescence of *A. prosthemius* is a vital part of filling the intellectual void surrounding the mesopelagic zone.

This research will closely examine the external and internal features of *A. prosthemius* to form a better understanding of its specific characteristics and adaptations. This research falls in line with Regis University's mission regarding environmental awareness, responsibility, and men and women in service to others. I firmly believe that each piece of scientific research improves our understanding of the biosphere and how we affect it.

## Purpose and Specific Aims

1) The primary goal of this project is to clarify in detail the gross and histological anatomy of the west Pacific luminous roughy (*A. prosthemius*). This fish was initially discovered and named by Jordan and Fowler in 1902, under the name *Paratrachichthys prosthemius*. Very little primary literature exists regarding this species of roughy, although it is known to exist in the mesopelagic zone of the Pacific Ocean between China and the United States. *Aulotrachichthys prosthemius* does exhibit bioluminescent areas on its abdomen, which produce light through a symbiotic

relationship with bacteria (Davis et al., 2016). Histological studies of the light organ will be critical to understanding how the light organ develops and functions. Quantifying the precise structures of these organs are important for developing an understanding of *A. prosthemius*' evolution, life-history, range, and role in deep-sea ecology.

2) I will compare the anatomy and histology of *A. prosthemius* to several other bioluminescent mesopelagic fishes. *Paratrachichthys fernandezianus* and *Paratrachichthys argyrophanus* are two luminescent fishes that are related to *A. prosthemius*. Additionally, *Hoplostethus occidentalis* and *Anoplogaster cornuta* will be compared to *A. prosthemius* as they are non-luminescent relatives.

## Methods

### *External and Internal Anatomy*

All specimens will be formaldehyde-fixed and ethanol-preserved specimens on loan from museum collections. All specimens will be photographed, including their external and internal anatomical structures using a high definition camera. The external anatomy will be photographed to produce a detailed, scientific description of *A. prosthemius* and allow for accurate illustration for figures and diagrams.

The examination of the internal anatomy of the fish will focus on the location and structure of the bioluminescent organ, and associated structures. Additionally, structures associated with the bioluminescent organ will be studied to better understand the organ system. The internal anatomy and structures will be photographed to understand the orientation of specific organs, and organ systems. A laterally-angled ventral right parasagittal cut in the body cavity will reveal the internal organs without damaging skeletal structures. After examining the general internal anatomy, the bioluminescent organ will be inspected to determine any visceral structures and tissues that are directly associated with it. The bioluminescent organ will be examined grossly in *A. prosthemius*, *Paratrachichthys fernandezianus*, *Paratrachichthys argyrophanus*, *Hoplostethus occidentalis*, and *Anoplogaster cornuta*. Three specimens of *A. prosthemius* will be sampled for histological study and one specimen each of the other species will be sampled for histological study.

### *Histology*

In the lab, the light organ and associated structures of *A. prosthemius* and related species will be dissected out. Dissected sections measuring ~0.5 $cm^3$ will be prepared for histological analysis through a series of dehydrations using ethanol, followed by xylene to make the cells clear. Then, the sections will be embedded with paraffin and sectioned using a rotary microtome at 10 µm. Finally, the 10 µm sections will be stained using Masson's Trichrome (MT) Stain Kit (Sheehan and Hrapchak, 1980; Bancroff and Stevens, 1982) to differentiate between collagen fibers and muscle tissues. The stained sections will be mounted on glass slides and examined using a Leica DM 2500 compound microscope and photographed with a Q Imaging MicroPublisher 5.0 RTC photodocumentation system (Ghedotti et al., 2015).

## Work Plan

The initial gross anatomical portions of this project will begin in February 2017 and will continue through early May. The histological examinations will be prepared and completed May through August. A research paper summarizing the results will be completed in the 2017-2018 academic year, and the research will be presented at a national conference.

# References

Appeltans, W., Ahyong, S. T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., … Costello, M. J. (2012). The magnitude of global marine species diversity. *Current Biology*, *22*(23), 2189–2202. http://doi.org/10.1016/j.cub.2012.09.036

Bancroff J, Stevens A. 1982. Theory and practice of histological techniques, 2nd ed. New York: Churchill-Livingston. 662p.

Belman, B. W. (1978). Respiration and the effects of pressure on the mesopelagic vertically migrating squid *Histioteuthis heteropsis*. *Limnology and Oceanography*, *23*(4), 735–739. http://doi.org/10.4319/lo.1978.23.4.0735

Berge, J., Cottier, F., Last, K. S., Varpe, Ø., Leu, E., Søreide, J., … Brierley, A. S. (2009). Diel vertical migration of Arctic zooplankton during the polar night. *Biology Letters*, *5*(1), 69–72. http://doi.org/10.1098/rsbl.2008.0484

Boden, B. P. (1950). *Plankton organisms in the deep scattering layer*. San Diego, CA: U.S. Navy Electronics Laboratory.

CalcTool: Pressure at depth calculator. (n.d.). Retrieved November 28, 2016, from http://www.calctool.org/CALC/other/games/depth_press

Costanza, R. (1999). The ecological, economic, and social importance of the oceans. *Ecological Economics*, *31*(2), 199–213. http://doi.org/10.1016/S0921-8009(99)00079-8

Davis, M. P., Sparks, J. S., & Smith, W. L. (2016). Repeated and widespread evolution of bioluminescence in marine fishes. *PLOS ONE, 11*(6). doi:10.1371/journal.pone.0155154

Dunlap, P. V., Gould, A. L., Wittenrich, M. L., & Nakamura, M. (2012). Symbiosis initiation in the bacterially luminous sea urchin cardinalfish Siphamia versicolor. *Journal of Fish Biology*, *81*(4), 1340–1356.

Eyring, C. F., Christensen, R. J., & Raitt, R. W. (1948). Reverberation in the sea. *The Journal of the Acoustical Society of America*, *20*(4), 462-475.

Ghedotti, M. J., Barton, R. W., Simons, A. M., & Davis, M. P. (2015). The first report of luminescent liver tissue in fishes: Evolution and structure of bioluminescent organs in the deep-sea naked barracudinas (Aulopiformes: Lestidiidae). *Journal of Morphology*, *276*(3), 310–318.

Haddock, S. H. D., Moline, M. A., & Case, J. F. (2010). Bioluminescence in the sea. *Annual Review of Marine Science*, *2*, 443–93.

Haneda, Y. (1957). Observations on luminescence in the deep sea fish, *Paratachichthys prosthemius*. *Science Report of the Yokosuka City Museum*, *2*, 15–23.

Irigoien, X., Klevjer, T. A., Røstad, A., Martinez, U., Boyra, G., Acuña, J. L., . . . Kaartvedt, S. (2014). Large mesopelagic fishes biomass and trophic efficiency in the open ocean. *Nature Communications, 5*.

Karspeck, A. R., Yeager, S., Danabasoglu, G., Hoar, T., Collins, N., Raeder, K., … Tribbia, J. (2013). An ensemble adjustment kalman filter for the CCSM4 ocean component. *Journal of Climate*, *26*(19), 7392–7413.

Kuwabara, S. (1955). Some observations on the luminous organ of the fish *Paratrachichthys prosthemius*. *Journal of the Shimonoseki College of Fisheries*, *4*(2), 197–202.

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., & Worm, B. (2011). How many species are there on earth and in the ocean? *PLoS Biology*, *9*(8), 1–8.

Nelson, J. S. (2006). *Fishes of the world*. Hoboken, NJ: John Wiley.

Poulsen, J. Y., Sado, T., Hahn, C., Byrkjedal, I., Moku, M., & Miya, M. (2016). Preservation obscures pelagic deep-sea fish diversity: doubling the number of sole-bearing Opisthoproctids and resurrection of the genus Monacoa (Opisthoproctidae, Argentiniformes). *PLOS ONE*, *11*(8).

Robinson, C., Steinberg, D. K., Anderson, T. R., Arístegui, J., Carlson, C. A., Frost, J. R., . . . Zhang, J. (2010). Mesopelagic zone ecology and biogeochemistry – a synthesis. *Deep Sea Research Part II: Topical Studies in Oceanography*, *57*(16), 1504-1518.

Sheehan D, Hrapchak B. (1980.) Theory and practice of histo- technology, 2nd ed. Ohio: Battelle Press. 262p.

Widder, E. A. (2010). Bioluminescence in the ocean. *Science*, *704*(5979), 704–708.

WoRMS Editorial Board (2016). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2016-11-28.

# CHAPTER 3. JOURNAL MANUSCRIPT: DEMOGRAPHIC INFLUENCES ON VISITOR USE OF THE DENVER BOTANIC GARDENS

## Abstract

The study of visitor use in museums, zoos, and aquariums is an important aspect of understanding visitor engagement and evaluating the function of the institution. Here, visitor demographics such as age, group size, and number of children were assessed to see their influence on time spent and number of stops visitors made while visiting the Denver Botanic Gardens in Denver, Colorado. This study evaluated differences in time spent and number of stops made among different gardens by observing visitors and mapping their paths and activities through each zone. The results show that older visitors and larger groups spent more time and made more stops than younger visitors and smaller groups. Spatially, visitors made many more stops within the first zones of the main gardens, and the physically separated children's garden. These results show how different visitors use the gardens and how features hold visitor attention, allowing better informed decisions to improve visitor engagement. For example, children were frequently observed in focal groups in the Mordecai Children's garden, but not in the main garden. This suggests that changes can be made to the main gardens, like added interactive activities throughout, would increase the engagement and improve the experience of groups with children. Finally, this study highlights the need for the framework used to be tailored to botanic gardens because of the unique structural differences, like the ability to enjoy the gardens without stopping, from other institutions like museums, zoos, and aquariums where visitors generally stop to appreciate what is being displayed.

# Introduction

Similar to science museums, art museums, zoos, and aquariums, botanic gardens are an informal educational institution that provides access to scientific and cultural education. These institutions are structured through movement in space, and the spatial layouts influence how visitors explore, interact, and understand the content being presented to them (Wineman and Peponis, 2010). The visitor experience includes the feelings, reactions, and perceptions while in the environment, but the expectations of visitors ranges greatly across environment types (Packer, 2008; Silverman, 1996). This represents the core of informal educational institutions because without visitors, most museums would not survive (Wallace, 2013). Obtaining data about how visitors use different exhibitions, galleries, or parts of a museum creates a picture of visitor engagement, which is vital to evaluating the function of a museum (Gilman, 1923; Levin, 1983; Montaner and Oliveras, 1986; Nurse Rainbolt et al, 2012). Curators and designers require this type of information to assist in making decisions regarding exhibition design, interpretive sign placement, and to understand the visitor experience.

When evaluating visitor movement and interaction, typically three questions are addressed: (1) Where are visitors going?, (2) To what are visitors paying attention?, (3) How are visitors learning? (Bitgood, 2010). Visitor movement in museums, art galleries, and similarly styled institutions can often be led by wayfinding and interpretive signage. However, open-plan institutions like the Denver Botanic Gardens provide the opportunity to investigate visitor movement and engagement in a less structured way since visitors are required to make more choices about the direction they travel (Kaynar, 2005). When a collection of movement data shows an area being ignored by most visitors, changes in signage, art, and other displays can be made to make the area more likely to be visited.

Botanic gardens, like zoos and museums, seek to capture visitor attention, focus it, and keep the visitor engaged (Bitgood, 2010). Each of these involves an interaction between personal, physical, psychological, and environmental input (Bitgood, 2010). The pieces with which visitors choose to interact have a high perceived value, the ratio of the utility of an object divided by the cost of the interaction (Bitgood, 2010). For example, an obscured showcase, or a display with inadequate interpretation, may be skipped over entirely, because the effort needed to interact with the object outweighs the benefit (Bitgood, 2010; Lanir et al, 2016). Exhibit designers make decisions regarding the theme of an exhibition, placement of objects, as well as the interpretive and wayfinding signage, all with the goal of increasing the likelihood of visitor engagement (Lanir et al, 2016). Enhancing the lighting, adding larger or bolder interpretive signs, moving other objects away from the focal piece, or grouping similar pieces together can dramatically change the objects a with which visitor chooses to interact.

In addition to the physical characteristics of the space, the audience must also be considered. Different demographic groups will move through the same exhibits but decode very different information and ultimately have very different experiences (McManus 1989). Children accompanied by adults are a prime example of this, where children may be unable to read or understand a sign. The adult must read, comprehend, and interpret it for the child in a more appropriate way, likely interacting with the sign for a longer period of time than if the adults were alone (McManus 1989). This can be used to the institution’s advantage because the creation of dialogue between guests, especially when it involves asking questions, significantly increases the information retained from the experience (Gutwill 2017; McManus 1989; Screven 1992). The length of time a visitor spends engaged with something is positively correlated to their learning (Falk, 1982; Kaynar, 2005; Peponis et al, 2004; Serrell, 1995; Sandifer, 1997). Visitor engagement and learning is fundamentally tied with their expectations and reasons for visiting the institution (Tröndle et al, 2014).

The visitor experience in science museums, art museums, and zoos has been widely studied, however, there are few publications on visitor use or experience in botanic garden spaces. Primarily, the reason given for visiting botanic gardens is enjoying the aesthetic value of the gardens (Ward 2010). Additionally, visitor movement studies are typically focused inside specific exhibitions instead of the institution in its entirety. Furthermore, these areas are usually set up with a wayfinding path, or a general direction in which visitors are expected to travel. The Denver Botanic Gardens provides an opportunity to analyze visitor experience and movement in an outdoor open-plan institution that focuses on botanical education. This open plan will allow visitors to enjoy the gardens without physically stopping to enjoy the displays, unlike zoos, museums, and aquariums where there are physical (or implied) barriers between visitors and the features.

