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IDENTIFYING REGIONAL CLIMATIC AND HUMAN INFLUENCES ON DENGUE FEVER
TRANSMISSION IN PUERTO RICO

BY:

EMILY A. SERMAN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE

IN

ENGINEERING

UNIVERSITY OF RHODE ISLAND

2017

MASTER OF SCIENCE THESIS

OF

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2017

ABSTRACT

Worldwide, approximately 2.5 billion people live in regions that are vulnerable to dengue fever. Dengue is primarily transmitted by *Aedes aegypti* mosquitoes who prefer urban habitats where they can live in close proximity to humans, their preferred source of blood meals. There is no cure for dengue fever and vaccines are still in early stages, years away from mass distribution. As a result, effective control of dengue is reliant on our ability to understand the complex relationship between humans, vectors, and the environment.

The purpose of this study is to assess temporal and spatial patterns of dengue transmission in Puerto Rico, as they relate to both climatic and anthropogenic factors, using linear models and Multiple Linear Regression (MLR) analysis. Unlike previous studies, this analysis is done at an intermediate spatial scale, considering 6 regions across the island. This regional approach allows for the impact of local variations in anthropogenic and environmental factors on dengue incidence to be considered. We examined the influence of Land Use Land Cover (LULC) and environmental factors, both separately and combined, at stationary points, and over time.

These analyses provide insight into the role of both anthropogenic and environmental variables as they relate to dengue incidence. The linear models between individual environmental factors and incidence showed the strongest correlations between the Central region and temperature. The MLR models showed higher incidence levels in the West region of the island and complex relationships between incidence and variables related to open space, such as shrub and herbaceous cover. The MLR model with the highest R^2 value was the best fit change analysis model

using both LULC and environmental factors. This best fit model (adjusted $R^2 = 0.343$) included: average annual maximum temperature, average annual minimum temperature, crop cover, pasture, and herbaceous cover.

ACKNOWLEDGMENTS

I could fill a book with the list of names of people in my life who have supported me along this journey. First and foremost, my family and friends who have listened to countless stories about dengue even when they didn't want to. If they had a dollar for every time they heard "do you want to hear something I learned about dengue?", they'd be billionaires. Thank you for listening, for feeding me, for putting a roof over my head, for encouraging, and believing in me. I would be lost without you.

Ali, thank you for introducing me to the world of water, climate, and health, and for funding my research for the past 3 years.

Dr. Thiem, thank you for serving on this committee.

Howie, you helped this entire project get started by graciously agreeing to guide me through an independent study class. Thank you for introducing us to Nelle and being a champion of this project.

Nelle, words can't describe how much I appreciated your advice (on graduate school and life), for talking me off the ledge when I felt overwhelmed, and for encouraging me at every step of the writing process.

Gavino, thank you for serving as my defense examination chair and for all your help with our modelling efforts. I am so excited to see what else we can add to the model you have already created for us!

Finally, I would like to thank the Rhode Island Water Resources Center and the Rhode Island Space Grant for funding my graduate program over the past three

years. The financial support that these programs provided allowed me to give my full attention to my research and classes.

Thank you all so much!

~Emily

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CHAPTER 1

INTRODUCTION

In recent decades, rapid changes in anthropogenic and environmental factors have influenced the transmission dynamics of vector-borne illnesses, such as dengue fever (Gubler 2011). Globally, approximately 2.5 billion people live in regions that are vulnerable to dengue (CDC 2014). Dengue is a febrile disease whose symptoms include, joint pain, headaches, mild bleeding from nose or gums, and rash (CDC 2014). Dengue virus is transmitted primarily by the mosquito species, *Aedes aegypti* and *Ae. albopictus*, both of which frequently bite humans for blood meals, especially *Ae. aegypti* (CDC 2014, Eisen and Lozano-Fuentes 2009, Gubler 2002). Each year, there are an estimated 50-100 million cases of dengue fever worldwide, roughly 30 times the number of cases as 50 years ago (WHO 2014), with some estimates even higher (Bhatt et al. 2013). Because a vaccine is not widely available beyond early trials, effective control is dependent on our ability to understand the complex relationship between environmental factors, mosquito vector ecology, and disease epidemiology (Gubler 2011).

As a U.S. island territory where dengue is endemic (CDC 2015), Puerto Rico provides an ideal study area to investigate the role of climate, land use patterns, and dengue epidemiology. Although other countries, such as India, Indonesia, China, and Brazil, have higher dengue incidence, they also have much larger populations. Disease burden uncertainty is inevitably influenced by population size, and these regions have

the greatest uncertainty in dengue incidence estimates (Bhatt et al 2013). In contrast, Puerto Rico has a population of nearly 3.6 million and is approximately three times the size of the state of Rhode Island (CIA World Factbook). Because it is a U.S. territory, and home to the Centers for Disease Control and Prevention's (CDC) Dengue Branch, dengue case data is well documented. Climate and Land Use Land Cover (LULC) datasets are also relatively complete and easily accessible through U.S. government agency websites (Figure 1). Because Puerto Rico has such a robust dataset, long term patterns between climate, land use, and vector-borne disease can be explored. Puerto Rico has experienced epidemic dengue activity since 1963 (CDC 2015). Since 1990 there have been 4 large epidemics, the most recent in 2010 where there were nearly 27,000 cases reported, amounting to almost 1% of the island's total population (Sharp et al. 2013, CDC 2015).

Overall, the climate in Puerto Rico is tropical marine, and the temperature difference between seasons is very small (CIA World Factbook). The Cordillera Central, or "Central Mountains", that run East-West across the territory, are partially responsible for the climate zones on the island. For example, the southern coast is on the leeward side of the mountains, and is therefore drier. The climate zones, as defined by the National Oceanic and Atmospheric Administration (USGS 2016) can be seen in Figure 1. Though the island has regional differences in climate, the entire island is warm and wet enough to provide the ideal habitat for *Ae. aegypti*. Since 2015, the island population has been heavily affected by the Zika virus, also carried by *Ae. aegypti* (Dirlikov et al 2017), thus raising the importance and future potential of this

study on understanding the environmental factors that influence their habitats and life cycle.

Figure 1: Climate zones of Puerto Rico (NOAA 1982)

Attempts to model dengue transmission in Puerto Rico have explored fine spatial scales at the community and individual municipality level (Barrera 2011, Little et al 2011, Johansson et al 2009, Morin 2015) and coarse spatial scales, considering trends over the entire island (Jury 2008), but not at an intermediate scale. This study builds upon the fine-scale work that has been done while still taking coarse scale factors into consideration to create an intermediate, regional, scale approach to dengue modeling in Puerto Rico, something that has not been done before. Johansson et al (2009) provides evidence for the impact of local climate on dengue incidence. Analyzing incidence at an intermediate scale allows for a more in-depth look at the role of regional differences in climate, population density, and land cover in transmission dynamics. We combined the 78 municipalities of Puerto Rico by their geographic, climatic, and land cover characteristics into 6 unique regions: North, Metro San Juan, East, South, Central, and West (Figure 2). Within these regions,

urban, suburban, and rural areas are represented as well as coastal plains, mountains, and foothills. Two of the 78 municipalities are separate islands off the coast of the mainland. These islands were grouped together into the Outlying Islands “region” and were excluded from this study.

Figure 2: Regions and National Climatic Data Center (NCDC) weather stations of Puerto Rico

The purpose of this study is to address: 1. the impacts of regional climate on dengue incidence 2. the role of anthropogenic factors, including LULC and population densities, on dengue incidence 3. whether environmental and anthropogenic factors have a combined influence on dengue incidence 4. the impact of change in LULC and climate over time on incidence. These questions are addressed at both monthly and annual time scales as well as an intermediate, regional spatial scale. This study

introduces a regional scale approach to dengue modeling in Puerto Rico. In addition, LULC for the entire island and changes in landscape are both addressed for the first time for this location. Identifying and analyzing these relationships will contribute to our understanding of the complex relationship between environmental factors, landscape, mosquito vector ecology, and disease epidemiology.

