June 2018

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Scholar Commons Citation
Laureano-Rosario, Abdiel Elias, "Evaluating Beach Water Quality and Dengue Fever Risk Factors by Satellite Remote Sensing and Artificial Neural Networks" (2018). *USF Tampa Graduate Theses and Dissertations.*
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Evaluating Beach Water Quality and Dengue Fever Risk Factors by Satellite Remote Sensing and Artificial Neural Networks

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
with a concentration in Biological Oceanography
College of Marine Science
University of South Florida

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Date of Approval
May 4, 2018

Keywords: *Aedes aegypti*, public health, beach water quality, machine learning, fecal indicator bacteria, ocean color, artificial neural networks

DEDICATION

Esta disertación es dedicada a mi madre y padre, Sol Rosario y Ramon Laureano, quienes hicieron todo en su poder para ayudarme y apoyarme durante este proceso. Gracias a ambos por sus sacrificios desde el primer día, por su apoyo, paciencia y amor durante el tiempo que trabajé mi grado. ¡Los amo!

This dissertation is dedicated to my mother and father, Sol Rosario and Ramon Laureano, who did everything in their power to help me and support me through this process. Thank you both for your sacrifices since day one, for all your support, patience, and love while I was working on my degree. I love you!

ACKNOWLEDGMENTS

I thank my major advisor, Dr. Frank E. Muller-Karger, for his support and encouragement during my time as a student at USF-CMS. Special thanks are also given to my committee members, Dr. Mya Breitbart, Dr. Mark Luther, Dr. Ricardo Izurieta, Dr. James Mihelcic, and Dr. Dragan Savic, for their constant support, help, and contributions to my dissertation work. Furthermore, I also thank my co-authors and collaborators from the Universidad Autonoma de Yucatan, University of Puerto Rico, University of Exeter, and the Instituto Costarricense de Acueductos y Alcantarillados. I acknowledge Mr. Bernard Batson, from the College of Engineering, for his constant support and encouragement through my time at USF. I thank the IMaRS team for their constant support and help.

There is a special place in my heart, and I cannot express how thankful I am for my support group during my Ph.D. journey: D. Chacin, Dr. Freytes-Ortiz, L. Martell, Dr. Vega-Rodriguez, N. Lopez, and Dr. Mendez-Ferrer. Lastly, this work would not have been possible without the financial support from USF-CMS Linton Tibbetts Graduate Endowed Fellowship, Alfred P. Sloan Foundation, DEX Imaging Fellowship, and the NASA Earth and Space Science Fellowship. Images were done with courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/). This material is based upon work supported by the National Science Foundation under Grant No. 1243510. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

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ABSTRACT

Climatic variations, together with large-scale environmental forces and human development affect the quality of coastal recreational waters, creating potential risks to human health. These environmental forces, including increased temperature and precipitation, often promote specific vector-borne diseases in the Caribbean and Gulf of Mexico. Human activities affect water quality through discharges from urban areas, including nutrient and other pollutants derived from wastewater systems. Both water quality of recreational beaches and vector-borne diseases can be better managed by understanding their relationship with local environmental forces.

I evaluated how changes in vector-borne diseases and poor recreational water quality were related to specific environmental factors through the application of satellite-derived observations, field observations, and public health records. Variability in dengue fever incidence rates in coastal towns of the Yucatan Peninsula (Mexico) was evaluated with respect to environmental factors in Chapter Two. Correlations between fecal indicator bacteria concentrations (i.e., culturable enterococci) at Escambron Beach (San Juan, Puerto Rico, USA) and regional environmental factors are discussed in Chapter Three. Predictions of dengue fever occurrences in the Yucatan Peninsula were tested using a nonlinear approach (i.e., Artificial Neural Networks) and are presented in Chapter Four. The Artificial Neural Network (ANN) model was also used to predict culturable enterococci concentration exceeding safe recreational water quality standards in Escambron Beach and results are presented in Chapter Five. Environmental factors assessed to understand their influence on dengue fever occurrences and culturable

enterococci concentrations included precipitation, mean sea level (MSL), air temperatures (e.g., maximum, minimum, and average), humidity, and satellite-derived sea surface temperature (SST), dew point, direct normal irradiance (DNI), and turbidity. These factors were combined with demographic data (e.g., population size) and compared with dengue fever incidence rates and culturable enterococci concentration using linear and nonlinear statistical approaches.

Dengue incidence rates in Yucatan (Mexico) generally increased in July/August and decreased during November/December. A linear regression model showed that previous dengue incidence rates explained 89% of dengue fever variability ($p < 0.05$). Dengue incidence two weeks prior (previous incidence) influences future outbreaks by allowing the virus to continue propagating. Yet dengue incidence was best explained by precipitation, minimum air temperature, humidity, and SST ($p < 0.05$). Dengue incidence variability was best explained by SST and minimum air temperature in our study region ($r = 0.50$ and 0.48, respectively). Increases in SST preceded increased dengue incidence rate by eight weeks. Dengue incidence time series were positively correlated to SST and minimum air temperature anomalies. This is related to the virus and mosquito behavior. Including oceanographic variables among environmental factors in the model improved modelling skill of dengue fever in Mexico.

Chapter Three shows that precipitation, MSL, DNI, SST, and turbidity explained some of the enterococci variation in Escambron Beach surface waters (AIC = 26.76; $r = 0.20$). Variation in these parameters preceded increased culturable enterococci concentrations, with lags spanning from 24 h up to 11 days. The highest influence on culturable enterococci was precipitation between 480 mm–900 mm. Rainy events often result in overflows of sewage systems and other non-point sources near Escambron Beach in Puerto Rico. A significant decrease in culturable enterococci concentrations was observed during increased irradiance (r = -0.24). This may be due to bacterial inactivation. Increased culturable enterococci concentrations were significantly associated with higher turbidity daily anomalies (r = 0.25), in part because bacteria were protected from light inactivation. Increased culturable enterococci concentrations were related to warmer SST anomalies (r = 0.12); this is likely due to increased bacterial activity and reproduction. Higher culturable enterococci concentrations were also significantly correlated to medium to high values of dew point daily anomalies (r = 0.19). A significant decrease in culturable enterococci during higher daily MSL anomalies (r = -0.19) is possibly due to dilution of bacteria in beach waters, whereas during lower MSL anomalies the back-washing promotes increased bacteria concentrations through mixing from sediments. These environmental variables improve our understanding of the ecology of these bacteria over time. The predictive capability increases by including more than one environmental variable.

Chapter Four explains a predictive model of dengue fever occurrences in San Juan, Puerto Rico (1994–2012), and Yucatan (2007–2012). The model was modified to predict dengue fever outbreak occurrences for two population segments: population at risk of infection (i.e., < 24 years old) and vulnerable population (i.e., < 5 years old and > 65 years old). There were a total of four predictive models, two sets for each location using the specified population segments. Model predictions showed previous dengue cases, minimum air temperature, date, and population size as the factors with the most influence to predict dengue fever outbreak occurrences in Mexico. Previous dengue cases, maximum air temperature, date, and population size were the most influential factors for San Juan, Puerto Rico. The models showed an accuracy around 50% and a predictive capability of 70%. These environmental and demographic variables are important primary predictors for dengue fever outbreaks in Puerto Rico and Mexico.

Chapter Five shows the application of the ANNs model to predict culturable enterococci exceedance based on the U.S. Environmental Protection Agency (U.S. EPA) Recreational Water Quality Criteria (RWQC) at Escambron Beach, San Juan, Puerto Rico. The model identified DNI, turbidity, 48 h cumulative precipitation, MSL, and SST as the most influential factors to predict enterococci concentration exceedance, based on the U.S. EPA RWQC at Escambron Beach from 2005–2014. The model showed an accuracy of 76%, with a predictive capability greater than 60%, which is higher than linear models. Results showed the applicability of remote sensing data and ANNs to predict recreational water quality and help improve early warning system and public health.

This work helps to better understand complex relationships between climatic variations and public health issues in tropical coastal areas and provides information that can be used by public health practitioners.

CHAPTER ONE

INTRODUCTION

Overview and objectives

The overall objective of this dissertation was to assess the influence of environmental factors on the variability of dengue fever incidence rates in Mexico and Puerto Rico, and culturable enterococci concentration in Escambron Beach, San Juan, Puerto Rico. The approach included the application of remotely sensed environmental observations, local meteorological information, and public health data in both locations. A nonlinear model, based on Artificial Neural Networks (ANN), was applied to predict dengue fever outbreak occurrences in Mexico and Puerto Rico, as well as culturable enterococci concentration exceedance at Escambron Beach. The work is presented in six chapters.

Chapter One is a general introduction to the dissertation. It describes how environmental factors can influence vector-borne diseases and fecal indicator bacteria in beach environments. The modelling of dengue fever incidence rates by including satellite-derived sea surface temperature (SST) and other environmental factors (i.e., precipitation, humidity, air temperatures) is discussed in Chapter Two. In Chapter Three, satellite-derived data (i.e., turbidity, SST, irradiance) and data on other environmental factors (i.e., mean sea level, dew point, precipitation) were used to better understand variability in fecal indicator bacteria (FIB; i.e., culturable enterococci concentration). In Chapter Four, a non-linear model based on ANN was applied to predict dengue fever occurrences in Mexico and Puerto Rico. The analysis examined specific population segments. Chapter Five used the ANN approach to model culturable enterococci concentration exceedance in Escambron Beach, San Juan, Puerto Rico. The model is based on the U.S. EPA Recreational Water Quality Criteria (RWQC). Chapter Six is a summary of dissertation findings and implications of this work. The research contributes to understanding how environmental factors affect temporal patterns of variability of dengue fever and culturable enterococci concentrations in Mexico and Puerto Rico.

The specific objectives of this dissertation were:

- **Objective 1**: Evaluate and model dengue fever incidence rates in Yucatan, Mexico using regional-scale satellite-derived sea surface temperature.
- **Objective 2**: Evaluate the influence of satellite-derived environmental factors and those measured *in situ* on culturable enterococci concentration at Escambron Beach, San Juan, Puerto Rico.
- **Objective 3**: Apply a nonlinear model based on artificial neural networks to predict dengue fever outbreak occurrences in Mexico and Puerto Rico based on specific population segments.
- **Objective 4**: Identify the most influential environmental factors to predict exceedances of culturable enterococci concentrations at Escambron Beach, San Juan, Puerto Rico.

Environmental forces influence on dengue fever occurrences and recreational water quality

Large-scale environmental forces influence infectious diseases. This is clearly the case in the Caribbean and Gulf of Mexico (Chretien et al. 2015, Dobson 2009). Variability of specific environmental factors affects dengue fever occurrence and water quality of recreational beaches

(Chowell and Sanchez 2006, Pednekar et al. 2005). Thus, it should be possible to develop better management, disease surveillance, and mitigation strategies by understanding the variability of environmental forces and their influence on public-health related issues. In this dissertation, I examined these problems in more detail in the northwest coast of the State of Yucatan, Mexico, and near San Juan, Puerto Rico, USA.

Environmental and demographic factors influence on vector-borne diseases

Human populations in the Caribbean Sea and the Gulf of Mexico have seen an increase in the incidence of vector-borne diseases. Dengue fever cases have increased especially since the 1970s (Dick et al. 2012, Laureano-Rosario et al. 2017, Mendez-Lazaro et al. 2014). This increase is in part due to the adaptation of the mosquito, *Aedes aegypti*, to live in urban areas (Gratz 1991, Gubler 2002). Previous studies have shown the influence of specific environmental and demographic factors on the occurrence of dengue fever cases in places like Yucatan State, Mexico and San Juan, Puerto Rico (Colon-Gonzalez et al. 2011, Colon-Gonzalez et al. 2013, Mendez-Lazaro et al. 2014). Furthermore, local environmental factors and population behavior play a key role in the epidemiology and phenology of dengue fever (Eastin et al. 2014). Consequently, the understanding of the local variability of environmental factors is important to understand their influence on dengue fever occurrences.

Dengue fever is mostly transmitted by *Aedes aegypti*, a mosquito found around tropical and subtropical areas (Gubler 2002). These mosquitoes use water containers (natural and artificial) to develop, being precipitation and temperature the main promoters of their development (Brady et al. 2013, Campbell-Lendrum et al. 2015, Descloux et al. 2012, Johansson et al. 2009). Warmer temperatures decrease mosquito development time, increasing mosquito egg production, hatching, and density (Dickerson 2007). Furthermore, increased temperatures lead to higher metabolic activity, which promotes more mosquito biting (by female mosquitoes) due to energetic demands (Paaijmans et al. 2013). Both Mexico and Puerto Rico have reported *Aedes albopictus* as another vector for dengue fever (Dantes et al. 2014, Dick et al. 2012, Mendez-Lazaro et al. 2014, Stramer et al. 2012). Dengue has four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4; Halstead 1988), which have been reported in both Mexico and Puerto Rico. More recently, studies have shown the emergence of sylvatic dengue 5 (DENV-5; Joob and Wiwanitkit 2016, Mustafa et al. 2015). Peaks in dengue cases usually take place after a shift from one serotype to another, since during this time the population would only be partially immune to the other serotypes (Gubler and Clark 1995, Rothman 2004). Relevant epidemiological studies in Yucatan and Puerto Rico have focused on understanding where *Aedes aegypti*'s larvae are found (e.g., schools, households) and how the disease is transmitted (Baak-Baak et al. 2014a, Baak-Baak et al. 2014b, Garcia-Rejon et al. 2008, Garcia-Rejon et al. 2011). In both tropical locations, dengue fever coincides with periods of higher precipitation, higher SST, higher mean sea level, and higher minimum air temperature along the coast.

Climatic variations are expected to influence the ecology and geographic distribution of vector-borne diseases. Studies have shown how vectors that transmit malaria (i.e., *Anopheles spp*.) have been found in higher altitudes in Africa due to warmer temperatures (Afrane et al. 2007, Afrane et al. 2012, Harvell et al. 2002). Similarly, studies have documented both increases and re-occurrences of vector-borne diseases in Europe due to recent warmer conditions (Medlock and Leach 2015). Nevertheless, these are also affected by human activities such as population movement, farming, dams, and changes in irrigations systems. Therefore, some of these climatic effects might be masked by human activities, including human population movement across the world, leading to further spreading and increasing incidence rates (Campbell-Lendrum et al. 2015).

Modelling dengue fever in endemic areas is important to better mitigate and manage these occurrences. The present work was driven by the hypothesis that variability and trends in environmental factors (e.g., precipitation, temperatures, and humidity) are primary drivers of dengue fever incidence, and that including satellite-derived SST improves dengue fever incidence rate predictions. The objective was to help improve epidemiological surveillance through the combination of oceanographic, meteorological, and long-term epidemiological data.

The influence of environmental factors on fecal indicator bacteria and recreational water quality

Water quality is a major concern to coastal communities due to the potential for exposure to pathogens in beaches downstream of watersheds with sources of fecal contamination (Garcia-Montiel et al. 2014, Pruss 1998, Soderberg 2012). Wastewater discharges are point sources. Other sources include septic tanks and open sewers that discharge directly to river streams. Likewise, resuspension of bacteria by winds and waves, and stormwater discharges are potential non-point sources of fecal contamination in coastal areas (Cordero et al. 2012, Quiñones 2012, Rochelle-Newall et al. 2015).

Fecal indicator bacteria (FIB) are used by the United States Environmental Protection Agency (U.S. EPA) to identify poor recreational water quality. Out of these FIB, culturable enterococci are commonly used in fresh and marine waters (U.S. EPA 2012). The U.S. EPA established the 2012 Recreational Water Quality Criteria (RWQC), where these culturable enterococci cannot exceed the geometric mean of 35 colony forming units (CFU) per 100 mL. This represents 36 illnesses per 1,000 primary contact recreators (U.S. EPA 2012). This value was modified in 2014 to the Beach Action Value (BAV) of 70 CFU/100 mL based on specific criteria for conducting research (U.S. EPA 2014). These guidelines were adopted by the Environmental Quality Board of Puerto Rico (PREQB). In Puerto Rico, the PREQB assesses bathing water quality at beaches throughout the island every two weeks, and if concentrations exceed those values set by the U.S. EPA (i.e., BAV of 70 CFU/100 mL; PREQB 2016), they issue beach advisories. These data are openly available but are only used for issuing public warnings.

FIB variability has been associated with environmental forces in both subtropical and tropical regions (Aranda et al. 2016, Lamparelli et al. 2015, Viau et al. 2011, Wright et al. 2011). These studies have shown how specific environmental factors (e.g., precipitation, turbidity, temperatures) influence higher or lower FIB concentrations in marine and fresh waters (Byappanahalli et al. 2010, He and He 2008, Nevers and Whitman 2005). Therefore, a series of statistical models (e.g., linear and multiple regression models) were used to better understand variability of culturable enterococci concentrations. This was guided by the hypothesis that changes in culturable enterococci concentration in surface waters at Escambron Beach (Puerto Rico) were related to variations of environmental factors (e.g., SST, turbidity, precipitation). The main objective was to improve early warnings for FIB and health risks.

Predicting vector-borne diseases and recreational water quality with Artificial Neural Networks

Predictive models can help improve management and mitigation of health-related matters (de Brauwere et al. 2014, Gonzalez and Noble 2014, Gubler 2010, Tabachnick 2010). In this dissertation, a nonlinear model was used to evaluate prediction of dengue fever outbreaks in endemic areas, as well as exceedances of FIB in tropical areas.

Modelling can help predict and understand the epidemiology of dengue fever in endemic areas (Medeiros et al. 2011, Racloz et al. 2012). Likewise, recreational water quality modelling helps protect humans from potential exposure to specific FIB (Colford et al. 2007, Pruss 1998). For example, some studies have applied Monte Carlo and support vector machine to predict dengue fever cases (Husin et al. 2008, Wu et al. 2008). Similarly, nonlinear modelling using ANNs, decisions trees, and Monte Carlo approaches helped model water quality (Jiang et al. 2013, Lin et al. 2008) and have supported beach management (Mavani et al. 2014, Zhang et al. 1998, Thoe et al. 2014).

Predicting health-related matters is a management goal. These ANN models do not assume functional relationships between predictor factors (e.g., environmental factors) and target variable (e.g., dengue fever, culturable enterococci concentration), thus they can identify nonlinear, complex relationships (Zhang et al. 1998). ANN models were applied to predict dengue fever outbreak occurrences in Mexico and Puerto Rico for specific population segments (i.e., population younger than 24 years and those younger than 5 years and older than 65 years). These ANNs models were also applied to predict culturable enterococci concentration exceedance in surface waters at Escambron Beach in Puerto Rico. The objective was to help management and mitigation of these two health-related matters.