To investigate this question, visitor movement data was analyzed across 12 different zones of the Denver Botanic Gardens to assess how the gardens are being used. Additionally, demographic information was collected to determine how different patron groups use the gardens. Evaluation of the number of stops made, and the observed behaviors at those stops will be used to identify what is attracting visitor attention. It is expected that smaller groups (one or two people) will spend more time in a zone of the gardens compared to larger groups (Ross & Gillespie, 2009). Groups that have children spend more time and make more stops than groups without children as adults interact with the children and interpret signs and features for them. Also, the goal of each garden zone will influence visitor use. Visitors of the gardens will more likely take photos or look at gardens that focus on aesthetic value, whereas visitors of gardens that have an educational focus are more likely to stop and read interpretive signs. Information interpreted from this study will allow Denver Botanic Gardens to better understand the usage of different zones. This will give the curators and designers the chance to make decisions about exhibition and signage placement based on visitor traffic to improve the overall visitor experience and increase visitor engagement.

# Methods

## *Visitor Movement Data Collection*

The York Street location of the Denver Botanic Gardens (DBG) in Denver, Colorado was the site of this observational study, which is in a heavily urbanized area and adjacent to Cheeseman Park. DBG hosted 1.2 million visitors in 2016, served over 34,000 school children from 394 different schools, and served 27,863 visitors in 213 outreach events (Denver Botanic Gardens, 2016). The first planting at the York Street location of DBG was in 1959, with simple displays of roses, irises, peonies, and daffodils. Now, the Gardens features North America's largest collection of plants from cold temperate climates, and a wide array of gardens inspired by vegetation from around the world (Dressel-Martin, 2006). The DBG mission is to connect people with plants from the Rocky Mountain Region and beyond, and feature gardens inspired by the Rocky Mountain alpine, the tropical rainforests, and the gardens of Japanese Shinto shrines.

The study was modeled after a study by Serrell (1997), tailored to museums and their visitors, and no changes were made to the general protocol. To assess movement patterns of visitors in this open-plan institution, the Denver Botanic Gardens was split into 14 different zones (Figure 1) by sightlines, major garden boundaries, and buildings. Each zone had predetermined areas for the volunteer observers to either sit or follow the visitor they were tracking. Data was collected by Denver Botanic Garden volunteers on June 17$^{th}$, June 24$^{th}$, and June 30$^{th}$, 2016. Each shift was 90 minutes long, beginning at 10:00 am or 12:00 pm. When observers began their shift, the first adult to enter their zone was chosen as the respondent. The data collection sheet contained a map of the zone, and areas for the time the visitor spent in the zone, date, the estimated visitor's age, number of people in the group, number of children in the group, and if the group was associated with a school (Figure 12, Appendix). The path the visitor took through the zone was drawn on the map, and any stops were noted with an "X." At each stop, the observer collected data on visitor activities when they stopped. These behaviors included talking, reading signs, looking at the plants, gardens, building, or art, taking photos, or using their phone. Behaviors outside of these were listed as "other" and if the visitor made no stops, stop 1 was reported as "none." Additionally, the observer noted when the visitor was no longer being tracked. If the visitor exited the zone or stopped in a location for more than ten minutes, the observer stopped tracking the patron. This process was repeated with the next adult that entered the zone. The data from the observation sheets was compiled by zone in Microsoft Excel spreadsheets for analysis. Zone C is the parking lot and Zone I was under construction during the time of this study and are therefore excluded from this set of data. The data was cleaned to ensure all demographic information and behavioral categories were consistent through all zones using Microsoft Excel and R (R Core Team, 2016) before analysis.

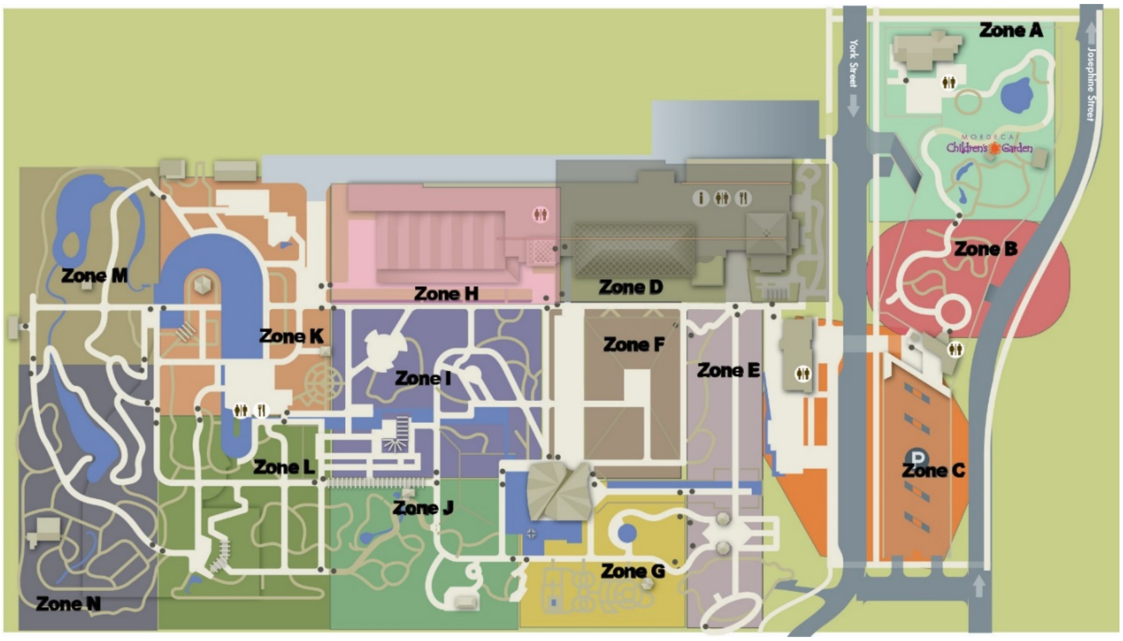



*Figure 3: Denver Botanic Garden map showing the 14 different zones.*

## *Analysis of Visitor Movement Data*

All visitor movement data was analyzed using R version 3.3.1 (R Core Team, 2016). A total of 361 visitors were observed across the 12 included zones. Zone use was first assessed by looking at the proportions of behaviors across the zones using ggplot2 (Wickham, 2009). The "other" category was excluded from this exploratory analysis because 33.34% of stops recorded did not fit into the described behavior categories. Additionally, histograms were created to show average number of stops per zone, average time spent per zone, average group size per zone, and average age of visitors per zone. Histograms were also used to determine which variables should be transformed prior to using them for analysis. Group size, number of children in the group, and zone path length were all $\log_2$ transformed, or $\log_2(x+1)$ transformed and time was $\log_{10}$ - transformed to normalize the distribution. Poisson generalized linear models were fit to describe how the number of stops made varied as a function of demographic factors like group size, age of visitors, and number of children. Gaussian generalized linear models were fit to describe how the

$log_{10}$ transformed time spent in a zone varied as a function of demographics factors. All linear models were offset by the $log_2$ length of the zone's path.

To assess which zones differed in the number of stops made and the amount of time spent by visitors, the differences in the mean number of stops made and mean time spent in each zone were compared using analysis of variance. Before conducting the analyses, the responses were normalized by dividing the log transformed variables by the zone area. The mean in each zone was compared to every other zone using Tukey's range test.

The stops made by each visitor were analyzed using a non-metric multidimensional scaline in two dimensions, using the ecodist and vegan pack in R, to assess how demographic variables influenced the types of stops made by each visitor (Goslee, 2007; Oksanen et al. 2017).

In addition to the statistical analysis of the data, a GIS analysis was performed to assess what gardens, features, or art instillations are drawing visitor attention and making them stop. For each zone, each stop was mapped using ArcMap and coded by the corresponding behavior (ESRI, 2011). Each point was also associated with a unique visitor code, the estimated age to the nearest decade, number of people in the group, number of adults, number of children, and whether the group was associated with a school. The time between each stop was not recorded or included in this analysis. A 500 $m^2$ hexagonal fishnet was generated over the entire gardens area to assess the overall use of the gardens. Points were joined with the fishnet based on different demographic information and activities. Counts within individual hexagons were used to create heatmaps across the gardens and see the hotspots where visitors are frequently making stops. Major gardens, structures, and art pieces were labeled on the map to give spatial reference. Due to time constraints, only the first 10 visitors with at least one stop were mapped per zone.

# Results

## *Exploratory Analysis*

Undefined behaviors listed as “other” comprised 33.34% of all behaviors observed across all zones and were excluded from this portion of the analysis. Exploring the behavioral data across zones revealed that the O’Fallon Perennial Walk (Zone E) had a high proportion of stops made where people were looking at plants (38.67%), the Romantic Gardens (Zone G) had a high occurrence of photos being taken (47.06%), and the Orangery (Zone H) had a high proportion of stops made to look at plants (56.94%). Interestingly, the UMB Bank Amphitheater (Zone F) had a high number of instances where the visitors passed through without making any stops (30.77%) (Figure 1). Looking at plants, talking, and taking photos were generally the most common behaviors across all zones, and using phones was the least common across many zones (Table 6, Appendix).

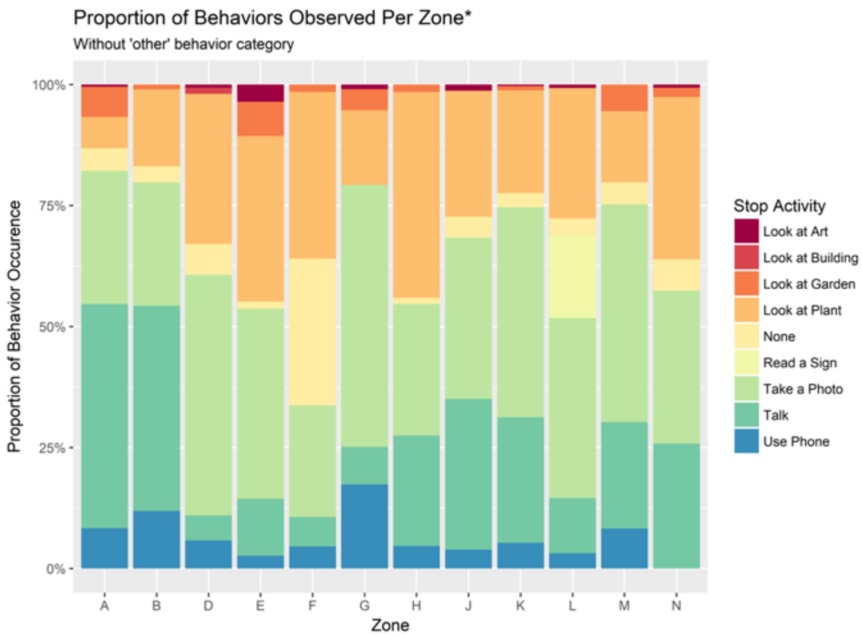



*Figure 2: Proportions of behaviors observed per zone. This is excluding the "other" category due to the overwhelming proportion (33.34%) of observations outside of the behavior key.*

The average age and group size did not differ among zones (Figure 2A and 2D), and while there were not significant differences in the average time spent or number of stops made across zones, there are definite trends. Particularly, in the O'Fallon Perennial Walk (Zone E) visitors spent more time on average (10.81 minutes, 95% CI: 7.83 - 13.80) and made more stops on average (9.43 stops, 95% CI: 5.97 – 12.90) than the other zones (Table 1).

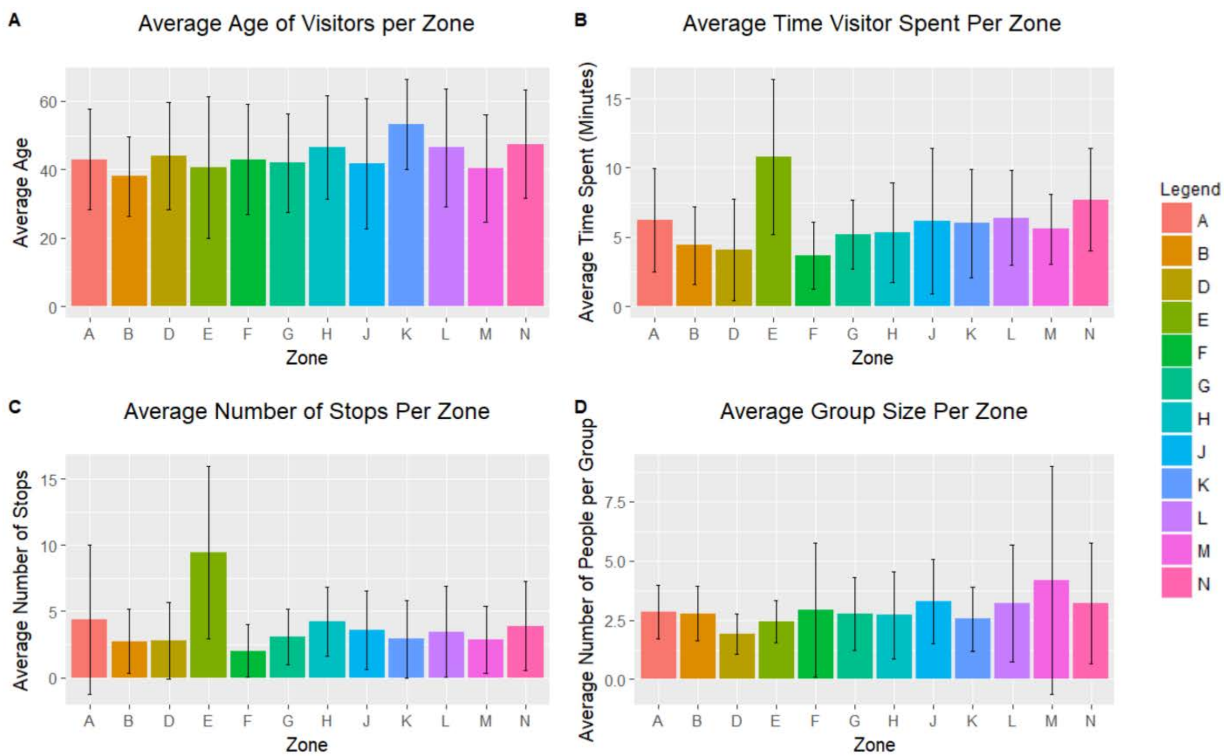



*Figure 3: Graphs showing the relationships between (A) Average age of visitors per zone, (B) Average amount of time visitors spent per zone, (C) Average number of stops per zone, and (D) Average group size per zone.*