CHAPTER 2

REVIEW OF LITERATURE

Research studies around the world have analyzed the impact of weather variables on dengue incidence at various spatial and temporal scales. However, relationships between these variables vary geographically (Johansson et al 2009). *Ae. aegypti* mosquitoes prefer tropical and subtropical climates that are warm, wet, and humid, (CDC 2014). Precipitation, temperature, and humidity are all key components in mosquito development and therefore impact the transmission of dengue (CDC 2014, Couret & Benedict 2014, Brady et al 2013). Precipitation is essential for mosquito hatching, *Ae. aegypti* oviposits in small containers above water lines, thus ensuring that eggs develop only when there is sufficient precipitation to submerge eggs and trigger hatching. As ectotherms, temperature and humidity influence all aspects of mosquito biology including aspects that directly relate to the vectorial capacity such as development rate (Couret & Benedict 2014), juvenile survival (Brady et al 2013), and daily survival rates (Harrington et al 2008). In addition, the extrinsic incubation period, the time needed for the dengue virus to replicate within the mosquito, is also influenced by temperature (Xiao et al 2014, Chan & Johansson 2012).

Several studies have also cited population dynamics and climate change as contributing factors in rising case numbers (Ali et al 2003, Colón-Gonzalez et al 2013,

Johansson et al 2009, Hii et al 2012, Lu et al 2009, Wu et al 2008). With global temperatures continuing to rise (IPCC/Stocker 2014), suitable habitat for *Ae aegypti* is increasing and these mosquitoes are being found in more northern locations and higher elevations (Colón-Gonzalez et al 2013, Banu et al 2014, Jury 2007). As their geographical range expands, the number of people at risk for contracting dengue increases (Banu 2014, Gubler 2011, Jury 2007). *Ae. aegypti* thrive in urban settings, such as in countries like Bangladesh and Indonesia, where rapid unplanned population growth has led to, millions living in urban slums, or shanty towns with a distinct lack of infrastructure (Banu 2014, Gubler 2011). In these settlements, water storage containers and trash provide excellent sites for larval development in close proximity to hosts, who have little to no protection from the mosquitoes their living situation attracts (Gubler 2002, Little et al 2011). Other population characteristics, such as population density, household income, percent of population living below the poverty line, Gross Domestic Product (GDP), number of clinics per 10,000 people, and others have been incorporated into models to account for the anthropogenic factors that contribute to dengue transmission (Ali et al 2003, Colón-Gonzalez et al 2013, Johansson et al 2009, Wu et al 2008).

Asian nations carried 70% of the global dengue burden in 2010 (Bhatt et al 2013). Banu et al (2014) projected the impact of climate change on dengue transmission in Dhaka, Bangladesh. Dhaka is the capital city of Bangladesh and has a population of 11.8 million people within its metropolitan area, many in slums with little to no infrastructure (Banu 2014). The climate is tropical and impacted by monsoon cycles, resulting in almost 80% of its annual rainfall occurring between May

and September (Banu 2014). This study assessed monthly dengue cases versus monthly mean, minimum, and maximum temperatures, as well as relative humidity and rainfall for the period of January 2000 to December 2010. Their best fit model included average monthly maximum temperature and average monthly relative humidity and they found that temperature and humidity both had positive associations with dengue incidence. These relationships were non-linear and the strongest correlations were found at two months' lag time. The Intergovernmental Panel on Climate Change (IPCC) estimates that Asia will see an increase in temperature of 3.3°C over the next 100 years (Banu et al 2014). Based on this projection, Banu estimates that there will be a 16,030-case increase in the annual number of cases in Dhaka by the end of the 21st century.

A similar study in Taiwan by Wu et al (2008) also found that rising temperatures and urbanization effected dengue incidence. Using daily dengue cases and temperature and precipitation information, GIS, and multiple logistic regressions, the authors assessed dengue risk for townships across the island. They found that higher temperatures and urbanization levels were two risk factors for determining areas that were at risk for dengue epidemic activity. They also tested whether an increase in temperature would impact dengue case numbers and found that with 1°C increase in temperature, the number of high risk townships rose from 48 to 86 and increased risk of contracting dengue by 1.95 times (Wu et al 2008). Spatially, this expansion would stretch from the south to the north of the island. In addition, townships with minimum temperatures above 18°C for at least 11 months out of the

year had considerable potential for sustaining year-round dengue transmission (Wu et al 2008).

Though Banu et al (2014) found that humidity was a strongly correlated with dengue incidence in Bangladesh, Hii et al (2012) did not find the same relationship to be true in Singapore. Singapore is a nation of 5.2 million people, and has a tropical climate influenced by monsoon rains (Hii et al 2012). Since 1980, Singapore has had a 5-6year cyclical pattern of dengue epidemics, however in a recent 8-year period, they experienced 4 separate epidemics (Hii et al 2012). In addition to an increased frequency in epidemics, dengue has spread to new regions of the island (Hii et al 2012). Hii et al (2012) used weekly dengue case numbers from 2000-2011 and daily mean temperature and rainfall in a piecewise regression to assess non-linear relationships between weather and dengue. They found that maximum and minimum temperatures were strong predictors of dengue while rainfall and humidity were weak indicators. The model that performed most consistently used a weather time cycle of 24 weeks for temperature and 20 weeks for precipitation at a 16-week lag (Hii et al 2012). This model predicted true outbreaks 90-98% of the time and had only a 10-20% risk of false alarm (Hii et al 2012).

In addition to temperature, precipitation, and humidity, Lu et al (2009) included wind velocity in their study in southern China. Dengue has frequently occurred in Southern China since a 1978 outbreak in Foshan City (Lu et al 2009). Dengue will likely continue to be a threat to China, due to the effects of climate change, widespread suitable habitat, and increasing shifts in populations (Lu et al

2009). This study focused on the city of Guangzhou which has a humid, subtropical climate influenced by monsoon cycles and has 10 million inhabitants in its metropolitan area (Lu et al 2009). Lu et al (2009) studied the relationship between monthly dengue cases and monthly maximum and minimum temperature, cumulative precipitation, minimum relative humidity, and wind velocity from 2001-2006 using generalized estimating equations with a Poisson distribution. They assessed these relationships at 0, 1, 2, and 3 months' lag times and found that minimum and maximum temperature, total precipitation, and minimum relative humidity were all positively associated with dengue at all lags, while wind was negatively correlated but not significant statistically (Lu et al 2009). The best fit model for this study used minimum temperature, wind velocity, and minimum relative humidity, though only the first two were significant, minimum relative humidity improves the model (Lu et al 2009).

In 2010 the Americas account for 14% of the global dengue burden (Bhatt et al 2013), and the economic burden, caused by dengue, for Latin America and the Caribbean alone, is estimated at \$2.1 billion USD per year (Colón-Gonzalez et al 2013). Colón-Gonzalez et al (2013) analyzed the impacts of climate change on dengue incidence for Mexico by first modeling historical data from 1985-2007, then projecting incidence for 2030, 2050, and 2080. Average monthly mean, minimum, and maximum temperatures, as well as cumulative monthly precipitation, as well as several non-climatic variables, such as yearly GDP, access to piped water, and proportion of people living in urban areas, were used in General Additive Models to assess these relationships at one and two month lags (Colón-Gonzalez et al 2013).

They found that associations between weather and dengue were non-linear, and that all environmental factors as well as access to piped water were significant indicators (Colón-Gonzalez et al 2013). In this scenario, as access to piped water increases, so does dengue incidence, which contradicts the idea that access to infrastructure reduces dengue (Gubler 2011). However, in Mexico, water is often brought into neighborhoods via tanker trucks (Colón-Gonzalez et al 2013). Because these piped water access points are not traditional well or public water systems, there is still a need to store water in containers (Colón-Gonzalez et al 2013).

Colón-Gonzalez et al (2013) also projected the impact of climate change on dengue incidence in Mexico. They projected incidence for 2030, 2050, and 2080 and found that there was a positive and increasing impact of climate change at a national and provincial level (Colón-Gonzalez et al 2013). There were significant rises in provinces where dengue is already endemic (Colón-Gonzalez et al 2013). Colón-Gonzalez et al (2013) found that there would be a 12-18% increase in cases by 2030, 22-31% increases by 2050, and 33-42% more cases by 2080. Minimum temperature had the biggest impact on dengue incidence in the overall 40% increase by 2080 due to climate change (Colón-Gonzalez et al 2013).

Using weekly dengue case data and mosquito sampling, Barerra et al (2011) studied dengue incidence for two neighborhoods located in the San Juan and Carolina municipalities. They found that water storage containers contributed to dengue outbreaks during dry seasons and that peaks in dengue followed peaks in female mosquito density. Incidence reached its lowest levels by the end of the drier season,

but mosquitoes density remained high, as mosquitoes adapted and began to breed in man-made environments. Though access to piped water may not have been associated with an increase in dengue incidence, it certainly did not prevent it. Puerto Rico has a reliable drinking water system, and tire-recycling program, and yet access to piped water sources did not decrease incidence, and discarded tires still contributed to mosquito habitats.