Study areas

Northwest coast, Yucatan State, Mexico

The study was focused on the mainland region in the northwest coastal area of the State of Yucatan, Mexico, located adjacent to the Gulf of Mexico (19.55°N–21.63°N, 87.53°W–90.40°W). The study area has nine municipalities: Chicxulub Pueblo, Dzemul, Hunucma, Ixil, Progreso, Telchac Pueblo, Telchac Puerto, Ucu, and Merida, which is the capital and largest municipality within this region (Figure 1). The highest precipitation occurs between July and October (with an average of 400–700 mm of precipitation over the season). The dry season occurs between March and June (0–50 mm for the season). A third season, the “Nortes” season, is characterized by strong (~80 km h^{-1}) winds coming from the continental mass of the U.S. and associated with cold fronts during November–February. Air temperatures generally range from 36–40 °C during the dry season, 30–35 °C during the rainy season, and 20–23 °C during “Nortes” (Herrera-Silveira 1994).

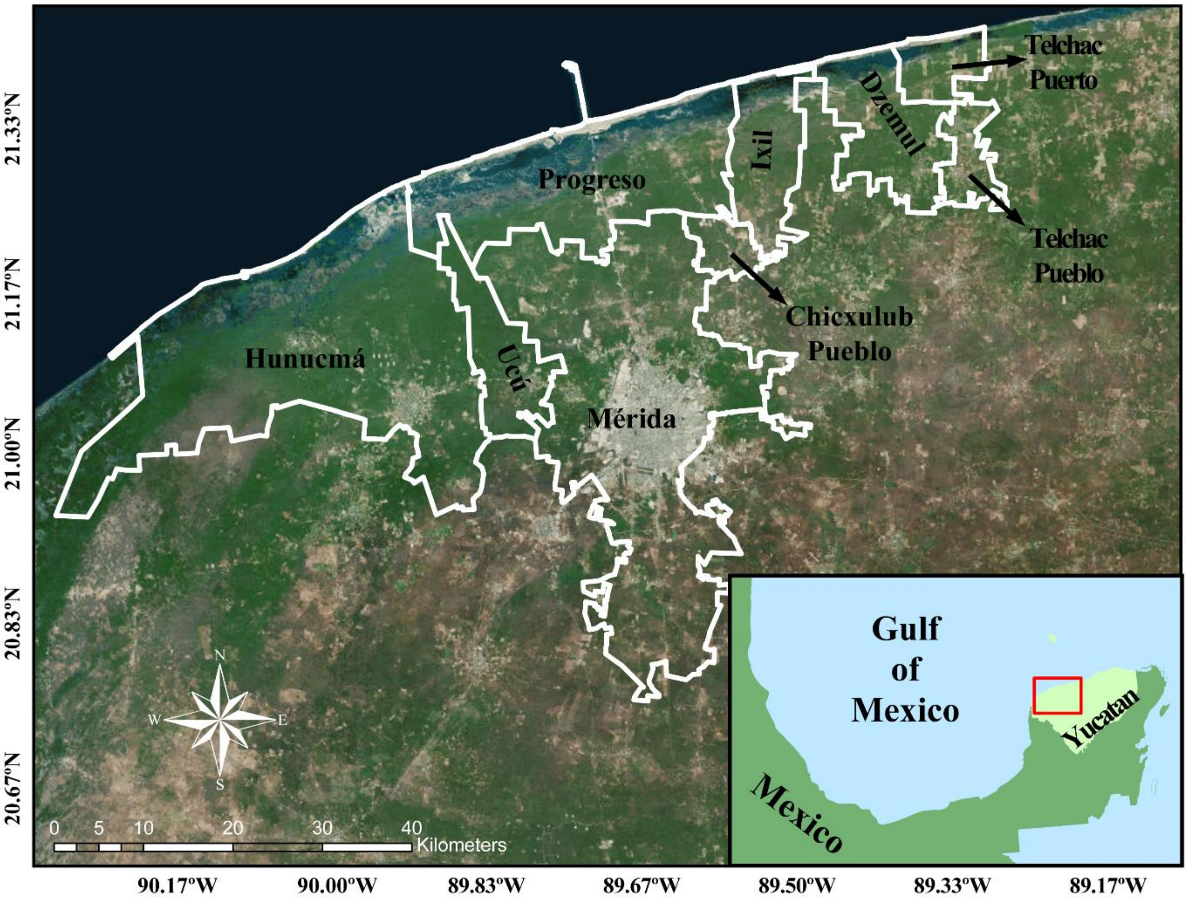

Figure 1.1 Northwest coast of the Yucatan Peninsula, Mexico. Map depicts the location of municipalities used in the study: Chicxulub Pueblo, Dzemul, Hunucma, Ixil, Progreso, Telchac Pueblo, Telchac Puerto, Ucu, and Merida. Modified from *Modelling dengue fever risk in the State of Yucatan, Mexico using regional-scale satellite-derived sea surface temperature* by Laureano-Rosario et al. 2017, published by Acta Tropica 172 pp. 50-57. ©2017 Laureano-Rosario CC-BY-NC-ND. Used with permission (Appendix E).

Escambron Beach, San Juan, Puerto Rico

Escambron beach is located on the north coast of Puerto Rico (18.47°N, 66.08°W, Figure 2). It has a year-long swimming season and the average annual air temperatures range between 24–29 °C (Murphy et al. 2011). Two sites, separated by ~100 m, were sampled by the PREQB (Figure 2). These sites may have been affected by: (1) stormwater drainage (18.46°N, 66.09°W) located immediately adjacent to one of the sampling sites, which includes urban runoff, precipitation, and other graywaters (e.g., showers, washing machines; Diaz 2007); (2) wastewater treatment plant (WWTP) ocean outfall (18.47°N, 66.14°W); (3) beach public bathrooms; and (4) Rio Grande de Loiza, a river that receives agricultural runoff, WWTP effluent (secondary treatment only), and septic system effluent and seepage (PREQB 2011, PREQB 2007, Ortiz-Zayas et al. 2006).

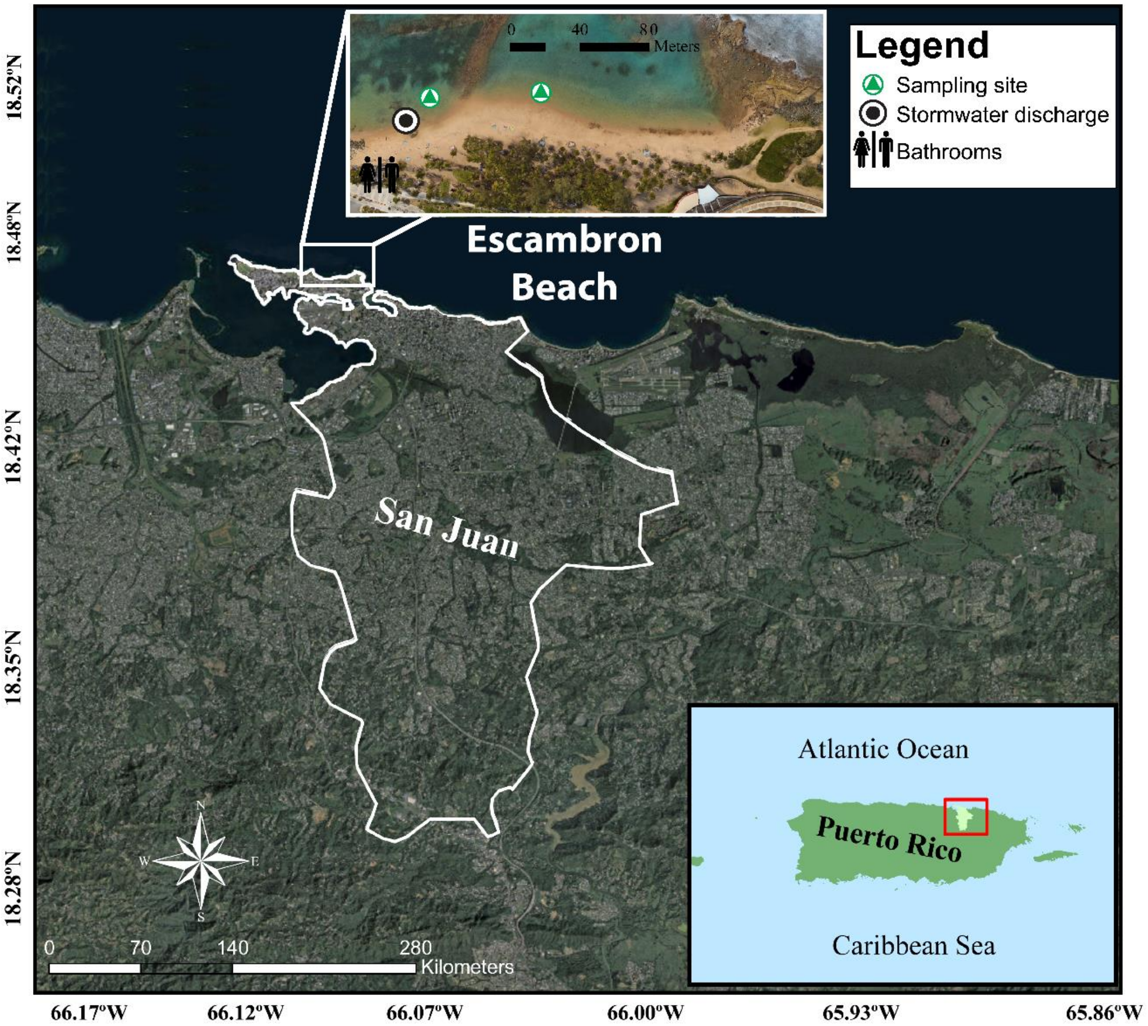

Figure 1.2 San Juan, Puerto Rico. The inset map depicts Escambron Beach study area, both sampling locations (green triangles), stormwater discharge drain (black circle), and public bathrooms (bathroom symbol). Modified from *Environmental Factors Correlated with Culturable Enterococci Concentrations in Tropical Recreational Waters: A Case Study in Escambron Beach, San Juan, Puerto Rico* by Laureano-Rosario et al. 2017, published by International Journal of Environmental Research and Public Health 14(12), 1602. ©2017 Laureano-Rosario CC-BY-4.0. Used with permission (Appendix E).

Literature cited

Afrane, Y. A., Githeko, A. K., & Yan, G. (2012). The ecology of Anopheles mosquitoes under climate change: case studies from the effects of deforestation in East African highlands. Annals of the New York Academy of Sciences, 1249(1), 204-210.

Afrane, Y. A., Zhou, G., Lawson, B. W., Githeko, A. K., & Yan, G. (2007). Life-table analysis of Anopheles arabiensis in western Kenya highlands: effects of land covers on larval and adult survivorship. The American journal of tropical medicine and hygiene, 77(4), 660-666.

Aranda, D., Lopez, J.V., Solo-Gabriele, H.M. and Fleisher, J.M. (2016) Using probabilities of enterococci exceedance and logistic regression to evaluate long term weekly beach monitoring data. J. Water Health 14(1), 81-89.

Baak-Baak, C.M., Arana-Guardia, R., Cigarroa-Toledo, N., Lorono-Pino, M.A., Reyes-Solis, G., Machain-Williams, C., Beaty, B.J., Eisen, L. and Garcia-Rejon, J.E. (2014a) Vacant lots: Productive sites for *Aedes* (Stegomyia) *aegypti* (Diptera: Culicidae) in Merida City, Mexico. J. Med. Entomol. 51(2), 475-483.

Baak-Baak, C.M., Arana-Guardia, R., Cigarroa-Toledo, N., Puc-Tinal, M., Coba-Tun, C., Rivero-Osorno, V., Lavalle-Kantun, D., Lorono-Pino, M.A., Machain-Williams, C., Reyes-Solis, G.C., Beaty, B.J., Eisen, L. and Garcia-Rejon, J.E. (2014b) Urban mosquito fauna in Merida City, Mexico: Immatures collected from containers and storm-water drains/catch basins. Southwest. Entomol. 39(2), 291-305.

Brady, O.J., Johansson, M.A., Guerra, C.A., Bhatt, S., Golding, N., Pigott, D.M., Delatte, H., Grech, M.G., Leisnham, P.T., Maciel-de-Freitas, R., Styer, L.M., Smith, D.L., Scott, T.W., Gething, P.W. and Hay, S.I. (2013) Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings. Parasites & Vectors 6, 12.

Campbell-Lendrum, D., Manga, L., Bagayoko, M., & Sommerfeld, J. (2015). Climate change and vector-borne diseases: what are the implications for public health research and policy?. Phil. Trans. R. Soc. B, 370(1665), 20130552.

Chowell, G. and Sanchez, F. (2006) Climate-based descriptive models of Dengue fever: The 2002 epidemic in Colima, Mexico. J. Environ. Health 68(10), 40-44.

Chretien, J.-P., Anyamba, A., Small, J., Britch, S., Sanchez, J.L., Halbach, A.C., Tucker, C. and Linthicum, K.J. (2015) Global climate anomalies and potential infectious disease risks: 2014-2015. PLoS currents 7.

Colford, J.M., Wade, T.J., Schiff, K.C., Wright, C.C., Griffith, J.F., Sandhu, S.K., Burns, S., Sobsey, M., Lovelace, G. and Weisberg, S.B. (2007) Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. Epidemiology 18(1), 27-35.

Colon-Gonzalez, F.J., Fezzi, C., Lake, I.R. and Hunter, P.R. (2013) The effects of weather and climate change on dengue. PLoS Negl. Trop. Dis. 7(11), 9.

Colon-Gonzalez, F.J., Lake, I.R. and Bentham, G. (2011) Climate variability and Dengue Fever in warm and humid Mexico. Am. J. Trop. Med. Hyg. 84(5), 757-763.

Cordero, L., Norat, J., Mattei, H. and Nazario, C. (2012) Seasonal variations in the risk of gastrointestinal illness on a tropical recreational beach. J. Water Health 10(4), 579-593.

Dantes, H.G., Farfan-Ale, J.A. and Sarti, E. (2014) Epidemiological trends of Dengue disease in Mexico (2000-2011): A systematic literature search and analysis. PLoS Negl. Trop. Dis. 8(11), 13.

de Brauwere, A., Ouattara, N.K. and Servais, P. (2014) Modelling fecal indicator bacteria concentrations in natural surface waters: A review. Crit. Rev. Environ. Sci. Technol. 44(21), 2380-2453.

Descloux, E., Mangeas, M., Menkes, C.E., Lengaigne, M., Leroy, A., Tehei, T., Guillaumot, L., Teurlai, M., Gourinat, A.C., Benzler, J., Pfannstiel, A., Grangeon, J.P., Degallier, N. and De Lamballerie, X. (2012) Climate-based models for understanding and forecasting Dengue epidemics. PLoS Negl. Trop. Dis. 6(2), 19.

Dick, O.B., San Martin, J.L., Montoya, R.H., del Diego, J., Zambrano, B. and Dayan, G.H. (2012) The history of Dengue outbreaks in the Americas. Am. J. Trop. Med. Hyg. 87(4), 584-593.

Dickerson, C. Z. (2007). The effects of temperature and humidity on the eggs of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Texas. Texas A&M University.

Dobson, A. (2009) Climate variability, global change, immunity, and the dynamics of infectious diseases. Ecology 90(4), 920-927.

Eastin, M.D., Delmelle, E., Casas, I., Wexler, J. and Self, C. (2014) Intra- and Interseasonal autoregressive prediction of Dengue outbreaks using local weather and regional climate for a tropical environment in Colombia. Am. J. Trop. Med. Hyg. 91(3), 598-610.

Garcia-Montiel, D.C., Verdejo-Ortiz, J.C., Santiago-Bartolomei, R., Vila-Ruiz, C.P., Santiago, L. and Melendez-Ackerman, E. (2014) Food sources and accessibility and waste disposal patterns across an urban tropical watershed: Implications for the flow of materials and energy. Ecol. Soc. 19(1).

Garcia-Rejon, J., Lorono-Pino, M.A., Farfan-Ale, J.A., Flores-Flores, L., Rosado-Paredes, E.D., Rivero-Cardenas, N., Najera-Vazquez, R., Gomez-Carro, S., Lira-Zumbardo, V., Gonzalez-Martinez, P., Lozano-Fuentes, S., Elizondo-Quiroga, D., Beaty, B.J. and Eisen, L. (2008) Dengue virus-infected *Aedes aegypti* in the home environment. Am. J. Trop. Med. Hyg. 79(6), 940-950.

Garcia-Rejon, J.E., Lorono-Pino, M.A., Farfan-Ale, J.A., Flores-Flores, L.F., Lopez-Uribe, M.P., Najera-Vazquez, M.D., Nunez-Ayala, G., Beaty, B.J. and Eisen, L. (2011) Mosquito infestation and Dengue virus infection in *Aedes aegypti* females in schools in Merida, Mexico. Am. J. Trop. Med. Hyg. 84(3), 489-496.

Gonzalez, R.A. and Noble, R.T. (2014) Comparisons of statistical models to predict fecal indicator bacteria concentrations enumerated by qPCR- and culture-based methods. Water Res. 48, 296-305.

Gratz, N.G. (1991) Emergency control of *Aedes aegypti* as a disease vector in urban areas. J. Am. Mosq. Control Assoc. 7(3), 353-365.

Gubler, D.J. (2002) Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol. 10(2), 100-103.

Gubler, D.J. (2010) Vector biology, ecology and control, pp. 39-62, Springer.

Gubler, D.J. and Clark, G.G. (1995) Dengue/dengue hemorrhagic fever - the emergences of a global health prolem. Emerg. Infect. Dis. 1(2), 55-57.

Halstead, S.B. (1988) Pathogenesis of Dengue - challenges to molecular-biology. Science 239(4839), 476-481.

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. Science, 296(5576), 2158-2162.

He, L.M. and He, Z.L. 2008 Water quality prediction of marine recreational beaches receiving watershed baseflow and stormwater runoff in southern California, USA. Water Res. 42(10-11), 2563-2573.

Herrera-Silveira, J.A. (1994) Spatial heterogeneity and seasonal patterns in a tropical coastal lagoon. J. Coast. Res. 10(3), 738-746.

Husin, N.A., Salim, N. and Ahmad, A.R. (2008) Modelling of Dengue outbreak prediction in Malaysia: A comparison of Neural Network and Nonlinear Regression Model. International Symposium of Information Technology 2008, Vols 1-4, Proceedings: Cognitive Informatics: Bridging Natural and Artificial Knowledge, 1796-1799.

Jiang, Y.C., Nan, Z.R. and Yang, S.C. (2013) Risk assessment of water quality using Monte Carlo simulation and artificial neural network method. J. Environ. Manage. 122, 130-136.

Johansson, M.A., Dominici, F. and Glass, G.E. (2009) Local and global effects of climate on Dengue transmission in Puerto Rico. PLoS Negl. Trop. Dis. 3(2).

Joob, B., and Wiwanitkit, V. (2016). Fifth serotype of dengue virus: What we should prepare for?. Medical Journal Armed Forces India, 72(2), 194-195.

Lamparelli, C.C., Pogreba-Brown, K., Verhougstraete, M., Sato, M.I.Z., de Castro Bruni, A., Wade, T.J. and Eisenberg, J.N. (2015) Are fecal indicator bacteria appropriate measures of recreational water risks in the tropics: A cohort study of beach goers in Brazil? Water Res. 87, 59-68.