*Table 1: General zone demographics across all zones, including average age, average time spent, average group size, and average number of stops. Values with an asterisk indicate the highest (**) and lowest (*) values for the demographic.*

| Zone | Average Age (Years) | Average Time Spent | Average Group Size | Average Number of Stops |
|---|---|---|---|---|
| **Zone A** | 42.94 | 6.23 | 2.85 | 4.38 |
| **Zone B** | 38.06* | 4.41 | 2.77 | 2.74* |
| **Zone D** | 43.94 | 4.07* | 1.92* | 2.79 |
| **Zone E** | 40.62 | 10.81** | 2.43 | 9.43** |
| **Zone F** | 42.95 | 3.66 | 2.92 | 2.01 |
| **Zone G** | 42.00 | 5.20 | 2.76 | 3.08 |
| **Zone H** | 46.45 | 5.35 | 2.71 | 4.22 |
| **Zone J** | 41.76 | 6.17 | 3.29 | 3.58 |
| **Zone K** | 53.33** | 6.00 | 2.56 | 2.91 |
| **Zone L** | 46.40 | 6.38 | 3.19 | 3.46 |
| **Zone M** | 40.37 | 5.59 | 4.18** | 2.88 |
| **Zone N** | 47.50 | 7.70 | 3.20 | 3.90 |

# Statistical Analysis

## *Demographic Effects on Number of Stops Made*

Several demographic variables influenced the number of stops visitors made in a zone (Table 7, Appendix). After accounting for $\log_2$ pathlength, older patrons made significantly more stops than younger patrons. For every 10-year increase in the visitor's age, the stopping rate increased by 7.91% ($p < 0.0001$, 95% CI: 4.3% – 11.4%) (Figure 4A). $\text{Log}_2$ group size positively correlated with the rate of stops such that for every doubling in group size, the average number of stops increased by 5.01% ($p < 0.0001$, 95% CI: 2.64 – 7.54%) (Figure 4B). The $\log_2$ number of children in a group negatively correlated with stop frequency such that when the number of children in the group doubled, the number of stops decreased by 15.61% ($p < 0.0001$, 95% CI: 14.47% – 19.51%) (Figure 4C). While groups with children did not have a significant effect on the rate of stops made ($p = 0.267$), groups without children positively correlated with the number of stops made. For each doubling of the $\log_2$ group size when no children were present, the rate of stops increased by 24.78% ($p < 0.0001$, 95% CI: 19.11% – 25.15%) (Figure 4D).

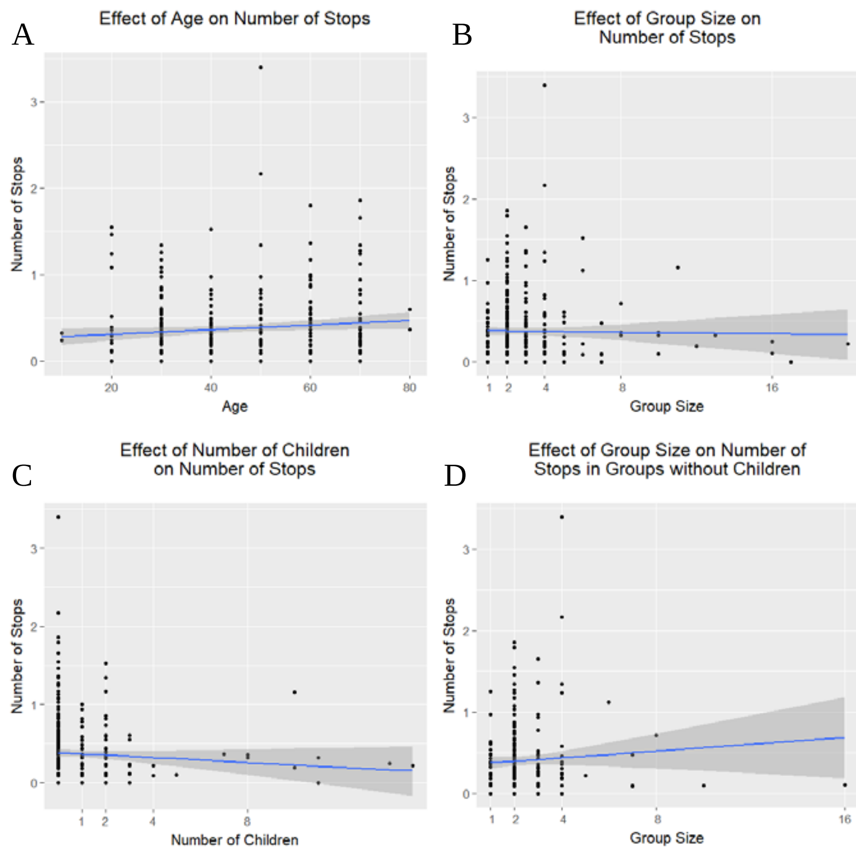



*Figure 4: The effects of various demographic variables on the average number of stops visitors made, all after offsetting by the zone's path length. A) Older visitors made more stops on average than younger visitors. B) Larger groups made more stops than smaller groups. C) Groups with more children made more stops than groups with fewer children. D) In groups without children, larger groups made more stops than smaller groups.*

### *Demographic Effects on Time Spent in a Zone*

Unlike the number of stops made per zone, the only demographic variable to significantly influence the $\log_{10}$ time spent in a zone was the number of children present (Table 8, Appendix). After accounting for $\log_2$ pathlength, visitor's age did not influence the amount of time they spent. For every 10 years a visitor's age increased, the median amount of time spent per zone increased by 0.012% (p = 0.092, 95% CI: 0.002 – 0.0269) (Figure 5A). $Log_2$ group size did not correlate with the amount of time spent in a zone (p = 0.698) (Figure 5B). Unlike for the number of stops, the $log_2$ number of children present positively correlated with the time spent in a zone. For every doubling in the number of children, the median amount of time spent increased by 0.323% (p = 0.0371, 95% CI: 0.283% – 0.361%) (Figure 5C). The presence of children in a group did not affect the amount of time visitors spent in a zone (p = 0.369) (Figure 5D). Groups without children did not have an affect as group size increased (p = 0.621) (Figure 5E).

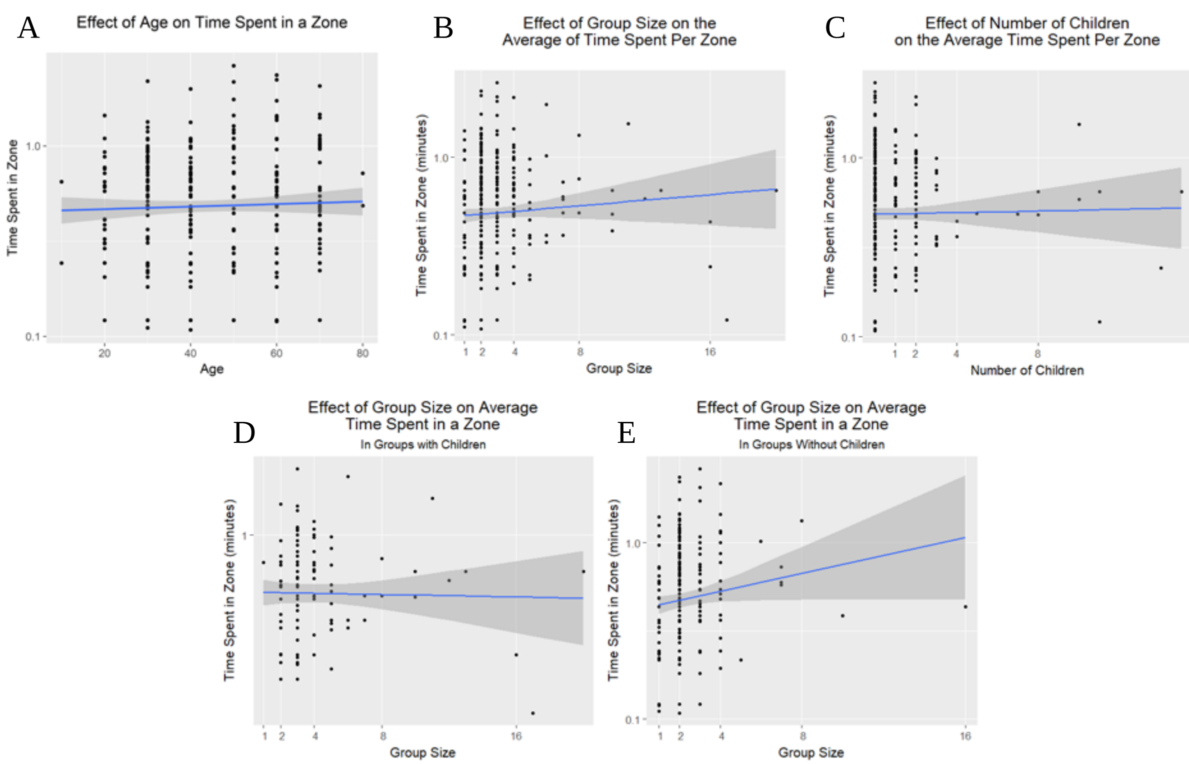



*Figure 5: The effects of various demographic variables on the average amount of time visitors spent in a zone, all after offsetting by the zone's pathlength. A) Older visitors spent more time on average than younger visitors. B) Larger groups on average spent more time in a zone than smaller groups. C) Groups with more children spent less time than groups with fewer children. D) Larger groups with children spent more time on average than smaller groups with children. E) Larger groups without children spent more time on average than smaller groups with children.*

*Differences in Number of Stops Between Zones*

On average, people made 0.974 fewer stops in the UMB Bank Amphitheater (Zone F) than the Orangery (Zone H), and they made more stops in the O'Fallon Perennial Walk (Zone E) across all other zones, except the Orangery (Zone H) (Table 2). Otherwise, the Tukey range test found that the number of stops did not differ significantly between zone.

*Table 2: Tukey range test results for the differences in the number of stops made per zone. Bolded values are statistically significant (p = >0.05). Reading from the top to the left, estimates are given in number of stops. (Example: On average, visitors made 0.363 more stops in Zone A than in Zone B.)*

| | A | B | D | E | F | G | H | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| B | 0.363 | | | | | | | | | | |
| D | 0.283 | -0.08 | | | | | | | | | |
| E | **-1.085** | **-1.448** | **-1.368** | | | | | | | | |
| F | 0.623 | 0.26 | 0.34 | **1.708** | | | | | | | |
| G | 0.021 | -0.342 | -0.262 | **1.106** | -0.602 | | | | | | |
| H | -0.351 | -0.714 | -0.634 | 0.734 | **-0.974** | -0.372 | | | | | |
| J | 0.01 | -0.372 | -0.293 | **1.075** | -0.633 | -0.031 | 0.341 | | | | |
| K | 0.207 | -0.156 | -0.076 | **1.292** | -0.416 | 0.186 | 0.558 | 0.217 | | | |
| L | 0.092 | -0.271 | -0.191 | **1.177** | -0.531 | 0.071 | 0.443 | 0.102 | -0.115 | | |
| M | 0.195 | -0.168 | -0.088 | **1.279** | -0.428 | 0.174 | 0.546 | 0.205 | -0.012 | 0.103 | |
| N | -0.065 | -0.428 | -0.348 | 1.02 | -0.688 | -0.086 | 0.286 | -0.055 | -0.272 | -0.157 | -0.26 |

*Overall Distribution of Stops Made*

Across zones, there were hotspots observed where a high number of stops were made compared to the surrounding areas and features (Table 3, Figure 6, Full size: Figure 13, Appendix). Hotspots occurred around highly interactive areas such as the pond in the Mordecai Children's Garden, or striking installations or structures (Chihuly Statue, and water feature in the Rock Alpine Garden), as well as educational gardens (Roads Water-Smart Garden and the Conservation Garden).