Johansson et al (2009) considered the effects of local climate on dengue transmission in Puerto Rico at the municipality level. They considered monthly mean, minimum, and maximum temperature, and total monthly precipitation as well as monthly suspected dengue cases for July 1986-December 2006. In addition to weather variables, Johansson et al (2009) also considered population data, household income, and percent of the population living below the poverty line. Temperature and precipitation were positively associated with dengue incidence in most municipalities and across the entire island at 0, 1, and 2 month lags (Johansson et al 2009). Monthly variations in cumulative precipitation were significantly associated with variation in dengue for some but not all municipalities at 1 and 2 month lags (Johansson et al 2009). Municipalities with higher poverty indexes had stronger short term associations between weather and dengue incidence (Johansson et al 2009). They predict that in municipalities where precipitation and temperature are already high, climate change will not have as great of an effect (Johansson et al 2009). Temperature is more likely to impact municipalities located in the central mountain range, while changes in precipitation will increase dengue incidence along the southern coast of the island (Johansson et al 2009).

Jury (2008) considered incidence trends and their association with weather variables across the entire island of Puerto Rico from 1979-2005. The study considered low dengue and high dengue seasons separately and found that some epidemics coincided with higher temperatures. Low pressure systems around Florida caused humidity to rise during epidemic years (Jury 2008). They also concluded that annual fluctuations in incidence were driven by rainfall while year to year variability was positively associated with temperature. Overall, Jury (2008) concluded, based on IPCC climate projections, that long term increases in dengue seem likely.

Land cover in Puerto Rico has also been associated with dengue transmission and mosquito abundance (Gubler 2011, Little et al 2011). Urban centers in Puerto Rico are typically located along the coasts and in valleys and cover approximately 16% of the island (Martinuzzi et al 2007). These urban areas are known to incorporate non-developed lands such as forests, parklands, and other vegetated areas (Martinuzzi et al 2007). This information was critical for a study conducted by Little et al (2011) that found that, in study areas along the southern coast of Puerto Rico, urban density and the number of tree patches predicted the distribution of *Ae. aegypti* populations. The largest concentrations of densely developed areas are typically along the coast, with the most densely populated being the capital city of San Juan along the northern coast of the island. LULC categories that were analyzed in this study include crop cover, pastures, evergreen forests, shrub land, herbaceous areas, developed open land, and barren land. This study expands on the LULC analysis done by Little et al (2011) in an effort to determine the impact of landscape on dengue fever incidence in

mainland Puerto Rico municipalities, and investigate how changing landscapes affect dengue incidence

The relationship between dengue, humans, and the environment is very complex. Because the associations between these factors vary by location, there is no clear understanding on determining the role each factor plays in dengue incidence. Most studies found that the relationship between environmental variables and dengue incidence was non-linear (Banu et al 2014, Hii et al 2012, Colón-Gonzalez et al 2013). Temperature was most commonly positively associated with dengue, but some locations had stronger correlations to maximum temperature and others to minimum temperature (Banu et al 2014, Wu et al 2008, Hii et al 2012, Lu et al 2009, Colón-Gonzalez et al 2013, Johansson et al 2009, Jury 2008). Relationships with humidity and precipitation varied the most from location to location. In Bangladesh, China, and Puerto Rico humidity had a significant impact on incidence (Banu et al 2014, Wu et al 2008, Jury 2008), while in Singapore the association was weak (Hii et al 2012).

In addition to determining the most influential variables, one must also resolve the best spatial scale at which to apply these relationships. These studies assessed dengue incidence at the city (Banu et al 2014, Barrera et al 2011, Little et al 2011, Lu et al 2009), township and municipality (Colón-Gonzalez et al 2013, Johansson et al 2009, Wu et al 2008) and national levels (Colón-Gonzalez et al 2013, Jury 2008, Hii et al 2012). The municipality and township scale, in particular, showed that local variations in climate impacted dengue incidence and that the effects of climate change would not be universal (Colón-Gonzalez et al 2013, Johansson et al 2009, Wu et al

2008). We are hoping to capture the fine scale, localized effects of dengue in an intermediate scale approach that combines municipalities with similar climate and population characteristics.

This study will build upon the work done in Puerto Rico by Barrera et al (2011), Johansson et al (2009), Jury (2008), and Little et al (2011). The analysis investigates trends over a 25-year period from 1990-2014, which makes it the most up to date study at this time. We will analyze the associations between maximum and minimum temperatures, and precipitation at an intermediate, regional, spatial scale. These relationships will be analyzed at both annual and monthly temporal scales at 0, 1, 2, and 3 month lags. In addition, we will consider the impacts of LULC on dengue incidence, both individually and combined with weather factors using multiple linear regression models. This study will contribute to the field of dengue incidence modeling by considering new spatial scales and anthropogenic factors on the island of Puerto Rico.

Table 1: Summary of literature review

Authors (Year Published)	Time period	Location	Variables Tested	Relationships Found
Banu et al (2014)	Jan 2000 – Dec 2010	Dhaka, Bangladesh	Monthly dengue, monthly mean, minimum, and maximum temperatures, monthly relative humidity	Positive association with max temp and humidity; strongest relationship at 2 month lag
Wu et al (2008)	1998 – 2002	Taiwan	Daily dengue, temperature, precipitation,	Temperature and urbanization

			and urbanization	positively associated with incidence
Hii et al (2012)	2000 – 2011	Singapore	Weekly dengue, daily temperature, precipitation, and humidity	Strong relationships between min/max temperature and dengue; rainfall and humidity were weak indicators
Lu et al (2009)	2001 – 2006	Guangzhou, China	Monthly dengue, maximum and minimum temperature, cumulative precipitation, minimum relative humidity, and wind velocity	Min/max temperature, total precipitation, and minimum relative humidity were all positively associated with dengue; wind negatively correlated
Colón-Gonzalez et al (2013)	1985 – 2007	Mexico	Monthly average minimum, mean, and maximum temperature, total precipitation; yearly GDP, access to piped water, proportion of people living in urban areas	All environmental variables and access to piped water were significant indicators
Barerra et al (2011)	November 2007 – December 2008	San Juan, Puerto Rico	Weekly dengue and mosquito sampling	Water storage containers contributed to dengue in dry season
Johansson et al (2009)	July 1986 – December 2006	Puerto Rico	Monthly mean, minimum, and maximum	Temperature and precipitation

			temperature, total precipitation, suspected dengue cases; household income, percentage of people living below poverty line	positively associated in most municipalities and entire island, precipitation significant in some locations; municipalities with higher poverty indexes had stronger short term associations between weather and dengue incidence
Jury (2008)	1979 – 2005	Puerto Rico	Annual and monthly dengue, temperature, and precipitation	Seasonal dengue was driven by precipitation and year-to-year changes in incidence were positively associated with temperature
Little et al (2011)	May 2010 – August 2010	Southern Puerto Rico	Land Use Land Cover (LULC)	Urban density and number of tree patches predicted the distribution of <i>Ae. aegypti</i>

CHAPTER 3

METHODOLOGY

I. Data Sources

Dengue case data at the municipality level was provided by the Center for Disease Control's (CDC) Passive Dengue Surveillance System. These data do not include personal identifying information (ie: age, sex, home address) but the monthly number of confirmed and suspected cases for each municipality from 1990-2014. Confirmed cases represent those cases where blood samples were sent off to a laboratory and tested using reverse transcription-polymerase chain reaction (RT-PCR) to confirm the presence of dengue virus (CDC 2016). Because the presence of dengue in the body changes over time, not all positive samples can be confirmed as a dengue infection (CDC 2016). Immunoglobulin (Ig)M identifies cases where a person has been exposed to the dengue virus and produced antibodies, but are not currently infected (CDC 2016). These samples are classified as a "recent probable dengue infection" or "laboratory-indeterminate case" and are represented in our dataset as suspected cases.

Environmental data were downloaded from the National Climatic Data Center (NCDC), which has approximately 35 weather stations spaced throughout the island (Figure 1). Data downloaded for this study included daily maximum temperature, minimum temperature, and precipitation measurements. In addition, the name, elevation, and geographic coordinates for each weather station were provided. Not all weather stations had complete datasets for the entire 1990-2014 time period.

Municipality level population data were downloaded from the U.S. Census Bureau. These included data from census years (1990, 2000, 2010) as well as estimates for non-census years from 1999-present. The years 1991-1998 were estimated using linear interpolation. In addition, number of houses, housing density, and population density were included in this download.

