Distribution Agreement:

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Marissa Cummings Date

Rodent Plague Surveillance and Species Diversity – Understanding Environmental Conditions Associated with an Epizootic Event, 2014 – 2018 Yosemite National Park, California

By

Marissa Cummings

Master of Public Health

Department of Environmental Health

Global Environmental Health

Lance Waller, M.S., PhD

Thesis Committee Chair

Julie Clennon, MSc., PhD

Committee Member

Karen Levy, MSc., MPH, PhD

Committee Member

Rodent Plague Surveillance and Species Diversity – Understanding Environmental Conditions Associated with an Epizootic Event, 2014 – 2018 Yosemite National Park, California

By

Marissa Cummings
Bachelor of Science
Oregon State University
2016

Thesis Committee Chair: Lance Waller, MSc., PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health in Global Environmental Health

2019

Abstract

Rodent Plague Surveillance and Species Diversity – Understanding Environmental Conditions Associated with an Epizootic Event, 2014 – 2018

Yosemite National Park, California

By Marissa Cummings

Background: Plague has been responsible for three known pandemics, and entered the United States through the Port of San Francisco in 1900 via urban rats. Plague has since spread across the western United States into wild rodent populations. The disease is considered endemic in parts of California due the maintenance in wild rodents. Several rodent species are moderately resistant to the disease, serving as the primary reservoir of plague. However, several species are highly susceptible to infection and mortality, serving as amplifying hosts, causing plague epizootics, or outbreaks. The California Department of Public Health does routine surveillance sampling of rodents for plague across the state.

Objective: This study aims to understand certain environmental conditions and species diversity that may be associated with a known epizootic event that occurred from 2014 through 2018 at Yosemite National Park. During this time, there was heightened plague activity, causing spillover from animals to humans, with two humans infected.

Methods: Maps for spatial distribution of plague results were completed and compared to climate covariates. The Shannon-Weiner Diversity Index was used to calculate rodent species diversity at rodent collection locations. A generalized linear mixed model was used to determine association of epizootic activity between test results and environmental and non-environmental factors.

Results: The California Department of Public Health obtained 604 samples by trapping efforts or contributed mammal carcasses between 2014 and 2018, with 29 samples positive for plague, and a peak of positive samples in 2015. Monthly minimum and maximum temperature, yearly temperature average, yearly dew point temperature, monthly and yearly precipitation and elevation environmental covariates were statistically associated with the epizootic event that occurred. The Shannon-Weiner Diversity Index was statistically associated with the epizootic event.

Discussion: Several environmental conditions were associated with epizootic activity that occurred from 2014 – 2018, though many factors were correlated with elevation. The Shannon-Weiner Diversity Index was associated with disease activity, where low diversity was associated with the epizootic event. However, sampling varied in intensity, with extensive sampling in 2015 occurring due to the human spillover event. More consistent sampling needs to occur within Yosemite National Park.

Keywords: plague, zoonotic, vector-borne, animal, rodent, one health

Rodent Plague Surveillance and Species Diversity – Understanding Environmental Conditions Associated with an Epizootic Event, 2014 – 2018 Yosemite National Park, California

By

Marissa Cummings
Bachelor of Science
Oregon State University
2016

Thesis Committee Chair: Lance Waller, MSc., PhD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health in Global Environmental Health

2019

Acknowledgements:

I extend my sincerest gratitude to Lance Waller for his encouragement, guidance and support from the initial to end stage of this thesis, which pushed me to work with a project than was far different than any work I have done previously.

I would also like to thank Julie Clennon, for helping me find a true interest in disease mapping over the past year, and assistance understanding certain ecological concepts.

I am thankful to Karen Levy for her availability and support during these past two years – and for her class to help me decide I wanted to have my thesis topic be on plague!

Thank you to Ian Buller, who was a great role model for his PhD work on plague in coyotes, and who provided advice and encouragement throughout this project. To the Waller Student Group (Brian Conroy, Behzad Kianian), thank you for our great, constructive weekly meetings that always wanted to push me further in my statistical knowledge.

To the California Department of Public Health, Vector-Borne Disease Section: Greg Hacker, Mark Novak, Mary Beth Danforth, James Tucker, Vicki Kramer: I offer my sincerest regards for the use of data for this project, as well as true and honest engagement of my thesis topic. This project has pushed me in my understanding of disease ecology and zoonotic diseases far beyond what I previously knew.

Thank you to Danielle Buttke at the National Park Service for the support of this project, and desire to see how this project could help with future park implications.

Lastly, I would like to thank my parents, grandparents and my friends for their love and support during my MPH program. This has trying two years and I wouldn't have been able to make it without the encouragement of you all!

Rodent Plague Surveillance and Species Diversity – Understanding Environmental Conditions Associated with an Epizootic Event, 2014 – 2018 Yosemite National Park, California

Marissa Cummings

Table of Contents

I. INTRODUCTION:

In the realm of infectious diseases, zoonotic diseases are becoming an increasingly important topic of concern in the world of public and human health. Zoonotic diseases are diseases that can be passed from animals to humans either by spillover from an animal outbreak, or due to proximity of humans to infected wildlife. Over 60% of infectious diseases and 75% of emerging infectious diseases currently are classified as zoonotic (1).

The most critical public health intervention for zoonotic diseases is quick detection during an epizootic event, also known as a large outbreak of communicable disease among animals. However, epizootics can be difficult to detect, sample, and enumerate; and zoonotic detection often does not become a priority until human cases occur nearby. Sampling and detection of zoonotic pathogens in the United States occurs with the partnership of local and state health departments, as well as federal agencies, including the United States Centers for Disease Control and Prevention (U.S. CDC), the Forest Service (USFS), the Department of Agriculture (USDA), and the National Park Service (NPS). These departments and agencies conduct surveillance of reservoir hosts for zoonotic diseases to determine baseline disease prevalence and whether any samples may indicate outbreaks occurring in animal populations (epizootics).

As humans continue to encroach on wildlife environments, it is imperative to understand the trends in zoonotic disease, with particular attention to diseases with high mortality, such as rabies and plague. With an average of 9 cases per year and average case fatality rate of 30 – 60% (2 - 3), plague is highly concerning disease found

worldwide, with domestic concern focused in the western United States, and has been responsible for three known pandemics. Plague arrived in the continental United States through the Port of San Francisco, California in March 1900. Concerns were high for spread of the disease due to urban risk factors, including poor sanitation and high urban rat populations. Following the San Francisco earthquake in 1906, the disaster-struck city had the ripe conditions for infectious disease spread, and a plague epidemic was recorded, with urban rat and wild squirrel infection, indicating plague never left once it entered the mainland U.S. territory. Between May 1907 and June 1908, there were 159 cases and 77 deaths attributed to plague reported (4). From this time on, *Y. pestis* was capable of surviving and thriving in urban, semi-urban and rural rodent species, and spread eastward from California, ending near the 100th meridian mark and not moving beyond since 1960 (5). Because of plague's introduction into California and expansion into the wild rodent communities, plague is now considered endemic in many foothill and mountainous areas of California (6).

One area of particular interest relating exposure of humans to wildlife is in National Parks within the United States. Each year, more and more humans are visiting National Parks throughout the United States, bringing them closer in proximity to wildlife and potentially at risk for contracting zoonotic disease. As humans increasingly visit wildlife areas and environmental factors such as temperature and precipitation are changing due to climatic shifts, understanding these factors as they pertain to the incidence of zoonotic diseases can help state, local and federal health departments implement prevention and control measures, as well as understand how to prioritize surveillance and sampling efforts. This thesis will focus on Yosemite National Park in

California, with the objective to understand environmental factors that may be associated with known epizootic activity that occurred within the park during 2014 – 2018. Further, this thesis will allow for deeper understanding of rodent host species influence on epizootic activity.

II. BACKGROUND:

Pathogen: Yersinia pestis:

The etiological agent responsible for plague is the bacterium *Yersinia pestis*. This pathogen, *Y. pestis*, is a gram-negative, non-motile, coccobacillus-shaped bacterium in the genus *Yersinia* and the family *Enterobacteriaceae* (4). Plague is a zoonotic and vector-borne infectious disease; zoonotic meaning that the disease can be transmitted between animals and humans, vector-borne meaning that the disease is primarily transmitted by the bite of an infected, blood-feeding arthropod species (i.e., fleas are the vector for plague).

Host-Vector Relationship:

Plague has been found in approximately 200 different species of rodents worldwide, with several rodent species having mild symptomatic plague infection and high survivability from the disease, therefore serving as the primary reservoir host (4). In addition to rodents, several other mammals can serve as incidental hosts of plague, including coyotes and companion animals (i.e., cats and dogs) (5). Generally, away from urban settings, the transmission of *Y. pestis* from fleas to rodents occurs in low numbers in wildlife, with occasional outbreaks, also known as the sylvatic plague cycle. However, aside from several rodent species that suffer only mild plague infection, there are several rodent species more seriously impacted by the disease; resulting often in higher mortality from plague and high transmission in rodent communities, and often spreading to other mammals including humans during epizootic events.

An epizootic event with plague dynamics is classified as increased plague activity due to a variety of factors. Factors identifying an epizootic event include increased rodent and flea populations compared to what is observed in a baseline environment, with

an abundance of amplifying, or susceptible, hosts increasing the scenario of an epizootic event. Additionally, the trophic cascade hypothesis has been identified as playing a role in the epizootic cycle. The trophic cascade hypothesis is a concept where increased precipitation increases the vegetation, thus increasing rodent food supply; therefore rodent host populations and greater risk of plague transmission and risk of epizootics (6).

When there are higher rodent populations from factors such as increased vegetation, breeding, etc., flea populations may subsequently rise, as fleas have an increased number of hosts to feed on. Further, certain rodent species that are predominant plague hosts can be infested with multiple flea vectors, or live in habitats (i.e., burrows) that harbor massive flea populations, thus increasing plague transmission among rodents and fleas (6). This dynamic between rodent populations and flea populations can be particularly problematic as it can increase plague transmission in the rodent species particularly susceptible to plague. In turn, increased transmission causes elevated plague incidence in rodents and increased rodent die-off, leaving fleas without their primary blood host, a key indicator for suspected epizootic events (7). As infected fleas need a host and continuous blood meals to survive, they may jump onto other mammalian species, thus infecting and introducing plague into additional host species (8). Humans are more likely at higher risk of flea bites and potential plague infection during and immediately following an epizootic event, since primary route of transmission for human plague is from the bite of a plague infected flea, which was infected by obtaining a blood meal from an infected host (2).