Laureano-Rosario, A.E., Garcia-Rejon, J.E., Gomez-Carro, S., Farfan-Ale, J.A. and Muller-Karger, F.E. (2017) Modelling dengue fever risk in the State of Yucatan, Mexico using regional-scale satellite-derived Sea Surface Temperature. Acta Trop. 172, 50-57.

Lin, B., Syed, M. and Falconer, R.A. (2008) Predicting faecal indicator levels in estuarine receiving waters - An integrated hydrodynamic and ANN modelling approach. Environ. Model. Software 23(6), 729-740.

Mavani, J., Chen, L., Joksimovic, D. and Li, S. (2014) *Development and testing of data driven nowcasting models of Beach water quality*; City University of New York: New York, NY, USA, 2014; p. 318.

Medeiros, L.C.D., Castilho, C.A.R., Braga, C., de Souza, W.V., Regis, L. and Monteiro, A.M.V. (2011) Modelling the dynamic transmission of Dengue Fever: Investigating disease persistence. PLoS Negl. Trop. Dis. 5(1).

Medlock, J. M., and Leach, S. A. (2015). Effect of climate change on vector-borne disease risk in the UK. The Lancet Infectious Diseases, 15(6), 721-730.

Mendez-Lazaro, P., Muller-Karger, F.E., Otis, D., McCarthy, M.J. and Pena-Orellana, M. (2014) Assessing climate variability effects on Dengue incidence in San Juan, Puerto Rico. Int. J. Environ. Res. Public Health 11(9), 9409-9428.

Murphy, D.J., Hall, M.H., Hall, C.A.S., Heisler, G.M., Stehman, S.V. and Anselmi-Molina, C. (2011) The relationship between land cover and the urban heat island in northeastern Puerto Rico. IJCli 31(8), 1222-1239.

Mustafa, M. S., Rasotgi, V., Jain, S., & Gupta, V. (2015). Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Medical Journal Armed Forces India, 71(1), 67-70.

Ortiz-Zayas, J.R., Cuevas, E., Mayol-Bracero, O.L., Donoso, L., Trebs, I., Figueroa-Nieves, D. and McDowell, W.H. (2006) Urban influences on the nitrogen cycle in Puerto Rico. Biogeochemistry 79(1-2), 109-133.

Paaijmans, K. P., Cator, L. J., & Thomas, M. B. (2013). Temperature-dependent pre-bloodmeal period and temperature-driven asynchrony between parasite development and mosquito biting rate reduce malaria transmission intensity. PLoS One, 8(1), e55777.

Pednekar, A.M., Grant, S.B., Jeong, Y., Poon, Y. and Oancea, C. (2005) Influence of climate change, tidal mixing, and watershed urbanization on historical water quality in Newport Bay, a saltwater wetland and tidal embayment in southern California. Environ. Sci. Technol. 39(23), 9071-9082.

Pruss, A. (1998) Review of epidemiological studies on health effects from exposure to recreational water. Int. J. Epidemiol. 27(1), 1-9.

Puerto Rico Environmental Quality Board (PREQB). 2007 Total maximum daily loads (TMDL) Rio Grande de Loiza watershed. Junta de Calidad Ambiental, San Juan, Puerto Rico. p. 281.

Puerto Rico Environmental Quality Board (PREQB). 2011 Total Maximum Daily Loads (TMDL) of fecal coliform for evaluation units, Puerto Rico. Division de Planes y Proyectos Especiales, Area de Evaluacion y Planificacion Estrategica. Junta de Calidad Ambiental, San Juan, Puerto Rico. p. 164.

Puerto Rico Environmental Quality Board (PREQB). 2016 Beach Monitoring and Public Notification Program - Performance criteria 2016-2017, p. 40.

Quiñones, F. (2012) Impacto ambiental de pozos sépticos en puerto rico y su diseño y control. Dimensión, Revista del Colegio de Ingenieros y Agrimensores de Puerto Rico 1, 16-22.

Racloz, V., Ramsey, R., Tong, S.L. and Hu, W.B. (2012) Surveillance of Dengue Fever Virus: A review of epidemiological models and early warning systems. PLoS Negl. Trop. Dis. 6(5).

Rochelle-Newall, E., Nguyen, T.M.H., Le, T.P.Q., Sengteheuanghoung, O. and Ribolzi, O. (2015) A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. Front. Microbiol. 6, 308.

Rothman, A.L. (2004) Dengue: Defining protective versus pathologic immunity. J. Clin. Investig. 113(7), 946-951.

Shuman, E.K. (2010) Global climate change and infectious diseases. N. Engl. J. Med. 362(12), 1061-1063.

Soderberg, C.-A.P. (2012) Agua potable y saneamiento para comunidades aisladas: Un imperativo social y ambiental para Puerto Rico. Revista Internacional de Desastres Naturales, Accidentes e Infraestructura Civil 8(1), 4.

Stramer, S.L., Linnen, J.M., Carrick, J.M., Foster, G.A., Krysztof, D.E., Zou, S.M., Dodd, R.Y., Tirado-Marrero, L.M., Hunsperger, E., Santiago, G.A., Munoz-Jordan, J.L. and Tomashek, K.M. (2012) Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. Transfusion 52(8), 1657-1666.

Tabachnick, W.J. (2010) Challenges in predicting climate and environmental effects on vector-borne disease episystems in a changing world. J. Exp. Biol. 213(6), 946-954.

Thoe, W., Gold, M., Griesbach, A., Grimmer, M., Taggart, M.L. and Boehm, A.B. (2014) Predicting water quality at Santa Monica Beach: Evaluation of five different models for public notification of unsafe swimming conditions. Water Res. 67, 105-117.

United States Environmental Protection Agency (U.S. EPA). 2012 Recreational Water Quality Criteria; US EPA:Washington, DC, USA. Available online: https://www.epa.gov/wqc/microbial-pathogenrecreational-water-qualitycriteria (accessed on 30 July 2017).

United States Environmental Protection Agency (U.S. EPA). 2014 National Beach Guidance and Required Performance Criteria for Grants. Available online: https://www.epa.gov/sites/production/files/2014-07/documents/beach guidance-final-2014.pdf (accessed on 30 July 2017).

Viau, E.J., Goodwin, K.D., Yamahara, K.M., Layton, B.A., Sassoubre, L.M., Burns, S.L., Tong, H.I., Wong, S.H.C., Lu, Y.A. and Boehm, A.B. (2011) Bacterial pathogens in Hawaiian coastal streams-associations with fecal indicators, land cover, and water quality. Water Res. 45(11), 3279-3290.

Wright, M.E., Abdelzaher, A.M., Solo-Gabriele, H.M., Elmir, S. and Fleming, L.E. (2011) The inter-tidal zone is the pathway of input of enterococci to a subtropical recreational marine beach. Water Sci. Technol. 63(3), 542-549.

Wu, Y., Lee, G., Fu, X.J. and Hung, T. (2008) Detect climatic factors contributing to dengue outbreak based on wavelet, support vector machines and genetic algorithm. World Congress on Engineering 2008, Vols I-Ii, 303-307.

Zhang, G.Q., Patuwo, B.E. and Hu, M.Y. (1998) Forecasting with artificial neural networks: The state of the art. Int. J. Forecasting 14(1), 35-62.

CHAPTER TWO

Modelling dengue fever risks in the State of Yucatan, Mexico using regional-scale satellite-derived sea surface temperature

Note to reader

This chapter was published in the peer-reviewed journal *Acta Tropica* and is included in Appendix A. The full citation is: Laureano-Rosario, A.E., Garcia-Rejon, J.E., Gomez-Carro, S., Farfan-Ale, J.A., Muller-Karger, F.E. (2017). Modelling dengue fever risk in the State of Yucatan, Mexico using regional-scale satellite-derived sea surface temperature. *Acta Tropica*, 172:50-57. Authorization for inclusion in this dissertation is found in Appendix E.

Research overview

Data on dengue fever incidence were obtained from Yucatan's National Health Information System. This included data from eight municipalities: Chicxulub Pueblo, Dzemul, Hunucma, Ixil, Progreso, Telchac Pueblo, Telchac Puerto, Ucu, and Merida. These cases were converted to incidence rates per 100,000 individuals using population size from 2007–2012. Nearshore satellite-derived SST was collected by the Advanced Very High Resolution Radiometer (AVHRR; 1 km spatial resolution) from 2006–2012. Dengue fever data were combined with precipitation, humidity, and minimum and maximum air temperature into a multiple regression model. Results showed that dengue incidence rates increased around the month of July and started to decrease in November, following the precipitation patterns. Linear regression model showed that previous dengue incidence rates explained 89% of dengue fever variation. Our model identified precipitation, minimum air temperature, humidity, and SST as the best variables to explain dengue incidence variability. Furthermore, results also showed increases in SST preceding increases in dengue incidence rates by eight weeks ($r = 0.50$; $p < 0.05$). Dengue incidence rates were positively correlated with SST and minimum air temperature anomalies. Combining environmental and oceanographic variables improved modelling of dengue fever in Mexico; this was shown by a smaller AIC value (AIC: -1410). This suggested that as the temperature anomalies, humidity, and precipitation change, dengue cases will also change as these variables were positively correlated.

CHAPTER THREE

Environmental factors correlated with culturable enterococci concentrations in tropical recreational waters: A case study in Escambron Beach, San Juan, Puerto Rico

Note to reader

This chapter was published in the peer-reviewed journal *International Journal of Environmental Research and Public Health* and is included in Appendix B. The full citation is: Laureano-Rosario, A.E., Symonds E.M., Rueda, D., Otis, D., Muller-Karger, F.E. (2017). Environmental factors correlated with culturable enterococci concentrations in tropical recreational waters: A case study in Escambron Beach, San Juan, Puerto Rico. *International Journal of Environmental Research and Public Health*, 14(12):1602. Authorization for inclusion in this dissertation is found in Appendix E.

Research overview

Culturable enterococci concentrations data were obtained from the U.S. Environmental Agency Storage and Retrieval data warehouse for Escambron Beach (2005–2012), and extended to 2015 using data obtained from the Puerto Rico Environmental Quality Board. Environmental data were measured *in situ* (i.e., daily mean sea level (MSL), precipitation) or derived from satellites (i.e., sea surface temperature, remote sensing reflectance (R_{rs} 645), direct normal irradiance (DNI), winds). These data were combined in a multiple regression model to better understand variability seen in culturable enterococci concentrations. Significant lags were also identified through Pearson's correlations, and environmental variables were divided into specific ranges (i.e., bins) to identify exceedances in culturable enterococci concentration among bins, based on U.S. EPA's safe bathing water quality criteria. Data showed that precipitation, mean sea level (MSL), DNI, SST, and turbidity explained some of the observed variation ($r = 20$) and these parameters preceded changes (i.e., increased or decreased) in culturable enterococci concentrations with lags spanning from 24 h up to 11 days. Increased culturable enterococci concentrations were observed during positive anomalies of turbidity, SST, and 481–960 mm of 4-day cumulative precipitation. Culturable enterococci concentrations decreased with elevated MSL anomalies and irradiance. Unsafe enterococci concentrations per U.S. EPA water quality guidelines occurred when precipitation ranged from 481–960 mm, irradiance < 667 W m^{-2}, turbidity daily anomaly > 0.005 sr^{-1}, SST daily anomaly > 0.8 °C, and MSL daily anomaly < -18.8 cm. Our model accounted for the combined effects of these environmental variables, which can help improve our understanding of the ecology of culturable enterococci and protect public health.

CHAPTER FOUR

Application of Artificial Neural Networks for dengue fever outbreak predictions in the northwest coast of Yucatan, Mexico and San Juan, Puerto Rico

Note to reader

This chapter was published in the peer-reviewed journal *Tropical Medicine and Infectious Diseases* and is included in Appendix C. The full citation is: Laureano-Rosario, A.E., Duncan, A.P., Mendez-Lazaro, P.A., Garcia-Rejon, J.E., Gomez-Carro, S., Farfan Ale, J., Savic, D.A., Muller-Karger, F.E. (2018). Application of Artificial Neural Networks for dengue fever predictions in the northwest coast of Yucatan, Mexico and San Juan, Puerto Rico. *Tropical Medicine and Infectious Diseases*, 3(1):5. Authorization for inclusion in this dissertation is found in Appendix E.

Research overview

Artificial Neural Networks (ANNs) were applied to predict dengue fever outbreak occurrences in Mexico and Puerto Rico. Models were trained with six years of dengue fever data for Yucatan, Mexico and 19 years for San Juan, Puerto Rico. Dengue fever data were obtained from the Yucatan's Health Department and Puerto Rico's Health Department. Cases were converted to incidence rates per 100,000 inhabitants, and thresholds based on the 75th percentile were calculated for the population considered at risks due to exposure (i.e., number of people younger than 24 years old) and the most vulnerable population (i.e., number of people younger than 5 years and older than 65 years). Predictor variables included were precipitation, air temperature (i.e., minimum, maximum, average), sea surface temperature (SST), humidity, previous dengue cases, and population size. A total of four models were run, where the predictive power was above 70% for both study areas. These models were divided as follow: 1) Mexico ages less than 24 years old, 2) Mexico ages less than 5 years old and greater than 65 years old, 3) Puerto Rico ages less than 24 years old, and 4) Puerto Rico ages less than 5 years old and greater than 65 years old. The most influential variables on predicting dengue fever occurrences identified by the models in Mexico were population size, previous dengue cases, minimum air temperature, and date. In San Juan, Puerto Rico, the most important variables identified were population size, previous dengue cases, maximum air temperature, and date. For both study areas, demographic factors were the top two most influential variables. By using a nonlinear approach, the models were able to better predict dengue fever occurrences as this approach considers complex and holistic interactions between dengue fever cases, demographics, and environmental variables.

CHAPTER FIVE

Artificial Neural Networks better predict exceedances of recreational water quality criteria at Escambron Beach, San Juan, Puerto Rico

Note to reader

This chapter is currently in review in the peer-reviewed *Journal of Water and Health* and is included in Appendix D. The full citation is: Laureano-Rosario, A.E., Duncan, A.P., Symonds E.M., Savic, D.A., Muller-Karger, F.E. (2018). Artificial Neural Networks better predict exceedances of recreational water quality criteria at Escambron Beach, San Juan, Puerto Rico. *Journal of Water and Health* (*in review*). Authorization for inclusion in this dissertation is found in Appendix E.

Research Overview

Culturable enterococci concentration exceedances were predicted in Escambron Beach surface waters using a nonlinear approach based on Artificial Neural Networks. Ten years of culturable enterococci data obtained from the U.S. Environmental Protection Agency (U. S. EPA) and the Puerto Rico Environmental Quality Board were used to train, validate, and test the model. In order to predict whether it was safe or unsafe to swim, a threshold of 70 colony forming units (CFU) per 100 mL was used based on the U.S. EPA 2014 Beach Action Value for safe recreational water quality. Predictor variables included in the model were satellite-derived sea surface temperature (SST), direct normal irradiance (DNI), turbidity, and dew point together with *in situ* cumulative precipitation from the previous 24 h up to 120 h and mean sea level (MSL). Based on the Receiving Operating Characteristic Curve and the F-Measure metrics, the model showed an accuracy of 76% and a power greater than 60%, which was higher than linear models. The factors identified as the most relevant for predicting culturable enterococci exceedances were DNI, turbidity, cumulative 48 h precipitation, MSL, and SST. The ANN model showed the importance of identifying how environmental conditions can influence culturable enterococci concentration, as well as the complexity of these relationships between FIB and environmental factors. By using a nonlinear approach, I was able to accurately predict culturable enterococci exceedances, which can help management and mitigation strategies for recreational water quality.

CHAPTER SIX

CONCLUSION

Summary

Environmental forces have been associated with dengue fever occurrences in endemic areas, as well as fecal indicator bacteria variability in recreational waters (Chowell and Sanchez 2006, Pednekar et al. 2005). These are important to model and understand to protect public health. Nevertheless, these interactions are complex and by just modelling them with linear models we might be missing important data (Chebud et al. 2012, He and He 2008). This research provides a better understanding of how environmental factors are related to dengue fever and culturable enterococci in a tropical setting, applying linear and nonlinear models with satellite-derived data and long-term epidemiological data.

Chapter Two showed that dengue incidence rates generally increased in July (wet season) and decreased in November (dry season) in Yucatan, Mexico. Changes in previous dengue fever cases explained the most variability and were positively correlated with current cases. Precipitation, minimum air temperature, humidity, and SST were selected as the best variables to explain dengue fever incidence. These results showed that increases in SST precede increased dengue incidence rates by eight weeks and that dengue incidence rates were positively correlated to SST changes. It is concluded, then, that dengue fever incidence rates can be modelled using environmental variables alone, and that by including satellite-derived regional-scale SST the modelling was improved. Nevertheless, it is important to note that even though seroprevalence studies are expensive, the inclusion of human immune background can allows to have more robust models.

Chapter Three showed that precipitation, mean sea level (MSL), direct normal irradiance (DNI), SST, and turbidity explained some of the observed variation. These parameters preceded changes in culturable enterococci concentrations with lags spanning from 24 h up to 11 days. The highest influence on culturable enterococci concentration was between 480 mm – 900 mm of 4-day cumulative precipitation. Higher culturable enterococci were observed during higher turbidity anomalies, warmer SST anomalies, and lower MSL anomalies. A significant decrease in culturable enterococci concentrations was observed during increased solar irradiance. Better monitoring of recreational water quality can be achieved by understanding the influence of environmental factors on culturable enterococci concentrations and how marine waters influence culturable enterococci decay rates (Anderson et al. 2005). It is concluded, then, that culturable enterococci concentration variability can be explained by looking at the combined effects of precipitation, SST, MSL, and turbidity.

In Chapter Four, a predictive model was applied to predict dengue fever outbreak occurrences in San Juan, PR and Yucatan, MX. These models were modified to predict dengue fever outbreak occurrences for the population at highest risk of infection (i.e., < 24 years old) and highest vulnerability of infection (i.e., < 5 years old and > 65 years old; Mendez-Lazaro et al. 2014). These groups were based on previous studies (Laureano-Rosario et al. 2017, Mendez-Lazaro et al. 2014) and data provided by the Department of Health of Mexico and Puerto Rico. Based on these predictions, the most influential variables to predict dengue fever outbreak occurrences in both Puerto Rico and Mexico were previous dengue incidence rates, minimum/maximum air temperatures, date, and population size. These models showed an accuracy of ~50%, with an overall power greater than 70%. Nonetheless, these results showed that the most influential variables to predict dengue fever occurrences are those related to demographics, followed by environmental factors such as temperatures (i.e., sea temperature, air temperature) for both Puerto Rico and Mexico. Therefore, it is concluded that, while demographic factors are important for prediction and mitigation, environmental factors should always be taken into account, and that these relationships are location-specific.