LULC data were downloaded from the Multi-resolution Land Characteristics Consortium, a joint effort by several federal agencies to create the National Landcover Database (NLCD) from Landsat satellite images and supporting datasets. LULC data are available for 2001 and 2011. Shapefiles for bodies of water (streams, rivers, lakes), municipality borders, and elevation were downloaded from the U.S. Census Bureau and Data.gov. Weather station locations were converted into shapefiles using the geographic coordinates provided by NCDC.

II. Data Processing

After the data were compiled, they were processed and organized. Monthly dengue case data were provided for each municipality in alphabetical order. Each municipality was then grouped into the appropriate region, and combined to create monthly and annual datasets. For this study, only lab confirmed dengue cases by either PCR or IgM detection are considered.

A unique aspect of this study is the use of a regional spatial scale to assess transmission dynamics of dengue across the island of Puerto Rico. The main factors in determining the regions were climate and geography. For example, the central mountain range has high elevations, high precipitation due to the presence of rainforest, and a lower population density compared to the surrounding areas of the

island, so municipalities in this area of the island were grouped together into the Central region. San Juan and the surrounding municipalities are very densely populated and were considered together in studies completed by Barrera (2011), therefore these municipalities are considered together as the Metro San Juan region. The southern and northern coasts of Puerto Rico have distinct climate, as can be seen in the NOAA climate regions, and were grouped together as the South and North regions. Municipalities along the east and west coasts of the island were grouped by their geographical location into the East and West regions, respectively.

Non-valid entries (ie: -9999) were removed from the environmental data and units were standardized. Once condensed into regions, monthly and annual values were calculated based upon the averages of the stations located in that region. Each region is represented by at least 3 weather stations. To adjust for gaps in weather station data, we only included years that were at least 75% complete (missing 4 months or less). For the East region, temperature data for April 2004-December 2005 and March 2008-August 2010 were missing, resulting in these incomplete years being excluded from annual calculations. Despite having missing years, the East region is considered in this study because there are still 20 complete years of data to be used for analysis. The environmental data used in the Multiple Linear Regression (MLR) model was interpolated from the weather stations around the island based upon the centroid location of each municipality. These data include average monthly precipitation, maximum temperature, and minimum temperature for each municipality, for the entire 1990-2014 time period. From these averages, annual cumulative

precipitation and average monthly precipitation were calculated, as well as annual average maximum and minimum temperatures.

Population data were available at the municipality level (US Census Bureau). As with the dengue case data, these were condensed into regions, and total regional population found by summing the municipalities together. Total annual population was used to represent population for each month of the year. For example, if the population of the San Juan municipality in 1990 was 350,000 that value was carried through for January through December of that year. Because the census is only completed every 10 years (1990, 2000, and 2010) and population estimates only go back to the year 1999, estimates from the years 1991-1998 were calculated linearly based on the change in population between censuses. Data processing was conducted in Microsoft Excel and RStudio (Version 1.0.136), and maps and spatial calculations were done in ArcMap (Version 10.2.2), a Geographic Information Systems (GIS) program.

Land use was analyzed using two different methods in ArcMap. Using the reclassify function, each 2001 LULC category was assigned a number and then, using zonal statistics, the area of each category was calculated by municipality. The 2010 land use statistics were calculated by municipality directly then summarized using zonal statistics as a table. The resulting areas for both data sets were then converted from square meters to square miles. The two data sets had slightly different LULC categories and so only the categories that directly matched were compared. These categories are listed in Table 1.

Table 2: List of LULC categories for the 2001/2011 datasets. () Denotes categories that were used in analysis*

LULC Categories	
2001	2011
Open Water*	Impervious Surface
Developed, Open Space*	Developed, Open Space*
Developed, Low Intensity	Cultivated Crops*
Developed, Medium Intensity	Pasture/Hay*
Developed, High Intensity	Grassland/Herbaceous*
Barren Land*	Deciduous Forest
Evergreen Forest*	Evergreen Forest*
Shrub/Scrub*	Shrub/Scrub*
Herbaceous*	Palustrine Forest Wetland
Hay/Pasture*	Palustrine Scrub/Shrub Wetland
Cultivated Crops*	Palustrine Emergent Wetland
Woody Wetlands	Estuarine Forested Wetland
Emergent Herbaceous Wetlands	Estuarine Scrub/Shrub Wetland
	Estuarine Emergent Wetland
	Unconsolidated Shoreline
	Bare Land*
	Open Water*
	Palustrine Aquatic Bed
	Estuarine Aquatic Bed

III. Linear Model of Dengue and Environmental Factors

Linear models were used to assess the influence of regional climate on dengue fever incidence. After the data were processed and organized, general trends were identified, and single variable correlation tests were run to identify if, and which, relationships were significant (α level 0.05). The Pearson Product-Moment Correlation Coefficient (r) was calculated between dengue incidence and each environmental variable. These relationships were analyzed at the annual, and monthly temporal scale as well as intermediate (regional) and coarse (whole island) spatial scales. Several variations of this model were run in order to consider a variety of variable combinations.

IV. Land Use Land Class and Dengue Incidence

Using a Multiple Linear Regression (MLR) the relationship between incidence and land use categories and population variables at the municipality scale was explored. Model selection was conducted using backward elimination and minimization of the Akaike Information Criteria (AIC). Based on the methods described in Farroway 2006.

First, the relationships between landscape and dengue incidence in the years surrounding 2000 and 2010 were modeled. Dengue incidence was the dependent variable and LULC and housing and population densities were the independent variables. For the 2000 analysis, the years 1999-2003 were averaged for dengue incidence. Population and housing densities from the 2000 census, and LULC from 2001 were used. The 2010 analysis considers dengue incidence for 2009-2013, population and housing densities from 2010, and LULC from 2011.

Next, changes in landscape and population were examined with respect to change in dengue incidence between 2000 and 2010. As with the first analysis, change in incidence is the dependent variable and change in LULC and housing and population densities are the independent variables. Changes for each variable category were calculated as follows:

(Equation 1):

$$\Delta Prevalence = [Average\ Incidence\ 2009 - 2013 \left(\frac{cases}{100k\ people} \right)] \\ - [Average\ Incidence\ 1999 - 2003 \left(\frac{cases}{100k\ people} \right)]$$

$$(2) \ \Delta LULC\ Categories = 2011\ Area\ (sq\ mi) - 2001\ Area\ (sq\ mi)$$

$$(3) \Delta \textit{Housing Density} \\ = 2010 \textit{ Density (per sq mi)} - 2000 \textit{ Density (per sq mi)}$$

$$(4) \Delta \textit{Population Density} \\ = 2010 \textit{ Density (per sq mi)} - 2000 \textit{ Density (per sq mi)}$$

V. Land Use, Climate, and Dengue Incidence

Finally, environmental data were added to the MLR so that the possibility of a combined environmental and LULC effect could be investigated. Once again, the time periods were first considered individually before analyzing change over time. Annual cumulative precipitation, annual average monthly precipitation, annual average maximum temperature, and annual average minimum temperature were included in these models. The environmental data were averaged in the same manner as the dengue incidence and the change in environmental data were calculated as follows:

$$(5) \Delta \textit{Cumulative Precipitation} \\ = [\textit{Average Cumulative Precipitation 2009} - 2013] \\ - [\textit{Average Cumulative Precipitation 1999} - 2003]$$

$$(6) \Delta \textit{Average Monthly Precipitation} \\ = [\textit{Average Monthly Precipitation 2009} - 2013] \\ - [\textit{Average Monthly Precipitation 1999} - 2003]$$

$$(7) \Delta \textit{Maximum Temperature (Tmax)} \\ = [\textit{Average Tmax 2009} - 2013] - [\textit{Average Tmax 1999} - 2003]$$

$$(8) \Delta \textit{Minimum Temperature (Tmin)} \\ = [\textit{Average Tmin 2009} - 2013] - [\textit{Average Tmin 1999} - 2003]$$

CHAPTER 4

RESULTS/FINDINGS

I. Introductory calculations:

During the initial processing, averages were calculated for the environmental variables and dengue case data. These monthly averages for incidence, precipitation, maximum and minimum temperature revealed some interesting regional variations. Starting with regional average monthly dengue incidence, displayed in Figure 3, one can see that the West region has a much higher incidence rate than the other regions. In addition, the curve itself is also different. Unlike the other regions, whose incidence rates peak and stay elevated through the summer, incidence in the West begins to decrease immediately after the peak. The West also increased much faster during June and July than the other regions. Other notable trends in Figure 3 include the much lower incidence in the East region, the similarity in incidence rates between all 6 regions during the first 5 months of the year, and the double peaks in the North and East region curves.