Transmission of plague within rodent populations occurs when one of approximately 31 globally-known flea vectors bites an infected host (primarily, a rodent)

(8). While dozens of known species are capable of plague transmission, factors such as flea population abundance, rodent population abundance, infective potential, infection potential, and transmission potential all serve as ways to increase transmission of plague from flea vectors (9-10). Primary transmission occurs when a flea bites an infected host and ingests *Y. pestis* bacterium, the bacteria activates a plasminogen, called "Pla", within the flea, growing *Y. pestis* bacterium in and blocking the proventriculus; a muscular expansion above the stomach necessary for blood feeding (11). Typically, the blockage of the proventriculus slowly leads the flea to starve and become insatiable, as they are unable to effectively ingest any blood they attempt to consume. As *Y. pestis* continues to reproduce and build in the flea's proventriculus, the flea then begins to transmit *Y. pestis* and infect other rodents and susceptible mammals when they bite a host. This occurs as they regurgitate contaminated blood into the host at the site of the bite wound. This pattern defines the standard core activity of the plague cycle: where fleas regurgitate contaminated blood into new hosts and subsequently expose and likely infect these hosts with plague, before the flea eventually starves to death (11).

A secondary component of transmission from fleas discussed in recent literature and research is the role of fleas and their transmission of plague prior to a blocked proventriculus, also known as "early-phase transmission". Eisen et al., 2006 and Eisen et al., 2007 found with multiple flea vector species that early-phase transmission of plague among fleas without a blocked proventriculus had similar efficiency to blocked proventriculus plague transmission among the same flea-vector species. Furthermore, Eisen et al was able to determine that the transmission efficiency was constant until fleas experienced a blocked proventriculus; indicating longer infectivity than previously

known (12-13). This research provides further insight and understanding into the complexity of plague dynamics, in that flea vectors play a larger and more constant role than previously thought.

Human Clinical Illness:

The incubation period for human plague infection is approximately 3 – 7 days, and as short as 1 – 3 for an individual exposed to *Y. pestis* by inhalation of infectious droplets (15). There are three different clinical representations of plague; bubonic, septicemic and pneumonic, with all representations experiencing a general sudden onset fever, chills, weakness and headache. Bubonic plague is the most common form and accounts for approximately 80% of all cases, with a hallmark "bubo", or swollen, painful and tender lymph node present after the bite of an infected flea (15). Septicemic plague can occur either as a first representation of plague or develop as a result of untreated bubonic plague. Clinical signs of septicemic plague are often seen as gangrene of fingers, toes and the nose. Pneumonic plague is the most serious and fatal form of plague, and the only form with documented human-to-human transmission (16). Pneumonic plague can develop from inhaling infectious *Y. pestis* droplets or can develop from untreated bubonic or septicemic plague. The case fatality rate of plague ranges from 30 – 100%, with pneumonic plague nearly always fatal.

Current treatment of human plague infection involves an intravenous antibiotic mix of streptomycin, tetracycline, and/or sulfonamides for 10 – 14 days. If unavailable, gentamicin and fluoroquinolones may alternatively be used for 10 – 14 days, or for two days after fever subsides, and there is currently no known antibiotic resistance present in

North America (17). Mortality rate for plague with antibiotic treatment is approximately 16% (2, 15).

Environmental Stability and Persistence:

Outside of the host-vector relationship, *Y. pestis* has also been found to persist in soil. In research conducted in 2008 by Eisen et al., *Y. pestis* was assessed for its persistence under natural soil conditions, with soil being collected as part of an ongoing investigation into a fatal human plague case that had occurred at Grand Canyon National Park in 2007. Soil was obtained from a site where a mountain lion died, with blood saturating the soil underneath the carcass, with blood testing confirming the mountain lion died from plague. Approximately 3 weeks after the mountain lion's death, soil was sampled and injected into four mice; one of which died within 12 hours, and a second dying within seven days (18). These results indicate that after three weeks, *Y. pestis* was still highly virulent and capable of fatality under natural soil conditions. This finding, in addition to other research projects, such as Savage et al., (19) indicates that the persistence of *Y. pestis* in soil could be an additional mode of transmission for rodent and flea infection, as both have the capability of ingesting or inhaling soil particles contaminated with *Y. pestis* from rodents or other animals who have succumbed from the disease. However, the work by Eisen et al. (18) was the first study to examine *Y. pestis* persistence in soil under natural soil conditions in North America, and further research should be conducted to determine soil as route of exposure for rodents and fleas, as well as the true longevity of *Y. pestis* bacterium in soil.

Environmental Suitability of Yersinia pestis:

There are several roles that the environment and climate may play in the impact and role of plague dynamics. Environmental and climate factors can include suitable soil conditions, precipitation, humidity, food supply, temperature, elevation gradients (20). These factors may play a key role in the occurrence of epizootic events and possible human plague cases and therefore addressing each factor and their subsequent impact on plague occurrence are of public health interest.

Temperature, precipitation and humidity can work with each other to influence plague dynamics, as both can influence rodent food supply, rodent and flea population dynamics, and, hence, epizootic activity. Increased precipitation and milder average temperatures may allow for increased rodent food supply and vegetation coverage. This increased local vegetation and food supply increases rodent populations, flea populations and subsequently increases available hosts for fleas to feed on and increases the plague transmission cycle and may increase plague activity (20). Elevation can play a role as climate change and increased temperatures may cause a change in more suitable habitat than previously recorded for plague (21).

Rodent Host Species Diversity:

Understanding the species diversity of rodent communities in a given rodent collection location, trapping protocols, and during appropriate sampling collection times, factors, etc., may provide a crucial link in understanding epizootic activity, as these factors can allow us to understand which species are present and absent from given trapping and sampling locations and hypothesize why given species may be absent. In the context of plague dynamics, if a resident rodent species is suddenly absent or the

population is drastically reduced in a given location during intensive surveillance sampling, this may suggest local rodent die-off from plague and provide a marker event to begin sampling to understand whether local plague transmission is occurring in higher numbers and if a local epizootic event may be occurring. However, intensive surveillance sampling such as this does not frequently occur, and typically does if spillover events occur where a human has contracted the disease in question.

When considering rodent species, there may be one or multiple species which maintain baseline levels of disease, in naturally occurring numbers (the enzootic cycle) in a given location. Additionally, there are species which amplify the disease, and are highly susceptible to mortality from plague, and can potentially trigger an epizootic event (4, 23). Enzootic rodent species involve several species that are moderately to highly resistant to plague, including Californian species such as: *Tamiasciurus douglasii* (pine squirrel, douglas squirrel, chickaree squirrel), *Tamias senex* (shadow chipmunk, Allen's chipmunk), and *Tamias quadrimaculatus* (long-eared chipmunk) (22, 25). Epizootic rodent species known to be moderately to highly susceptible to plague and therefore potential species to trigger epizootic events in California include: *Tamias amoenus* (yellow-pine chipmunk), *Callospermophilus lateralis* (golden-mantled ground squirrel), *Tamias speciosus* (lodgepole chipmunk), *Marmota flaviventris* (yellow-bellied marmot), *Neotoma* spp. (pack rat), *Otospermophilus beecheyi* (california ground squirrel) and *Peromyscus maniculatus* (deer mouse). (22). Identifying which rodent species are residents in a given location and study site, as well as which are known to be enzootic and epizootic species in the area may allow for further investigation into rodent surveillance. Further, understanding species diversity at trapping and carcass collection

locations may allow us to identify epizootic intensity within Yosemite National Park. For instance, homogeneity of species diversity may mean we have all enzootic species present, possibly maintaining plague in low numbers. There may also be heterogeneity of rodent species both within a population and between species, therefore allowing for more (or less) diversity – therefore possibly altering plague dynamics.

Study Site:

Due to previous epizootic events in wild rodent communities, California's classification as being plague endemic, and previous plague cases in humans in California, Yosemite National Park represents the focus of this research. Attention was drawn to this area in particular due to two cases of humans contracting plague after visiting the park in 2015; the first cases in California since 2006 (23). These two cases were different clinical representations (bubonic and septicemic) and neither resulted in fatality. Different travel itineraries and pathogenic strain testing indicated at least two different *Y. pestis* strains circulating throughout the park. This spillover event from rodents to humans was the motivating cause for a deeper exploratory analysis into understanding what factors may have been associated with the epizootic spillover of plague and how these factors and epizootic activity may increase human risk of contracting the disease, or other zoonotic diseases, at Yosemite National Park.

Yosemite National Park is located in Mariposa and Tuolumne counties in California, U.S.A. The size of the park is approximately 3,028 km^2, with 2,852 km^2 of designated wilderness and ranges in elevation from 914 to 3,962 meters (28). The climate varies throughout the park, with temperatures reaching 100˚F in Yosemite Valley, and falling to 20˚F or lower across the park. There is an average of 37.2 inches of

precipitation, and, of these, 30 inches are snowfall, with greater snowfall in higher elevation areas of the park. There are over 90 species of mammals within Yosemite National Park, with the highest proportion of mammalian species being rodents. Enzootic and epizootic species mentioned above and present in Yosemite National Park include: *T. senex, T. quadrimaculatus, M. flaviventris, Neotoma* spp., *O. beecheyi*, *P. maniculatus, T. amoenus, C. lateralis,* and *T. douglasii.* (22, 29). There are three major camping areas with 13 total campgrounds at Yosemite National Park; Yosemite Valley (Upper, Lower and North Pines), South of Yosemite Valley (Wawona, Bridalveil Creek) and North of Yosemite Valley (Hodgdon Meadow, Crane Flat, Tamarack Flat, Yosemite Creek, White Wolf, Tuolumne Meadows and Porcupine Flat). Additionally, there are backcountry campgrounds in the High Sierra Camps and Little Yosemite Valley (30).

Annual visitors to Yosemite National Park have drastically increased in recent years, with 4,336,890 in 2017, including 2,064,032 overnight stays (29). As the annual visitor and park usage at Yosemite National Park increases each year and there is opportunity for more interaction with wildlife, it is important to examine zoonotic disease prevalence, especially with a potentially highly fatal disease such as plague.

Primary Objective:

The objective of this study is to examine and understand how certain environmental covariates such as meteorological variables, elevation and normalized difference vegetation index (NDVI) are associated with epizootic activity and intensity, as well as rodent diversity indices at rodent collection sites, during 2014 – 2018 in Yosemite National Park. These findings may provide insight into which types of environmental conditions may be associated with epizootic events that cause excessive

rodent die-off. Examining and analyzing data from 2014 – 2018 specifically focuses on known epizootic activity that occurred, which led to a spillover event where two human plague cases occurred within Yosemite National Park. We will be focusing and providing a detailed, descriptive and geographic analysis and summary of activity during this epizootic period.

Hypothesis:

I believe that rodent species diversity plays a pivotal role in intensity of epizootics, and that low species diversity among different collection locations suggests epizootic activity is present, and strong heterogeneity of rodent species across different collection locations is associated with increased epizootic activity. I predict that we will see a relationship between the interaction of climate and elevation covariates influencing rodent species diversity within Yosemite National Park, as a shift in increased temperatures will influence a shift in species diversity at higher elevations. I believe that low rodent species diversity will be associated with increased epizootic activity, as lower diversity may signify plague activity occurring.