The predictive model was also applied in Chapter Five to predict culturable enterococci concentration exceedance at Escambron Beach surface waters. The model showed the following as the most influential factors: 48 h cumulative precipitation, turbidity anomalies, DNI, MSL anomalies, and SST anomalies. These predictions had an accuracy greater than 70%, higher than the predictive capability of only using a simple linear regression model. Thus, modelling culturable enterococci concentration exceedance at Escambron Beach was achieved by the predictive nonlinear model, where it identified the combined effects of these environmental factors influencing culturable enterococci concentrations.

The results of this dissertation can be integrated into future models to better understand the burden of water-related pathogens correlated with fecal indicators and vector-borne diseases in specific locations. The World Health Organization (WHO) estimates about 720,000 deaths per year related to 12 vector-borne diseases, where 80% of the world's population is at risk and those younger than 5 years old are considered more susceptible (WHO 2018). Understanding the relationship and seasonality of these vectors, as shown in Chapter Two and Four with dengue fever, can help achieve better predictions and further develop disease surveillance and prevention strategies. In terms of water, sanitation, and hygiene (WASH), WHO reports about 840,000 deaths per year with 361,000 of those being children younger than 5 years old, and where 58% of these deaths could be averted through better sanitation practices (WHO 2018). While these statistics include both freshwater (i.e., drinking water) and marine waters, the results of this dissertation can help better understand patterns of specific indicators and how those are related to human activities and climate. Consequently, this dissertation supports and expands on efforts to understand diseases occurrence on specific population segments and seasonal variability of vector-borne diseases and water indicators related to poor recreational water quality.

This study demonstrated that the combined effects of environmental factors can improve our understanding of the ecology and epidemiology of diseases and microbial indicators over time, which would have been missed by just looking at just one environmental variable. Combining environmental and oceanographic variables improved modelling of dengue fever in Mexico and recreational water quality in Puerto Rico. Thus, this research contributes to the understanding of the influence of environmental factors on public health issues through the comparison of linear and nonlinear modelling as well as predictive models targeting specific population segments and geographic locations.

Future research

This dissertation shows the importance of understanding the influence of large-scale environmental, human, and pathogen factors on specific public health issues in coastal and non-coastal areas. Results show that these interactions are complex, and that there is a combined effect of environmental factors, thus looking at them separately might not provide a complete understanding. Therefore, the combination of these factors should be taken into consideration in future work, as well as those other factors that were not included due to data limitations (discussed below). Nevertheless, this study contributes to the understanding of environmental and demographic factors that should be included for early warning systems and to improve mitigation and management strategies.

Predictive models used for Mexico and Puerto Rico looking at dengue fever occurrences and FIB exceedances showed high predictive capabilities. Models can be further improved by including data that was not considered in this dissertation. For example, for dengue fever predictions, seroprevalence and human population movement should be considered to better understand occurrences and peaks in dengue fever. Likewise, different populations segments (i.e., age groups) were considered for this study, but these age groups could be either expanded or divided differently for better predictions, according to information available on the limitations to their immune system. In terms of the FIB, models can be improved by including sanitation infrastructure, river and stormwater discharge, and wastewater treatment plant outflows. These FIB can also be found in sediments/sand and vegetation, which should also be considered in the future. Lastly, time series length can influence outcomes due to lack of data, overfitting, and underfitting. Those Puerto Rico models used 19 years (dengue) and 11 years (fecal indicator bacteria) of data, while Mexico models used years of data (dengue). Nevertheless, these models yielded high predictive capabilities, and future studies should consider expanding time series to better predict specific health-related occurrences.

The application of remote sensing data should be considered in future efforts to better understand phenology of vector-borne diseases and recreational water quality. Results of this work provide managers and public health practitioners the data needed to better model and understand public-health related issues in coastal areas. Also, this dissertation provides specific limitations such as epidemiological, demographic, and environmental data not being available to further improve management, targeted sampling, and early warning systems.

Literature cited

Anderson, K. L., Whitlock, J. E., and Harwood, V. J. (2005). Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Appl Environ Microbiol, 71(6), 3041-3048.

Chebud, Y., Naja, G.M., Rivero, R.G. and Melesse, A.M. (2012) Water quality monitoring using remote sensing and an Artificial Neural Network. Water Air Soil Pollut 223(8), 4875-4887.

Chowell, G. and Sanchez, F. (2006) Climate-based descriptive models of Dengue fever: The 2002 epidemic in Colima, Mexico. J. Environ. Health 68(10), 40-44.

He, L.M. and He, Z.L. (2008) Water quality prediction of marine recreational beaches receiving watershed baseflow and stormwater runoff in southern California, USA. Water Res. 42(10-11), 2563-2573.

Laureano-Rosario, A.E., Garcia-Rejon, J.E., Gomez-Carro, S., Farfan-Ale, J.A. and Muller-Karger, F.E. (2017) Modelling dengue fever risk in the State of Yucatan, Mexico using regional-scale satellite-derived Sea Surface Temperature. Acta Trop. 172, 50-57.

Mendez-Lazaro, P., Muller-Karger, F.E., Otis, D., McCarthy, M.J. and Pena-Orellana, M. (2014) Assessing climate variability effects on Dengue incidence in San Juan, Puerto Rico. Int. J. Environ. Res. Public Health 11(9), 9409-9428.

Pednekar, A.M., Grant, S.B., Jeong, Y., Poon, Y. and Oancea, C. (2005) Influence of climate change, tidal mixing, and watershed urbanization on historical water quality in Newport Bay, a saltwater wetland and tidal embayment in southern California. Environ. Sci. Technol. 39(23), 9071-9082.

World Health Organization (WHO). 2018 Vector Control. Available online: http://www.who.int/vector-control/en/ (accessed on 10 May 2018).

World Health Organization (WHO). 2018 Water Sanitation Hygiene (WASH). Available online: http://www.who.int/water_sanitation_health/diseases-risks/en/ (accessed on 10 May 2018).

APPENDIX A

Modelling dengue fever risks in the State of Yucatan, Mexico using regional-scale satellite-derived sea surface temperature

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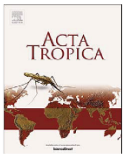

Modelling dengue fever risk in the State of Yucatan, Mexico using regional-scale satellite-derived sea surface temperature

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ARTICLE INFO

Keywords:
Satellite imagery
Aedes aegypti
Public health
Sea surface temperature

ABSTRACT

Accurately predicting vector-borne diseases, such as dengue fever, is essential for communities worldwide. Changes in environmental parameters such as precipitation, air temperature, and humidity are known to influence dengue fever dynamics. Furthermore, previous studies have shown how oceanographic variables, such as El Niño Southern Oscillation (ENSO)-related sea surface temperature from the Pacific Ocean, influences dengue fever in the Americas. However, literature is lacking on the use of regional-scale satellite-derived sea surface temperature (SST) to assess its relationship with dengue fever in coastal areas. Data on confirmed dengue cases, demographics, precipitation, and air temperature were collected. Incidence of weekly dengue cases was examined. Stepwise multiple regression analyses (AIC model selection) were used to assess which environmental variables best explained increased dengue incidence rates. SST, minimum air temperature, precipitation, and humidity substantially explained 42% of the observed variation ($r^2 = 0.42$). Infectious diseases are characterized by the influence of past cases on current cases and results show that previous dengue cases alone explained 89% of the variation. Ordinary least-squares analyses showed a positive trend of 0.20 ± 0.03 °C in SST from 2006 to 2015. An important element of this study is to help develop strategic recommendations for public health officials in Mexico by providing a simple early warning capability for dengue incidence.

1. Introduction

Worldwide, dengue fever is a prominent vector-borne disease that has led to more than 500,000 cases reported per year (Bhatt et al., 2013; Murray et al., 2013; Shepard et al., 2011). The main vector is *Aedes aegypti*, a tropical/subtropical mosquito. Dengue virus cases are prominent in urban areas where the mosquito has adapted to develop effectively (Cheong, 1967; Gratz, 1991; Gubler, 2002). Their development typically occurs in both artificial and natural water containers. Increased precipitation, air temperature, and humidity have been shown to promote their development (Brady et al., 2013; Colon-Gonzalez et al., 2011; Descloux et al., 2012; Johansson et al., 2009a; Shuman, 2010). Dengue outbreaks are also influenced by virus serotypes and socio-economic factors including patterns of population distribution, behavior of different age groups, and previous dengue cases (Teurlai et al., 2012; Thomas et al., 2003). Previous dengue cases are defined as those cases that took place weeks before an outbreak. These periods vary depending on geographic location and the dynamics of dengue transmission, and contribute to increasing risk of infection with the virus. The occurrence of previous low or high dengue cases is an indication of the currently circulating virus serotype and the immune status of a population (Focks and Barrera, 2006). Previous studies have shown that humans travelling from areas with known outbreaks and that have been infected with dengue virus also promote the spread of the virus (Hales et al., 2002; Teurlai et al., 2012).

Since the late 1970s Mexico, and the rest of the Caribbean and Latin America, have reported thousands of dengue fever cases annually (Dick et al., 2012). Mexico provides weekly epidemiological panoramas. In particular, the Yucatan State showed a 45% increase in dengue cases from 2014 to 2015 (629 cases in 2014 and 1129 cases in 2015) (Salud, 2016), where serotypes DENV-1, DENV-2, and DENV-4 had been reported. Most of the studies done in the Yucatan State are focused on the development of mosquito larvae and potential breeding sites of *Aedes* species (Dantes et al., 2011; Lorono-Pino et al., 2004). Other

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http://dx.doi.org/10.1016/j.actatropica.2017.04.017
Received 30 January 2017; Received in revised form 21 April 2017; Accepted 21 April 2017

studies have included environmental variables (i.e., air temperature, precipitation, and humidity) to understand and predict dengue-fever outbreaks in Mexico (Colon-Gonzalez et al., 2013; Hurtado-Diaz et al., 2007). Some of these studies were done at larger geographic areas (i.e., State level) using monthly resolution of dengue data (Colon-Gonzalez et al., 2011; Hurtado-Diaz et al., 2007) or up to one year of data at smaller geographic areas (Garcia-Rejon et al., 2008; Garcia-Rejon et al., 2011). Studies including oceanographic variables (e.g., water temperature) were done at larger geographic areas and used data from the Pacific Ocean and the influence of El Niño Southern Oscillation (ENSO) on dengue fever in the Americas (Brunkard et al., 2008; Colon-Gonzalez et al., 2011; Hurtado-Diaz et al., 2007). It is known that the influence of these parameters on dengue fever is location-dependent (Eastin et al., 2014), and that ENSO influences can be obscured by local variability (Johansson et al., 2009b). Moreover, literature is lacking on studies using long-term epidemiological data together with regional-scale satellite-derived sea surface temperature (SST), and those environmental variables mentioned above, to model dengue fever in Yucatan, Mexico.

Oceanographic variables such as SST are important to include due to their influence on coastal weather patterns (Goddard and Mason, 2002; Thomson et al., 2005; Xie et al., 2010). It has been shown that SST in the Gulf of Mexico region has been increasing (Lluch-Cota et al., 2013; Muller-Karger et al., 2015). Therefore, incorporating SST, together with meteorological parameters, could help improve predictions of dengue cases in coastal areas, especially in the Caribbean-Gulf of Mexico region.

The main objective of this study was to assess the use of regional-scale satellite-derived SST data to model dengue fever incidence rates in the northwest region of the Yucatan Peninsula, Mexico. It was hypothesized that variability and trends in precipitation, air temperature, and humidity were primary drivers of dengue incidence; and that satellite-derived SST would improve dengue risk predictions. An important element of this study is to help develop strategic recommendations for public health officials in Mexico by providing a simple early warning capability for dengue incidence. Epidemiological surveillance has helped track dengue fever patterns in the past and such information, together with inter-disciplinary research and inclusion of oceanographic parameters such as SST, can significantly improve dengue fever surveillance and predictive power of future outbreaks.

2. Material and methods

2.1. Northwest coast, Yucatan Peninsula, Mexico

The study took place in the northwest coastal area of the State of Yucatan, Mexico, located adjacent to the Gulf of Mexico (19.55°N–21.63°N, 87.53°W–90.40°W). The study area has nine municipalities: Chicxulub Pueblo, Dzemul, Hunucma, Ixil, Progreso, Telchac Pueblo, Telchac Puerto, Ucu, and Merida, which is the capital and largest municipality within this region (Fig. 1).

The highest precipitation occurs during the rainy season between July and October (with an average of 400–700 mm of precipitation over the season). The dry season occurs between March and June (0–50 mm for the season). A third season, the “Nortes” season, is characterized by strong (~ 80 km h^{-1}) winds coming from the continental mass of the U.S. and associated with cold fronts during November–February. Air temperatures generally range from 36 to 40 °C during the dry season, 30–35 °C during rainy season, and 20–23 °C during “Nortes” (Gonzalez et al., 2008; Herrera-Silveira, 1994).

2.2. Satellite-derived oceanographic data and in-situ meteorological data

Day- and night-time SST data were obtained from the U.S. National Oceanic and Atmospheric Administration’s Advanced Very High Resolution Radiometer sensor (AVHRR; 1 km^2 spatial resolution). These images were mapped using cylindrical equidistant projection and are available online at the Institute for Marine Remote Sensing webpage (http://imars.marine.usf.edu/). Data were extracted from January 2006 to February 2015 using the average of three 3 × 3 pixel boxes (centered on 21.37°N, 90.10°W; 21.47°N, 89.72°W; and 21.50°N, 89.24°W) for the northwest coastal area of the Yucatan Peninsula. Interactive Data Language (IDL; v. 7.2) was used to extract data. Monthly and weekly time series were created using the average values of day- and night-time SST images, and climatologies and its anomalies were calculated from 2006 to 2015, which coincides with the time period of the dengue fever data.

Meteorological data (i.e., air temperature, humidity, and precipitation) from 2006 to 2012 were obtained from the National Water Commission (CONAGUA) of the Yucatan State. Both minimum and maximum air temperatures were used to calculate (arithmetic) mean air temperature. Due to the lack of dengue fever data for some of the municipalities for some years, we pooled the nine municipalities in our study area. Moreover, only Merida, Progreso, Telchac Puerto, and Chicxulub Puerto had meteorological data available for the study period. These data sets (i.e., meteorological data) from the aforementioned municipalities were compared and parameters (e.g., precipitation and air temperatures) followed the same patterns across municipalities. There were no significant difference across municipalities of the relatively small geographical area studied (~ 2500 km^2). Therefore, we assumed that data from Merida (the largest city in the region) were representative for the entire study area. Climatologies, anomalies, and monthly and weekly means of meteorological data were calculated for the purpose of this study. Environmental and oceanographic data were collected from years prior and after dengue data timeframe in order to calculate trends and lags between dengue incidence rates and environmental/oceanographic data.

2.3. Dengue fever and demographic data

Daily data of confirmed dengue fever cases were obtained from Yucatan’s National Health Information System (Subdirección de Salud Pública, Servicios de Salud de Yucatan; January 2007–December 2010) and from the Universidad Autónoma de Yucatan (January 2011–December 2012). Due to inconsistencies in the data, dengue cases before 2007 were not included in this study. Since we were only interested in dengue cases regardless of the serotype, data for both dengue fever and dengue hemorrhagic fever were pooled for a total of 312 weekly observations. Dengue cases by age class were calculated to determine which age groups were more susceptible to dengue fever. These age-groups were: less than 5 years old, 5–9, 10–19, 20–24, 25–29, 30–34, 35–39, 40–49, and greater than 50 years old. To assess variability and susceptibility to infections during 2007–2012, weekly dengue incidence rates by municipality and age were calculated by dividing number of cases by population each year and multiplying it by 100,000 (i.e., incidence per 100,000 inhabitants). These weekly dengue incidence rates were used in the analyses and not the number of cases. Geographic and demographic data, such as population size, were obtained from Yucatan’s National Institute of Statistics and Geography (2007–2012).

2.4. Non-parametric statistical analyses

Data were analyzed with non-parametric permutation-based statistics, which are a distribution free method. Permutation-based Pearson’s correlation analyses were used to identify lags between predictor variables (i.e., precipitation, SST, humidity, minimum, maximum and mean air temperature, and previous dengue cases), and target variable (i.e., dengue incidence rates). The goal was to identify significant time-lagged (positive or negative) correlations between predictor variables and weekly dengue incidence rates. These time-lags were for all the cases in the study area and these lags comprise the mean of two

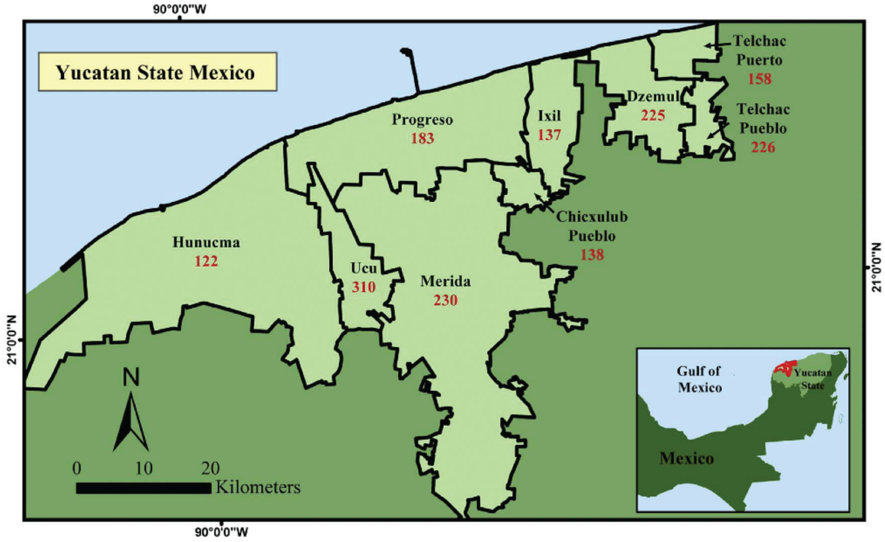

Fig. 1. The mainland region used for our study area located in the northwest coast of Yucatan State (inset). Numbers are mean dengue incidence rates (cases per 100,000 inhabitants) from 2007 to 2012.

consecutive weeks (e.g., if the lag was one week, we used the mean of week one and two for the analyses).

Several separate analyses were conducted to assess the relative importance of each independent environmental variable: 1) a linear regression to assess the percentage of the variation of new dengue cases (as incidence rates) explained only by previous rates of dengue incidence using the identified time-lag from the correlation analyses; 2) a multiple linear regression to asses which of the environmental parameters explains some of the variation of new dengue cases (as incidence rates). We used a stepwise selection of explanatory variables via forward addition, based on Akaike Information Criteria (AIC). AIC selects (optimal) environmental variables that significantly explain variation in dengue incidence rates (Blanchet et al., 2008; Burnham and Anderson, 2001; Godinez-Dominguez and Freire, 2003); 3) ordinary least-squares analyses were used to identify trends, and their significance, for the SST data; 4) a single linear regression model to assess variation explained only by SST in terms of dengue incidence rates; and finally, 5) two one-way ANOVAs were done to assess differences in dengue incidence rates among municipalities and years. We used MATLAB Fathom toolbox for data analyses (Jones, 2015).