Figure 3: Average monthly disease incidence for each region of Puerto Rico from 1990-2014

The monthly average precipitation curves also reveal regional differences. The Central region receives the highest rainfall amounts during the wet season compared to the other 5 regions. It is also important to note the spike in rainfall during the month of May, this spike may be biologically relevant to mosquito development as mosquitoes that emerge during this time may begin to spread dengue in the early summer months. All the regions appear to have similar rainfall amounts in the first half of the year, while there is more variation in their monthly averages in the second half of the year.

Figure 4: Average monthly rainfall for each region of Puerto Rico from 1990-2014

The temperature profiles have less variation than precipitation and incidence curves. The regions with the highest average monthly maximum temperature are the West and Metro San Juan regions, though it is important to note that all the regions have monthly maximum temperatures that can support mosquito development. The lowest mean maximum temperatures are in the Central region, due to its higher elevation. The South region has a slightly different maximum temperature signature that levels off during November and December instead of decreasing like the other regions. The monthly average minimum temperatures revealed a larger variation in temperature throughout the year, approximately 9-11 degrees, as opposed to the 5-7 degree range seen in the maximum temperatures. The Central region, once again, has the lowest temperatures while the East region has the highest monthly minimum. The

minimum temperatures are also evenly spaced, not grouped together as were some of the monthly average maximum temperatures. The inferences that can be made from these simple monthly averages are helpful in interpreting the results from the remaining rounds of analysis.

Figure 5: Average monthly maximum temperature for each region of Puerto Rico from 1990-2014

Figure 6: Average monthly minimum temperature for each region of Puerto Rico from 1990-2014

II. Linear Model of Dengue and Environmental Factors

Based on a linear model, relationships between environmental factors (just precipitation or just temperature) and dengue incidence were, in most cases, weak. One of the highest correlations was between minimum temperature and dengue incidence in the Central region. Since temperature affects mosquito development (Couret & Benedict 2014, Brady et al 2013, Harrington et al 2008), and the high elevations of the Central region mean that this region sees the lowest minimum temperatures on the island.

Various spatial and temporal scales were used in the linear models between environmental factors and dengue incidence. At the coarsest spatial and temporal scales (whole island and annual, respectively), the overall correlation coefficients (r -values) were very weak ($r=0.284$, Table 3). When the temporal scale was reduced to

the monthly level, the coefficients were much higher. Here the strongest relationship was between dengue and minimum temperature at a lag of two months ($r=0.401$, Table 4).

Table 3: Correlation coefficients for the linear model of cumulative annual dengue for the entire island vs annual average Precipitation (Precip), Maximum Temperature (Tmax), and Minimum temperature (Tmin) for the entire island for each year from 1990-2014

Dengue vs.	Correlation coefficient (r)
Precip	0.218
Tmax	0.254
Tmin	0.284

Table 4: Correlation coefficients for the linear model of monthly dengue for the entire island vs monthly average precipitation (Precip), Maximum Temperature (Tmax), and Minimum temperature (Tmin) for the entire island for each month from 1990-2014

	Number of Months Lagged			
Dengue vs	0	1	2	3
Precip	0.175	0.234	0.238	0.165
Tmax	0.255	0.353	0.388	0.345
Tmin	0.260	0.369	0.401	0.340

Refining the spatial scale into the six regions improved some correlation coefficients, but not all. There were distinct differences in each region and the strongest relationships were at a two-month lag. The Central region had the highest correlations between dengue incidence and maximum and minimum temperatures, with $r=0.406$ and $r=0.446$, respectively (Table 5). While none of these relationships are particularly strong, there were regional differences in the correlations between environment and incidence.

Table 5: Correlation coefficients for the linear model of monthly dengue vs monthly average precipitation (Precip), Maximum Temperature (Tmax), and Minimum temperature (Tmin) for each region and month from 1990-2014 at 0,1,2, and 3 months lag.

Region	Dengue vs	Number of Months Lagged			
		0	1	2	3
North	Precip	0.094	0.139	0.174	0.146
	Tmax	0.187	0.276	0.315	0.289
	Tmin	0.252	0.347	0.374	0.325
Metro San Juan	Precip	0.085	0.174	0.156	0.104
	Tmax	0.094	0.191	0.274	0.293
	Tmin	0.177	0.293	0.359	0.345
East	Precip	0.162	0.233	0.244	0.154
	Tmax	0.157	0.241	0.320	0.320
	Tmin	0.001	0.158	0.230	0.219
South	Precip	0.135	0.195	0.181	0.100
	Tmax	0.263	0.278	0.242	0.192
	Tmin	0.185	0.261	0.267	0.224
Central	Precip	0.180	0.174	0.175	0.101
	Tmax	0.206	0.354	0.406	0.368
	Tmin	0.228	0.379	0.446	0.400
West	Precip	0.162	0.195	0.184	0.100
	Tmax	0.250	0.290	0.273	0.208
	Tmin	0.245	0.297	0.291	0.223

III. Land Use Land Class and Dengue Incidence

The first step of the MLR analysis was investigating the relationships between LULC and dengue incidence at two separate time periods (2000/2001 and 2010/2011). This analysis explores the impacts of anthropogenic factors at a stationary point in time at the municipality scale. The stepwise selection process for the 2000/2001 model can be seen in Table 5.

Table 6: Stepwise model selection for dengue incidence as relates to LULC for the years 2000/2001

Model	AIC	Δ AIC
Inc: Reg, W, Dev, B, F, S,	502.65	14.61

Herb, P, C, Hous, Pop, Hous:Pop,		
Inc: W, Dev, B, F, S, Herb, P, C, Hous, Pop, Hous:Pop,	500.09	12.05
Inc: W, Dev, B, F, S, Herb, P, C, Hous, Pop	498.09	10.05
Inc: W, Dev, F, S, Herb, P, C, Hous, Pop	496.09	8.05
Inc: W, Dev, F, S, P, C, Hous, Pop	494.23	6.19
Inc: Dev, F, S, P, C, Hous, Pop	492.49	4.45
Inc: Dev, F, S, P, C, Pop	491	2.96
Inc: Dev, F, S, P, C	489.26	1.22
Inc: Dev, F, S, C	488.03	-0.01
Inc: Dev, S, C	487.69	-0.35
Inc: S, C	487.85	-0.19
Inc: S	488.04	0

For the years surrounding 2000, the backwards model selection resulted in a best fit model (AIC= 488.04) of shrub and crop cover (Table 6). The resulting incidence equation is as follows:

$$\begin{aligned}
 \text{Dengue Incidence} \\
 &= 49.913 - 6.302(\text{Area of Shrub land}) \\
 &\quad - 1.888(\text{Area of Crop Cover})
 \end{aligned}$$

In this model, shrub and crop cover were related to lower dengue incidence. For shrub cover, dengue incidence decreased by ≈ 6 cases per 100k people for every additional square mile of shrub land. Adjusted R^2 for this model was 0.153 and shrub was a significant predictor of dengue incidence.

Table 7: Model summary for dengue incidence as relates to LULC for the years 2000/2001

Model	Multiple R Square	Adjusted R Square	AIC	
Incidence ~ Shrub + Crops	0.176	0.153	488.04	

Coefficient	Estimate (β)	Std. Error	t-value	Pr ($> t$)
(Intercept)	49.913	3.818	13.073	0.000
Shrub	-6.302	1.959	-3.217	0.002
Crop	-1.888	1.242	-1.520	0.133

The same process was used to analyze the 2010/2011 data. The stepwise selection for this model can be found in Table 7, and the summary of the model coefficients in Table 8. The best fit model for the 2010/2011 data included region, forest, shrub and population density (AIC= 676.64) and had an adjusted R^2 value of 0.293. As with the previous model, coefficients with positive β -estimates indicate positive associations with dengue incidence, and negative β -estimates are related with decreasing incidence. In this analysis, all models compare individual regions with the Central region. For example, in this model, municipalities in the West have 173 times more cases than those in the Central region. The North, South, West, and Metro San Juan regions all have higher cases than the Central region, while the East experiences fewer cases. Forest cover correlated with increased incidence, but shrub cover and population density corresponded to decreased incidence. The South region, West region, and area of forest and shrub cover were all significant predictors of dengue incidence.