Analysis Plan (Specific Aims:)

The first aim of this project is to examine rodent species abundance and diversity, as well as plague serology via rodent surveillance conducted from 2014 – 2018 at rodent collection sites within Yosemite National Park. Particularly focusing on rodent diversity at collection locations within Yosemite National Park can aid understanding the intensity of epizootic activity during the 2014 – 2018 study period, seeing how diversity plays into the dynamics of an epizootic event.

The second aim is to map plague serology within Yosemite National Park to describe the geographical distribution of plague samples, and how the distribution differs in terms of species composition, elevation, vegetation and areas that may have more positive or negative samples.

The third aim of the project is to model associations between plague prevalence from specimen types (i.e., Nobuto, mammal carcass and flea pool) and the covariates of interest (climate, elevation, NDVI and non-environmental factors), and understand which conditions may be associated with known epizootic activity that occurred during the study period (i.e., increased plague prevalence and known spillover to humans) at specific collection locations. Here we define epizootic activity as elevated prevalence of plague during the epizootic event in locations where trapping effort occurred and/or were mammal carcasses were collected. We assess associations via generalized linear mixed model regression in SpaceStat to take into account random effects of repeat location sampling. The significance level for covariates in the model will be set at an alpha level of α=0.10 to provide a broad assessment of potentially relevant associations.

III. METHODS:

Dataset:

The rodent dataset for this project comes from the California Department of Public Health, Vector-Borne Disease Section (CDPH-VBDS), with the U.S. National Park Service as a partnering agency. Rodent data were collected from September 9, 2014 through August 23, 2018 from various rodent collection locations within Yosemite National Park and include the location, latitude, longitude, number of traps laid out, number of captures, rodent species type, number of fleas collected on each rodent, and elevation. Rodent were trapped with Sherman (H.B. Sherman Traps, Tallahassee, FL, U.S.A.) and Tomahawk (Tomahawk Live Trap, Hazelhurst, WI, U.S.A.) live traps, where traps had a grain-mix bait (corn, rolled oats, and barley). Rodent traps were placed early morning through midday, or overnight through midday and then collected to observe the number and types of rodent species captured (23). In addition to trapping, any reported rodent carcass was included in the data, for analysis of potential death due to plague.

Rodent blood sampling was also conducted by CDPH-VDBS, and the NPS to determine presence of *Y. pestis*. Specimen testing type was recorded, and included carcass testing (i.e., reported animal carcass being collected and sampled), flea pool (collecting fleas off rodent and sampling), and Nobuto (paper strips that specifically test for plague-specific antibodies), as well as the final test result (positive or negative). For the purposes of this analysis and the small sample size, any antibody tests with serology equal to or great than 1:16 were considered a positive result. Any samples not tested or animal carcasses that were unable to be sampled were excluded from the analysis, as well as repeat carcass samples).

Because the data does not include sensitive identifiers, human data or clinical investigation, Emory IRB review and approval was not required to be obtained.

Covariate data were compiled from multiple resources for the purpose of this project. Normalized Difference Vegetation Index (NDVI) data for Yosemite National Park were obtained from Climate Engine via Google Earth Engine APIS (https://app.climateengine.org). Meteorology data were obtained from Oregon State University's PRISM Climate Group (http://prism.oregonstate.edu/). PRISM (Parameter-elevation Regressions on Independent Slopes Model) depends on weighted regression framework which relies on digital elevation models. PRISM's framework and dataset is a series of time series interpolations with data from weather stations in given areas to provide estimated measures for specific areas of interest (31). PRISM data were collected from 2014 – 2018 at a 4-kilometer resolution within or as close to possible each collection location for monthly minimum temperature (averaged over all days in the month), monthly maximum temperature (averaged over all days in the month), monthly average temperature ((maximum temperature + minimum temperature) ÷ 2), monthly total precipitation (rain + snow + melted snow), and monthly average dew point temperature (averaged over all days in the month) (31). Additionally, data were obtained for annual average minimum temperature, annual average maximum temperature, annual average temperature, average total precipitation and annual average dew point temperature.

Variable Description:

Environmental covariates included monthly average precipitation (inches) during sampling months, monthly minimum temperature during sampling months, monthly

temperature average during sampling months, minimum and maximum temperature during sampling months, and monthly average dew point temperature during sampling month as an indicating for humidity. In addition to monthly weather data, annual weather covariate data was also included for the same factors. Additional landscape environmental factors included elevation, which was generally correlated with climate covariates, and normalized difference vegetation index (NDVI). Covariates that were not environmental but also included in the model analysis were: number of fleas on rodent, rodent host type and location as the random effect. A full and detailed description of all variables can be found in Table 1.

Shannon-Weiner Diversity Index:

In order to analyze rodent species abundance and diversity for possible correlation with epizootic event triggers, the Shannon-Weiner Diversity Index was used. Rather than using Simpson's Index to measure species richness, or the Sørenson-Dice Index to calculate similarity between samples, the Shannon-Weiner Diversity Index was used to be able to understand abundance and diversity within each given collection location. The Shannon-Weiner Diversity Index is an ecological tool used to be able to examine species abundance and diversity, given the number of species found per location and the total sum of all species by:

$$H = -\sum_{j=1}^{S} p_i \ln p_i$$

where p_i is the proportion of species, multiplied by the natural log of p_i. In areas rich in species composition, diversity and distribution, the Shannon-Weiner Index aims to correctly predict the certainty of diversity from one community to the next.

Generalized Linear Mixed Model (GLMM):

A generalized linear mixed model (GLMM) will be used for the analysis of multiple covariates against positive or negative plague tests, and to take into account random effects of repeat location sampling that occurred over the time period of data collection. This GLMM analysis will be conducted via SpaceStat software (BioMedware, Ann Arbor, MI, U.S.A.). This GLMM used a binomial, logistic, minimal-estimate model as our response variable was a binary outcome of a positive or negative test. The GLMM equation is given by:

$$\boldsymbol{y}|\boldsymbol{u} \sim \mathrm{N}(\boldsymbol{X}\boldsymbol{\beta} + \boldsymbol{Z}\boldsymbol{u}, \boldsymbol{R})$$

where $u \sim N(0, G)$, X, and Z are classified as known (i.e., host species and number of fleas on rodents), and R and G can change depending on known or changing variances (i.e., environmental components, NDVI). Analysis of rodent host type will be conducted via chi-square between epizootic and enzootic species and their test result.

Because of the small sample size, repeat sampling, and sparse, sometimes missing data due to locations not being sampled, or locations being extensively sampled more than others, a significance level of α=0.10 was to provide a broad collection of potentially associated factors for closer future follow up.

Spatial Distribution of Rodent Collection Locations:

To understand distribution of rodent species and plague samples among Yosemite National Park, maps were created using ArcGIS software (ESRI, Redlands, CA, U.S.A.) to visually represent positive and negative rodent samples in their respective environmental conditions (elevation, NDVI). Additionally, each rodent's respective taxa were also mapped to represent species distribution found in rodent collection locations among Yosemite National Park from 2014-2018.

V. RESULTS:

The data include 604 rodent samples representing 15 various rodent species and 8 rodent collection locations sampled between 2014 and 2018. There were 0 cases identified in 2014, 24 cases in 2015, 6 cases in 2016, 1 case in 2017, and 0 cases identified in 2018 (Figure 1).

Rodent Host Species:

P. maniculatus were the most commonly collected and trapped rodent species, comprising 257 of the 604 rodent samples (42.5%). *O. beecheyi* were the second most common, at 133 samples (22.0%), followed by *T. speciosus* at 98 samples (16.2%), and *C. lateralis* at 61 samples (10.1%). The rodents least frequently found in traps or as carcasses were: *T. senex* and *Spermophilus spp.* (indistinguishable *O. beecheyi* or *C. lateralis*), captured twice per host (0.3%). *S. griseus, M. flaviventris, Scapanus spp.,* and *M. longicaudus* were all collected as mammal carcasses, found once per species (0.2%) (Table 2). When testing for plague was conducted, the most common form of laboratory testing was the Nobuto method, or *Y. pestis* antibody test, which comprised 63.5% of all rodent samples, followed by 35.0% for flea pools (fleas collected off the rodent), and 6.3% for rodent carcasses (a rodent found deceased within Yosemite National Park and submitted for PCR testing for detection of *Y. pestis*). (Table 2). Samples that were not tested or samples were unable to be obtained were excluded in the analysis, as well as confirmed 2nd carcass tests that were duplicates. The specimen types with the highest positive *Y. pestis* identification with seropositive rodents were the mammal carcass at 44.8%, followed by Nobuto samples at 41.4%. The specimen type with the highest

prevalence of current or recent *Y. pestis* infection was the mammal carcass (34.2%), followed by flea pools (19.9%), and the Nobuto method (3.3%) (Table 2).

Positive, negative and total seroprevalence of samples:

Of the rodent samples, 29 (4.80%) were positive for current or recent *Y. pestis* infection, and 575 (95.20%) were negative for *Y. pestis*. Positive *Y. pestis* samples were found at an average elevation of 2,513 meters, while negative *Y. pestis* samples were sampled at an average of 2,130 meters. The species with the highest prevalence of positive current or recent *Y. pestis* infection based on all serology (Nobuto) and mammal carcass testing were *Tamias speciosus* (lodgepole chipmunk), which had 10 *Y. pestis* currently or recently infected rodents out of 98 total sampled, comprising 35.7% of the total *Y. pestis*-positive rodents. *T. speciosus* was followed by *C. lateralis* (golden-manteled ground squirrel) with 7 samples positive for *Y. pestis*, all of which were mammal carcasses, which accounted for 24.1% of the 29 active or recent infections. The remaining species with prevalence of infection from highest to lowest was: *O. beecheyi* (california ground squirrel), *Tamias spp* (chipmunk), *P. maniculatus* (deer mouse), *T. douglasii* (pine squirrel, chickaree squirrel, douglas squirrel), and *T. senex* (shadow chipmunk, Allen's chipmunk) (Table 2). Of the total prevalence, *Tamias* spp. had the highest prevalence, with 57.1% positive, though this is a small numbers issue as only 7 rodents were sampled. For a more accurate measurement, we can look at samples such as *T. speciosus*, with 98 samples and a plague prevalence of 10.2%, or *C. lateralis* with 61 samples and a plague prevalence of 11.5%.