3. Results

3.1. Oceanographic and meteorological patterns and dengue fever incidence rates

Within the Yucatan Peninsula, all of the explanatory variables (i.e., precipitation, humidity, SST, and air temperature) showed similar patterns where they increased towards May–June and decreased in October–November from 2000 to 2012 (Fig. 2A–D). Dengue incidence rates followed this pattern from 2007 to 2012 (Fig. 2E). Precipitation, air temperatures (minimum, maximum, and mean), SST, and humidity preceded dengue fever incidence rates with lags varying from 1 to 13 weeks ($p < 0.05$; Table 1). The most significant correlation was found with previous dengue cases ($r = 0.94$; $p < 0.05$). These occurrences (previous dengue cases) were calculated using the mean of the past two consecutive weeks of dengue cases and converted into incidence rates. Those results (calculated means of previous dengue cases) help explain whether past rates of dengue incidence affect new rates of dengue incidence over the course of a few weeks.

3.2. Non-parametric ANOVAs and AIC model analyses

Permutation-based one-way ANOVAs were done to look for significant differences between municipalities and years (Fig. 3A). Dengue incidence rates were significantly different among municipalities and years ($p = 0.0002$, $F = 271$; $p = 0.0002$, $F = 158$; respectively). Merida, the largest municipality in our study area (population > 850,000 as of 2012), did not show the highest incidence rates of dengue (Fig. 3A). The highest dengue incidence rates were reported in 2012 in the municipalities of Telchac Pueblo and Ucu (population of ~3200 each) (Fig. 3A). Individuals between 10 and 24 years old predominantly showed higher incidence of dengue fever among these municipalities, with an increase after 50 years old (Fig. 3B).

The AIC analyses revealed that minimum air temperature, precipitation, humidity, and SST were the best explanatory variables for dengue incidence rates in the northwest coast of Yucatan ($p = 0.001$; Table 2). These parameters explained 42% of the total variation. Only environmental variables were included in this model to assess how much of the variability could be explained by environmental factors alone. There was an increase in dengue incidence rates about two weeks after the initial dengue cases had occurred (Table 1). This lag was subsequently tested through a single linear regression analysis, where it explained 89% of the overall dengue incidence ($p = 0.001$; $F = 2512$). By first using the Pearson's correlation analyses and then a single linear regression and multiple linear regression analyses, we were able to identify not only the time lag, but also the strength of the relationship between the variables.

3.3. Sea surface temperature trend analyses and dengue fever incidence

Trend analyses were done using ordinary least-squares. Trend and uncertainty were calculated for SST along the coast of the Yucatan Peninsula. A significant and positive trend for SST (0.20 ± 0.03 °C/10 years) was found for 2006–2015. In general, the SST preceded dengue incidence by five weeks. This warming trend was compared

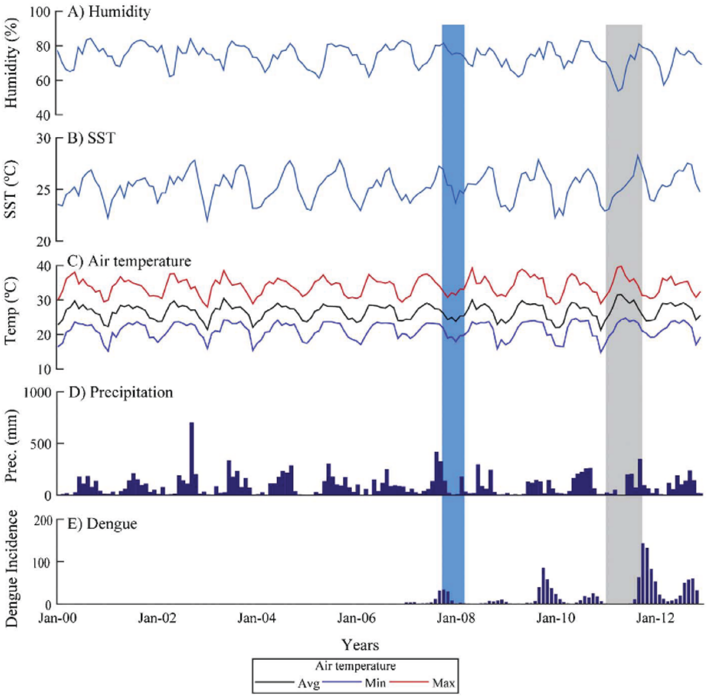

Fig. 2. A) Humidity; B) sea surface temperature (SST); C) air temperature; D) precipitation; and E) dengue incidence (per 100,000 inhabitants) for the northwest coast of the Yucatan Peninsula. Time period of 2000–2012 and 2007–2012. Blue box represents when these variables show a decrease and gray box represents when they show an increase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Pearson's correlation coefficients for identification of week-long lags between dengue incidence rates and explanatory variables. Values are significant at an alpha level = 0.05.

Parameter	Pearson's correlation coefficient (r)	Dengue incidence lag[a]
Mean air temperature	−0.15	1-week
Maximum air temperature	−0.22	1-week
Humidity	0.37	3-weeks
Precipitation	0.34	6-weeks
SST	0.51	5-weeks
Minimum air temperature	0.49	13-weeks
Previous dengue incidences	0.94	2-weeks

[a] All lags comprise the mean of 2 consecutive weeks.

with dengue fever cases by linear regression, and it showed that 26% of the variation in dengue was explained by SST (p = 0.001; F = 110). Minimum air temperature and SST were selected as the two variables that explained most of the total variation and plotted their monthly means with dengue incidence. Both of these variables showed an increased trend (warming) in the study area. Dengue trends showed an increase with increased air temperature and SST (warmer), and they decreased with cooler temperatures (Fig. 4).

4. Discussion

This study investigated the applicability of regional-scale satellite-derived SST and assessed how it helps to better predict dengue fever in the northwest coast of the Yucatan Peninsula. Data showed that regional-scale satellite-derived SST can help explain up to 42% of dengue fever variability, where 26% is explained by SST itself and preceded a peak in dengue fever cases by five weeks. This modelling was achieved by using a stepwise multiple linear regression (AIC model selection), which selected those optimal explanatory variables that explained dengue fever variability from 2007 to 2012 in the northwest coast of the Yucatan Peninsula. This work builds upon those studies using ENSO-related SST from the Pacific Ocean (e.g., Colon-Gonzales et al., 2011; Dantes et al., 2014; Garcia-Rejon et al., 2008) and contributes to the gaps in the literature with the use of regional-scale SST to model dengue fever in Yucatan, Mexico.

Dengue incidence rates showed seasonal trends in the Yucatan Peninsula. There is generally an increase of the disease in July–August and a decrease during November–December. Data showed that these seasonal trends were related to increased precipitation, humidity, and warmer temperatures, which also relate to mosquitoes' laying eggs period (Costa et al., 2010; Dickerson 2007). These correlations are due to the development of the mosquito, considering time to develop, grow, and transmit the virus. These conditions favor decreased development time of mosquitoes and incubation time of the virus (Depradine and Lovell 2004; Hales et al., 2002; Lu et al., 2009; Phung et al., 2015; Wu et al., 2007). Results also revealed how previous dengue cases may influence future outbreaks, which can be due to propagation of the virus through other mosquitoes, by human movement, or by local environmental factors conducive to mosquito breeding (Teurlai et al., 2012). Huang et al. (2013) reported how previous confirmed dengue cases help predict new (current/future) dengue cases. Therefore, including dengue incidence rates over the previous two weeks of current cases decreases model prediction error for historical dengue events.

The time series of dengue incidence rates by municipality and age

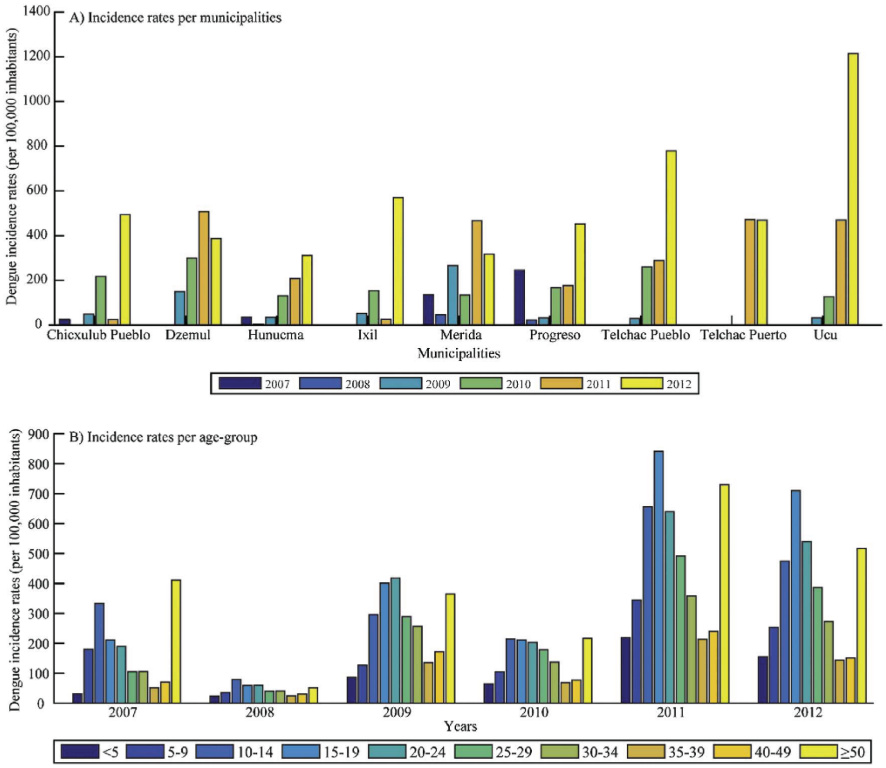

Fig. 3. Northwest coast of Yucatan, Mexico. Dengue incidence rates by: A) municipality; and B) age-group for the time period of 2007–2012. These incidence rates were calculated using the population size for each municipality during those years.

Table 2
Variables that significantly improved prediction of dengue incidences for our study area according to an Akaike information criterion (AIC) model.

AIC- based stepwise forward selection with sequential variable addition			
Variable	r^2	r^2-adjusted	AIC
Minimum air temperature	0.35	0.35	−1386
SST	0.40	0.40	−1409
Humidity	0.42	0.42	−1416
Precipitation	0.43	0.42	−1421

groups showed how they have increased from 2007 to 2012. We propose two possible explanations for this. One possibility is that dengue fever incidence has been increasing in this region as a function of air and water temperature. Another is that there is an increase in reporting of confirmed dengue cases, which has also been the case in areas such as the Americas from 2000 to 2010 (Guzman et al., 2013). The data identified 2009 and 2011 as epidemic years (Dick et al., 2012; Liao et al., 2015; Vázquez-Pichardo et al., 2011). Serotypes circulating in the years of 2009 and 2011 might have influenced these epidemics (Garcia-Rejon et al., 2011; Sanchez-Casas et al., 2013; Vázquez-Pichardo et al., 2011). Only Merida showed 2009 and 2011 as epidemic years, whereas the other eight municipalities showed a general increase in dengue incidence rates over the period of 2007–2012 with some peaks in years 2010 and 2012. These differences can be due to Yucatan being a developing state, an increased reporting of cases, population movement, and secondary infections. For instance, there is an increased population movement into Yucatan of individuals that have not been exposed to dengue fever, increasing susceptible population and dengue fever cases (Dantes et al., 2014; Eisen et al., 2014; Torres-Galicia et al., 2014). Furthermore, Merida is considered one of the centers for virus propagation in this region. For example, individuals become infected while visiting the area (e.g., work, school, tourism) and promote the spread of the virus through secondary infections (Garcia-Rejon et al., 2011; Teurlai et al., 2012). Nevertheless, Merida was representative of dengue fever epidemics in the region, as it contained more than 60% of the cases for the period of 2007–2012.

In addition, the one-way ANOVAs results found a significant difference among towns and years in terms of dengue incidence rates; however, these were aggregated together as they were inconsistencies with the data and the time series analyses from 2007 to 2012 could not have been done for most of the municipalities. Ucu, smallest municipality, showed the highest dengue incidence, while Merida, largest municipality, showed the lowest. These differences could be attributed to Merida being a developing city and having a better action plan against dengue fever, compared to other smaller communities. Studies have shown how other factors such as number of individuals working outside (higher in rural areas; i.e. smaller town), movement of infected working population, as well as transmissions taking place in schools, zoos, churches, abandoned lots, and population size can influence dengue fever dynamics (Baak-Baak et al., 2014a, 2014b; Eisen et al., 2014).

The age groups with the predominantly highest incidence rates were 10–24 years old from 2007 to 2012, with an increase after 50 years old.

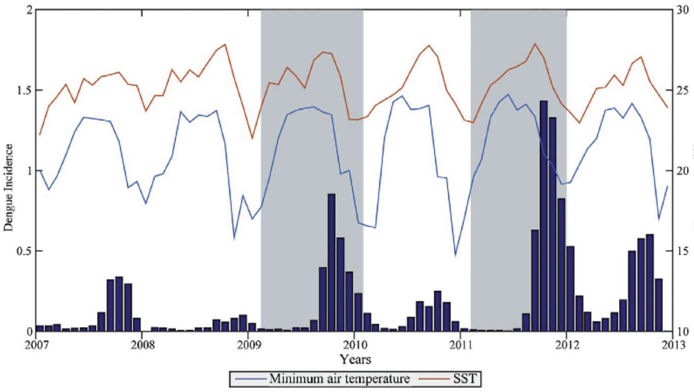

Fig. 4. Northwest coast of Yucatan, Mexico. Orange line is monthly mean of sea surface temperature (SST), blue line is monthly mean of minimum air temperature, and blue bars are monthly dengue incidence rates. Gray boxes are epidemic years 2009 and 2011. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

This could be due to young children (< 10 years old) showing symptoms for a short period of time, being asymptomatic, or cases not being reported (Cavalcanti et al., 2011). Moreover, older adults (> 24 years) could have partial immunity for specific virus serotypes after being previously infected, which could explain a decreased number of cases. Lastly, increased incidence with age (e.g., > 50 years) can occur due to secondary infection, loss of immunity with time, or the increased susceptibility to infections with aging (Cavalcanti et al., 2011; Garcia-Rivera and Rigau-Perez 2003; Guzman et al., 2002; Rigau-Perez et al., 1998; Thomas et al., 2008). Nevertheless, all age groups were affected as has been previously shown for the Americas and these patterns are location-dependent (Rigau-Perez 1997).

AIC analyses showed that the best model to explain dengue incidence rates in this region includes precipitation, minimum air temperature, humidity, and SST. Data showed that, on average, an increase in SST (warmer) precedes an increase in dengue incidence by five weeks (i.e., highest correlation); however, previous studies have shown different lags for SST and dengue incidence. This indicates that these relationships are location-dependent (Colon-Gonzalez et al., 2011; Eastin et al., 2014; Hurtado-Diaz et al., 2007). Dengue incidence rates were preceded by SST peaks by five weeks and precipitation by six weeks, showing a one-week difference between the oceanic (SST) and atmospheric (precipitation) variables. The observed one-week difference could be explained through the work of Kumar et al. (2013), where warming peaks in SST preceded peaks in precipitation by 5–10 days. Identifying these lags can help to better predict dengue incidence in these regions. Increased dengue incidence rates seemed to also track the positive trend in SST. Ordinary least-squares analyses showed that SST has increased 0.20 ± 0.03 °C for this time period (2006–2015). Lluch-Cota et al. (2013) and Muller-Karger et al. (2015) reported similar increases in SST on previous years. Mosquitoes' life cycle, extrinsic incubation period, and intrinsic incubation period play a role in these identified lags (Chan and Johansson, 2012). The life cycle of an *Aedes aegypti* mosquito (i.e., development from larvae to adult) is, on average, about 12–21 days. The time between taking a (viremic) blood meal and becoming infectious (i.e., extrinsic incubation period) is around 6–15 days. The intrinsic period, which is between a human becoming infected and showing symptoms, is around six days. These account for six weeks and there is also likely a factor that accounts for the time it takes to report confirmed cases (Chan and Johansson, 2012; Thomas et al., 2003; Wilder-Smith et al., 2010). The lags identified in this study were within those six weeks. Extrinsic incubation period and mosquito development time are shorter with warmer temperatures, thus increasing mosquitoes' density and influencing peaks in cases in shorter times (Chan and Johansson, 2012). Continuous epidemiological surveillance is currently done as an early warning system technique to protect public health from dengue fever. Our results show that by including additional oceanographic parameters, such as SST, we could improve surveillance of dengue fever by improving modelling of the disease in coastal areas.

As SST and minimum air temperature increase (warmer) or decrease (colder), dengue incidence also increase and decrease, respectively. Increased warming of air temperatures has led to a subsequent warming of the lowest seasonal air temperatures, which suggests that the winter air temperatures could be getting warmer. This has positive feedback leading to an increase of dengue incidence rates in Mexico. These general trends are followed during epidemic years as well as non-epidemic years. It has been well documented that SST is increasing (Lluch-Cota et al., 2013; Muller-Karger et al., 2015), along with minimum air temperatures. While there is no clear causality, there is likelihood that dengue incidence rates are related to warmer temperatures. However, even with warmer temperatures there are other factors that influence dengue fever cases such as population mobility, socio-economic factors, irregular circulation of virus serotypes, and actions being taken to prevent the disease (Banu et al., 2011; Gubler, 2002; Hales et al., 2002; Keating, 2001; Lorono-Pino et al., 2004; ten Bosch et al., 2016). For example, transmission dynamics are affected by population susceptibility to circulating serotypes. Individuals who were previously infected with a specific serotype (i.e., DENV-1, DENV-2, DENV-3, or DENV-4) will develop immunity against that particular serotype. Nonetheless, if there is a shift from one serotype to another on the following year, there would be an increase in dengue fever cases as the population would only have partial immunity to the other serotypes (Gubler and Clark, 1995; Rothman, 2004).

5. Conclusion

Our study indicates that adding satellite-derived SST helps to better model dengue fever outbreaks for the northwest coast of the Yucatan Peninsula. Implementing results of SST on early warning systems for dengue fever can help reduce future outbreaks. Environmental data from most of the municipalities were not available, which could have influenced the results, especially when identifying epidemic years. These epidemic years were mostly based on cases reported in Merida due to dengue fever data limitation. Future research should look into individual municipalities to identify whether these epidemics occur during the same time periods. The application of remote sensing should be considered for future efforts to improve prediction and mitigation of dengue fever outbreaks in coastal and non-coastal areas, as this data is readily available. It is important to predict and understand the influence of environmental variables, such as regional-scale SST, on dengue fever as this virus is transmitted by the same species of mosquitoes (*Aedes* spp.) of upcoming diseases such as chikungunya and Zika.