Table 8: Stepwise model selection summary for dengue incidence as relates to LULC for the years 2010/2011

Model	AIC	ΔAIC
Inc: Reg, W, Dev, B, F, S, Herb, P, C, Hous, Pop, Hous:Pop,	685.87	-2.42
Inc: Reg, W, Dev, F, S, Herb, P, C, Hous, Pop,	683.93	-4.36

Hous:Pop,		
Inc: Reg, W, Dev, F, S, Herb, P, Hous, Pop, Hous:Pop,	682.03	-6.26
Inc: Reg, W, Dev, F, S, P, Hous, Pop, Hous:Pop,	680.17	-8.12
Inc: Reg, W, Dev, F, S, P, Hous, Pop	678.38	-9.91
Inc: Reg, W, F, S, P, Hous, Pop	677.09	-11.20
Inc: Reg, F, S, P, Hous, Pop	675.78	-12.51
Inc: Reg, F, S, P, Pop	675.98	-12.31
Inc: Reg, F, S, Pop	675.99	-12.30
Inc: Reg, F, S	676.64	-11.65
Inc: Reg, S	679.11	-9.18
Inc: Reg	688.29	0

Table 9: Model summary for dengue incidence as it relates to LULC for the years 2010/2011

Model	Multiple R Square	Adjusted R Square	AIC	
Incidence ~ Region+ Forest+ Shrub + Population density	0.3686	0.2932	676.64	
Coefficient	Estimate (β)	Std. Error	t-value	Pr (> t)
(Intercept)	134.628	34.230	3.933	0.000
East Region	-13.521	32.569	-0.415	0.679
Metro San Juan Region	108.691	67.043	1.621	0.110
North Region	72.733	36.826	1.975	0.052
South Region	90.754	35.022	2.591	0.012
West Region	173.095	32.022	5.405	0.000

Forest	1.599	0.755	2.118	0.038
Shrub	-14.277	4.128	-3.458	0.000
Population density	-0.01667	0.014	-1.209	0.231

Next, the impact of changes in LULC on dengue incidence over time was analyzed. The model that best fit change in dengue incidence over time included the factors region, water, herbaceous, and crop land cover (AIC= 657.64). In this model, the adjusted R^2 value was 0.316, and the South and West regions, area of water, and crop cover were all significant indicators of incidence (Table 10).

In the change analysis models, positive β -estimates have positive correlations between change in LULC and change in incidence, and negative β -estimates have negative correlations between the two. For example, a reduction in the area of water would result in a decrease in incidence, where as an increase in water leads to an increase in incidence. According to this model there is an increase of ≈ 55 cases per 100k people for every 1 sq mile increase in the area of water. As with the 2010/2011 model, the West region has a much higher rate of dengue incidence compared with the Central region.

Table 10: Stepwise selection summary for change in dengue incidence as it relates to LULC over the time period of 2000-2010

Model	AIC	ΔAIC
Inc: Reg, W, Dev, B, F, S, Herb, P, C, Hous, Pop, Hous:Pop,	670.28	-10.35
Inc: Reg, W, B, F, S, Herb, P, C, Hous, Pop, Hous:Pop,	668.52	-12.11
Inc: Reg, W, F, S, Herb, P, C, Hous, Pop, Hous:Pop,	666.81	-13.82

Inc: Reg, W, S, Herb, P, C, Hous, Pop, Hous:Pop,	663.72	-16.91
Inc: Reg, W, Herb, C, Hous, Pop, Hous:Pop,	662.25	-18.38
Inc: Reg, W, Herb, C, Hous, Pop	661.04	-19.59
Inc: Reg, W, Herb, C, Pop	659.07	-21.56
Inc: Reg, W, Herb, C,	657.64	-22.99
Inc: Reg, W, C	658.94	-21.69
Inc: Reg, C	661.33	-19.30
Inc: Reg	680.63	0

Table 11: Model summary for change in dengue incidence as it relates to LULC over the time period of 2000-2010

Model	Multiple R Square	Adjusted R Square	AIC	
Δ Incidence ~ Region + Water + Herbaceous + Crops	0.389	0.316	657.64	
Coefficient	Estimate (β)	Std. Error	t-value	Pr (> t)
(Intercept)	90.106	24.147	3.732	0.000
East Region	8.874	30.736	0.289	0.774
Metro San Juan Region	52.946	35.725	1.482	0.143
North Region	42.661	31.085	1.372	0.175
South Region	48.427	27.302	1.774	0.081
West Region	139.740	25.035	5.582	.000
Water	55.370	24.276	2.281	.026

Herbaceous	2.090	1.212	1.724	0.089
Crops	14.875	6.324	2.352	0.022

I. Land Use, Climate, and Dengue Incidence

Adding the environmental factors to the MLR analysis allows us to analyze the combined effect of climate and land cover. For 2000/2001, the best fit model included: shrub, and open development (AIC= 489.22) and had an adjusted R^2 value of 0.1513. The stepwise selection process for this can be seen in Table 11. For this model, shrub was a significant indicator of dengue incidence. Shrub, and developed open cover corresponded with decreases in incidence.

Table 12: Stepwise selection summary of model relating dengue to LULC and environmental factors for the years 2000/2001

Model	AIC	ΔAIC
Inc: Reg, W, Dev, B, F, S, Herb, P, C, Hous, Pop, Hous:Pop, AvgPrcp, Tmax, Tmin	501.9	12.68
Inc: Reg, W, Dev, B, F, S, Herb, P, C, Hous, Pop, AvgPrcp, Tmax, Tmin	499.91	10.69
Inc: Reg, W, Dev, B, F, S, P, C, Hous, Pop, AvgPrcp, Tmax, Tmin	498.05	8.83
Inc: Reg, W, Dev, B, F, S, C, Hous, Pop, AvgPrcp, Tmax, Tmin	496.37	7.15
Inc: Reg, W, Dev, F, S, C, Hous, Pop, AvgPrcp, Tmax, Tmin	494.7	5.48
Inc: Reg, Dev, F, S, C,	493.06	3.84

Hous, Pop, AvgPrcp, Tmax, Tmin		
Inc: Dev, F, S, C, Hous, Pop, AvgPrcp, Tmax, Tmin	491.9	2.68
Inc: Dev, F, S, C, Pop, AvgPrcp, Tmax, Tmin	490.31	1.09
Inc: Dev, F, S, C, Pop, Tmax, Tmin	489.28	0.06
Inc: Dev, F, S, C, Pop, Tmax,	487.81	-1.41
Inc: Dev, F, S, C, Tmax	486.49	-2.73
Inc: Dev, S, C, Tmax	487.16	-2.06
Inc: Dev, S, Tmax	487.56	-1.66
Inc: Dev, S	488.03	-1.19
Inc: S	489.22	0

Table 13: Summary of model relating dengue to LULC and environmental factors for the years 2000/2001

Model	Multiple R Square	Adjusted R Square	AIC	
Incidence ~ Shrub + DevOpen	0.1739	0.1513	489.22	
Coefficient	Estimate (β)	Std. Error	t-value	Pr (> t)
(Intercept)	55.188	5.829	9.468	0.000
Shrub	-7.125	1.916	-3.719	0.000
Developed Open	-4.515	3.101	-1456	0.150

The best fit model for the 2010/2011 data included: region, average annual maximum temperature, forest, and shrub cover (AIC= 676.29). This model had an adjusted R^2 value of 0.32 and the South and West regions as well as maximum

temperature, forest, and shrub were all significant indicators of dengue incidence. According to this model, for every 1°F increase in average annual maximum temperature there is an increase of ≈ 40 cases of dengue per 100k people. As with previous models, the West region continues to have a significantly higher incidence and decreases in shrub cover correlate with decreases in incidence. The summary of the stepwise selection process can be seen in Table 13 and the summary of the coefficients can be seen in Table 14.