Rodent Collection Locations:

The locational with the most trapping effort was Tuolumne Meadows, with a combinations of traps and/or mammal carcasses totaling 287 samples (47.5%), followed by Yosemite Valley, with traps and/or mammal carcasses totaling 113 samples (18.7%), and Glacier Point with trapping efforts totaling 92 samples (15.2%). Crane Flat Campground had trapping efforts totaling 56 samples (9.3%), Hodgdon Meadow had trapping efforts and/or mammal carcass collection totaling 30 samples (5.0%), and Tamarack Flat Campground had trapping efforts total 17 samples (2.8%). Wawona and White Wolf Campgrounds had the least samples either by trapping efforts or mammal carcass collection, at 4 (0.7%) and 5 (0.8%) times, respectively (Table 2).

Of positive samples, Tuolumne Meadows had the highest prevalence, with 21 samples (75.9%) seropositive, followed by Glacier Point having 4 seropositive samples (13.8%), Crane Flat with 2 seropositive samples (6.9%), and Tamarack Flat Campground with 1 seropositive sample (3.5%). Wawona, White Wolf and Yosemite Valley all had zero seropositive samples between 2014 and 2018 (Table 2). Of total prevalence, Tuolumne Meadows was also the highest prevalence, with 7.7%, followed by Tamarack Flat (5.9%), Glacier Point (4.3%), and Crane Flat (3.6%).

Rodent Host Species and Elevation Interaction:

The diversity of species caught in traps varied across elevation gradients throughout the park. Yosemite National Park ranges from 1,199 to 2,659 meters in elevation, with rodents found in traps among the entire elevation range. *P. boylii*, *Spermophilus spp.*, *S. griseus*, and *Scapanus spp*, were generally found in elevations below 1,600 meters. *O. beecheyi* and *P. maniculatus* were found at an average of 1830 -

2,070 meters, and *U. beldingi, Tamias spp., T. speciosus, T. senex, C. lateralis, T. douglasii, M. flaviventris, M. longicaudus,* and *N. cinerea* were found at the upper elevation range of 2,600 meters.

Rodent Host Species and Number of Fleas:

Analysis was conducted on the average number of fleas found on each rodent species. *O. beecheyi* had the highest average number of fleas, with an average of 14 fleas per rodent. The remainder of the rodent species had low flea counts, such as: *T. douglasii* with an average of 4 fleas per host, *T. senex* with an average of 3 fleas per host, *N. cinerea* with an average of 2 fleas per host, and *T. speciosus* with an average 2 fleas per host. Several rodent species (*P. maniculatus, C. lateralis, and M. longicaudus*) had an average of 1 flea collected per host (Table 3).

Shannon-Weiner Diversity Index:

To examine the diversity of rodent species among Yosemite National Park, we used the Shannon-Weiner Diversity Index to calculate diversity index scores based on the quantity (abundance) of rodent species present within each rodent collection location. Our results show an overall Shannon-Weiner Index in Yosemite National Park of 1.73. Tuolumne Meadows had the highest diversity, with a Shannon-Weiner Diversity Index (H) of 1.46, followed by Tamarack Flat with an H of 1.15, and Crane Flat with an H of 1.06. Wawona had the lowest diversity index, with an H of 0.56, followed by White Wolf (0.67), and Hodgdon Meadow (0.77). A full table can be found in Table 4.

Spatial Distribution of Plague Serology and Rodent Species:

When spatially examining the negative rodent samples based on elevation, we can see that the distribution is predominately near the western side of the park, which is in the

lower elevation of sampling and collection efforts, with spread of negative samples in the center of the park and to the eastern half of the park in increasing elevations (Figure 2). Positive rodent samples were found to be concentrated primarily on the eastern half of the park, in a higher elevation category for the park (~2,600 meters), though there were several samples found ~1,000 meters lower (~1,800) in the western half of the park (Figure 3). Mapping of NDVI data showed distribution of negative samples among the lower half of Yosemite National Park (Figure 4), whereas positive samples were primarily located along the middle section of Yosemite National Park (Figure 5). These distributions with NDVI look to be across areas with lush to moderate vegetation.

Next, we determined the distribution rodent species at rodent collection locations (i.e., campgrounds where trapping or carcass collection occurred) across the park. Chipmunk species (*Tamias spp.*, *T. speciosus* and *T. senex*) were generally concentrated to the eastern half of the park, with several samples in the central and western areas of the park (Figure 6). Mouse species (*P. boylii, P. maniculatus, M. longicaudus*) were distributed evenly across the middle section of the park (Figure 7). Squirrel species (*U. beldingii, O. beecheyii, C. lateralis, S. griseus, Spermophilus spp.*, and *T. douglasii*) were found to be concentrated in the central and eastern parts of the park, with a few samples found in the western and southern areas of the park (Figure 8). Lastly, the remaining rodent species that did not fall into chipmunk, mouse or squirrel species (*M. flaviventris, Scapanus spp., N. cinerea*) were predominately in the eastern section of the park, with one point found in the central region of the park (Figure 9).

Generalized Linear Mixed Model (GLMM) Analysis:

A generalized linear mixed model (GLMM) was used for the analysis of multiple covariates against positive or negative plague tests, and to take into account random effects of repeat location sampling that occurred over the time period of data collection.

When examining environmental factors, we can see that at an α=0.10 level, several factors are statistically significant for positive *Y. pestis* test results in rodents, with p<0.10. Monthly temperature minimum and maximum, yearly temperature minimum, yearly temperature average, yearly average dew point temperature, yearly and monthly precipitation, and elevation were all found to be statistically significant, at values p<0.10. Precise interpretation of associations is challenging due to correlations between various landscape and climate features (e.g., associations between elevation and temperature, and elevation and precipitation) and will require continued study. Examining additional for significance that were not environmental factors found that the Shannon-Weiner Diversity Index was statistically significantly associated epizootic activity (i.e., increased plague activity at rodent collection sites). Chi square analysis of epizootic and enzootic species and their test results resulted in a p-value of 0.1696 and therefore did not provide significant findings between test results and maintenance or amplification species. GLMM results can be found in Table 5.

V. DISCUSSION:

Public Health Implications:

As modern trade and commerce continues to expand, more and more people are traveling across the world. As globalization continues to increase, many countries, including the United States, experience globalization in the form of tourism. Tourism in the national parks and other wilderness areas in the United States has become increasingly popular, leading to more contact with nature, the environment and wildlife.

A newly emerging concept, known as One Health, addresses the interconnectedness of human health, animal health and the environment (32). This concept has become crucial in the realm of public health, especially as humans continue to encroach into wildlife areas at an increased rate, whether by urbanization or by tourism. The present analysis and thesis aim to address plague, a zoonotic disease with high mortality from a One Health perspective, by understanding animal and environmental factors into potential triggers of plague epizootics to better protect human health in Yosemite National Park. Public health implications of this analysis show importance of address disease surveillance and sampling efforts among animal rodents within National Parks and other areas of high tourism, to ensure the safety and protection of visitors and prevention spillover of zoonotic disease.

We found that environmental and climatic factors such monthly and yearly temperature minimum, monthly and yearly temperature averages, yearly average dew point temperature, yearly and monthly precipitation as well as elevation were all found to be statistically significantly associated with the epizootic event that occurred between 2014 and 2018, and were associated with increased positive plague results. It is important

to note that monthly precipitation was analyzed in relation to precipitation during sampling or carcass collection, primarily during late spring and early summer months; but, monthly precipitation can be highly sporadic during these periods in California, as the climate is primarily dry during this time and receives most of its precipitation during October through April, aside from occasional summer monsoons (33). More precise local climate data (precipitation and temperature) would provide more focused insight into this relationship.

Regarding temperature and elevation, our results suggest that as trapping efforts or carcass collection elevation increases, the temperature decreases, and positive plague results were more likely to be found during the epizootic event. When we see increased annual average dew point temperature over the study period, this indicates increased humidity in the rodent collection locations, which was also associated with positive plague results over the epizootic event. Lastly, we find that monthly and yearly precipitation resulted in an association with plague positive results at rodent collection locations and therefore with epizootic activity. These results can involve both rainfall and snowfall, and higher elevations would have received more snowfall throughout the years.

With unstable climate shifts resulting in more extreme weather events such as colder and wetter seasons, or hotter and drier seasons, these results may signify a shift in epizootic activity occurring in higher elevations and during periods of increased temperature instability, increased precipitation (snowfall and/or rain) and maximum temperatures. These shifts in increased temperatures have been known to cause species to shift into higher elevation (17).

When examining rodent species diversity and its association with the epizootic activity that occurred between 2014 and 2018, we found statistically significant association between the Shannon-Weiner Diversity Index and positive plague results. These findings indicate that low species diversity was associated with the epizootic event that occurred, and therefore an indicator of its presence. This diversity shows relatively low heterogeneity of rodent species among rodent collection locations within the park.

Limitations:

While these data were able to provide us with ample information surrounding 2014 through 2018, the overall infection rate for *Y. pestis* is low, making it a difficult and elusive disease to study in most small areas, even in a location that is considered endemic for plague, such as Yosemite National Park. Our small sample size of positive rodents made our analysis difficult, as there were only 29 positive samples of *Y. pestis* over a 4-year period; which was a known epizootic event, meaning the numbers were higher than what would be observed at baseline. Additionally, rodent surveillance sampling is an expensive and labor-intensive method conducted by the California Department of Public Health, with surveillance sampling and the ability of widespread sampling contingent on funding, personnel staffing, and timing availability to conduct trapping efforts. Factors for surveillance sampling can limit where trapping efforts will occur, which often end up being in areas with historically positive samples, or campgrounds most frequently used in summer months, as these are locations with high potential for human-wildlife interactions. Because of this, spatial distribution maps and our statistical analysis showed that this sampling technique may not be able fully cover the multiple other campgrounds

and high-traffic locations in the park that may also be at high risk for plague transmission during the summer.

In our analyses, rodent species and trapping location samples or carcass collections varied from location to location, species to species and year to year. Location of samples generally differed year to year due to where best to prioritize sampling based on areas of highest concern and previous epizootic activity. However, variations in sampling can impact data from year to year and by location. Lastly, this dataset had its largest sample period occur in 2015, in response the two human cases of plague in Yosemite National Park. The increased data collection provides additional insight insight into real-time prevalence of *Y. pestis* during the human case occurrence, but it can be challenging to compare results to those of previous years.

Future Directions:

These results show clear importance for prioritizing plague as a risk consideration among visitors of Yosemite National Park. Environmental factors and certain rodent host species type were found to be significant for positive plague outcomes. Species diversity differed by location in our data, with general low species diversity among each location; which may be a result of ongoing epizootic events (34). Statistically significantly associated low species diversity indicated the epizootic activity that occurred over the 2014-2018 time period, with evidence given by the spillover of the two human cases in 2015.

By understanding location specific information, species diversity, environmental factors this information allows state public health officials to design and prioritize trapping procedures for upcoming trapping seasons each year within Yosemite National

Park. This could entail more trapping during wetter and hotter months, as well as trapping into different areas within the park at varying elevations, with more traps.