*Table 3: The 12 zones mapped with the features found at the hotspots where visitors stops accumulated.*

| Zone | Hotspot Features |
|---|---|
| Zone A | Pond area of Mordecai Children’s Garden |
| Zone B | Opening of Mordecai Children’s Garden |
| Zone D | Information desk, restrooms, Offshoots Restaurant |
| Zone E | Chihuly Statue |
| Zone F | Roads Water-Smart Garden |
| Zone G | Gloria Falkenberg Herb Garden |
| Zone H | Orangery |
| Zone J | June’s PlantAsia Garden |
| Zone K | The Hive Restaurant |
| Zone L | Conservation Garden |
| Zone M | South end of the Japanese Garden |
| Zone N | Near water feature of Rock Alpine Garden |

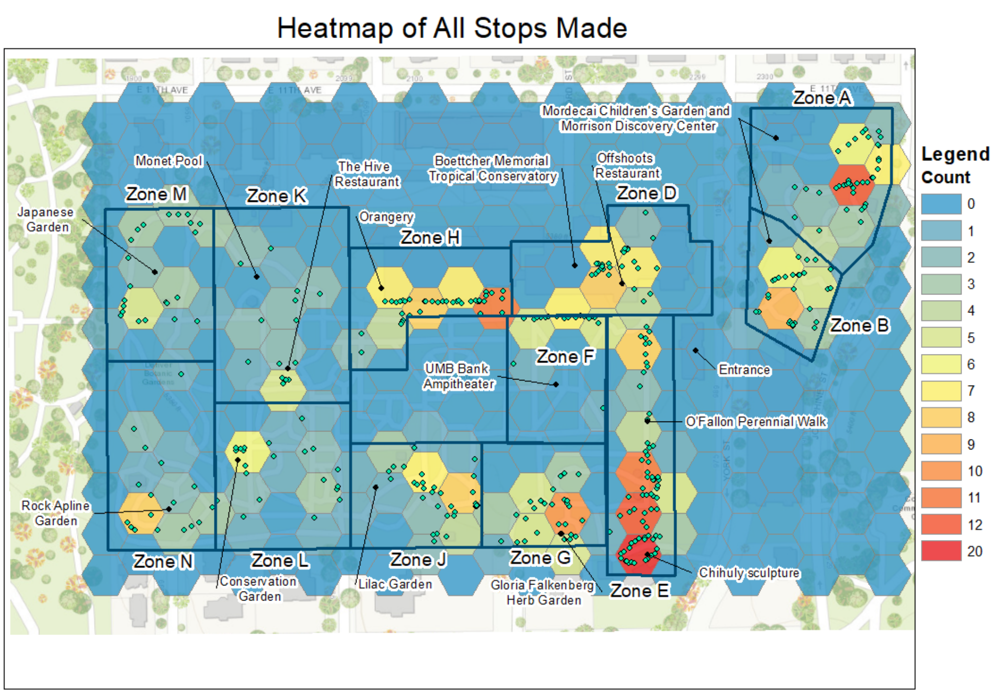



*Figure 6: 500 m² hexagonal heatmap of all stops mapped across the gardens.*

*Distribution of Small and Large Group Sizes Across the Gardens*

Different group sizes were seen across the gardens (Figure 8). One interesting result is that group size did not change in the Mordecai Children's Garden (Zones A & B). Small groups consisting of one or two people made more stops around the Orangery (Zone H) than the Chihuly statue (Zone E), and the opposite was true for groups of three or more. Large groups made more stops along the O'Fallon Perennial Walk (Zone E) and June's PlantAsia garden (Zone J) than smaller groups did.

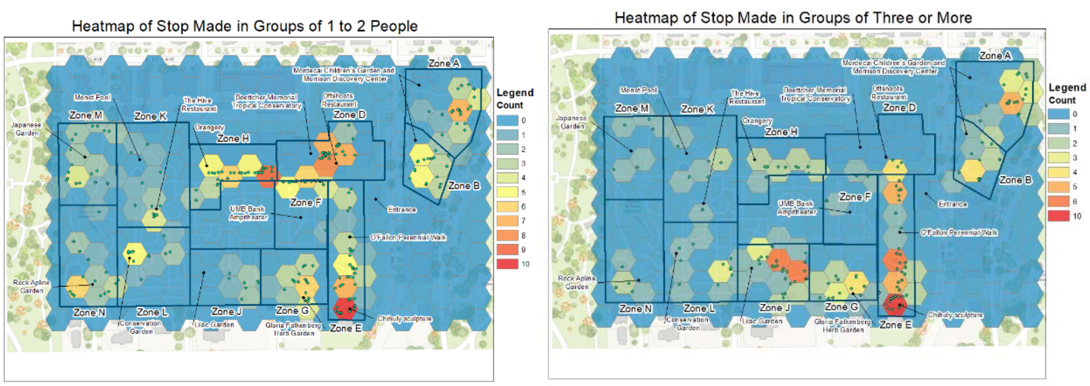



*Figure 7: 500 m² hexagonal heatmaps with different group sizes. A) Group sizes of 1 – 2 people. B) Group sizes of 3 or more. Smaller groups made more stops throughout the front half of the main gardens while larger groups frequently stopped along the O'Fallon Perennial Walk.*

*Distribution of Groups With and Without Children Across the Gardens*

The difference in the stops made in groups with and without children was striking. The Mordecai Children's Garden (Zone A and B) had the highest number of stops made in groups with children but were scarce throughout the rest of the gardens. Groups without children were rarely present in the Mordecai Children's Garden but had high numbers of stops throughout the Boettcher Memorial Tropical Conservatory, the O'Fallon Perennial Walk, and the Orangery (Zones D, E, and H).

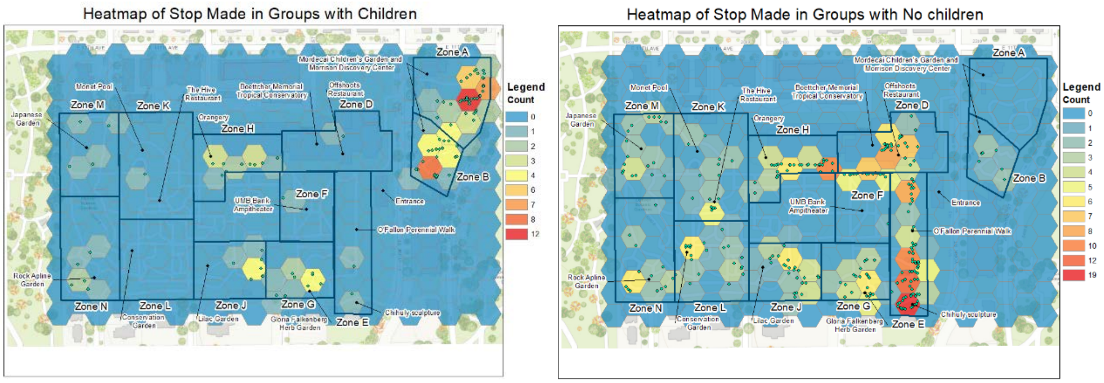



*Figure 8: 500 m² hexagonal heatmaps with different group compositions. A) Groups with children, B) Groups without children. Groups with children primarily stayed withing the Mordecai Children's Garden, whereas groups without children were distributed across the main gardens, but not observed in the Mordecai Children's Garden.*

## *Differences in Time Spent Between Zones*

The amount of time spent in a zone differed across more zones than the number of stops did (Table 4). However, as seen with the number of stops made, visitors in the O'Fallon Perennial Walk (Zone E) spent significantly more time than visitors in many other zones.

*Table 4: Tukey range test results for the differences in the amount of time spent in each zone. Bolded values are statistically significant (p = >0.05). Reading from the top to the left, estimates are given in minutes. (Example: On average, visitors spent 0.568 minutes less in Zone A than in Zone B.)*

| | A | B | D | E | F | G | H | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| B | -0.568 | | | | | | | | | | |
| D | **-0.909** | 0.341 | | | | | | | | | |
| E | -0.763 | **-1.33** | **-1.671** | | | | | | | | |
| F | **-0.859** | 0.291 | 0.05 | **1.621** | | | | | | | |
| G | -0.258 | -0.31 | 0.651 | **1.02** | -0.601 | | | | | | |
| H | -0.242 | -0.326 | **0.667** | **1.004** | **0.617** | 0.016 | | | | | |
| J | -0.174 | -0.394 | 0.735 | 0.936 | -0.685 | -0.084 | -0.068 | | | | |
| K | 0.128 | -0.44 | **-0.781** | **-0.89** | **-0.731** | -0.13 | -0.114 | -0.046 | | | |
| L | -0.044 | -0.612 | **-0.953** | -0.718 | **-0.903** | -0.302 | -0.286 | -0.218 | 0.172 | | |
| M | -0.108 | -0.46 | **-0.801** | **-0.87** | **-0.751** | -0.15 | -0.134 | -0.066 | 0.445 | -0.152 | |
| N | -0.318 | **-0.886** | **-1.226** | 0.445 | **-1.176** | -0.575 | -0.559 | -0.491 | 0.02 | -0.273 | -0.425 |

*Behavioral Pattern Analysis*

The NMDS ordination showed that the total time spent, number of stops, number of children in the group, overall group size, and age significantly correlated with the behavioral patterns of visitors (Figure 9). The stress of the final ordination was 0.157, indicating a decent fit in two dimensions. Axis 1 explained 61.80% of the variation in behavioral patterns, whereas 29.80% of the variation was explained by Axis 2. Visitors that spent more time and made more stops were more likely to take photos and look at the gardens, while older visitors were more likely to look at specific plants.

The permutational analysis of variance used to analyze the drivers of difference in behavioral patterns showed that the zone, number of stops, age, and group size all explained a significant proportion of the variation in the behavioral patterns of individual visitors. Zone explained 10.3% of the variation ($p = 0.002$), the number of stops explained 6.80% ($p = 0.001$), a visitors age explained 1.34% ($p = 0.031$), and group size explained 1.19% ($p = 0.047$) of the variation in behaviors observed in visitors. Total time in zone, number of children in a group, and whether or not the group was associated with a school did not explain a significant amount of variation after other factors were taken into account ($p = 0.85$, 0.61, and 0.67, respectively).

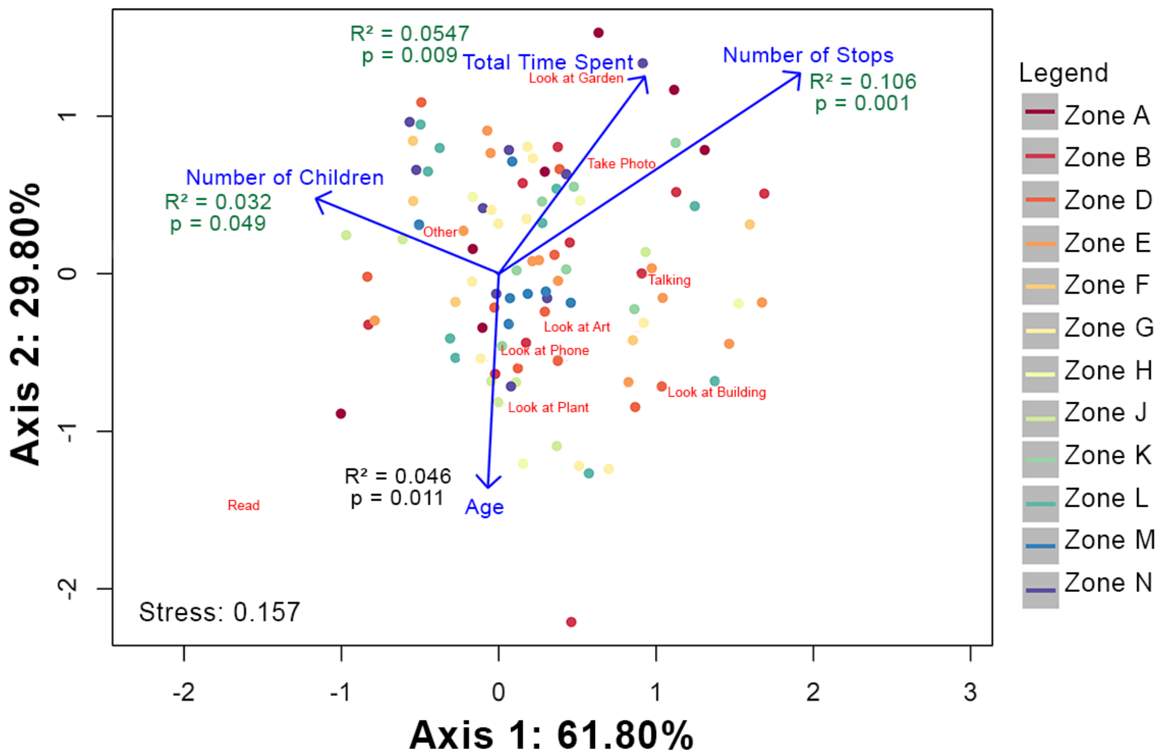



*Figure 9: Bray-Curtis Dissimilarity test plot showing the influence of visitor demographics on types of behaviors, with the colored dots representing the different zones, and blue arrows representing the variables that significantly influenced the behavior of visitors, and red text representing the different behaviors.*

*Table 5: Permutational analysis of variance for the drivers of behavioral differences between visitors. Asterisks indicate variables that explained a significant difference in the distances between behavioral patterns of visitors.*

| | **Df** | **Sum of Squares** | **Mean Sum of Squares** | **F Model** | **$R^2$** | **P-Value** |
|---|---|---|---|---|---|---|
| **Zone*** | 11 | 4.691589 | 0.426508 | 1.985592 | 0.102986 | 0.002 |
| **Number of Stops*** | 1 | 3.097406 | 3.097406 | 14.41985 | 0.067992 | 0.001 |
| **Age*** | 1 | 0.611588 | 0.611588 | 2.847223 | 0.013425 | 0.031 |
| **Group Size** | 1 | 0.543678 | 0.543678 | 2.531073 | 0.0182 | 0.186 |
| **Number of Children** | 1 | 0.295411 | 0.295411 | 1.375277 | 0.006485 | 0.672 |
| **Total Time Spent** | 1 | 0.081238 | 0.081238 | 0.378202 | 0.001783 | 0.850 |
| **School Group Status** | 1 | 0.148075 | 0.148075 | 0.689357 | 0.00325 | 0.607 |
| **Residuals** | 168 | 36.08665 | 0.214801 | | 0.792145 | |
| **Total** | 185 | 45.55563 | | | 1 | |

*Distribution of Behaviors of Interest Across the Gardens*

Different behaviors showed different trends throughout the zones. The most commonly read sign was found in the Conservation Garden, followed by the sign for the Monet Pool (Figure 10A). Looking at plants was unsurprisingly evenly distributed throughout most zones (Zones D – N), except along the Orangery (Zone H) (Figure 10B). Photos were most commonly taken along the O'Fallon Perennial Walk (Zone E), the Orangery (Zone H), and the Rock Alpine Garden (Zone N).