Table 14: Stepwise selection summary of model relating dengue to LULC and environmental factors for the years 2010/2011

Model	AIC	ΔAIC
Inc: Reg, W, Dev, B, F, S, Herb, P, C, Hous, Pop, Hous:Pop, AvgPrcp, Tmax, Tmin	501.9	12.68
Inc: Reg, W, Dev, B, F, S, Herb, P, C, Hous, Pop, AvgPrcp, Tmax, Tmin	499.91	10.69
Inc: Reg, W, Dev, B, F, S, P, C, Hous, Pop, AvgPrcp, Tmax, Tmin	498.05	8.83
Inc: Reg, W, Dev, B, F, S, C, Hous, Pop, AvgPrcp, Tmax, Tmin	496.37	7.15
Inc: Reg, W, Dev, F, S, C, Hous, Pop, AvgPrcp, Tmax, Tmin	494.7	5.48
Inc: Reg, Dev, F, S, C, Hous, Pop, AvgPrcp, Tmax, Tmin	493.06	3.84
Inc: Dev, F, S, C, Hous, Pop, AvgPrcp, Tmax, Tmin	491.9	2.68
Inc: Dev, F, S, C, Pop, AvgPrcp, Tmax, Tmin	490.31	1.09

Inc: Dev, F, S, C, Pop, Tmax, Tmin	489.28	0.06
Inc: Dev, F, S, C, Pop, Tmax	487.81	-1.41
Inc: Dev, F, S, C, Tmax	486.49	-2.73
Inc: Dev, S, C, Tmax	487.16	-2.06
Inc: Dev, S, Tmax	487.56	-1.66
Inc: Dev, S	488.03	-1.19
Inc: S	489.22	0

Table 15: Summary of model relating dengue to LULC and environmental factors for the years 2010/2011

Model	Multiple R Square	Adjusted R Square	AIC	
Incidence ~ Region + Tmax + Forest + Shrub	0.3927	0.3202	676.29	
Coefficient	Estimate (β)	Std. Error	t-value	Pr (> t)
(Intercept)	-3276.691	1659.885	-1.974	0.053
East Region	-32.158	33.276	-0.966	0.337
Metro San Juan Region	8.757	42.615	0.205	0.838
North Region	55.832	35.772	1.561	0.123
South Region	84.436	34.446	2.451	0.017
West Region	145.736	33.793	4.313	0.000
Maximum Temperature	39.6087	19.3815	2.044	0.045
Forest	2.029	0.753	2.695	0.009
Shrub	-12.548	3.987	-3.148	0.002

Finally, we modeled the impact of change in LULC and environmental factors on the change in dengue incidence. The best fit model for this analysis (AIC= 648.82) included: average annual maximum temperature, average annual minimum temperature, crop cover, pasture, and herbaceous cover, and had an R^2 value of 0.343 (Table 16). For this model, an increase in minimum temperature and a decrease in maximum temperature by 1°F both increase incidence by ≈ 140 cases per 100k people. In addition, incidence is positively correlated with crop, herbaceous, and pasture LULC. Minimum and maximum temperature, crop, herbaceous, and pasture cover are all significant indicators of incidence in this model. The stepwise selection summary for this model can be found in Table 15.

Table 16: Stepwise selection summary for change in dengue incidence as it relates to LULC and environmental factors over the time period of 2000-2010

Model	AIC	ΔAIC
Inc: Reg, W, Dev, B, F, S, Herb, P, C, Hous, Pop, Hous:Pop, AvgPrcp, Tmax, Tmin	658.26	1.17
Inc: W, Dev, B, F, S, Herb, P, C, Hous, Pop, Hous:Pop, AvgPrcp, Tmax, Tmin	652.41	-4.68
Inc: W, Dev, B, F, Herb, P, C, Hous, Pop, Hous:Pop, AvgPrcp, Tmax, Tmin	650.45	-6.64
Inc: W, Dev, F, Herb, P, C, Hous, Pop, Hous:Pop, AvgPrcp, Tmax, Tmin	648.54	-8.55
Inc: W, F, Herb, P, C, Hous, Pop, Hous:Pop, AvgPrcp, Tmax, Tmin	647.2	-9.89
Inc: W, Herb, P, C, Hous, Pop, Hous:Pop, AvgPrcp, Tmax, Tmin	647.48	-9.61
Inc: W, Herb, P, C,	647.84	-9.25

AvgPrcp, Tmax, Tmin		
Inc: W, Herb, P, C, Tmax, Tmin	648.36	-8.73
Inc: Herb, P, C, Tmax, Tmin	648.42	-8.67
Inc: Herb, C, Tmax, Tmin	651.21	-5.88
Inc: C, Tmax, Tmin	651.90	-5.19
Inc: Tmax, Tmin	654.50	-2.59
Inc: Tmin	657.09	0

Table 17: Model summary for change in dengue incidence as it relates to LULC and environmental factors over the time period of 2000-2010

Model	Multiple R Square	Adjusted R Square	AIC	
Δ Incidence ~ Tmin + Tmax + Crops + Herbaceous + Pasture	0.3869	0.3431	651.21	
Coefficient	Estimate (β)	Std. Error	t-value	Pr (> t)
(Intercept)	80.891	20.055	4.033	0.000
Minimum Temperature	142.195	28.370	5.012	0.000
Maximum Temperature	-144.488	33.797	-4.275	0.000
Crops	15.782	6.598	2.392	0.019
Herbaceous	6.638	2.327	2.853	0.006
Pasture	18.467	6.071	3.042	0.003

CHAPTER 5

DISCUSSION & CONCLUSION

The purpose of this study is to assess the impact of environmental and anthropogenic factors on dengue fever epidemiology at various spatial and temporal scales on the island of Puerto Rico. Specifically, we addressed the influence of regional climate and landcover on dengue transmission, at both stationary periods of time, and over the course of a decade.

I. Linear Model of Dengue and Environmental Factors

We considered the impact of climate on dengue transmission over the entire island and at the regional scale. The linear model analysis resulted in weak correlation coefficients. This result is supported by previous studies that have shown that there is not one single environmental variable driving dengue transmission, but a combination of factors (Barrera 2011, Johansson et al 2009, Morin 2015, Jury 2008, Lu et al 2007, Stoddard et al 2014). However, regional divisions, showed stronger correlations. The most notable example of this is the relationship between maximum and minimum temperatures and the Central region. Because the Central region has a much higher elevation than the rest of the island, it experiences lower temperatures. This is the only place on the island where temperatures could get low enough to impact mosquito development and survival and therefore dengue transmission. It is likely that the relationship between environmental variables and dengue transmission are not linear,

which may account for the weakness of the correlation coefficients. The purpose of these linear models was to assess preliminary relationships between environmental factors and dengue incidence at a regional scale. Future studies should investigate non-linear modeling techniques.

The linear models had the strongest correlation coefficients at a two-month lag time. Based on the time required for a mosquito to develop from eggs to the first signs of symptoms in a human, we can estimate that the strongest correlations take place between 6-8 weeks' lag (CDC 2012, CDC 2016). However, since the dengue case data are only available at the monthly scale, this is the smallest resolution possible for our analysis. For subsequent studies, being able to analyze all variables at a weekly level would be ideal as this would allow for more precise analyses. It is also important to note that this study does not consider autocorrelation.

The regional definitions used in this study may also impact the strength of the correlations between environmental variables and dengue incidence. The regions presented in this analysis were defined mainly on geographical location and general climate trends. A more qualitative definition, that used specific climatic or LULC criteria to group together municipalities that have similar characteristics, may result in stronger results. Based on the NOAA climate zones presented in Figure 1, the West region, in particular, has a diverse climate. It may be best to split the West into at least two separate regions, Northwest and Southwest, to better capture these differences.

II. Land Cover, Climate, and Dengue Incidence

Table 18: Summary of MLR model results

Final Best Fit Model	Years Considered	Variables Considered	Adjusted R²
Incidence ~ Shrub + Crops	2000/2001	LULC only	0.153
Incidence ~ Region+ Forest+ Shrub + Population density	2010/2011	LULC only	0.293
Δ Incidence ~ Region + Water + Herbaceous + Crops	2000-2011	LULC only	0.316
Incidence ~ Shrub + DevOpen	2000/2001	LULC and Environmental	0.151
Incidence ~ Region + Tmax + Forest + Shrub	2010/2011	LULC and Environmental	0.320
Δ Incidence ~ Tmin + Tmax + Crops + Herbaceous + Pasture	2000-2011	LULC and Environmental	0.343

First, we considered LULC and dengue incidence without environmental factors to determine the role these play in transmission dynamics. Increased shrub cover was consistently related to decreases in incidence during the stationary single year analyses. However, increased herbaceous landcover corresponded to increased dengue. These two factors are both related to open space and seem to contradict one another. However, the way that people interact with these spaces could lead to a clearer understanding of these relationships. Future analyses should consider the interaction between patches of landcover to determine, for example, if herbaceous landcover is more frequently found near densely populated areas.