The U.S. Centers for Disease Control and Prevention One Health office conducts a zoonotic disease prioritization workshop (OHZDP), in which they partner with countries to assist in prioritizing zoonotic diseases to help develop prevention and control measures, with goals for disease eradication. Representatives of federal agencies in the United States recently underwent the OHZDP workshop and determined that plague was the fourth highest disease in the United States to prioritize (35). These findings, in combination with the United States and Centers for Disease Control prioritization for plague in the western United States show a clear indication for key efforts in education, prevention and control measures in reservoir hosts, with a major prevention and control effort being surveillance sampling in rodents.

A future analysis on environmental factors that would be important to conduct for Yosemite National Park is a risk and prediction map of plague within the park based on habitat suitability, to allow for understanding of higher risk areas of positive plague results based on known positive rodent collection locations previously recorded.

Additionally, an examination of wildfire's impact on plague and rodent host species should be conducted to understand possible correlation between habitat and land disturbance and following disease events. This topic comes at a particularly important time, as the Ferguson Fire recently burned more than 96,000 acres within and surrounding Yosemite National Park in summer 2018. Little research has been conducted on zoonotic diseases and their incidence immediately following and over time after destructive wildfires. Through extensive literature review, one study conducted by

MacDonald *et al* in 2018 found tick exposure initially increasing following wildfires, then declining dramatically over the following two-year study period. However, studying species composition, flea abundance, NDVI and other environmental factors following wildfires within Yosemite National Park boundaries could make for interesting analyses to understand disease correlation with plague (36). It is a possible hypothesis that plague prevalence could increase temporarily following a wildfire event due to potential low species diversity given possible high mortality or mass evacuation of rodent species, leaving few rodent hosts and potentially high flea populations behind. Understanding an environmental factor such as this and its role on zoonoses may help state health officials prepare for an upcoming tourist season following a fire near or within National Parks.

These results and discussion hope to provide insight into planning future federal and state policy surrounding budget and funding for crucial public health measures such as routine surveillance sampling, including sampling currently conducted within Yosemite National Park. Providing an increased budget to scale up sampling efforts will allow for increased surveillance for understanding baseline prevalence of a variety of zoonotic diseases, including plague. This surveillance is critical to public health as humans continue to come ever closer in contact with animals and the wild environment and ensuring a constant awareness of disease prevalence in the wild animal population will allow for the safety and health of tourists within Yosemite National Park.

VI. REFERENCES

1. Taylor, L. H., Latham, S. M., & Woolhouse, M. E. (2001). Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *356*(1411), 983-9

2. Kugeler, K. J., Staples, J. E., Hinckley, A. F., Gage, K. L., & Mead, P. S. (2015). Epidemiology of human plague in the United States, 1900-2012. *Emerging infectious diseases*, *21*(1), 16–22. doi:10.3201/eid2101.140564

3. World Health Organization. (2017, October 31). Plague. Retrieved from https://www.who.int/news-room/fact-sheets/detail/plague

4. Anderson E. T. (1978). Plague in the continental United States, 1900-76. *Public health reports (Washington, D.C. : 1974)*, *93*(3), 297–301.

5. Adjemian, J. Z., et al. (2007). "Initiation and spread of traveling waves of plague, Yersinia pestis, in the western United States." Am J Trop Med Hyg 76(2): 365-375

6. California Department of Public Health, Vector-Borne Disease Section. (2016, September). California Compendium of Plague Control. Retrieved from https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH Document Library/CAPlagueCompendium.pdf

7. Perry, R. D., & Fetherston, J. D. (1997). Yersinia Pestis - Etiologic Agent of Plague. *Clinical Microbiology Reviews, 10*(1), 35-66

8. Brown, H. E., Ettestad, P., Reynolds, P. J., Brown, T. L., Hatton, E. S., Holmes, J. L., . . .Eisen, R. J. (2010). Climatic predictors of the intra- and inter-annual distributions of plague cases in New Mexico based on 29 years of animal-based surveillance data. *American Journal of Tropical Medicine & Hygiene, 82*(1), 95-102. doi:https://dx.doi.org/10.4269/ajtmh.2010.09-0247

9. Gage, K. L. and M. Y. Kosoy (2005). "Natural History of Plague: Perspectives from More than a Century of Research." 50(1): 505-528.

10. Collinge, S. K., et al. (2005). "Testing the Generality of a Trophic-cascade Model for Plague." **2**(2): 102-112.

11. Wimsatt, J. and D. E. Biggins (2009). "A review of plague persistence with special emphasis on fleas." J Vector Borne Dis 46(2): 85-99.

12. Douglas, J. R., and C. M. Wheeler. 1943. Sylvatic plague studies. II. The fate of Pasteurella pestis in the flea. J. Infect. Dis. 72:18–30.

13. Wheeler, C. M., and J. R. Douglas. 1945. Sylvatic plague studies. V. The determination of vector efficiency. J. Infect. Dis. 77:1–12

14. Hinnebusch BJ, Erickson DL. Yersinia pestis biofilm in the flea vector and its role in the transmission of plague. *Curr Top Microbiol Immunol*. 2008;322:229-48. PubMed PMID: 18453279; PubMed Central PMCID: PMC3727414.

15. Eisen RJ, Bearden SW, Wilder AP, Montenieri JA, Antolin MF, Gage KL. Early-phase transmission of Yersinia pestis by unblocked fleas as a mechanism explaining rapidly spreading plague epizootics. *Proc Natl Acad Sci U S A*. 2006 Oct 17;103(42):15380-5. doi: 10.1073/pnas.0606831103. Epub 2006 Oct 10.

16. Rebecca J. Eisen, Aryn P. Wilder, Scott W. Bearden, John A. Montenieri, Kenneth L. Gage, Early-Phase Transmission of *Yersinia pestis* by Unblocked *Xenopsylla cheopis* (Siphonaptera: Pulicidae) Is as Efficient as Transmission by Blocked Fleas, *Journal of Medical Entomology*, Volume 44, Issue 4, 1 July 2007, Pages 678–682, https://doi.org/10.1093/jmedent/44.4.678

17. Brown, H. E., Levy, C. E., Enscore, R. E., Schriefer, M. E., DeLiberto, T. J., Gage, K. L., & Eisen, R. J. (2011). Annual seroprevalence of Yersinia pestis in coyotes as predictors of interannual variation in reports of human plague cases in Arizona, United States. *Vector Borne & Zoonotic Diseases, 11*(11), 1439-1446. doi:https://dx.doi.org/10.1089/vbz.2010.0196

18. Stenseth, N. C., Atshabar, B. B., Begon, M., Belmain, S. R., Bertherat, E., Carniel, E., Gage, K. L., Leirs, H., … Rahalison, L. (2008). Plague: past, present, and future. *PLoS medicine*, *5*(1), e3

19. Runfola, J. K., House, J., Miller, L., Colton, L., Hite, D., Hawley, A., . . . Douglas, J. (2015, May 01). Outbreak of Human Pneumonic Plague with Dog-to-Human and Possible Human-to-Human Transmission - Colorado, June–July 2014.

20. Wagner, D. M., Runberg, J., Vogler, A. J., Lee, J., Driebe, E., Price, L. B., … Keim, P. (2010). No resistance plasmid in Yersinia pestis, North America. *Emerging infectious diseases, 16*(5), 885–887. doi:10.3201/eid1605.090892

21. Eisen, R. J., Petersen, J. M., Higgins, C. L., Wong, D., Levy, C. E., Mead, P. S., . . . Beard, C. B. (2008). Persistence of Yersinia pestis in Soil Under Natural Conditions. *Emerging Infectious Diseases, 14*(6), 941-943

22. Savage, L. T., Reich, R. M., Hartley, L. M., Stapp, P. and Antolin, M. F. (2011), Climate, soils, and connectivity predict plague epizootics in black-tailed prairie dogs (*Cynomys ludovicianus*). Ecological Applications, 21: 2933-2943. doi:10.1890/10-1946.1

23. Ben Ari, T., Gershunov, A., Gage, K. L., Snäll, T., Ettestad, P., Kausrud, K. L., & Stenseth, N. C. (2008). Human plague in the USA: the importance of regional and local climate. *Biology letters, 4*(6), 737-40

24. Eisen, R. J., et al. (2007). "Human plague in the southwestern United States, 1957-2004: spatial models of elevated risk of human exposure to Yersinia pestis." J Med Entomol 44(3): 530-537.

25. Smith, C. R., Tucker, J. R., Wilson, B. A., & Clover, J. R. (2010). Plague studies in California: a review of long- term disease activity, flea-host relationships and plague ecology in the coniferous forests of the Southern Cascades and northern Sierra Nevada mountains. *Journal of Vector Ecology, 35*(1), 1-12. doi:10.1111/j.1948-7134.2010.00051.x

26. Danforth, M., Novak, M., Petersen, J., Mead, P., Kingry, L., Weinburke, M., … Kramer, V. (2016). Investigation of and Response to 2 Plague Cases, Yosemite National Park, California, USA, 2015. *Emerging infectious diseases*, *22*(12), 2045–2053. doi:10.3201/eid2212.160560

27. Danforth, M., et al. (2018). "The Deer Mouse (Peromyscus maniculatus) as an Enzootic Reservoir of Plague in California." **15**(3): 566-576.

28. Yosemite National Park (U.S. National Park Service). (n.d.). Retrieved from https://www.nps.gov/yose/index.htm

29. Integrated Resource Management Application (U.S. National Park Service). (n.d.). Retrieved from https://irma.nps.gov/Portal/

30. National Park Service. (n.d.). Yosemite National Park Campgrounds. Retrieved from https://www.nps.gov/yose/planyourvisit/campgrounds.htm

31. PRISM Climate Group, Oregon State University. (n.d.). Retrieved from http://prism.oregonstate.edu/

32. Karesh, W. B., Dobson, A., Lloyd-Smith, J. O., Lubroth, J., Dixon, M. A., Bennett, M., . . . Heymann, D. L. (2012). Ecology of zoonoses: natural and unnatural histories. *Lancet, 380*(9857), 1936-1945. doi:10.1016/s0140-6736(12)61678-x

33. California-Nevada Climate Applications Program. California Precipitation. Southwest Climate Science Center.

34. Achee, N. (2006). Disease Ecology: Community Structure and Pathogen Dynamics. Potential effects of a keystone species on the dynamics of sylvatic plague. Oxford.

35. Centers for Disease Control and Prevention. (2019). *Prioritizing Zoonotic Diseases for Multisectoral, One Health Collaboration in the United States: Workshop Summary*. Retrieved from Unpublished Manuscript.

36. MacDonald, A. J., et al. (2018). "Risk of vector tick exposure initially increases, then declines through time in response to wildfire in California." 9(5): e02227.

VII. APPENDIX

Table 1. CDPH-VBDS and Covariate Dataset Variable Descriptions.