Acknowledgments

We would like to thank Dr. Pablo Méndez-Lázaro (University of Puerto Rico) for his invaluable input on the dengue fever data analyses, as well as Dr. David Jones and Dr. Don Chambers (USF-College of Marine Science) for their help with the multivariate and time series analyses of these datasets. I would also like to thank Maria Vega-Rodriguez, Loraine Martell-Bonet, Ileana Freytes-Ortiz, Dinorah Chacin, Dimitre Guenov, and the IMaRS team for their help with manuscript revisions. This material is based upon work supported by the National Science Foundation Partnerships for International Research (PIRE) under Grant No. 1243510, by NASA Headquarters under the NASA Earth and Science Fellowship Program Grant No. NNX15AN60H, and the USF College of Marine Science Linton Tibbetts Endowed Fellowship. Partial support was provided by EPA STAR Grant No. 83519.

References

Baak-Baak, C.M., Arana-Guardia, R., Cigarroa-Toledo, N., Lorono-Pino, M.A., Reyes-Solis, G., Machain-Williams, C., Beaty, B.J., Eisen, L., Garcia-Rejon, J.E., 2014a. Vacant lots: productive sites for *Aedes* (Stegomyia) *aegypti* (Diptera: Culicidae) in Merida city, Mexico. J. Med. Entomol. 51 (2), 475–483.

Baak-Baak, C.M., Arana-Guardia, R., Cigarroa-Toledo, N., Puc-Tinal, M., Coba-Tun, C., Rivero-Osorno, V., Lavalle-Kantun, D., Lorono-Pino, M.A., Machain-Williams, C., Reyes-Solis, G.C., Beaty, B.J., Eisen, L., Garcia-Rejon, J.E., 2014b. Urban mosquito fauna in Merida City, Mexico: immatures collected from containers and storm-water drains/catch basins. Southwest. Entomol. 39 (2), 291–305.

Banu, S., Hu, W.B., Hurst, C., Tong, S.L., 2011. Dengue transmission in the Asia-Pacific region: impact of climate change and socio-environmental factors. Trop. Med. Int. Health 16 (5), 598–607. http://dx.doi.org/10.1111/j.1365-3156.2011.02734.x.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, G.R.W., Simmons, C.P., Scott, T.W., Farrar, J.J., Hay, S.I., 2013. The global distribution and burden of dengue. Nature 496 (7446), 504–507. http://dx.doi.org/10.1038/nature12060.

Blanchet, F.G., Legendre, P., Borcard, D., 2008. Forward selection of explanatory variables. Ecology 89 (9), 2623–2632. http://dx.doi.org/10.1890/07-0986.1.

Brady, O.J., Johansson, M.A., Guerra, C.A., Bhatt, S., Golding, N., Pigott, D.M., Delatte, H., Grech, M.G., Leisnham, P.T., Maciel-de-Freitas, R., Styer, L.M., Smith, D.L., Scott, T.W., Gething, P.W., Hay, S.I., 2013. Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings. Parasit. Vectors 6, 12. http://dx.doi.org/10.1186/1756-3305-6-351.

Brunkard, J.M., Cifuentes, E., Rothenberg, S.J., 2008. Assessing the roles of temperature, precipitation, and ENSO in dengue re-emergence on the Texas-Mexico border region. Salud Publica Mex. 50 (3), 227–234.

Burnham, K.P., Anderson, D.R., 2001. Kullback-Leibler information as a basis for strong inference in ecological studies. Wildl. Res. 28 (2), 111–119. http://dx.doi.org/10.1071/wr99107.

Cavalcanti, L.P., Viler, D., Souza-Santos, R., Teixeira, M.G., 2011. Change in age pattern of persons with dengue, northeastern Brazil. Emerg. Infect. Dis. 17 (1), 132–134. http://dx.doi.org/10.3201/eid1701.100321.

Chan, M., Johansson, M.A., 2012. The incubation periods of dengue viruses. PLoS One 7 (11).

Cheong, W.H., 1967. Preferred *Aedes aegypti* larval habitats in urban areas. Bull. WHO 36 (4), 586.

Colon-Gonzalez, F.J., Lake, I.R., Bentham, G., 2011. Climate variability and dengue fever in warm and humid Mexico. Am. J. Trop. Med. Hyg. 84 (5), 757–763. http://dx.doi.org/10.4269/ajtmh.2011.10-0609.

Colon-Gonzalez, F.J., Fezzi, C., Lake, I.R., Hunter, P.R., 2013. The effects of weather and climate change on dengue. PLoS Negl. Trop. Dis. 7 (11), 9. http://dx.doi.org/10.1371/journal.pntd.0002503.

Costa, E.A.P.d.A., Santos, E.M.d.M., Correia, J.C., Albuquerque, C.M.R.d., 2010. Impact of small variations in temperature and humidity on the reproductive activity and survival of *Aedes aegypti* (Diptera, Culicidae). Rev. Bras. Entomol. 54 (3), 488–493.

Dantes, H.G., Farfan-Ale, J.A., Sarti, E., 2014. Epidemiological trends of dengue disease in Mexico (2000–2011): a systematic literature search and analysis. PLoS Negl. Trop. Dis. 8 (11), 13. http://dx.doi.org/10.1371/journal.pntd.0003158.

Depradine, C.A., Lovell, E.H., 2004. Climatological variables and the incidence of dengue fever in Barbados. Int. J. Environ. Health Res. 14 (6), 429–441. http://dx.doi.org/10.1080/09603120400012868.

Descloux, E., Mangeas, M., Menkes, C.E., Lengaigne, M., Leroy, A., Tehei, T., Guillaumot, L., Teurlai, M., Gourinat, A.C., Benzler, J., Pfannstiel, A., Grangeon, J.P., Degallier, N., De Lamballerie, X., 2012. Climate-based models for understanding and forecasting dengue epidemics. PLoS Negl. Trop. Dis. 6 (2), 19. http://dx.doi.org/10.1371/journal.pntd.0001470.

Dick, O.B., San Martin, J.L., Montoya, R.H., del Diego, J., Zambrano, B., Dayan, G.H., 2012. The history of Dengue outbreaks in the Americas. Am. J. Trop. Med. Hyg. 87 (4), 584–593. http://dx.doi.org/10.4269/ajtmh.2012.11-0770.

Dickerson, C.Z., 2007. The Effects of Temperature and Humidity on the Eggs of *Aedes Aegypti* (L.) and *Aedes Albopictus* (Skuse) in Texas. Texas A & M University.

Eastin, M.D., Delmelle, E., Casas, I., Wexler, J., Self, C., 2014. Intra- and interseasonal autoregressive prediction of dengue outbreaks using local weather and regional climate for a tropical environment in Colombia. Am. J. Trop. Med. Hyg. 91 (3), 598–610. http://dx.doi.org/10.4269/ajtmh.13-0303.

Eisen, L., Garcia-Rejon, J.E., Gomez-Carro, S., Vazquez, M.D.N., Keefe, T.J., Beaty, B.J., Lorono-Pino, M.A., 2014. Temporal correlations between mosquito-based dengue virus surveillance measures or indoor mosquito abundance and dengue case numbers in Merida City. Mex. J. Med. Entomol. 51 (4), 885–890.

Focks, D.A., Barrera, Robert, 2006. Dengue Transmission Dynamics: Assessment and Implications for Control. In: Paper Presented at the Special Programme for Research and Training in Tropical Diseases. Geneva. .

Garcia-Rejon, J., Lorono-Pino, M.A., Farfan-Ale, J.A., Flores-Flores, L., Rosado-Paredes, E.D., Rivero-Cardenas, N., Najera-Vazquez, R., Gomez-Carro, S., Lira-Zumbardo, V., Gonzalez-Martinez, P., Lozano-Fuentes, S., Elizondo-Quiroga, D., Beaty, B.J., Eisen, L., 2008. Dengue virus-infected *Aedes aegypti* in the home environment. Am. J. Trop. Med. Hyg. 79 (6), 940–950.

Garcia-Rejon, J.E., Lorono-Pino, M.A., Farfan-Ale, J.A., Flores-Flores, L.F., Lopez-Uribe, M.P., Najera-Vazquez, M.D., Nunez-Ayala, G., Beaty, B.J., Eisen, L., 2011. Mosquito infestation and Dengue virus infection in *Aedes aegypti* females in schools in Merida, Mexico. Am. J. Trop. Med. Hyg. 84 (3), 489–496. http://dx.doi.org/10.4269/ajtmh.2011.10-0654.

Garcia-Rivera, E.J., Rigau-Perez, J.G., 2003. Dengue severity in the elderly in Puerto Rico. Rev. Panam Salud Publica 13 (6), 362–368.

Goddard, L., Mason, S.J., 2002. Sensitivity of seasonal climate forecasts to persisted SST anomalies. ClDy 19 (7), 619–631. http://dx.doi.org/10.1007/s00382-002-0251-y.

Godinez-Dominguez, E., Freire, J., 2003. Information-theoretic approach for selection of spatial and temporal models of community organization. Mar. Ecol. Prog. Ser. 253, 17–24. http://dx.doi.org/10.3354/meps253017.

Gonzalez, F.U.T., Ilveira, J., Aguirre-Macedo, M.L., 2008. Water quality variability and eutrophic trends in karstic tropical coastal lagoons of the Yucatan Peninsula. ECSS 76 (2), 418–430. http://dx.doi.org/10.1016/j.ecss.2007.07.025.

Gratz, N.G., 1991. Emergency control of *Aedes aeypti* as a disease vector in urban areas. J. Am. Mosq. Control Assoc. 7 (3), 353–365.

Gubler, D.J., Clark, G.G., 1995. Dengue/dengue hemorrhagic fever − the emergences of a global health prolem. Emerg. Infect. Dis. 1 (2), 55–57.

Gubler, D.J., 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol. 10 (2), 100–103. http://dx.doi.org/10.1016/s0966-842x(01)02288-0.

Guzman, M.G., Kouri, G., Bravo, J., Valdes, L., Vazquez, S., Halstead, S.B., 2002. Effect of age on outcome of secondary dengue 2 infections. Int. J. Infect. Dis. 6 (2), 118–124. http://dx.doi.org/10.1016/s1201-9712(02)90072-x.

Guzman, M.G., Brathwaite, O., Enria, D., Hunsperger, E., Kouri, G., Montoya, R., San Martín, J., 2013. Dengue in the American region. An update. J. Clin. Virol. 27, 1–13.

Hales, S., de Wet, N., Maindonald, J., Woodward, A., 2002. Potential effect of population and climate changes on global distribution of dengue fever: an empirical model. Lancet 360 (9336), 830–834. http://dx.doi.org/10.1016/s0140-6736(02)09964-6.

Herrera-Silveira, J.A., 1994. Spatial heterogeneity and seasonal patterns in a tropical coastal lagoon. J. Coast. Res. 10 (3), 738–746.

Huang, X.D., Clements, A.C.A., Williams, G., Milinovich, G., Hu, W.B., 2013. A threshold analysis of dengue transmission in terms of weather variables and imported dengue cases in Australia. Emerg. Microbes Infect. 2, 7. http://dx.doi.org/10.1038/emi.2013.85.

Hurtado-Diaz, M., Riojas-Rodriguez, H., Rothenberg, S.J., Gomez-Dantes, H., Cifuentes, E., 2007. Short communication: impact of climate variability on the incidence of dengue in Mexico. Trop. Med. Int. Health 12 (11), 1327–1337.

Johansson, M.A., Cummings, D.A.T., Glass, G.E., 2009a. Multiyear climate variability and dengue-El Niño southern oscillation, weather, and dengue incidence in Puerto Rico, Mexico, and Thailand: a longitudinal data analysis. PLoS Med. 6 (11). http://dx.doi.org/10.1371/journal.pmed.1000168.

Johansson, M.A., Dominici, F., Glass, G.E., 2009b. Local and global effects of climate on Dengue transmission in Puerto Rico. PLoS Negl. Trop. Dis. 3 (2). http://dx.doi.org/10.1371/journal.pntd.0000382.

Jones, D., 2015. Fathom Toolbox for Matlab: Software for Multivariate Ecological and Oceanographic Data Analysis. Retrieved from. http://www.marine.usf.edu/user/djones/.

Keating, J., 2001. An investigation into the cyclical incidence of dengue fever. Soc. Sci. Med. 53 (12), 1587–1597. http://dx.doi.org/10.1016/s0277-9536(00)00443-3.

Kumar, A., Zhang, L., Wang, W., 2013. Sea surface temperature–precipitation relationship in different reanalyses. MWRv 141 (3), 1118–1123.

Liao, C.M., Huang, T.L., Lin, Y.J., You, S.H., Cheng, Y.H., Hsieh, N.H., Chen, W.Y., 2015. Regional response of dengue fever epidemics to interannual variation and related climate variability. Stoch. Environ. Res. Risk Assess. 29 (3), 947–958. http://dx.doi.org/10.1007/s00477-014-0948-6.

Lluch-Cota, S.E., Tripp-Valdez, M., Lluch-Cota, D.B., Lluch-Belda, D., Verbesselt, J., Herrera-Cervantes, H., Bautista-Romero, J.J., 2013. Recent trends in sea surface temperature off Mexico. Atmosfera 26 (4), 537–546.

Lorono-Pino, M.A., Farfan-Ale, J.A., Zapata-Peraza, A.L., Rosado-Paredes, E.P., Flores-

Flores, L.F., Garcia-Rejon, J.E., Diaz, F.J., Blitvich, B.J., Andrade-Narvaez, M., Jimenez-Rios, E., Blair, C.D., Olson, K.E., Black, W., Beaty, B.J., 2004. Introduction of the American/Asian genotype of dengue 2 virus into the Yucatan State of Mexico. Am. J. Trop. Med Hyg. 71 (4), 485–492.

Lu, L., Lin, H.L., Tian, L.W., Yang, W.Z., Sun, J.M., Liu, Q.Y., 2009. Time series analysis of dengue fever and weather in Guangzhou, China. BMC Public Health 9, 5. http://dx.doi.org/10.1186/1471-2458-9-395.

Muller-Karger, F.E., Smith, J.P., Werner, S., Chen, R., Roffer, M., Liu, Y., Muhling, B., Lindo-Atichati, D., Lamkin, J., Cerdeira-Estrada, S., Enfield, D.B., 2015. Natural variability of surface oceanographic conditions in the offshore Gulf of Mexico. Prog. Oceanogr. 134, 54–76. http://dx.doi.org/10.1016/j.pocean.2014.12.007.

Murray, N.E.A., Quam, M.B., Wilder-Smith, A., 2013. Epidemiology of dengue: past, present and future prospects. Clin. Epidemiol. 5, 299–309. http://dx.doi.org/10.2147/clep.s34440.

Phung, D., Huang, C.R., Rutherford, S., Chu, C., Wang, X.M., Nguyen, M., Nguyen, N.H., Do Manh, C., 2015. Identification of the prediction model for dengue incidence in Can Tho city, a Mekong Delta area in Vietnam. Acta Trop. 141, 88–96. http://dx.doi.org/10.1016/j.actatropica.2014.10.005.

Rigau-Perez, J.G., Clark, G.G., Gubler, D.J., Reiter, P., Sanders, E.J., Vorndam, A.V., 1998. Dengue and dengue haemorrhagic fever. Lancet 352 (9132), 971–977.

Rigau-Perez, J.G., 1997. Clinical manifestations of dengue hemorrhagic fever in Puerto Rico, 1990–1991. Puerto Rico Association of Epidemiologists. Rev. Panam. Salud Publica 1 (5), 381–388. http://dx.doi.org/10.1590/s1020-49891997000500007.

Rothman, A.L., 2004. Dengue: defining protective versus pathologic immunity. J. Clin. Invest. 113 (7), 946–951.

Salud, S.D., 2016. Panoramas Históricos Epidemiológicos De Dengue. Retrieved from http://www.epidemiologia.salud.gob.mx/dgae/panodengue/historicos_dengue.html (Accessed 14 January 2016).

Sanchez-Casas, R.M., Alpuche-Delgado, R.H., Blitvich, B.J., Diaz-Gonzalez, E.E., Ramirez-Jimenez, R., Zarate-Nahon, E.A., Sanchez-Rodriguez, O.S., Laguna-Aguilar, M., Alvarado-Moreno, M., Ibarra-Juarez, L.A., de la Garza, C.E.M., Lorono-Pino, M.A., Dominguez-Galera, M., Mis-Avila, P., Fernandez-Salas, I., 2013. Detection of dengue virus serotype 2 in *Aedes aegypti* in Quintana Roo, Mexico, 2011. Southwest. Entomol. 38 (1), 109–117.

Shepard, D.S., Coudeville, L., Halasa, Y.A., Zambrano, B., Dayan, G.H., 2011. Economic impact of dengue illness in the Americas. Am. J. Trop. Med. Hyg. 84 (2), 200–207. http://dx.doi.org/10.4269/ajtmh.2011.10-0503.

Shuman, E.K., 2010. Global climate change and infectious diseases. N. Engl. J. Med. 362 (12), 1061–1063. http://dx.doi.org/10.1056/NEJMp0912931.

ten Bosch, Q.A., Singh, B.K., Hassan, M.R.A., Chadee, D.D., Michael, E., 2016. The role of serotype interactions and seasonality in dengue model selection and control: insights from a pattern matching approach. PLoS Negl. Trop. Dis. 10 (5). http://dx.doi.org/10.1371/journal.pntd.0004680.

Teurlai, M., Huy, R., Cazelles, B., Duboz, R., Baehr, C., Vong, S., 2012. Can human movements explain heterogeneous propagation of dengue fever in Cambodia? PLoS Negl. Trop. Dis. 6 (12), 8. http://dx.doi.org/10.1371/journal.pntd.0001957.

Thomas, S.J., Strickman, D., Vaughn, D.W., 2003. Dengue epidemiology: virus epidemiology, ecology, and emergence. Adv. Virus Res. 61, 235–289. http://dx.doi.org/10.1016/s0065-3527(03)61006-7.

Thomas, L., Verlaeten, O., Cabie, A., Kaidomar, S., Moravie, V., Martial, J., Najioullah, F., Plumelle, Y., Fonteau, C., Dussart, P., Cesaire, R., 2008. Influence of the dengue serotype, previous dengue infection, and plasma viral load on clinical presentation and outcome during a dengue-2 and dengue-4 co-epidemic. Am. J. Trop. Med. Hyg. 78 (6), 990–998.

Thomson, M.C., Mason, S.J., Phindela, T., Connor, S.J., 2005. Use of rainfall and sea surface temperature monitoring for malaria early warning in Botswana. Am. J. Trop. Med. Hyg. 73 (1), 214–221.

Torres-Galicia, I., Cortes-Poza, D., Becker, I., 2014. Dengue in Mexico: an analysis of two decades. Gac. Med. Mex. 150 (2), 122–127.

Vázquez-Pichardo, M., Rosales-Jiménez, C., Núñez-León, A., Rivera-Osorio, P., Cruz-Hernández, S.D.L., Ruiz-López, A., González-Mateos, S., López-Martínez, I., Rodríguez-Martínez, J.C., López-Gatell, H., Alpuche-Aranda, C., 2011. Serotipos de dengue en México durante 2009 y 2010. [Dengue serotypes in México during 2009–2010]. Bol. Med. Hosp. Infant. Mex. 68 (2), 103–110.