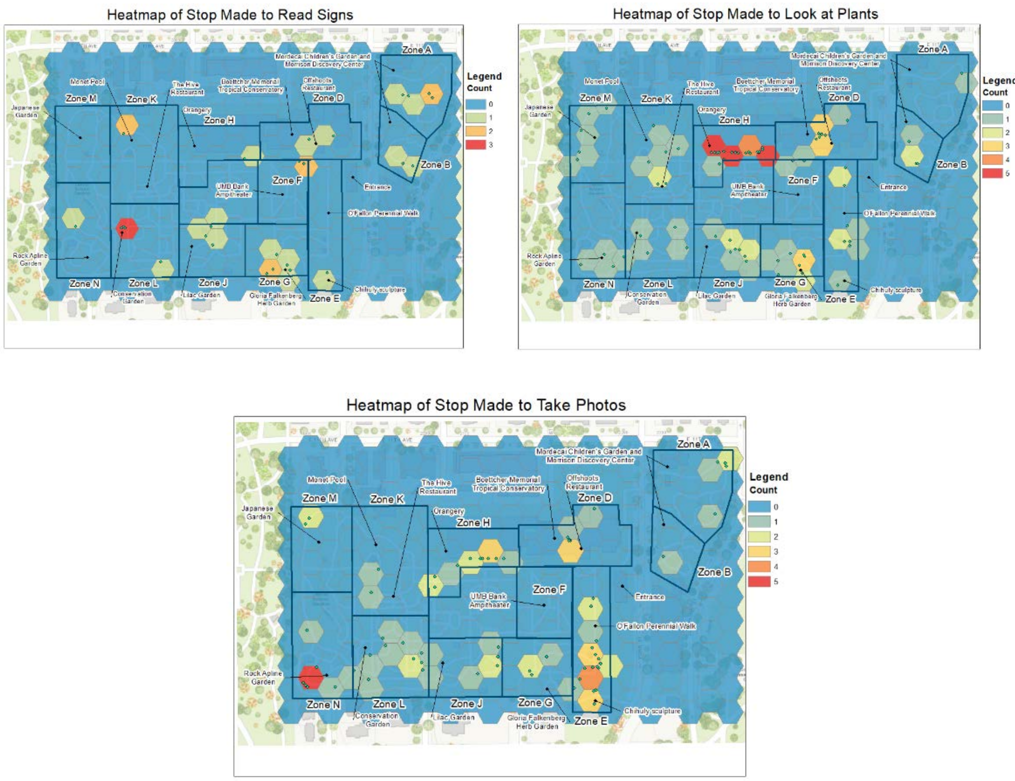



*Figure 10: 500 m² hexagonal heatmaps showing different behaviors of interest. A) Stops made to read signs or maps, B) Stops made to look at plants, C) Stops made to look at photos.*

## Discussion

Results from this analysis suggest behaviors and activity are influenced by the demographic variables of visitors at the Denver Botanic Gardens. The various areas of the Denver Botanic Gardens are used differently, particularly based on group size and whether children are in the group. Different areas of the gardens elicited different behaviors, which is likely influenced by the structure and goal of the garden, objects and features within it, and potentially factors like weather and season. However, despite the weakness of the correlations, the results partially agree with Serrell's (1997) conclusion that visitor movement patterns are not random. They are patterned and depend less on what the institution offers, but of the choices the visitor makes. Although similar studies concluded that these choices are also driven by the perceived value of the interaction with the exhibit, the object, or the signage (Bitgood 2010; Lanir et al. 2016). The drivers of visitor choice may be a combination of both patterns and perceived value, and may be unique to this type of institution.

In the overall map of stops made throughout the gardens, a few trends stand out. First, there are higher numbers of stops made in the eastern portion of the gardens closer to the entrance and the middle, compared the to the western side towards the far back of the gardens (Figure 7). This follows the pattern of the “Entrance Gradient Decrease” which describes the phenomenon of visitors making more stops and spending more time in the early parts of the exhibit than the later parts (Serrell 2016). Additionally, this could indicate that visitors are making fewer stops as they move deeper into the gardens because they are growing tired (Petrelli et al, 1999). Alternatively, many of these are strolling gardens like the Monet Pool, the Japanese Garden (Zones K and M) where people look at plants and the gardens overall without stopping, unlike the Orangery where there is a physical barrier between the visitors and the plants.

These patterns show how many demographic variables significantly affected the average number of stops visitors made per zone. The older the visitor is, the more likely the visitor is to stop to rest, or to enjoy the gardens. Larger groups without children gave the largest increase in the average number of stops, suggesting that groups of adults take more stops to enjoy the gardens. Small and large group sizes made many stops along Zone E with the O’Fallon Perennial Walk and the Chihuly sculpture. Smaller groups made more stops in the Boettcher Memorial Tropical Conservatory than large groups. Additionally, contrary to my hypothesis, smaller groups did not spend more time in a zone compared to larger groups. The difference in the effect of group size on the number of stops made but not on the amount of time spent could be explained by how the data were collected. Each observation only looked at one person in the group. A group with two people may move more closely together and make the same stops, whereas a larger group may have individuals acting more independently from the focal visitor, in both cases affecting the time spent. Groups of three or more made more stops in a heavily shaded area (Zone J), possibly to find relief from the summer heat and sun.

Despite the influence of group size on the number of stops, groups with children did not influence the number of stops made, indicating that the presence of children changes the reason or focus of a group's visit. This is consistent with the idea that while families are oriented towards learning, they are more likely to interact with each other, or with hands-on activities rather than activities that require stopping like reading text and signs (Borun et al. 1997). The effect of children overall would not be significant in this case because of the lack of learning-oriented activities for children. With the addition of the Science Pyramid, the interactive opportunities for children in the main gardens have significantly increased. It is important to note that this study did not include visitor interaction inside the Science Pyramid, so its influence on visitor attention is unknown.

While the number of stops was not influenced by the number of children in the group, groups with and without children differed in the locations where they made stops. Adults with children had an extremely high concentration of stops in the Mordecai Children's garden, and very few stops throughout the rest of the gardens. On the other hand, groups without children had higher concentrations throughout the rest of the gardens, with only a few stops at the entrance of the Mordecai Children's Garden (Zone B), but no adults were observed without children in Zone A, the main part of the garden. If the remaining observations after the first 10 in each zone were to be mapped, the distribution would be expected to change, but the overall confidence in the trends would increase.

Contrary to my hypothesis, groups without children did not differ in the amount of time spent from groups with children. This conflicts with Sandifer's (1997) results that families always spent more time than non-families, both in the two interactive exhibits in the study, and the Ruben Fleet Science Center overall. These exhibits were a step beyond "hands-on", and allowed visitors to explore and exercise control over one or more of the exhibits features. This suggests that the type of institution and the interactive nature of these exhibits may affect how families and nonfamilies use the space. If the gardens want to attract more children to the main gardens, installations and signage should be designed and distributed to be more interactive for young visitors.

The O'Fallon Perennial Walk (Zone E) had significantly more stops on average than all other zones except for the Orangery (Zone H) and more time spent on average than the Mordecai Children's Garden, Boettcher Memorial Tropical Conservatory, Gloria Falkenberg Herb Garden, and the Orangery (Zones A, B, D, G, and H). These results are also consistent with the "Entrance Gradient Decrease" (Serrell 2016). Mapping the stops in GIS showed a gradient of stops increased as visitors approached the Chihuly statue "Colorado." However, the Monet Pool (Zones K) and the Japanese Garden (Zone M) had significantly more time spent on average than the O'Fallon Perennial Walk (Zone E). This difference at the Monet Pool (Zone K) could be because of the Hive restaurant, and because the area is common for picnics. Between the O'Fallon Perennial Walk (Zone E) and the Japanese Garden (Zone M), in the midday hours, the O'Fallon Perennial Walk (Zone E) would not have much shade, but the Japanese Garden (Zone M) would have more shade with larger trees. Visitors could be traveling slower through the shadier garden, while also enjoying its aesthetics (Chan et al, 2017). The differences shown in the O'Fallon Perennial Walk (Zone E) are consistent with results from Melton (1935) that explained when all is equal, visitors will turn right. In this case, the three directions are not equal. Looking forward is a long walk toward the Monet Pool, to the right is a building going to the information desk and restrooms, and to the left is the O'Fallon Perennial Walk, which is designed to draw the visitors in. The eastern portion of the gardens generally had a low number of stops compared to the rest of the gardens. This again, could be due to the strolling type gardens, as well as visitor energy as they move deeper into the gardens (Petrelli et al, 1999). If the rest of the observations across all zones were plotted, it is very likely the distribution would not be as different.

UMB Bank Amphitheater (Zone F) had very significantly less time spent on average when compared to the O'Fallon Perennial Walk, the Monet Pool, the Conservation Garden, and the Japanese Garden (Zones E, K, L, M, and N). There are two gardens, the Ponderosa Border garden, and the Roads Water-Smart garden found in Zone F, but the majority of the area is filled with the amphitheater. This gives visitors few choices in where to spend their time and in many cases, the Roads Water-Smart garden was visited before exiting the zone. In this case, the right turn theory is supported (Melton 1935). When entering Zone F from the entrance, the Roads Water-Smart garden, which is small, is immediately to the right, whereas the Ponderosa Border garden is discreetly tucked to the left, and the UMB Bank Amphitheater was unused at the time of the study.

The amount of time spent, and the number of stops made influenced visitor behavior alongside age, group size, and number of children present in the group. Older visitors in smaller groups were more likely to look at specific plants, which could potentially be explained by hobby gardeners with an interest in plants to add to their collection. Group size and the number of children in the group influenced many "other" behaviors, which should be further assessed to better describe the behaviors seen for future studies. Visitors spending more time and making more stops generally took more photos and looked more at the gardens. This is further influenced by the number of children and less so by the group size or age. This could suggest that the time of year may have played a role in how visitors behaved due to many of the plants being in full bloom. Repeating this study in a different season or outside of the peak blooming time could dramatically change the influences of age, group size, number of children, time spent and number of stops on visitor behaviors.

In addition to the demographic and activity variables, the frequency of behaviors observed in each zone were influenced by the exhibits, objects, and signage found in the zone. In the Mordecai Children’s Garden and the Morrison Discovery Center (Zones A and B), there was a high proportion of talking and taking photos. These gardens are focused on education and exposure to plant life. They are generally used by parents or grandparents with young children in tow. The adults would frequently talk with the children and take photos of them while they played in the garden. The Boettcher Memorial Tropical Conservatory, O’Fallon Perennial Walk, UMB Bank Amphitheater, and Gloria Falkenberg Herb Garden (Zones D, E, F, and G) all had high frequencies of photos being taken, which is expected because these gardens are ornamental and aesthetically focused gardens, and during June many flowers are in full bloom. Interestingly, the UMB Bank Amphitheater (Zone F) also had many instances where visitors did not stop at all. Upon inspecting the paths these visitors took, most of them were travelling west to east, from the inner part of the gardens toward the exit. Zones J through N did not have any behaviors that were more or less common than the others.

Looking at plants was the most common activity seen when visitors stopped at the Orangery (Zone H), which again, is likely because a visitor must stop to look past the glass to view the plants inside. In other areas, visitors can view plants as they walk by without having to specifically stop. Alternatively, it could be a derivative of plant blindness, or the inability to see plants in one's own environment (Allen 2003). Instead of being blind to all plants, visitors may visually pass over plants that are not in full bloom or are not otherwise striking enough to draw their attention. This is a constant challenge for botanists and botanical conservationists, but facilities like DBG that provide a direct, plant focused experience, could help visitors develop an empathetic connection with plants (Balding & Williams 2016). Few signs were read across the gardens, which agrees with Ward et al. (2010) that visitors more often visit the gardens for recreational or restorative reasons, and infrequently for educational or horticultural reasons. However, the sign for the Conservation Garden was the most read of the observations mapped, which could show a positive influence of DBG on visitor's interest and empathy toward plants and plant conservation.

The results of this study are limited by the modest correlations with the data, implying the study has many undescribed factors like time between stops, variation in the time of day, day of the week, or season (Yalowitz & Bronnenkant, 2009), as well as the time limitations for plotting the GIS data. The behaviors that were described were very limited and the overwhelming number of observations were listed as "other." This suggests the list of possible behaviors observed needs to be expanded in future studies. Additionally, the data were collected by volunteers that received limited training. These two issues combined suggest that the observers may not have been able to visually distinguish between behaviors that look very similar. For example, looking at a specific plant, and reading the sign for the plant would be difficult to judge from a distance.

The timing of this study should be considered when repeating it. The days that data was collected had maximum temperatures between 82°F and 92°F, with trace or no precipitation. If data were collected in the spring or fall with milder temperatures, it could influence how visitors use the gardens. Additionally, data were collected on two Fridays, and a Thursday. Different days of the week could yield significantly different results. A similar study was done at the Lincoln Park Zoo, but they conducted the study over a 12-month period and found that visitor density was highest in the summer, and negatively impacted visitor duration with visitors moving more quickly through the hot and crowded exhibit (Ross & Lukas 2005). If DBG repeated the study, seasonality would likely influence visitors in a similar fashion, both in the case of weather, crowds, and blooming periods.

To repeat this study, volunteers should complete a training session that included a sample observation where a staff member or volunteer would travel through a zone and preform the same behaviors along a specific path. Volunteers would observe the person as they would a visitor and track their movements, stops, and behaviors. This would increase the inter-observer reliability among the volunteers, as well as increase their confidence in data collection. Additionally, observers should be provided with a behavior key, with the behaviors observed in this study as well as “resting” to indicate a stop with no particular activity, “touching/smelling/interacting with plant,” “sitting on bench/steps,” “interacting with staff/volunteers,” and “participating in activity” for activities within the Mordecai Children’s Garden and the Science Pyramid. Ideally, the times between stops would be recorded. This was done by a few observers, but it would be interesting to see how long visitors moved between stops. More collection days across the year would also be required and would allow DBG to look at differences across seasons. However, if DBG wanted to conduct the study passively without observers, Bluetooth and GPS technology could be used to show visitor paths, timing, and features attracting visitor attention similar to a study at the Louvre Museum, which showed that patrons that stayed a short amount of time visited similar features that long-stay patrons visited (Yoshimura et al. 2014). This would allow DBG to passively track visitor paths through the entire gardens, track the overall time as well as time at stops and between stops, and show which features are drawing attention. Followed by a demographic and visitor experience survey, would give DBG an excellent view of visitor use in the gardens, visitor demographics and how use and demographic variables interact.