Variable Name	Description
Location	Location where trapping occurred.
Rodent Species	Identifies rodent species by scientific and common name, allowing for determining hallmark rodent species presence or absence at trapping/carcass collection locations. These data will be used in both the statistical and spatial analysis.
Number of Fleas Collected	The number of fleas collected off the trapped rodent.
Specimen Type	Nobuto, flea pool, mammal carcass, confirmed second carcass test.
Elevation	Recorded in feet for each location, converted to meters. Elevation will be used for both statistical and spatial analysis.
Normalized Difference Vegetation Index	Vegetation index score ranging from -1 to 1
Monthly Precipitation Average	The average precipitation (inches) for the month of sample collection.
Monthly Temperature Average	The average temperature (ºF) at each location during the month of sample collection.
Monthly Temperature Minimum	The minimum temperature (ºF) recorded at each location during the month of sample collection.
Monthly Temperature Maximum	The maximum monthly temperature (ºF) at each location during the month of sample collection.
Monthly Dew Point Temperature Average	The average dew point temperature (ºF) at each location during the month of sample collection.
Annual Precipitation Average	The average precipitation (inches) for the year of sample collection occurrence.
Annual Temperature Average	The average temperature (ºF) for the year of sample collection occurrence.
Annual Temperature Minimum	The minimum temperature (ºF) for the year of sample collection occurrence.
Annual Temperature Maximum	The maximum temperature (ºF) for the year of sample collection occurrence.
Annual Dew Point Temperature Average	The average dew point temperature (ºF) for the year of sample collection occurrence.

Table 2. Distribution and Prevalence of Rodent Species, Specimen Type and Sampling Locations – Number of *Y. pestis* Positive and *Y. negative* Rodents

Characteristic	*Y. pestis*⁺ Rodents (N = 29) *n* (%)	*Y. pestis*⁻ Rodents (N = 575) *n* (%)	Prevalence	Total Rodents (N = 604) *n* (%)
Rodent Host				
Tamias speciosus	10 (35.7%)	88 (15.3%)	10.2%	98 (16.2%)
Callospermophilus lateralis	7 (24.1%)	54 (9.4%)	11.5%	61 (10.1%)
Otospermophilus beecheyi	3 (10.3%)	130 (22.6%)	2.3%	133 (22.0%)
Tamias spp	4 (13.8%)	3 (0.5%)	57.1%	7 (1.2%)
Peromyscus maniculatus	2 (6.9%)	255 (44.3%)	0.8%	257 (42.5%)
Tamiasciurus douglasii	2 (6.9%)	13 (2.3%)	13.3%	15 (2.5%)
Tamias senex	1 (3.5%)	1 (0.2%)	50%	2 (0.3%)
Peromyscus boylii	0 (0%)	12 (2.1%)	0%	12 (2.0%)
Neotoma cinerea	0 (0%)	7 (1.2%)	0%	7 (1.2%)
Urocitellus beldingi	0 (0%)	4 (0.7%)	0%	4 (0.7%)
Spermophilus spp	0 (0%)	2 (0.3%)	0%	2 (0.3%)
Sciurus griseus	0 (0%)	1 (0.2%)	0%	1 (0.2%)
Marmota flaviventris	0 (0%)	1 (0.2%)	0%	1 (0.2%)
Scapanus spp	0 (0%)	1 (0.2%)	0%	1 (0.2%)
Microtus longicaudus	0 (0%)	1 (0.2%)	0%	1 (0.2%)
Fleas, table	0 (0%)	2 (0.3%)	0%	2 (0.3%)
Specimen Type:				
Mammal Carcass	13 (44.8%)	25 (4.3%)	34.2%	38(6.3%)
Nobuto	12 (41.4%)	353 (61.4%)	3.29%	365 (63.5%)
Flea Pool	4 (13.8%)	197 (34.3%)	19.9%	201 (35.0%)
Location:				
Tuolumne Meadows	22 (75.9%)	265 (46.2%)	7.7%	287 (47.5%)
Glacier Point	4 (13.8%)	88 (15.3%)	4.3%	92 (15.2%)
Crane Flat Campground	2 (6.9%)	54 (9.4%)	3.6%	56 (9.3%)
Tamarack Flat Campground	1 (3.5%)	16 (2.8%)	5.9%	17 (2.8%)
Hodgdon Meadow	0 (0%)	30 (5.2%)	0%	30 (5.0%)
Wawona	0 (0%)	4 (0.7%)	0%	4 (0.7%)
White Wolf	0 (0%)	5 (0.9%)	0%	5 (0.8%)
Yosemite Valley	0 (0%)	113 (19.6%)	0%	113 (18.7%)

Table 3. Average Number of Fleas Collected per Rodent at Collection Locations, 2014 – 2018

Rodent Host	Avg # Fleas/Rodent
Otospermophilus beecheyi	14.16
Tamias senex	3.00
Neotoma cinerea	2.14
Tamias speciosus	1.76
Peromyscus maniculatus	1.14
Callospermophilus lateralis	1.11
Microtus longicaudus	1.00
Urocitellus beldingi	0.50
Peromyscus boylii	0.33
Tamias spp	0.00
Sciurus griseus	0.00
Spermophilus spp	0.00
Marmota flaviventris	0.00
Scapanus spp	0.00
Tamiasciurus douglasii	0.00

Table 4. Shannon-Weiner Diversity Index for Rodent Host Species

Location	Shannon-Weiner
Crane Flat Campground	1.06
Glacier Point	0.97
Hodgdon Meadow	0.77
Tuolumne Meadows	1.46
Wawona	0.56
Yosemite Valley	0.91
White Wolf	0.67
Tamarack Flat Campground	1.15
Yosemite National Park	1.73

Table 5. Generalized Linear Mixed Model Analysis Results for Environmental and Non-Environmental Factors

Independent Variable	Estimate	Standard Error	p-value
Monthly Temperature Minimum	0.338468	0.132856	**0.010846***
Monthly Temperature Average	0.075553	0.065211	0.246622
Monthly Temperature Maximum	0.097443	0.053907	**0.070667***
Monthly Dew Point Temperature Average	-0.27183	0.08993	**0.002505***
Monthly Precipitation (in)	-2.29876	1.057963	**0.029794***
Yearly Temperature Minimum	0.49759	0.245563	**0.042732***
Yearly Temperature Average	-0.40143	0.178656	**0.024645***
Yearly Temperature Maximum	-0.13337	0.528165	0.80064
Yearly Precipitation	-0.25972	0.111605	**0.019955***
Yearly Dew Point Temperature Average	2.043863	1.078844	**0.05816***
Elevation	0.002155	0.000669	**0.00128***
Normalized Difference Vegetation Index	0.164073	1.769856	0.926138
Number of Fleas Collected	-0.02444	0.040912	0.550252
Shannon-Weiner Diversity Index	2.884155	0.925309	**0.001827***

*all covariates p<0.10 considered statistically significant

Figure 1. Number of Total Positive *Y. pestis* Rodents in Yosemite National Park Rodent Collection Locations, 2014 - 2018

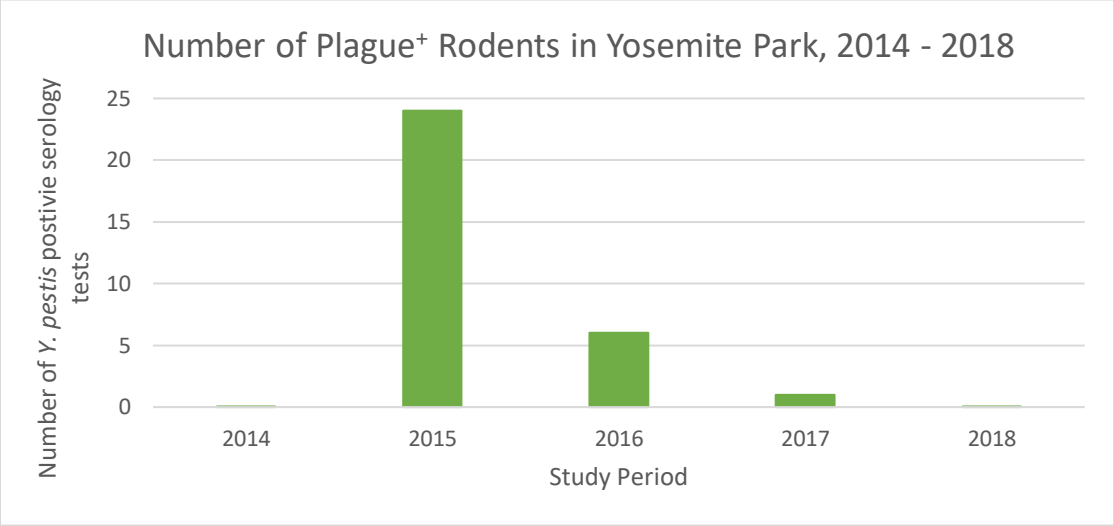

Figure 2. Elevation and Negative Plague Samples among Rodent Collection Locations 2014-2018.

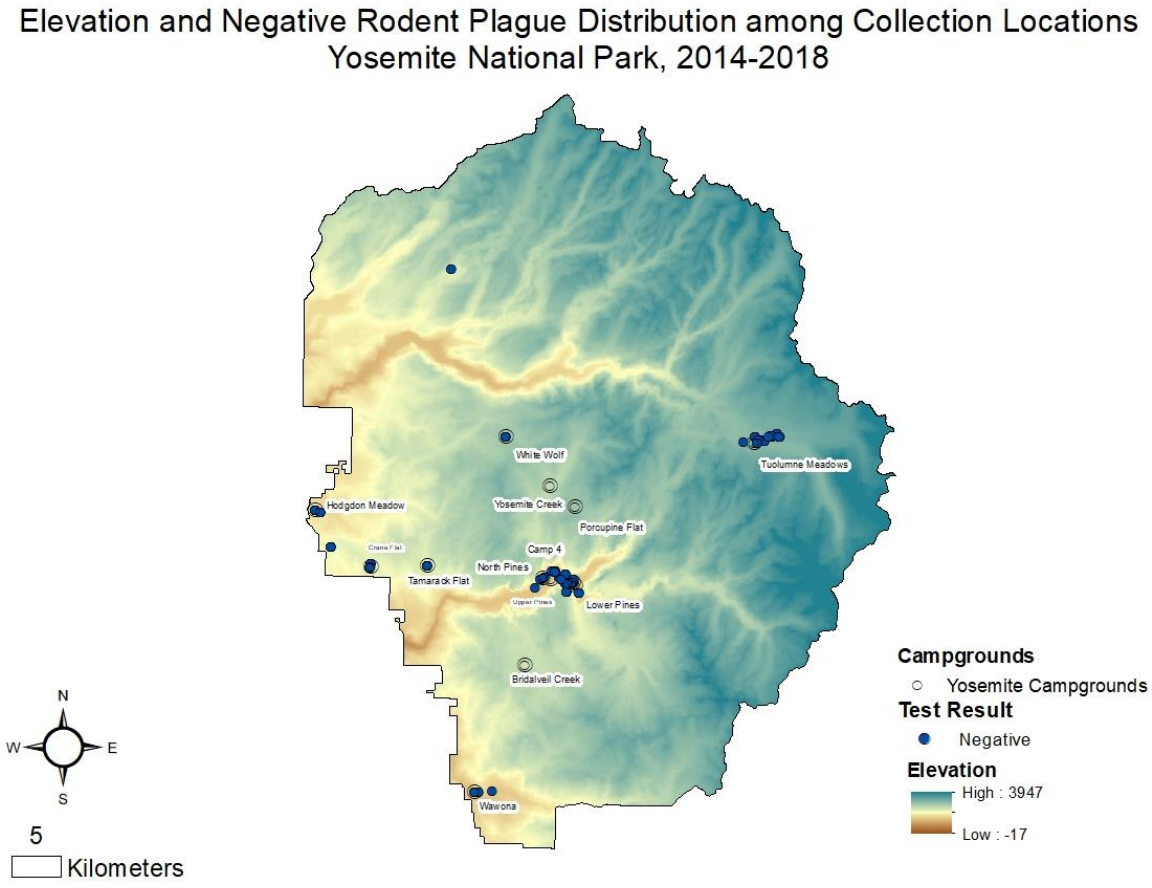

Figure 3. Elevation and Positive Plague Samples among Rodent Collection Locations 2014-2018.