Wilder-Smith, A., Ooi, E.-E., Vasudevan, S.G., Gubler, D.J., 2010. Update on dengue: epidemiology, virus evolution, antiviral drugs, and vaccine development. Curr. Infect. Dis. Rep. 12 (3), 157–164. http://dx.doi.org/10.1007/s11908-010-0102-7.

Wu, P.C., Guo, H.R., Lung, S.C., Lin, C.Y., Su, H.J., 2007. Weather as an effective predictor for occurrence of dengue fever in Taiwan. Acta Trop. 103 (1), 50–57. http://dx.doi.org/10.1016/j.actatropica.2007.05.014.

Xie, S.P., Deser, C., Vecchi, G.A., Ma, J., Teng, H.Y., Wittenberg, A.T., 2010. Global warming pattern formation: sea surface temperature and rainfall. J. Clim. 23 (4), 966–986. http://dx.doi.org/10.1175/2009jcli3329.1.

APPENDIX B

Environmental factors correlated with culturable enterococci concentrations in tropical recreational waters: A case study in Escambron Beach, San Juan, Puerto Rico

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International Journal of
Environmental Research and Public Health

Article

Environmental Factors Correlated with Culturable Enterococci Concentrations in Tropical Recreational Waters: A Case Study in Escambron Beach, San Juan, Puerto Rico

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Received: 8 November 2017; Accepted: 16 December 2017; Published: 19 December 2017

Abstract: Enterococci concentration variability at Escambron Beach, San Juan, Puerto Rico, was examined in the context of environmental conditions observed during 2005–2015. Satellite-derived sea surface temperature (SST), turbidity, direct normal irradiance, and dew point were combined with local precipitation, winds, and mean sea level (MSL) observations in a stepwise multiple regression analyses (Akaike Information Criteria model selection). Precipitation, MSL, irradiance, SST, and turbidity explained 20% of the variation in observed enterococci concentrations based upon these analyses. Changes in these parameters preceded increases in enterococci concentrations by 24 h up to 11 days, particularly during positive anomalies of turbidity, SST, and 480–960 mm of accumulated (4 days) precipitation, which relates to bacterial ecology. Weaker, yet still significant, increases in enterococci concentrations were also observed during positive dew point anomalies. Enterococci concentrations decreased with elevated irradiance and MSL anomalies. Unsafe enterococci concentrations per US EPA recreational water quality guidelines occurred when 4-day cumulative precipitation ranged 481–960 mm; irradiance < 667 $W \cdot m^{-2}$; daily average turbidity anomaly >0.005 sr^{-1}; SST anomaly >0.8 °C; and 3-day average MSL anomaly <-18.8 cm. This case study shows that satellite-derived environmental data can be used to inform future water quality studies and protect human health.

Keywords: recreational beach water quality; fecal indicator bacteria; coastal water quality; ocean color; remote sensing

1. Introduction

Fecal pollution is a threat to coastal ecosystems in many countries around the world that carries important public health and economic consequences. The city of San Juan, the capital of the island of Puerto Rico in the Caribbean Sea, is located within the Rio Piedras watershed, which receives the discharge of two centralized wastewater treatment plants (WWTPs; Puerto Nuevo Regional and Bayamon Regional WWTPs). The Rio Piedras watershed also catches the runoff from agricultural areas further upstream [1,2] as well as septic seepage [3–5]. While 56% of the Puerto Rican population is connected to these sewer systems [4,5], the remaining population, especially those located at higher elevations in San Juan, typically uses septic tanks. These septic tanks discharge approximately 165 million gallons per day directly to streams that empty into coastal waters [3–5]. Inadequate wastewater treatment prior to ocean outfall discharge, ineffective and old stormwater systems, and septic systems that leak into the karst geology and streams in the region are a constant and present danger to the public, especially because of the large numbers of people that enjoy visiting these beaches year-round for recreational purposes.

It is impractical to measure the concentrations of all wastewater-associated pathogens. Therefore, allochthonous gastrointestinal bacteria known as fecal indicator bacteria (FIB; e.g., fecal coliforms, *Escherichia coli*, and *Enterococcus* spp.), are used to characterize water quality [6,7]. While enterococci have correlated with public health risks in coastal areas with known point sources of fecal contamination in temperate and sub-tropical regions [7–12], this correlation has only been suggested in tropical regions [13] and has not been identified in areas exposed to non-point sources of fecal pollution [14,15]. Since FIB persist in the environment in the absence of active fecal pollution events, particularly in tropical climates, it is often difficult to differentiate between actual events that pose a threat to public health and the natural resuspension and growth of FIB in coastal waters [16,17]. Despite the environments important role on enterococci concentrations in tropical surface waters, few studies have investigated the relationship between environmental conditions and enterococci concentrations for beaches located in tropical climates [2,6,9,10,13].

The 2012 United States Environmental Protection Agency (US EPA) Recreational Water Quality Criteria (RWQC) recommends that culturable enterococci concentrations not exceed geometric means of 35 colony forming units (CFU) per 100 mL for safe recreation [18]. The Puerto Rico Environmental Quality Board (PREQB) has adopted this recommendation in their coastal recreational water quality monitoring program. Since October 2015, the public notification has been issued based on the Beach Action Value (BAV) of 70 CFU/100 mL, recommended by the US EPA National Beach Guidance and Required Performance Criteria for Grants [19,20]. The PREQB assesses beach water quality throughout the island every two weeks per the 2000 US Beaches Environmental Assessment and Coastal Health Act [14,21] and water quality standards of Puerto Rico [22].

Previous water quality studies in Puerto Rico have mostly focused on infrastructure; however, there have been a handful of short-term studies (i.e., weeks to months) on the relationship between water quality and environmental conditions [14,23–25]. These investigations showed that environmental parameters can influence the persistence and concentration of enterococci in recreational waters. For example, increased precipitation contributed to elevated enterococci concentrations [14]. Enterococci thrived in warmer waters [26] and were inhibited by increased irradiance [27]. Increased turbidity protected enterococci from ultraviolet (UV) light [28,29]. Enterococci concentrations in beach water also increased during low tide [30]. It is not known if enterococci co-occurred or correlated with the presence of wastewater-related pathogens or incidence of infection in these studies. Whether there is a relationship between FIB and long-term environmental change is a question that has not yet been explored.

The current case study seeks to identify environmental factors that influence the variability of culturable enterococci concentrations in Escambron Beach surface waters, and specifically seeks to assess when water quality issues exceed the US EPA recommended rate of 36 illnesses per 1000 primary contact recreators (BAV 70 CFU/100 mL; [18,20]). The approach included an analysis of 11 years of culturable enterococci concentrations with respect to the spatial and temporal variation of environmental factors observed locally (i.e., mean sea level, precipitation, winds) and via satellite (i.e., turbidity, sea surface temperature, dew point, direct normal irradiance). Previous studies in tropical areas have suggested a relationship between *Enterococcus* spp. and public health risks in recreational beach waters. However, the extra-intestinal sources of enterococci in the tropics as well as the presence of non-point sources of fecal pollution can obscure this relationship. Thus, it is important to understand how environmental factors influence culturable enterococci concentrations in tropical settings.

This case study demonstrates how the environmental factors significantly correlated with culturable enterococci surface water concentrations and considered the specific lags and ranges where these factors correlated with unsafe culturable enterococci concentrations at Escambron Beach. This study serves as an important point of reference for future water quality studies at Escambron beach as well as for other Caribbean beaches located in a similar context. The results of this case study begin to fill the existing knowledge gaps specific to water quality in the tropics and set the stage for targeted beach monitoring aimed at identifying a correlation between enterococci and health risks in the tropics. Additionally, the results can be used to develop microbial water quality forecasting systems, which would provide early information to local authorities, avoid unnecessary beach closures, and effectively balance the need to protect public health with the economic consequences associated with beach closures.

2. Materials and Methods

2.1. Escambron Beach, San Juan, Puerto Rico

The study took place at Escambron Beach (18.47° N, 66.08° W, Figure 1). This is one of the most popular, well-visited beaches of San Juan, Puerto Rico and it has a year-long swimming season. Escambron Beach is generally visited by residents between the months of May to September, whereas during October to December it is mostly visited by non-residents and tourists. The beach is surrounded by hotels, business, residences, and governmental buildings [2]. The average annual air temperatures range between 24 and 29 °C. During the timeframe of this study, average precipitation was ~1800 mm per year, with lowest precipitation during February–March. Escambron Beach has mixed semidiurnal tides, and is classified as a low wave action along beach.

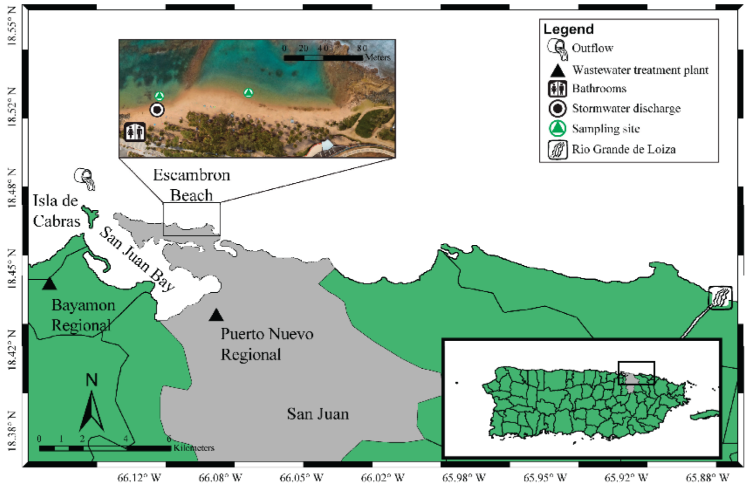

Figure 1. Location of Escambron Beach with respect to the combined ocean outfall that discharges primary-treated domestic wastewater from the Puerto Nuevo Regional and Bayamon Regional treatment plants (black triangles). The ocean outfall discharges at a depth of approximately 40 m; it is located 1 km north of Isla de Cabras and about 5 km from the study site. The inset map details Escambron Beach and depicts both sampling locations (green triangles), stormwater discharge drain (black circle), Rio Grande de Loiza (river symbol), and public bathrooms (bathroom symbol).

Two sites, separated by a distance of ~100 m, were sampled by the PREQB (Figure 1). These sites may have been affected by: (1) stormwater drainage (18.46° N, 66.09° W) located immediately adjacent to one of the sampling sites, which includes urban runoff, precipitation, and other graywaters (e.g., showers, washer machines; [2]); (2) WWTP ocean outfall (18.47° N, 66.14° W); (3) beach public bathrooms; and (4) Rio Grande de Loiza, a river that receives agricultural runoff, WWTP effluent (secondary treatment only), and septic system effluent and seepage (Figure 1; [31–33]). Non-point source pollution throughout San Jan Bay also likely affects water quality at Escambron Beach.

2.2. Culturable Enterococci Data

Data from biweekly measurements of culturable enterococci surface water concentrations were obtained for 2005–2012 from the US EPA Storage and Retrieval data warehouse for Escambron Beach (https://www.epa.gov/waterdata/water-quality-data-wqx). The enterococci time series was extended from 2012 to 2015 with data provided by PREQB. Two methods were used to quantify enterococci concentrations: US EPA method 1600 from January 2005–March 2015 [34] and IDEXX Enterolert (IDEXX Laboratories, Inc., Westbrook, ME, USA) from April 2015 through December 2015 [19]. The US EPA method 1600 quantifies culturable enterococci using membrane filtration and had a detection limit of 4 CFU/100 mL [34]. The IDEXX Enterolert Quanti-Tray methods determined the culturable enterococci concentrations (most probable number (MPN) per 100 mL); this method had a detection limit of 10 MPN/100 mL [19]. Over the 11-year period, a total of 642 culturable enterococci data points were generated, with the surface waters from two sites being sampled approximately once every two weeks (PREQB RW-20A at 18.47° N, 66.08° W and PREQB RW-20B at 18.46° N, 66.09° W; Figure 1). One site had 334 and the other site had 308 data points from 2005 to 2015. Daily geometric means for each sampling date, considering both sampling sites, were calculated for the beach; thus, the total combined number of unique enterococci data points was $n = 376$ for the 2005–2015 period. These geometric means were used in all further analyses. All samples were collected between 9:00 a.m. and 1:00 p.m. (AST). Puerto Rico's climate does not show a significant season variability through the entire year. Therefore, samples collection considered sunlight amount, timing before people go to the beach, traveling time to the Puerto Rico Environmental Sciences Research Laboratory for sample processing, and timing of beach advisories if bacterial levels exceed thresholds [19,20]. If a sample had concentrations above US EPA guidelines, a subsequent sample was taken within seven days.

2.3. Satellite-Derived and In Situ Environmental Data

Daily precipitation data for San Juan, Puerto Rico were obtained from the US National Oceanic and Atmospheric Administration National Centers for Environmental Information for 2000–2015. Accumulated precipitation was calculated for different intervals of days prior to each surface water sampling date. Direct Normal Irradiance (DNI) and dew point were obtained at a 30-min temporal resolution and 4 km spatial resolutions from the National Solar Radiation Database (1998–2014; http://rredc.nrel.gov/solar/old_data/nsrdb/). Mean sea level (MSL) was obtained from the University of Hawaii Sea Level Center for 2000–2015 (https://uhslc.soest.hawaii.edu/). Maximum values, in a 24-h period, were identified for both MSL and DNI and included in the analyses due to their known influence on enterococci [29,30]. Wind speed and direction were obtained from the Caribbean Coastal Observing System (CariCOOS; http://www.caricoos.org/) buoy located north of San Juan (18.47° N, 66.09° W). East (u) and nort (v) wind components were calculated due to their mixing potential and influence on enterococci concentrations [35]. Since the CariCOOS buoy was deployed in 2010, satellite-derived wind data was also included from the Cross-Calibrated Multi-Platform (CCMP; ~28 km spatial resolution) surface winds (2010–2015). Both data sets were compared and followed the same patterns regarding wind speed, direction, and east/west components.

Sea surface temperature (SST) data were obtained from the Advanced Very High Resolution Radiometer (AVHRR; 1 km spatial resolution) from 2000 to 2015. These images were mapped using a cylindrical equidistant projection at the University of South Florida Institute for Marine Remote Sensing (http://imars.usf.edu/). Interactive Data Language (IDL; v. 7.2) was used to extract SST data from 3 × 3-pixel boxes centered on three points for the northern coast of San Juan, Puerto Rico (18.47° N, 66.09° W; 18.48° N, 66.08° W; 18.46° N, 66.07° W). Data from those three boxes showed similar temporal patterns; therefore, they were averaged into one SST time series for further analyses.

Remote sensing reflectance at 645 nm (R_{rs} 645) [36,37] from the Moderate Resolution Imaging Spectroradiometer (MODIS-Terra; 250 m spatial resolution) was used as a proxy for water turbidity (2005–2015). Remote sensing reflectance represents the ratio of upwelling "water-leaving" radiance to downwelling irradiance measured in per steradian (sr^{-1}) units. Reflectance in the red (645 nm) is used here as a proxy for turbidity, an approach which has been used in several previous studies [36,38,39]. R_{rs} was extracted using MATLAB (v. 2014b; The MathWorks Inc., Natick, MA, USA, 2000); the average value of two 3 × 3-pixel boxes was used for turbidity for this coastal region (centered on: 18.47° N, 66.10° W; 18.46° N, 66.08° W; these included sampling sites and adjacent areas). Turbidity measurements from these boxes followed similar temporal patterns; thus, the data were averaged into a single time series for further analyses. Daily and weekly time series, climatologies, and anomalies were calculated for all the variables mentioned above for the period of 2005–2015. Both SST and turbidity, as extracted, covered the entire study site; however, for SST we added a third sampling point to cover the overall variability due to its lower spatial resolution (1 km) in comparison to turbidity (250 m). Additionally, 3-day averages of SST and turbidity anomaly images were also computed for the coastal region of the municipality of San Juan (l8.51–18.42° N, 66.16–65.85° W) to examine the spatial distribution of SST and turbidity before beach advisories on 9 March 2007 and 16 December 2011 (dates were selected based on satellite images availability/clarity to show data).

2.4. Data Identified as Below the Limit of Detection

Sixty-two enterococci data points out of the 376 (combined sampling sites) were described as below the limit of detection (LOD); consequently, it was necessary to accommodate these data to be able to use the 2005–2015 data set for downstream analyses. To determine the most appropriate substitution [40–42], the use of three different, previously-used substitutions were evaluated: the maximum concentration after the LOD (i.e., 3 CFU/100 mL and 9 CFU/100 mL for method 1600 and IDEXX Enterolert, respectively), minimum concentration (i.e., 1 CFU/100 mL), and half the detection limit (i.e., 2 CFU/100 mL and 5 CFU/100 mL). When comparing the three methods, all the correlations coefficients showed a difference less than 0.10 and were considered not significantly different. Based on this, it was concluded that the results of the downstream Akaike Information Criteria analyses were not significantly different among the three aforementioned substitution approaches (Supplementary Materials, Tables S1–S3). Therefore, a conservative approach was selected, such that all that left-censored data were substituted by the next highest concentration; 3 CFU per 100 mL for those samples analyzed before April 2015 and 9 MPN per 100 mL for samples analyzed after April 2015. These were substituted in the raw data (1999–2015) and then filtered to obtain a total of 376 points from 2005 to 2015.

2.5. Non-Parametric Statistical Analyses

Data were analyzed with non-parametric, permutation-based statistics, which are a distribution-free method. Significant time-lagged correlations between explanatory variables (i.e., SST, precipitation, DNI, dew point, MSL, and turbidity) and the dependent variable (i.e., culturable enterococci concentrations) were identified using Pearson's correlation analyses. A MATLAB function was created to identify different lags between explanatory variables and dependent variable, where those who showed the highest and significant Person's correlation coefficient were selected.

Lagged environmental factors, with the lag-periods showing significant correlations (Pearson's correlations) with culturable enterococci were divided into three to six bins using the histogram function of MATLAB (v.2014b); bins sizes were selected based upon sample size. Bins were divided as follow: (A) precipitation (mm) six bins: ≤240, 241–480, 481–720, 721–960, and ≥961; (B) DNI ($W \cdot m^{-2}$) five bins: ≤667, 668–732, 733–798, 799–864, and ≥865; (C) turbidity anomaly (sr^{-1}) three bins: ≤0.001, 0.002–0.004, and ≥0.005; (D) SST anomaly (°C) four bins: ≤−3.7, −3.6–−1.5, −1.4–0.7, and ≥0.8; (E) dew point anomaly (°C) five bins: ≤−1.6, −1.5–−0.7, −0.6–−0.3, −0.2–1.2, and ≥1.3; and (F) MSL anomaly (cm) six bins: ≤−78.8, −78.7–−18.8, −18.7–41.2, 41.3–101.2, 101.3–161.2, and ≥161.3. Surface water sampling dates that matched those specific ranges in environmental conditions were identified and the average geometric means (of enterococci concentrations) were extracted for each bin. Confidence intervals for each of the bins were calculated using bootstrapping (random sampling with replacement) with 5000 iterations. Subsequently, permutation-based one-way ANOVAs were executed for each explanatory variable to test for significance across different environmental parameter ranges. For those intervals that showed a significant or marginally non-significant difference, a series of a posteriori, multiple-comparison (pair-wise) tests were run to identify those bins that were significantly different.