The establishment of specific goals for each of the gardens, even in simple terms would help assess if the activities being observed in the garden are reflecting that goal. These goals could be built upon the Interpretive Engagement Conceptual Framework from DBG. Currently, there are three types of gardens “ornamental,” “strolling,” and “picnic” gardens. To expand on this, gardens like the Roads Water-Smart garden and the Birds and Bees Walk could be classified as “educational” gardens to fall in line with the *Understanding* portion of the framework. The Conservation Garden and the Boettcher Memorial Tropical Conservatory could be “conservation” gardens to reflect the *Understanding* and *Stewarding* parts of the framework. Other classifications could be “rest and rejuvenate” for the Appreciation framework, and “interactive” for the Participation framework. Including the garden type and goal to the assessment would give curators and designers the opportunity to see how the structure and objects placed within a garden affect behavior observed and how that relates to the garden’s goal.

These limitations should be taken with the consideration that the results of similar studies in zoo and museum setting yielded different results (Ross & Gillespie 2009; Sandifer 1997). Structurally, both are very different from botanic gardens. Zoos have an open plan like botanic gardens, but to see the animals, visitors must stop and look past a barrier, much like the Orangery in Zone H. Like museums, many can be enjoyed passively, reading signs of interest, but museum exhibits are highly structured by design, with a defined path and order of operations (Serrell 1997).

This implies that behaviors, environmental factors, structure, and visitor demographics affect botanic gardens in a unique way, requiring studies such as this one to be specifically tailored to the setting.

In conclusion, visitor behavior and use of the gardens were influenced by demographic variables, as well as the type and feature of each garden. This study is the first to use visitor movement, demographic information, and behavioral observations to better understand the use of botanic gardens. Although demographic information weakly predicted visitor's use of the gardens, it showed that different kinds of groups used areas of the gardens differently. Trends shown using GIS reflected the influence of demographic variables and behavioral patterns in spatial relation to various garden features. With more data, and perhaps a visitor questionnaire, this study design could easily be used for future assessments to give more informative results. The ordination used to assess behavioral patterns appears to be the first to use visitor data to evaluate the effect of an informal educational institution's environmental factors on the differences in observed behaviors. This could be applied in informal educational institutions to assess how visitor behavior and choices are influenced by the space around them. This study provides important information about how visitors use the different areas of DBG, giving curators and designers the chance to make better informed decisions about improving the visitor experience and increase visitor engagement across all demographics by changing signage, focal areas, or features across the gardens.

# Appendix

*Table 5: Key of behaviors recorded in this study.*

| **Behavior Key** | | | |
|---|---|---|---|
| TK - Talking | RD – Reading a sign, brochure, or label | LP – Looking at plants | LG – Looking at garden |
| LB – Looking at building | PH – Looking at phone (not counted if taking a photo) | Pic – Taking a picture | LA – Looking at art |
| O - Other | None – No stops made | | |

*Table 6: The most common and least common behaviors observed by zone, as well as the total number of stops made in the zone.*

| **Zone** | **Most Common Behavior** | | **Least Common Behavior** | | **Total Number of Stops in Zone** |
|---|---|---|---|---|---|
| **Zone A** | TK | 25 | LA | 2 | 122 |
| **Zone B** | TK | 16 | LG | 1 | 93 |
| **Zone D** | LP/Pic | 12 | TK/LB/PH/LA | 1 | 94 |
| **Zone E** | LP | 29 | PH | 1 | 98 |
| **Zone F** | LP | 34 | LG/PH | 2 | 140 |
| **Zone G** | Pic | 16 | TK/LA | 1 | 76 |
| **Zone H** | LP | 41 | LG/PH | 2 | 123 |
| **Zone J** | LP | 15 | PH | 1 | 62 |
| **Zone K** | Pic | 21 | LG/LA | 1 | 88 |
| **Zone L** | LP | 19 | PH | 1 | 84 |
| **Zone M** | Pic | 14 | PH | 2 | 79 |
| **Zone N** | LP | 13 | LG/LA | 1 | 74 |

*Table 7: Models assessing the effects of different demographics on the number of stops made. S, number of stops made in zone; A, age of visitor; G, $log_2$ group size; C, $log_2$ number of children in the group, GNC, $log_2$ groups without children; GWC, $log_2$ groups with children. Factor is the unit of change by which the estimate of the predictor affects the response. An asterisk indicates models that are statistically significant.*

| Model | Estimate | p-value | 95% CI | Pseudo $R^2$ | Factor |
|---|---|---|---|---|---|
| S ~ A* | 7.91% | $2.00*10^{-16}$ | 6.71% – 9.03% | 0.017 | Every 10 years |
| S ~ G* | 5.01% | $3.15*10^{-5}$ | 2.64% – 7.54% | 0.0016 | Double |
| S ~ C* | -15.61% | $2.00*10^{-16}$ | -19.51% – -14.47% | 0.0185 | Double |
| S ~ GNC* | 24.78% | $2.00*10^{-16}$ | 19.11% – 25.15% | 0.0267 | Double |
| S ~ GWC | 2.92% | 0.257 | -2.13 – 7.83 | 0.0001 | Double |

*Table 8: Models assessing the effects of different demographics on the amount of $log_{10}$ time spent in a zone. T, time spent in zone; A, age of visitor; G, $log_2$ group size; C, $log_2$ number of children in the group, GNC, $log_2$ groups without children; GWC, $log_2$ groups with children. Factor is the unit of change by which the estimate the predictor affects the response. An asterisk indicates models that are statistically significant.*

| Model | Estimate | p-value | 95% CI | Pseudo $R^2$ | Factor |
|---|---|---|---|---|---|
| T ~ A | 0.013% | 0.092 | -0.002% – 0.026% | 0.0079 | Every 10 years |
| T ~ G | 6.25% | 0.689 | -0.42% – 20.56% | 0.0004 | Double |
| T ~ C* | 3.46% | 0.0371 | 1.86% – 77.82% | 0.012 | Double |
| T ~ GNC | 10.41% | 0.621 | -38.36% – 72.19% | 0.001 | Double |
| T ~ GWC | -2.63% | 0.369 | -4.75% – 28.82% | 0.0072 | Double |

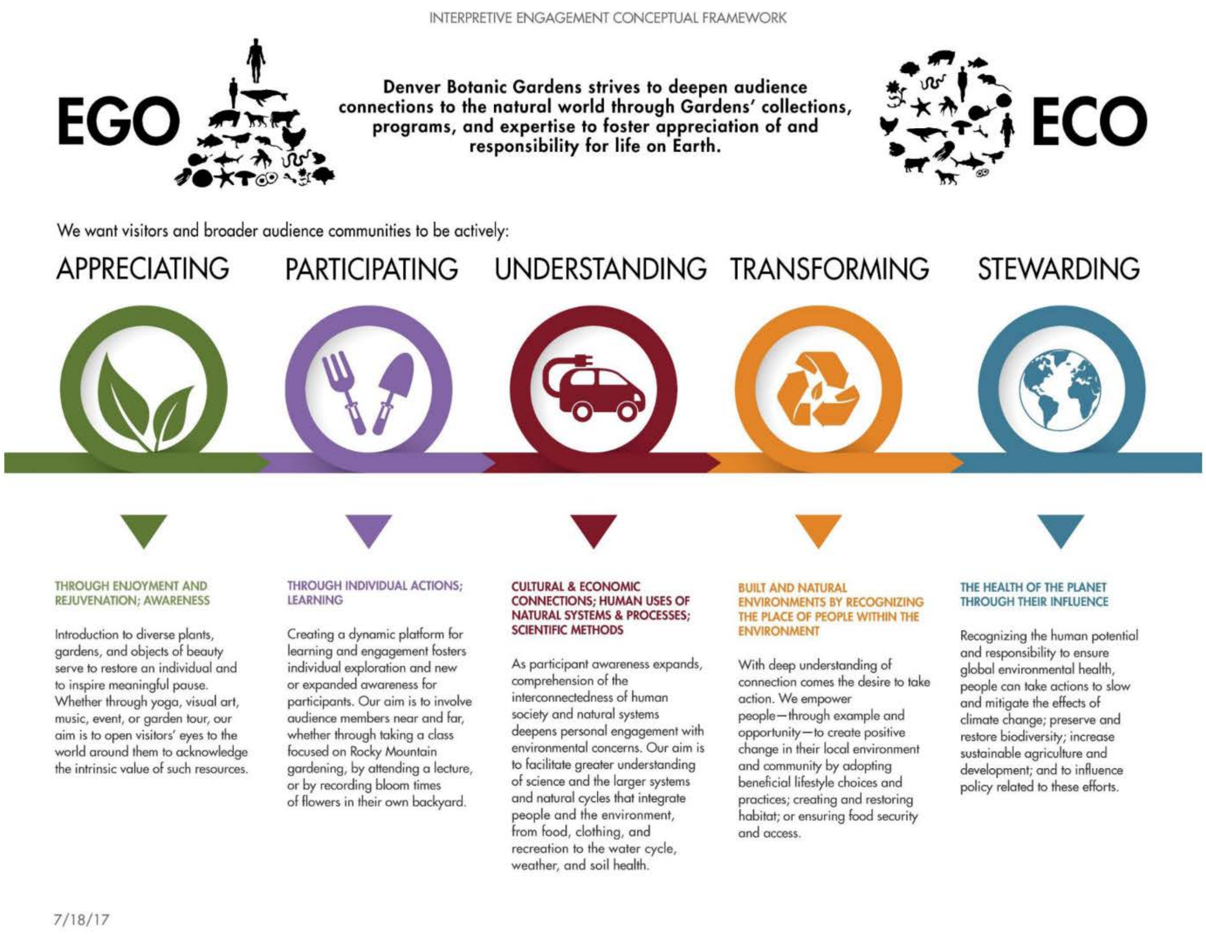
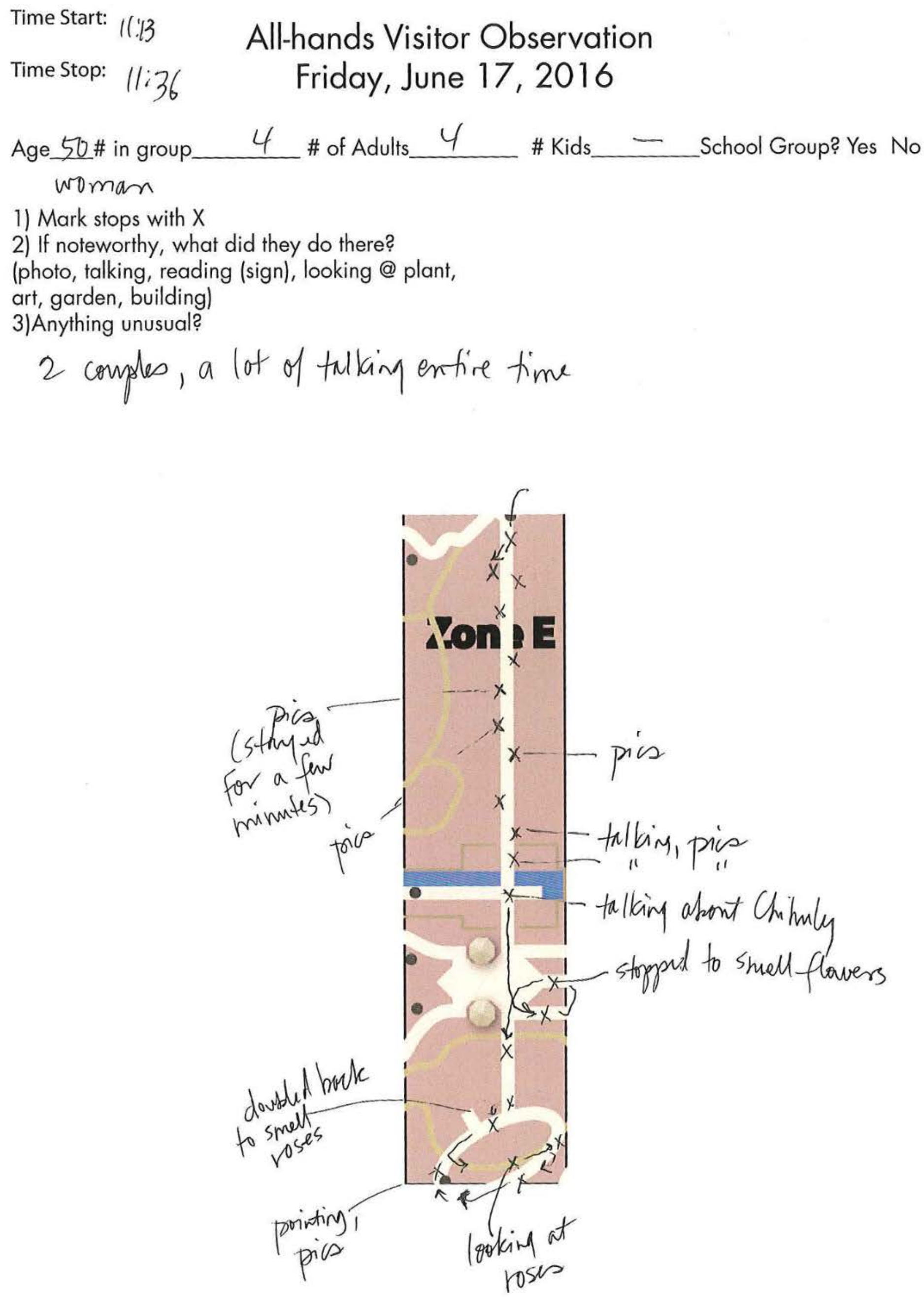



*Figure 11: The interpretation framework for the Denver Botanic Gardens expressing goals for active visitor engagement.*


Time Start: 11:13

Time Stop: 11:36

All-hands Visitor Observation

Friday, June 17, 2016

Age 50 # in group 4 # of Adults 4 # Kids — School Group? Yes No

woman

1) Mark stops with X
2) If noteworthy, what did they do there?
(photo, talking, reading (sign), looking @ plant, art, garden, building)
3)Anything unusual?