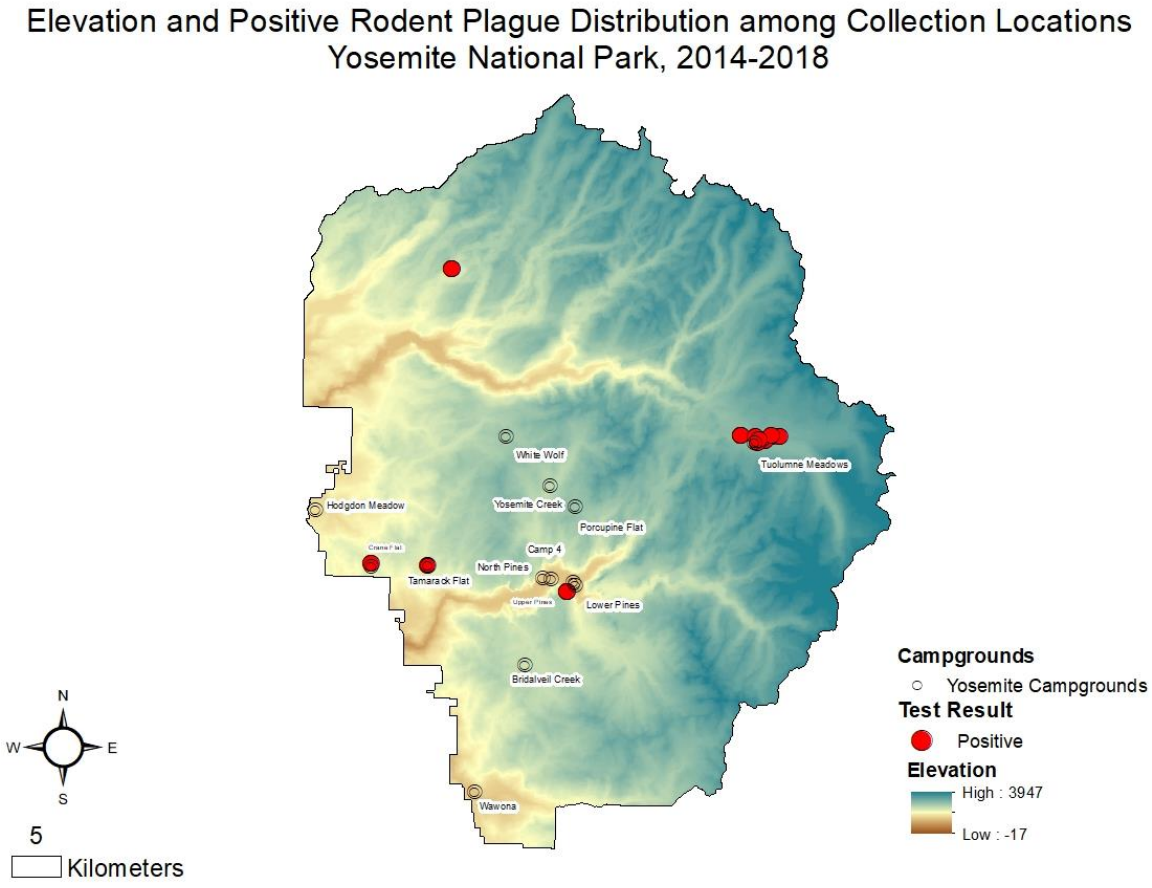

Figure 4. NDVI and Negative Plague Samples among Rodent Collection Locations 2014-2018.

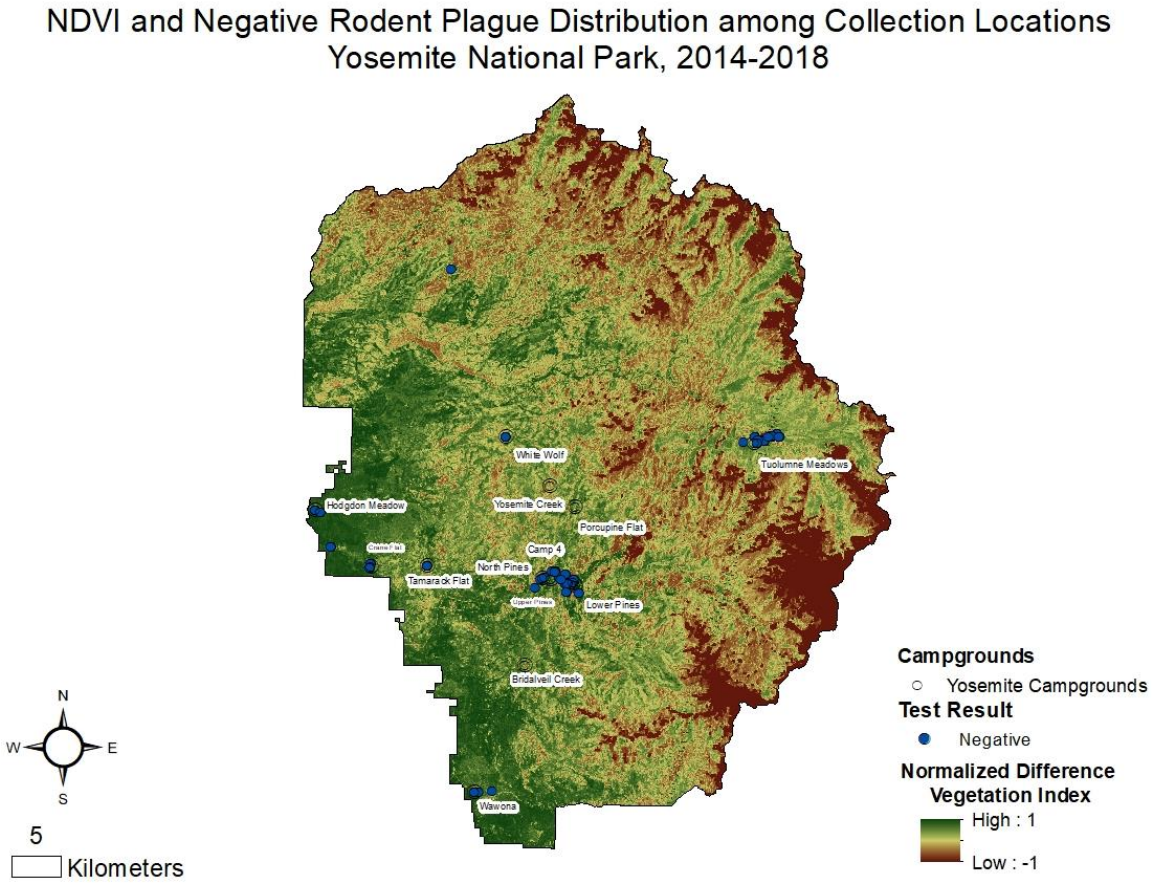

Figure 5. NDVI and Positive Plague Samples among Rodent Collection Locations 2014-2018.

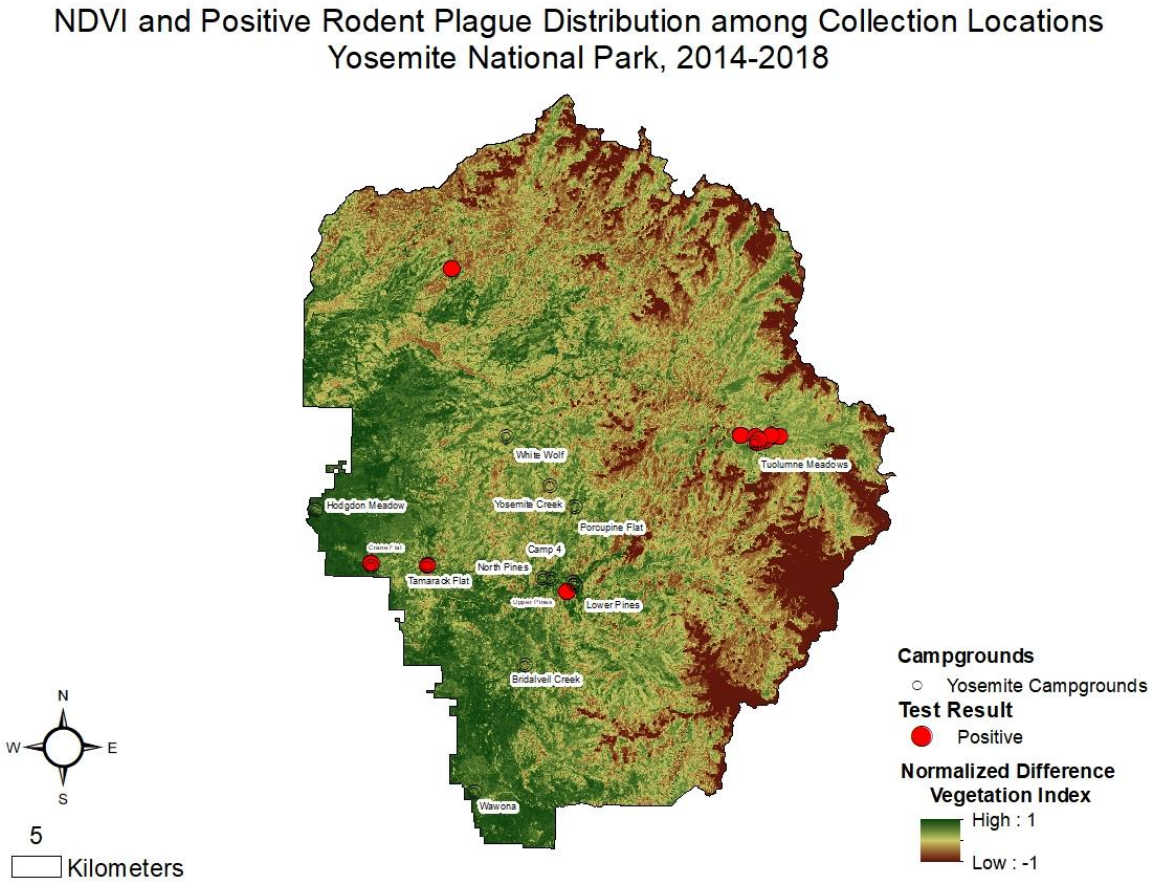

Figure 6. Chipmunk Species Distribution among Trapping Locations 2014-2018.