An unexpected result from the 2010/2011 LULC analysis was the relationship between dengue incidence and population density. In the best fit model, population

density had a negative β -estimate meaning that an increase in population density would lead to a decrease in dengue incidence. This is surprising because, as previously discussed, *Ae. aegypti* are known to live in residential areas. The coefficient for this relationship is very small and it was not a significant indicator for incidence, so it may not have a substantial impact on transmission modeling, but was an unanticipated result, nevertheless.

After adding the environmental variables to the MLR analysis, LULC factors were still significant. This is important because it shows that LULC plays a role in transmission, and its factors were not completely masked by the inclusion of environmental variables that are already known to impact dengue epidemiology. The combined LULC and environmental models also produced the best fit model with the highest R^2 value of 0.343. Because environmental factors are known to impact dengue incidence, it is not unexpected that LULC and environmental factors combined would result in the stronger relationships than LULC alone.

The role of the environmental factors in the MLR analysis was varied. Overall, precipitation seemed to have a greater impact on the stationary/single year models, while temperature was more significant in the change analysis model. This relationship suggests that the effect of precipitation on dengue incidence is more immediate, while long term changes in temperature effect transmission dynamics over time. The precipitation coefficients, however, seemed extreme in comparison to the other factors. For example, in the 2000/2001 stationary model the β -estimate for average monthly precipitation was 2763, meaning that for every 1mm increase in average monthly precipitation for a single year, there would be an increase of 2763

cases per 100k people. This number is an order of magnitude larger than any other β -estimate in this study and may be a source of error. In addition, for the 2000/2001 analysis, average monthly precipitation corresponded with an increase in incidence, but cumulative precipitation corresponded with decreased incidence.

There was a similar contradictory result for maximum temperature. In the 2010/2011 analysis, every increase of 1°F resulted in an increase of ≈ 40 cases per 100k people. But, in the change analysis maximum temperature had a negative β -estimate meaning a decrease in maximum temperature over time resulted in higher incidence, and an increase in maximum temperature resulted in a decrease in incidence. Because of the impact of temperature on mosquito survival rates, one explanation for this result is that a slightly lower maximum temperature would cause the mosquito to go through its life cycle at a slower rate. If the mosquitoes live longer, they will take more blood meals, and have the opportunity to infect more people. Subsequent studies could try and determine the optimal temperature for mosquito populations and dengue transmission in Puerto Rico.

Region was included in the best fit models for both 2010 analyses and the change analysis without environmental variables. It is possible that the introduction of environmental variables masked the effect of the regions in the final change analysis. The West and South region were both significant indicators of incidence. The MLR analysis also confirmed the result from the initial incidence averages which showed a higher incidence rate in the West compared to the other regions (Figure 3). Since region is significant in half of the models, future studies should perform a MLR analysis for each region separately to identify whether there are differences in the

relationships between LULC, environmental variables, and dengue incidence when regions are isolated.

This analysis did not exclude epidemic years from the models. One of the largest epidemics in Puerto Rico's history took place in 2010 when nearly 1% of the island's inhabitants were infected. Due to the vast variability between outbreak and no-outbreak years, relationships between LULC and environmental factors and dengue incidence may be masked. In the future, studies could exclude this year or find an alternative way to include it in the analysis.

Other sources of error include human error in initial data processing as well as the variations in the classifications used by the two LULC products. The main difference between the LULC datasets was the specificity of the categories. The 2001 categories were more general, especially the wetlands descriptions. The 2011 category had six extra class descriptions for wetlands, and less categories for urban development. Though both maps were created by the same group, and are intended for LULC comparison, it is possible that the change in classification titles could impact the calculated change over time since one is more general and the other very specific.

III. Future Directions

The results from these analyses show the need for more refined studies on the relationships between LULC and climate at an intermediate scale. There are several other environmental factors that could be included, such as soil moisture, humidity, sea surface temperature (SST), diurnal temperature range, and normalized difference vegetation index (NDVI) that would take advantage of remote sensing (RS)

opportunities that are available. RS has been used in a limited capacity to study dengue fever (Little et al 2011, Palaniyandi 2012, Eisen and Lozano-Fuentes 2009), but is a potentially valuable tool whose applications should be further explored. One of the challenges of using remote sensing data for tropical regions, such as Puerto Rico, is finding satellites that can take measurements through cloud cover. While there are a few satellites that are capable of taking ground measurements through cloud cover, such as the Soil Moisture Active Passive (SMAP) instrument, the resolution is too coarse for a small island like Puerto Rico.

One of the most obvious additions would be mosquito population data. We followed the same procedure as Johansson and Jury (2008) and only used confirmed dengue cases, but having data on mosquito populations and their locations would likely allow for more precise models of transmission. Mosquito data would also allow us to capture the impact of regional climate on mosquito populations and therefore dengue transmission.

Future studies would also benefit from the addition of serotype information. Limited serotype information is available through the CDC's Passive Dengue Surveillance System, from which we acquired our case data sets. We were unable to separate dengue serotypes in these analyses, but it would be interesting if transmission patterns differed for different serotypes.

Though not addressed in this study, social, political, and economic factors can also play a role in the transmission of vector-borne illnesses (Bhatt et al 2013, Gubler 2011, Banu et al 2014, Wu et al 2008, Colón-Gonzalez et al 2013, Johansson et al 2009, Moreno-Banda 2017). Low disease priority, changing life styles, and decaying

public health infrastructure have all been identified as elements in the increase of dengue incidence (Gubler 2011). These points have been highlighted during Puerto Rico's ongoing financial crisis. The island is over \$70 billion in debt, sales tax has been raised to 11.5%, and residents are paying up to three times what the average mainland American pays for utilities like water and electricity (A.A.K. 2016). The Bureau of Labor (2017) reports unemployment numbers in Puerto Rico as 12.4% as of December 2016, compared to the continental U.S. average of 4.7% for the same period.

The combined effects of the financial crisis, which have been going on for over a decade, have impacted health systems and infrastructure on the island (A.A.K. 2016, Pérez-Guerra et al. 2009). Some of these issues were discussed in a 2009 study by Pérez-Guerra et al. where survey participants expressed a desire for the government to improve drinking water systems, solid waste collection services, and develop better recycling strategies to help combat mosquito breeding grounds on the island. The survey also revealed that compared to other diseases, dengue was not perceived as important, and therefore citizens were less apt to remove potential breeding sites from in, and around, their homes (Pérez-Guerra et al. 2009). In addition, government budget cuts have impacted the island's public health systems and, in some cases, reduced vector control (Gubler 2011, Pérez-Guerra et al. 2009). While this study does not address these social and political issues quantitatively, it is important to be aware of them and their role in disease transmission.

IV. Conclusion

These analyses provide insight into the role of both anthropogenic and environmental variables as they relate to dengue incidence. Initial averages showed a much higher incidence level in the West region (Figure 3). The linear models between each environmental factor and incidence showed the strongest correlations between the Central region and temperature at a two month lag, but overall these relationships were weak (Table 4).

This study showed that the impacts of LULC are not masked by the inclusion of environmental variables in MLR models and are significant indicators of dengue incidence. The MLR models confirmed the higher incidence levels in the West region of the island. The MLR model with the highest R^2 value (0.343) was the best fit change analysis model using both LULC and environmental factors. This best fit model (AIC= 651.21) included: average annual maximum temperature, average annual minimum temperature, crop cover, pasture, and herbaceous cover. Precipitation had a more immediate impact on incidence, while temperature was significant over time. Factors relating to open space were both related to changes in incidence but require further investigation to better understand their role in dengue transmission. The results of this analysis also show the significance of an intermediate regional spatial scale and land cover in dengue epidemiology.

Ensuing studies can refine the variables and factors used here to focus on specific relationships found in these results. Identifying spatial patterns in the LULC data would help to explain the relationship between open space and dengue incidence. Adding additional environmental variables, serotype information, and mosquito

population data would all provide further incites to associations between anthropogenic and environmental factors on dengue transmission in Puerto Rico.

Understanding how changes in land cover and climate impact the spread of dengue can aid government officials in urban planning and vector control. Using current weather data, scientists and health officers can work together to warn the public about impending dengue outbreaks and efficiently administer aid to communities at the greatest risk. Furthermore, the techniques from this analysis, particularly the intermediate spatial scale and use of LULC in dengue incidence modeling, could be applied to other areas of the world. Until a vaccine is available for mass distribution, the world's understanding of dengue transmission will rely on our ability to predict dengue outbreaks using our knowledge of anthropogenic and environmental factors and their impact on dengue fever epidemiology.

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