2 couples, a lot of talking entire time



*Figure 12: Example observation sheet from Zone E.*

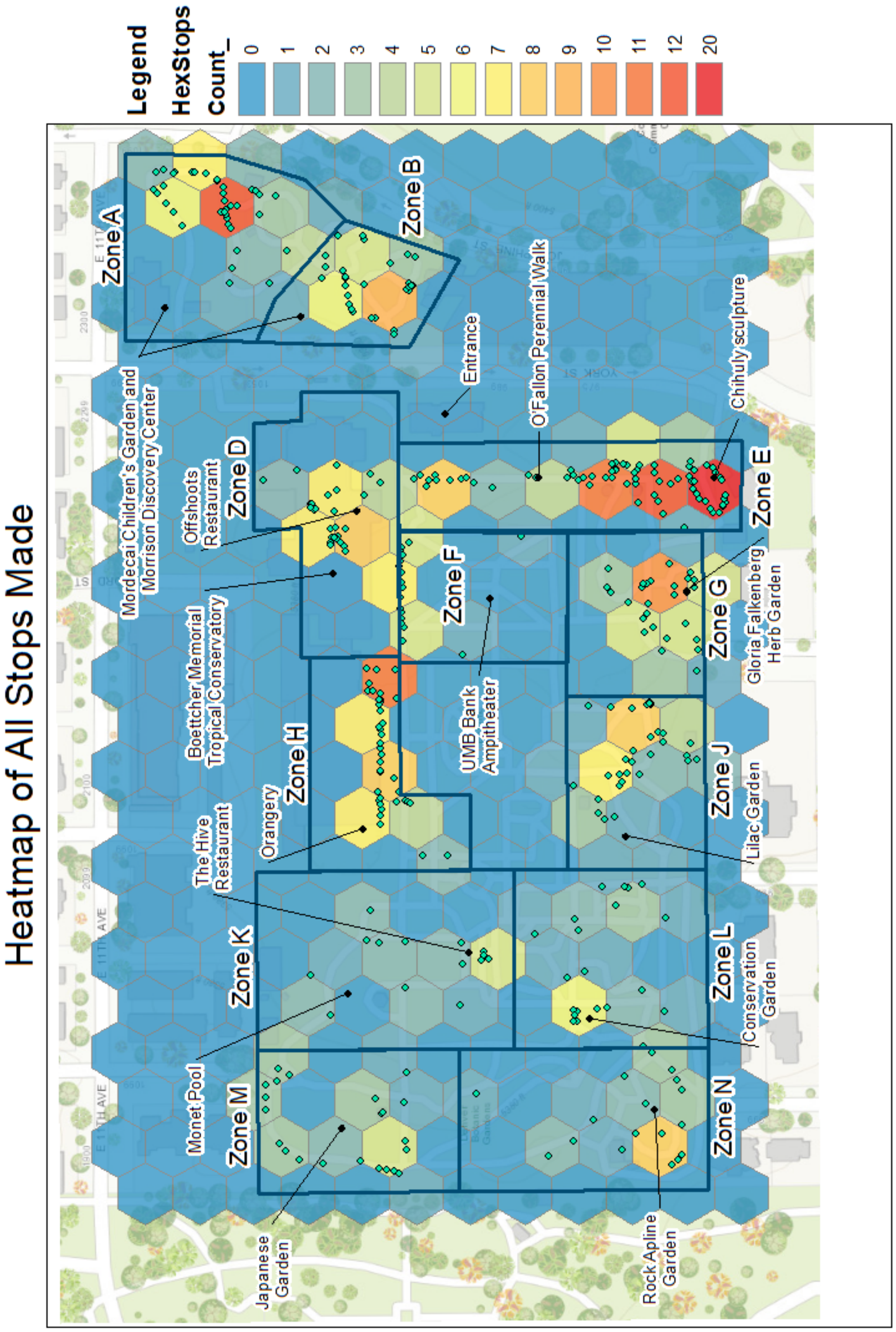



*Figure 13: Map of stops mapped across all zones.*

# References


Allen, W. (2003). Plant blindness. *AIBS Bulletin*, *53*(10), 926-926.

Balding, M., & Williams, K. J. (2016). Plant blindness and the implications for plant conservation. *Conservation Biology, 30*(6), 1192-1199.

Bitgood, S. (2010). An attention-value model of museum visitors. *Center for Advancement of Informal Science Education: Washington, DC, USA*.

Borun, M., Chambers, M. B., Dritsas, J., & Johnson, J. I. (1997). Enhancing family learning through exhibits. *Curator: The Museum Journal*, *40*(4), 279-295.

Chan, S. Y., Chau, C. K., & Leung, T. M. (2017). On the study of thermal comfort and perceptions of environmental features in urban parks: A structural equation modeling approach. *Building and Environment*, *122*, 171-183.

Denver Botanic Gardens. (2016). *Annual Report 2016* Retrieved https://www.botanicgardens.org/sites/default/files/blog-photos/2016_annualreport_1.pdf

Dressel-Martin, S. (2006). *Gardening with altitude: Cultivating a new western style*. Denver, CO: Denver Botanic Gardens.

ESRI 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

Falk J. H. (1982) The use of time as a measure of visitor behavior and exhibit effectiveness, *Roundtable Reports: The Journal of Museum Education*, 7(4), p. 10-13.

Gilman, B. (1923). *Museum ideals of purpose and method.* Cambridge, MA: Harvard University Press.

Goslee, S.C. and Urban, D.L. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* 22(7):1-19.

Gutwill, J. P., & Dancstep, T. (2017). Boosting metacognition in science museums: simple exhibit label designs to enhance learning. *Visitor Studies*, *20*(1), 72-88.

Kaynar, I. (2005, June). Visibility, movement paths and preferences in open plan museums: An observational and descriptive study of the Ann Arbor Hands-on Museum. In *Proceedings of the 5th International Space Syntax Symposium, Delft, The Netherlands* (Vol. 1317).

Lanir, J., Kuflik, T., Yavin, N., Leiderman, K., & Segal, M. (2016). Visualizing museum visitors' behavior. In *Advanced Visual Interfaces for Cultural Heritage* (pp. 44-47).

Levin, M. (1983). *The modern museum: Temple or showroom*. Jerusalem: Dvir Publishing House.

McManus, P. M. (1989). Oh, yes, they do: How museum visitors read labels and interact with exhibit texts. *Curator: The Museum Journal*, *32*(3), 174-189.

Melton, A. W. (1996). *Problems of installation in museums of art* (102-104). Washington, D.C.: American Association of Museums.

Montaner, J., & Oliveras, J. (1986). *The museum of the last generation.* London: Academy Editions.

Nurse Rainbolt, G., Benfield, J. A., & Loomis, R. J. (2012). Visitor self-report behavior mapping as a tool for recording exhibition circulation. *Visitor Studies*, *15*(2), 203-216.

Oksanen, J., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., Mc Glinn, D., Minchin, P., O’Hara, R.B., Simpson, G. L., Solymos, P., Stevens, M., Szoecs, E., Wagner, H. (2017).

vegan: Community Ecology Package. R package version 2.4-2. https://CRAN.R-project.org/package=vegan

Packer, J. (2008). Beyond learning: Exploring visitors' perceptions of the value and benefits of museum experiences. Curator: The Museum Journal, 5(1), 33–54

Peponis, J., Dalton, R. C., Wineman, J., & Dalton, N. (2004). Measuring the effects of layout upon visitors' spatial behaviors in open plan exhibition settings. *Environment and Planning B: Planning and Design*, *31*(3), 453-473.

Petrelli, D., Not, E., & Zancanaro, M. (1999). Getting engaged and getting tired: What is in a museum experience. In *User Modelling: Workshop on Attitude, Personality and Emotions in User-Adapted Interaction*.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ross, S. R., & Lukas, K. E. (2005). Zoo visitor behavior at an African ape exhibit. *Visitors Studies Today*, *8*(1), 4-12.

Ross, S. R., & Gillespie, K. L. (2009). Influences on visitor behavior at a modern immersive zoo exhibit. *Zoo Biology*, *28*(5), 462-472.

Sandifer, C. (1997). Time-based behaviors at an interactive science museum: Exploring the differences between weekday/weekend and family/nonfamily visitors. *Science Education*, *81*(6), 689-701.

Screven, C. G. (1992). Motivating visitors to read labels. *ILVS Review: A Journal of Visitor Behavior*, *2*(2), 183-211.

Serrell, B. (1995). The 51% solution research project: A meta-analysis of visitor time/use in museum exhibitions. *Visitor Behavior*, *10*(3), 6-9.

Serrell, B. (1997). Paying attention: The duration and allocation of visitors' time in museum exhibitions. *Curator: The Museum Journal*, *40*(2), 108-125.

Serrell, B. (2016, November). *Visitor Behavior in Museum Exhibitions and the Implications for Improving Exhibits*. Lecture presented at XXIX Jornada de la Xarxa de Museus Local, Usuaris de museus. Avaluacio i identificacio d'oportunitats de millora, Museu Molí Paperer in Spain, Capellades.

Tröndle, M., Greenwood, S., Kirchberg, V., & Tschacher, W. (2014). An integrative and comprehensive methodology for studying aesthetic experience in the field: Merging movement tracking, physiology, and psychological data. *Environment and Behavior*, *46*(1), 102-135.

Ward, C. D., Parker, C. M., & Shackleton, C. M. (2010). The use and appreciation of botanical gardens as urban green spaces in South Africa. *Urban Forestry & Urban Greening*, *9*(1), 49-55.

Wallace, V. (2013). *Successful visitor experience - Getting it right* [Guide]. Ludrow, Shropshire: AIM Association of Independent Museums.

Wickham, H. (2009). ggplot2: Elegant graphics for data analysis. *Springer-Verlag, New York.*

Wineman, J. D., & Peponis, J. (2010). Constructing spatial meaning: Spatial affordances in museum design. *Environment and Behavior*, *42*(1), 86-109.

Yalowitz, S. S., & Bronnenkant, K.  (2009). Timing and tracking: Unlocking visitor behavior. *Visitor Studies*, *12*(1), 47-64.

Yoshimura, Y., Sobolevsky, S., Ratti, C., Girardin, F., Carrascal, J. P., Blat, J., & Sinatra, R. (2014). An analysis of visitors' behavior in the Louvre Museum: A study using Bluetooth data. *Environment and Planning B: Planning and Design*, *41*(6), 1113-1131.

# CHAPTER 4. ENVIRONMENTAL STAKEHOLDER ANALYSIS: TREATMENT OF THE DOUGLAS FIR TUSSOCK MOTH (*ORYGIA PSEUDOTSUGATA*) IN PIKE NATIONAL FOREST

Pike National Forest (PNF) is a 1.1 million acre forest established in 1906 as a National Forest within Clear Creek, Teller, Park, Jefferson, Douglas, and El Paso counties. Several of Colorado's "fourteeners" are found within the forest, including Mount Evans and Mount Bierstadt. There are extensive recreational resources available within PNF, including hiking, biking, camping, fishing, hunting, and picnicking in a near-natural environment since most of the forest remains undeveloped. PNF is also home to a wide variety of plant and animal species, including two threatened and one endangered animal species. The Douglas fir forests within PNF are at risk of mass defoliation from the Douglas fir tussock moth, which would directly impact the usability and aesthetics of the forest. It is recommended that the United States Forest Service treats the tussock moth infestation of the Douglas Fir forests in Pike National Forest with the TM-Biocontrol because it specifically targets the tussock moth, would not endanger the endangered Uncompahgre Fritillary butterfly, and does not have restrictions to the size of the treatment area.

The Douglas fir tussock moth (*Orgyia pseudotsugata*) is a native defoliator of spruce, and Douglas fir trees in Colorado (Colorado State Forest Service, 2016). Outbreaks of this species are usually marked by rapid defoliation of spruce and fir trees, typically starting at the top of the tree and moving down. Normal infestations, or the early stages of an outbreak, generally do not cause tree mortality. However, recurring attacks or a sustained outbreak over multiple growing seasons could weaken trees and predispose them to other infestations that could kill the tree (Colorado State Forest Service, 2016). Tussock moth infestations will usually grow rapidly before abruptly subsiding after 2-3 years due to a nucleopolyhedrosis virus (NPV) that spreads throughout isolated populations (Williams et al., 2011).