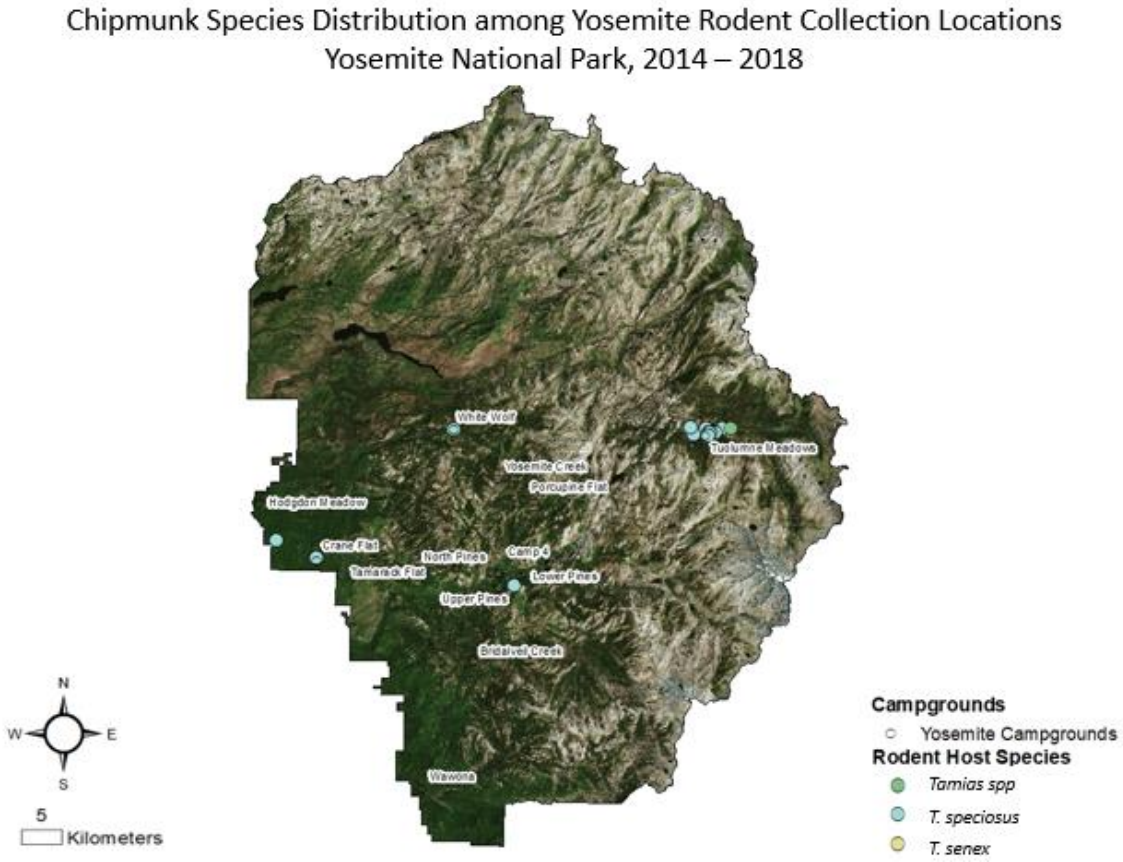

Figure 7. Mouse Species Distribution among Trapping Locations 2014-2018.

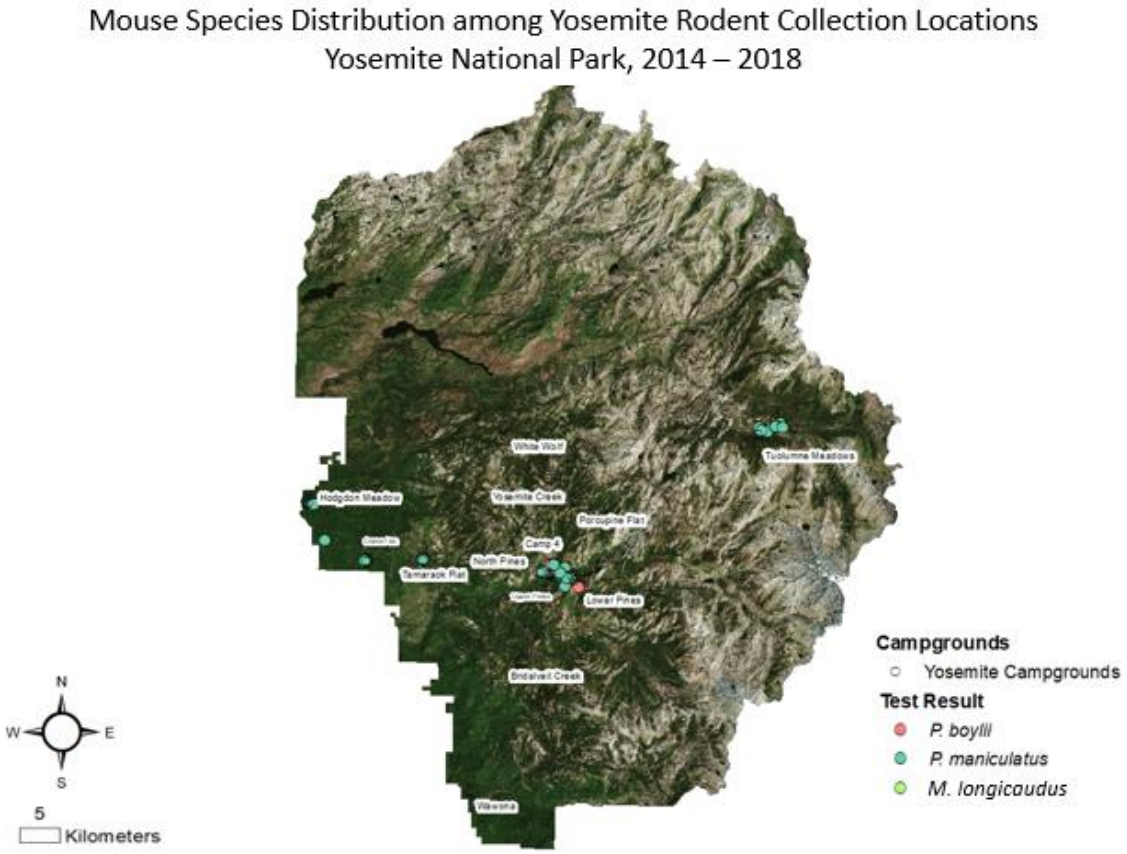

Figure 8. Squirrel Species Distribution among Trapping Locations 2014-2018.

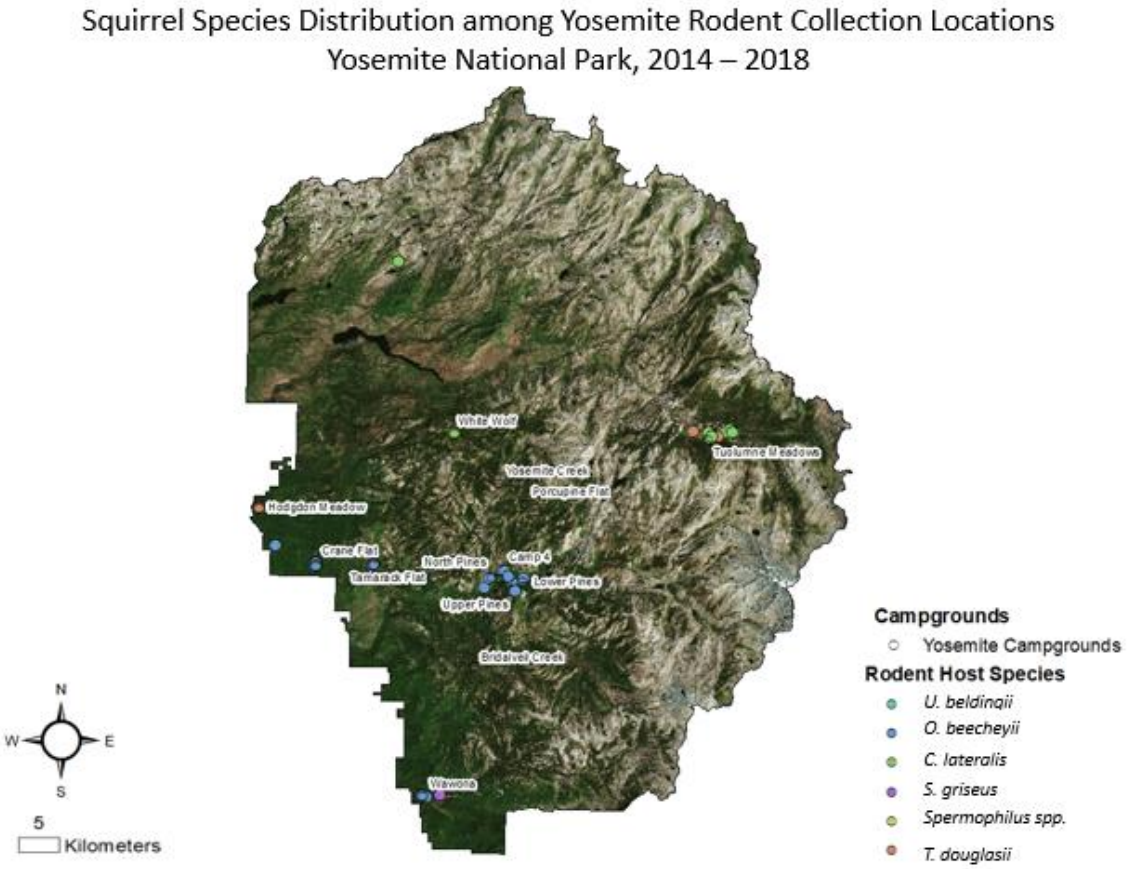

Figure 9. Marmot, Mole & Woodrat Species Distribution among Trapping Locations 2014-2018.