Within Pike National Forest, the United States Forest Service (USFS) and the South Platte Ranger District operates a Christmas Tree Cutting area approximately 18,500 acres in size, just south of Buffalo Creek, Colorado. The area is also used for recreation (hiking, camping, fishing, hunting, and sight-seeing), and houses the threatened Mexican spotted owl (*Strix occidentalis lucida*) and Pawnee montane skipper (*Hesperia leonardus montana*), as well as the endangered Uncompahgre Fritillary butterfly (*Boloria acrocnema*) (U.S. Fish and Wildlife, 2018). A mass die-off of the Douglas firs would have negative impacts on the environmental value, socioeconomic value, and the value of the wilderness or undeveloped area. One significant environmental concern is regarding fire because this site is situated between the burn scars of the Buffalo Creek fire (May 1996) that burned nearly 12,000 acres, and the Hayman Fire (June 2002) that burned over 137,000 acres. If the tussock moth infestation is not addressed, the tree defoliation and tree mortality would increase fuels and therefore increase the risk of a large-scale wildfire (United States Department of Agriculture, 2017). This risk, as well as the aesthetic effects of defoliation would have direct impacts on the socioeconomic value of the area. The reduction of trees could reduce the number of viable trees to cut down during the Christmas tree cutting season and decrease the suitable camping and hiking area, directly affecting the revenue the USFS gains from recreation in the area.

An environmental assessment (EA) was completed by master's students from Regis University's environmental biology program in the Fall of 2016. The proposed action by the USFS is to eradicate the tussock moth using Foray 48B, a biological insecticide that uses *Bacillus thuringiensis* to target and eliminate lepidopteran larvae. This insecticide must be ingested to be effective, and after the initial application via an airplane with spray equipment, Foray 48B must be reapplied every 3 – 14 days depending on the severity of the infestation. The toxic nature and safety precautions indicated by the Foray 48B label would require the entire area to be closed to civilians for the day of application and at least three days after. On the third day, an assessment of the infestation would determine if another application is needed. If more than one application is required, the area will remain closed. The alternative developed by the Regis University students used NPV (marketed as TM-Biocontrol) due to its ability to solely target the tussock moth, instead of all lepidopteran species.

## Pike National Forest and the United States Forest Service

The USFS's mission statement is to, "sustain the health, diversity, and productivity of the nation's forests and grasslands to meet the needs of present and future generations" (United States Forest Service, 2018) This raises concern about the impact of the tussock moth on Douglas fir trees as it relates to the ecology of the area, potential decrease in tourism, revenue from recreation, and maintaining the integrity of the forest ecosystem. Defoliation of Douglas firs by the tussock moth can cause permanent damage to the trees themselves, while reducing habitat for the animals including the threatened and endangered species.

Part of USFS's mission to sustain the productivity of the nation's forests brings economic concerns ranging from camping, hunting, and fishing, to the annual Christmas Tree cutting season. The latter is particularly important for revenue because permits sell out quickly every year, and a reduction of Douglas fir trees that are good candidates for cutting would negatively impact this activity. If the infestation is not addressed, there could be a 50-90 percent reduction in Douglas fir trees to harvest (Ciesla, n.d). Everyday recreation activities could be impacted by the changing aesthetics and thinning of the forest. Thinning forests would also reduce cover for deer and elk, causing them to leave the area, and reducing the viable hunting grounds for civilians.

The USFS’s mission also includes sustaining the health and diversity of the nation’s forests. Dead needles and branches, especially in mass quantities due to an uncontrolled infestation of the tussock moth, could significantly increase the risk of forest fire. Half a dozen major forest fires have ripped through PNF including the Hayman fire in 2002, and the Waldo Canyon Fire in 2012, both of which were the most destructive fires in Colorado history at the time they happened. The burn scars from both fires are constant reminders of how important it is to maintain healthy forests, and how long it can take to recover from such a devastating event, if recovery ever happens.

## The Audubon Society of Greater Denver

The Audubon Society of Greater Denver’s (ASGD) mission statement is to, “advocate for the environment, connecting people with nature through conservation, education, and research” (Audubon Society of Greater Denver, 2018). This raises concerns about the effects of the tussock moth infestation on the estimated 75 bird species found in the area. ASGD actively follows legislative activities and projects that affect birds. A reduction in Douglas firs would impact the habitat for birds using the area, as well as the availability of prey items, cover from predators or the ability to hide from potential prey. However, the primary concern of the ASGD is regarding the area being designated as critical habitat for the threatened Mexican Spotted owl (U.S. Fish & Wildlife, 2018). Defoliation of the tree tops opens the canopy and affects the shelter for this species, and could impact its ability to forage, and mate (Ganey et al, 1999). These effects could be seen across many species that use the area, including golden eagles, western and mountain bluebirds, and broad-tailed hummingbirds. Birdwatchers would be concerned with the potentially reduced number of species, as well as the negative impacts on the bird communities.

## Recreational Users of Pike National Forest

Recreational users hold concern with aesthetic value of the area, and the ability to use the area for hiking, biking, hunting, fishing, camping, and snowshoeing. An extremely popular activity is the yearly Christmas Tree cutting that allows visitors to enter the forest and cut a fresh tree for the holiday season. Recreational users value the opportunities for solitude, the aesthetic value of the landscape, as well as the full outdoor experience. The defoliation of the fir trees in the area reduces the aesthetic value and make it less appealing for recreational users. If the tussock moth population does not naturally collapse, chronic defoliation could dramatically change the scenery, but also the landscape. Debris from the defoliation could impact the ability to use trails and waterways and make the designated campsites unusable. Much of the area remains undeveloped, only being accessible by four-wheel drive, by horse, or by foot. Remnants of historic mining sites and railways adds to the cultural value of the area. The Lost Creek Wilderness is a 106,000 acre area where motorized vehicles of any kind are prohibited. This is a valuable resource for people to find solitude in nature, and away from the sights and sounds of the city. Defoliation of the Douglas firs in the area would reduce the recreational value, full outdoor experience, and the opportunities for solitude in Pike National Forest. Not addressing the infestation could pose concerns for permanent damage to the forest, but there are additional concerns with the cost of treatment for such a large area. Furthermore, the temporary closures for the treatments would directly affect the ability of the public to use the area for any type of recreation, although the lengths of closures vary depending on the treatment type.

# Treatment Options

Three options are considered for addressing the infestation of the tussock moth in PNF. First, is the no-treatment option, letting the infestation run its course in hopes that it naturally collapses due to NPV. Second, the USFS would use the insecticide Foray 48B on the affected areas, and finally, the use of TM-Biocontrol on the affected areas.

Each of these options poses certain risks, and benefits. The no-treatment option is inexpensive in the short term and could be extremely beneficial if the infestation collapses after one or two seasons. The risk of the infestation not collapsing could results in major defoliation, and a larger infestation to deal with.

The use of Foray 48B could be beneficial because it is extremely effective and can be applied aerially. However, there are several risks associated with this method of treatment. The USFS holds a categorical exclusion under Title VI of the Healthy Forests Restoration Act of 2003, allowing the use of Foray 48B, but this is limited to 3,000 acres to maximize the retention of old-growth trees. With the affected area being over 18,000 acres, this leaves over 15,000 acres of forest untreated, which could render the effort ineffective in managing the infestation. Additionally, the label for Foray 48B clearly states that it should not be sprayed within ¼ mile of any habitat containing a threatened or endangered lepidopteran species, of which there are two. Furthermore, because this insecticide does not discriminate across lepidopteran species, there could be a collapse in food availability and respiratory damage to the animals living in the area (Petrie et al, 2003).

Due to the toxicity of Foray 48B, the area of application would not be usable for 3 days after application, or longer if reapplication is necessary (Valent BioSciences Corportation, 2008). This would affect the ability of the public to use the area during treatment, significantly impacting revenue. It costs $6.00 USD to gain access to the site for any recreational activity; if a busy day includes 30-100 civilians, as well as camping groups, shutting down for 3-14 days even once per year would result in a significant (E. Biery, personal communication, December 1, 2016). Additionally, Foray 48B has been shown to remain in water bodies, increasing the risk of exposure to people using the waterways recreationally (Menon and De Mestral, 1985). Human exposure for Foray 48B has been associated with sleep problems, dizziness, throat irritation, and stomach discomfort (Petrie et al, 2003).

The use of TM-Biocontrol would be beneficial because it only targets the tussock moth, and would not affect the threatened Pawnee montane skipper, or the endangered Uncompahgre Fritillary butterfly. Additionally, because the TM-Biocontrol uses tussock moth DNA for replication, when the larvae burst, the NPV virus can spread throughout the population, reducing the number of applications required (Hughes, 1970). TM-Biocontrol has no limits for application area, and does not affect humans or other animal species.

There are concerns with both the use for Foray 48B or TM-Biocontrol due to the potential for repeated aerial application. Treatment with either method (Foray 48B or TM-Biocontrol) could be expensive, since they both require being applied via aerial spray. While the reapplication of Foray 48B could be cheaper due to a smaller area (limited to 3,000 acres), this could ultimately be more expensive if the treatment is not effective in managing the tussock moth. It is unclear how the potentially repeated application of Foray 48B or TM-Biocontrol would affect the Mexican spotted owl's environment, or ability to hunt or breed due to potential increased stress. The target time for application of both methods is when tussock moth larvae are hatching and feeding, which overlaps partially with the Mexican Spotted owl's breeding season (March – May) (United States National Park Service, n.d.). There is a risk of repeated applications disturbing a breeding pair to the extent that they do not lay eggs, or successfully raise their chicks that season (Delaney et al, 1999).

## Conflicts

Several conflicts arise when assessing these three options. First, the USFS is internally conflicted. The mission statement of the USFS includes maintaining the health of the forests, as well as the productivity. One department may want to proceed with spray treatments to maintain the health of the forests, while another department may have concerns with the economic impacts of treatment. Second, is the conflict between the USFS and the recreational users of PNF. Without treatment, the degradation of the forest could harm both groups, but treatment could be costly, shut down the area for extended periods of time, and be potentially toxic if the Foray 48B remains in the waterbodies. Third, the USFS and the ASGD have conflicting ideas on the effects of treatment on the threatened Mexican spotted owl. The designation of this area as critical habitat for the species implies special care should be taken to maintain the health and integrity of the forest, but treatment could affect their life cycles. With both conflicts, the USFS must make a decision between allowing the colony to collapse on its own and potentially saving money and time or disturb the visitors and animals within the area to treat the infestation before it gets worse.

The TM-Biocontrol has more benefits associated with its use over the Foray 48B. TM-Biocontrol does not affect any species besides the Douglas fir tussock moth, including the threatened and endangered lepidopteran species, unlike the Foray 48B (Syracuse Environmental Research Associates, Inc. (SERA), 1999). Additionally TM-Biocontrol, does not have an affect on the threatened Mexican Spotted owl, or humans. Additionally, there are no restrictions in the acreage available for application and reduces the closure times from a few days with Foray 48B, to just a few hours after application. This would allow recreational users to return to the area much more quickly, while falling in line with the USFS's mission to maintain the health and integrity of the nation's forests, with very little risk compared to the Foray 48B. With these considerations, it is recommended that the USFS treats the tussock moth infestation with the TM-Biocontrol over the Foray 48B.

# References

Audubon Society of Greater Denver. (2018) Retrieved from http://www.denveraudubon.org/about/

Biery. E. GIS and Vegetation Inventory Specialist Pike/San Isabel National Forest. Personal communication. Dec 1, 2016.

Ciesla, W. M., (n.d.) Defoliation of Douglas-Fir forest in the South Platte River Basin. Forest health management international publication. Fort Collins, CO. USA.

Colorado State Forest Service. (2016). *Treatment Options for Douglas-Fir Tussock Moth* [Brochure]. Fort Collins, CO.

Delaney, D. K., Grubb, T. G., Beier, P., Pater, L. L., & Reiser, M. H. (1999). Effects of helicopter noise on Mexican spotted owls. *The Journal of Wildlife Management*, 60-76.

Ganey, J. L., Block, W. M., Jenness, J. S., & Wilson, R. A. (1999). Mexican spotted owl home range and habitat use in pine-oak forest: implications for forest management. *Forest Science*, *45*(1), 127-135.

Hughes, P.R., Wood. H. A., A synchronous peroral technique for the bioassay of insect viruses. Journal of Invertebrate Pathology (37) S 154-159.

Menon, A. S., & De Mestral, J. (1985). Survival of Bacillus thuringiensis var. kurstaki in waters. *Water, Air, and Soil Pollution*, *25*(3), 265-274.

Petrie, K., Thomas, M., Broadbent, E. (2003) Symptom complaints following aerial spraying with biological insecticide Foray 48B. The New Zealand Medical Journal (116) (1170), 1-7.

Syracuse Environmental Research Associates, Inc. (1999). TM-BIOCONTROL: A preparation of polyhedral inclusion bodies of the Douglas fir tussock moth (*Orgyia pseudotsugata*) Nuclear Polyhedrosis Virus Final Report. USDA/FS Order No. 43-3187-9-0319

United States Department of Agriculture. (2017) Douglas-fir tussock moth *Orgyia pseudotsugata*. Retrieved from http://apps.fs.usda.gov/r6_decaid/views/douglas_fir_tussock_moth.html.

United States Forest Service. (2018). About the agency. Retrieved from https://www.fs.fed.us/about-agency

U.S. Fish and Wildlife. (2018). Listed animals. Retrieved from https://ecos.fws.gov/ecp0/reports/ad-hoc-species-report?kingdom=I&status=SAT&mapstatus=3&fcrithab=on&fstatus=on&fspecrule=on&finvpop=on&fgroup=on&header=Listed Animals

United States National Park Service (n.d.) Mexican spotted owl. Retrieved from https://www.nps.gov/articles/mexican-spotted-owl.htm

Valent BioSciences Corportation. (2008). *Foray 48B usage label* [Brochure]. Retrieved from https://dnr.wi.gov/topic/ForestHealth/documents/Foray48B_US-OrganicLabel.pdf

Williams, H. L., Monge-Monge, K. S., Otvos, I. S., Reardon, R., & Ragenovich, I. (2011). Genotypic variation among Douglas-fir tussock moth nucleopolyhedrovirus (OpNPV) isolates in the western United States. Journal of Invertebrate Pathology, 108(1), 13–21.