A stepwise selection of explanatory variables via forward addition based on Akaike Information Criteria (AIC) was executed. AIC analyses identified the optimal environmental factors that substantially explained variation in culturable enterococci concentrations [43–45]. The variables included in the AIC analysis were precipitation, SST, dew point, MSL, DNI, and turbidity. The MATLAB Fathom toolbox was used for all data analyses [46].

3. Results

3.1. Modeling Culturable Enterococci Using Akaike Information Criterion Model and Correlation Analyses

The environmental variables used in the AIC model were selected based on their significant, time-lagged correlations identified by the Pearson's correlation analyses ($p < 0.05$; Table 1). The stepwise AIC analyses showed that precipitation, MSL, DNI, SST, and turbidity were the optimal explanatory variables for culturable enterococci concentrations in Escambron Beach surface waters during 2005–2015 ($p < 0.05$; $r^2 = 0.20$; Table 2); dew point was not identified as an optimal explanatory variable by the AIC analyses.

Table 1. Pearson's correlation coefficient to identify significant lags in enterococci concentrations in surface waters at Escambron Beach with respect to the environmental parameters: Mean sea level (MSL), direct normal irradiance (DNI), sea surface temperature (SST), dew point, turbidity, and precipitation. Values are considered significant at $\alpha < 0.05$.

Variable	Pearson's Correlation Coefficient (r)	Lag
Mean sea level	−0.19	9th to 11th day (mean)
Direct normal irradiance	−0.24	1 day
Sea surface temperature	0.12	5th to 9th day (mean)
Dew point	0.19	7 days (mean)
Turbidity	0.25	1 day
Precipitation	0.22	4 days (accumulated)

Table 2. Akaike Information Criterion (AIC) model with those environmental variables that explained enterococci concentration variability in surface waters at Escambron Beach.

Variable	r^2	r^2-Adjusted	AIC
Precipitation	0.08	0.08	59.81
Mean sea level	0.13	0.12	48.76
Direct normal irradiance	0.16	0.15	39.19
Sea surface temperature	0.19	0.17	32.79
Turbidity	0.21	0.19	26.76

3.2. Environmental Variables Influence on Culturable Enterococci

All the variables were divided into three to six bins to characterize how culturable enterococci concentrations were influenced across specific ranges of environmental variables (Figure 2A–F). Precipitation, SST, dew point, and turbidity anomalies showed a positive correlation with enterococci concentrations in Escambron Beach surface waters (Table 1). Enterococci concentrations were above the 2014 US EPA BAV and exceeded the 2012 US EPA RWQC recommendation 1 (36 estimated illnesses per 1000 recreators, [18,20]) after 481 mm–960 mm of rainfall in four days, SST greater than 0.8 °C for at least 5 days, or turbidity anomalies greater than 0.005 sr^{-1} after 24 h (Figure 2). During warmer anomalies of high dew points sustained over seven consecutive days, there were higher concentrations of culturable enterococci. (Table 1). While these conditions did not automatically lead to levels exceeding the 2014 US EPA BAV, they did lead to values above the original 2012 US EPA RWQC recommendation 1 (Figure 2E).

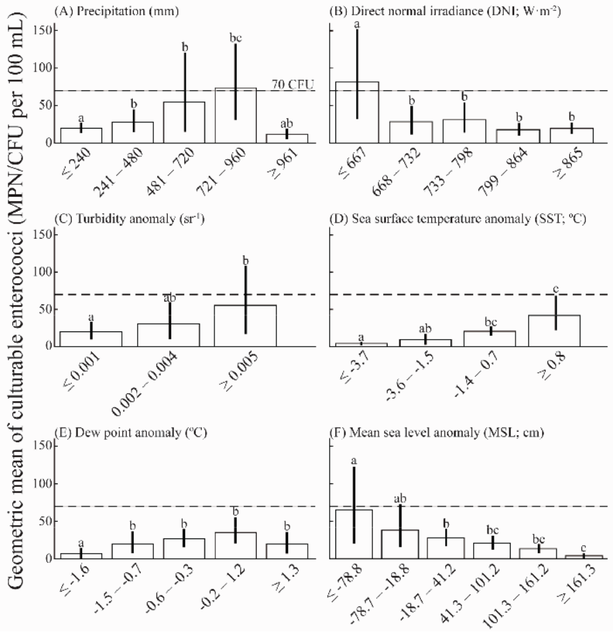

Figure 2. Geometric mean of enterococci concentrations in Escambron Beach surface waters at different ranges of (**A**) precipitation; (**B**) direct normal irradiance (DNI); (**C**) turbidity anomaly; (**D**) sea surface temperature anomaly (SST); (**E**) dew point anomaly; and (**F**) mean sea level (MSL) anomaly at Escambron Beach during 2005–2015. Dashed lines are the 2014 US EPA beach action value (BAV) of 70 CFU/100 mL (US EPA 2012; 2014). Vertical lines represent the 95% confidence interval. Lower-case letters above the vertical lines identify statistically significant differences among bins ($\alpha = 0.05$).

Conversely, DNI and MSL showed a strong, negative correlation with enterococci concentrations in Escambron Beach surface waters. The highest correlations were observed after a 24-h lag for DNI, and a nine-day lag for MSL anomalies (Table 1). Higher culturable enterococci concentrations, exceeding the 2014 US EPA BAV and 2012 US EPA RWQC recommendation 1, were observed during the lowest DNI (≤667 $W \cdot m^{-2}$; Figure 2B) as well as during the lowest negative MSL anomalies (≤−18.8 cm; Figure 2F). Culturable enterococci concentrations decreased as DNI increased (>~668 $W \cdot m^{-2}$; Figure 2B). Wind data (i.e., average wind speed, u-component, and v-component) showed no significant correlation with culturable enterococci during 2005–2015 (data not shown).

3.3. Satellite-Derived SST and Turbidity Anomaly Images to Anticipate Potential Beach Advisories

The PREQB issued beach advisories on 9 March 2007 (>35 enterococci CFU/100 mL) and 16 December 2011 (48 enterococci CFU/100 mL), where the possible sources were identified as sewer line and runoff, respectively (US EPA 2016). The mean satellite-derived turbidity over three consecutive days prior to these events was higher than normal in adjacent areas (Figure 3; gray boxes). Higher than normal river discharge (i.e., >~0.2 $m^3 \cdot s^{-1}$ based on anomalies from 1998 to 2015; data not shown) was observed on 16 December 2011 (Figure 3B; Rio Grande de Loiza; dark blue box). Warmer than normal waters were also observed on both dates (Figure 4) over the whole region. SST on 16 December 2011 was even warmer (~0.5–1.0 °C) than on 9 March 2007 (Figure 4).

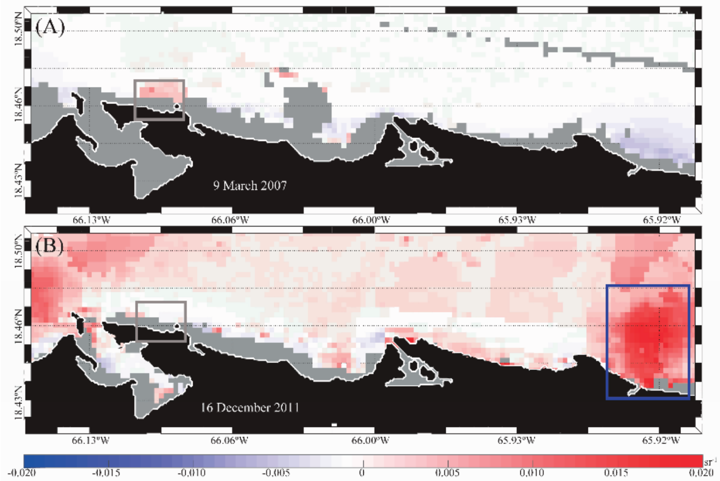

Figure 3. Anomalies of remote sensing reflectance (R_{rs} 645 nm, 250 m spatial resolution, from the Moderate Resolution Imaging Spectroradiometer MODIS-Terra) showing Escambron Beach (gray box) water clarity anomalies three days before the beach advisories of (**A**) 9 March 2007 and (**B**) 16 December 2011. Blue box on (**B**) shows the high discharge of Rio Grande de Loiza on 16 December 2011.

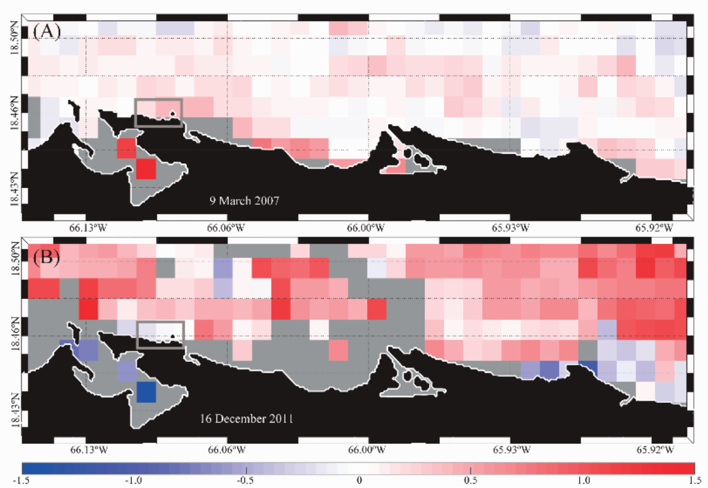

Figure 4. Sea Surface Temperature (SST) anomalies (from the Advanced Very High Resolution Radiometer-AVHRR; 1km spatial resolution) showing Escambron Beach (gray box) three days before the beach advisories of (**A**) 9 March 2007; and (**B**) 16 December 2011.

4. Discussion

In US recreational waters, enterococci continue to be the recommended fecal pollution indicator despite their natural presence in tropical waters [6,7,47,48]. Thus, it is necessary to understand the environmental factors related to elevated FIB concentrations to begin to differentiate pollution events from FIB ecology as well as to forecast water quality. The current case study investigated the environmental conditions that are related to exceeding the recommended enterococci concentrations at a Caribbean beach. While similar studies have been executed in other tropical [13,49] and subtropical locations [50–53] using in situ environmental data, this study also incorporated satellite-derived environmental data. In addition to identifying the time-lagged correlations of environmental parameters and culturable enterococci concentrations, the environmental parameter ranges and patterns were also identified when enterococci concentrations indicated unsafe recreational water quality (>70 CFU/100 mL; 2014 US EPA BAV). While future research is needed to identify if enterococci correlate with human health risks, this study provides an overview of how long-term data can be used to understand the most influential environmental variables on culturable enterococci concentrations and sets the stage for future investigations to distinguish the cause of high enterococci concentrations (fecal pollution events vs. enterococci ecology).

4.1. Environmental Factors Associated with Culturable Enterococci Variability

Of the environmental factors analyzed in this study, only precipitation, DNI, MSL, SST, and turbidity were significantly associated with enterococci concentrations; these associations may have been due to enterococci ecology and/or fecal pollution events. The strong correlation between precipitation, particularly 4-day accumulated rain events, and enterococci concentrations may indeed be explained by increased sewage and septic tank discharge [2,14,54–56], or runoff with animal feces [9,25,57,58]. However, it is also possible that precipitation increased the presence of non-fecal sources of enterococci via resuspension of sediments as well as runoff of bacteria in soil [35,36]. With respect to lower enterococci concentrations outside the 481 mm–960 mm precipitation range, it is possible that drier conditions promoted decreased bacterial replication due to lower nutrient additions and/or reduced stormwater inputs decreased the input of enterococci into beach surface waters. With respect to the wettest conditions, it is possible that the excess rainfall diluted enterococci concentrations in surface waters [59,60].

Similarly, the significant decrease observed in enterococci concentrations during periods of high solar irradiance was likely due to production of reactive oxygen species (ROS) that cause bacterial dieoff [27,30,61]; however, enterococci concentrations may have been low due to a lack of fecal pollution inputs (e.g., stormwater, runoff). There was also an inverse correlation between enterococci concentrations and MSL anomalies. It is possible that bacterial dilution occurs during higher MSL anomalies, and concomitant back-washing mixing and enhanced drainage from coastal sources may promote increased bacteria concentrations during lower MSL anomalies [30,62,63]. While winds have been previously correlated with increased enterococci concentrations [35,64], no correlation between wind components and culturable enterococci concentrations was identified for Escambron Beach surface waters during 2005–2015. It is possible that the limited local wind data obscured the identification of significant correlations between winds and enterococci concentrations. Additionally, increased enterococci concentrations were observed during warmer SST anomalies, which could be due to an increased bacterial growth and replication (e.g., metabolism) due to warmer water temperatures [35,48]. Finally, this study did not consider how the presence of aquatic plants, such as seagrass, or algae, such as the green alga *Cladophora*, could have decreased and increased enterococci concentrations, respectively [65–68].

Given the combined effects between many of the environmental parameters analyzed, it is difficult to tease apart their independent influence on coastal enterococci concentrations. For example, higher turbidity anomalies are the result of increased runoff [69,70], but increased enterococci concentrations could also be the result of protection from UV exposure [28,29,71]. Furthermore, it is possible that dew point was not identified as an optimal explanatory variable due to its relationship with rainfall as well as to SST [72] as the AIC model reduces multi-collinearity (i.e., higher correlations between predictor variables). Finally, winds are known to be associated with increased precipitation, which also leads to increased wave action that stimulate sediment resuspension, which can increase non-fecal enterococci concentrations [35,58,64]. While it is difficult to discern the confounding influence of each environmental parameter, this study identified the environmental conditions that should be considered for microbial water quality modeling at Escambron Beach.

Even though this study was not able to tease apart the combined effects of environmental variables nor enterococci ecology from actual fecal pollution events, the results of this investigation demonstrated that precipitation, DNI, MSL, SST, and turbidity were strongly and significantly associated with culturable enterococci concentrations at a tropical, Caribbean beach. Similar results have been shown in freshwaters, where enterococci concentrations were modeled in the great lakes and parameters, such as river discharge, temperature, turbidity, and winds were significantly correlated with enterococci variability [73,74]. Liu et al. [75] showed that human fecal pollution is also transported in river tributaries, where discharges into the great lakes influence enterococci concentrations and create public health concerns. Now that the environmental conditions associated with enterococci concentrations exceeding the limits for safe recreation have been identified for Escambron Beach, future research is needed to tease apart the influence of enterococci ecology versus fecal pollution events and should include microbial source tracking and measurement of human pathogens.

4.2. Sanitation Infrastructure, Human Fecal Pollution, and Culturable Enterococci Variability

Since only 20% of the enterococci variation was explained by the environmental parameters in the AIC model in this study, it is possible that the remaining 80% could have been attributed to sanitation infrastructure (which was not included in the analyses), other environmental factors (e.g., extra-intestinal, environmental enterococci sources; animal feces), as well as stochastic variability [35]. About 42% of the people living in Puerto Rico use septic tanks and many of these systems do not work properly or lack maintenance [4]. Consequently, improperly functioning systems and the porous karst-geology facilitate the movement of domestic wastewater into surrounding surface waters [1,4]. Over the last 50 to 60 years, there has been a shift from septic tanks to centralized WWTP (primary treatment) to accommodate San Juan's growing population and increasing urbanization. Escambron Beach also has public bathrooms located next to its stormwater discharge. While toilets are connected to the centralized sewer system, there may be leaks that can influence enterococci concentrations at the study site [2].

The combined Puerto Nuevo Regional and Bayamon Regional WWTP ocean outfall is located approximately 5 km northwest from Escambron Beach. Yet this discharge can impact beach water quality under specific current regimes. The outfall discharges ~200 MGD of primary-treated domestic wastewater at 40 m depth [76], which vertically mixes due to buoyancy forces and causes the development of an ocean outfall surface and sub-surface boils [77]. Following initial mixing, ocean currents can transport and dilute the outfall boil. Currents in Puerto Rico are generally westward and influenced by the westerlies; however, the CariCOOS buoy current data shows very weak south-southeast semi-diurnal tidal currents on Puerto Rico's northern coast between 2 and 30 m depth. Any episode that strengthens this eastward flow can carry outfall boils toward Escambron Beach. Additionally, the Rio Grande de Loiza discharge is another potential source of contamination due to westward movement of currents. Studies have shown that this river's tributaries were impaired due to fecal contamination [32,33,78]; thus, the Rio Grande de Loiza discharge could have impacted the study area, particularly when flow rates were high during rain events [79].

4.3. Satellite-Derived SST and Turbidity Anomaly Images, and PREQB Beach Advisories

Beach advisories are issued by PREQB two to three days after the sampling date when culturable enterococci concentrations exceeded the PREQB water quality criteria. Consequently, it is important to understand the lags, ranges, and spatial distributions of precipitation, DNI, MSL, SST, and turbidity, that are correlated with culturable enterococci concentrations to identify conditions that lead to such advisories [80]. Additionally, it is well-known that enterococci may not be the most appropriate water quality indicator for tropical regions due to its presence in secondary, non-fecal related reservoirs that confound the identification of health risks (e.g., soil, sediments) [17,49]. Thus, this understanding can inform future studies that seek to decipher when enterococci might exceed US EPA guidelines and represent an actual health risk versus when no health risk is present [17].

For example, high turbidity and SST anomalies occurred at Escambron beach during the days preceding the 9 March 2007 and 16 December 2011 beach closures. Prior to the 16 December 2011 advisory, there was a significant discharge from the Rio Grande de Loiza, which was likely transported toward the beach by westward ocean currents [1] and likely caused the high turbidity anomalies observed. While there was no anomalous river discharge during 9 March 2007, higher than normal turbidity was observed north of the study area. Additionally, the images showed warmer than normal water temperatures (~1.0–1.5 °C) for most of the region, which also likely influenced increased culturable enterococci concentrations. Since these satellite-derived data preceded the beach closures, satellite-derived data can help identify conditions for poor water quality in advance and guide sampling efforts.

4.4. Future Work

A better understanding of enterococci variability with respect to environmental conditions and fecal pollution events is needed to identify accurately public health risks and minimize public exposure to such risks [10,81,82]. Ideally, these risks should be forecasted by beach-specific predictive models to prevent the public's exposure to waterborne pathogens [28,83]. To create such models, future investigations at Escambron Beach should consider this study's results, which identified that precipitation, DNI, MSL, SST, and turbidity significantly influence enterococci concentrations, as well as the following: (1) the relationship between enterococci and human health risks (e.g., illness, reference pathogens) and (2) the impact of human (e.g., WWTP ocean outfall, leaky septic systems) and non-human (e.g., animal feces) fecal pollution sources that can influence enterococci variability and/or health risks. Additionally, data were not analyzed in terms of wet/dry season because the dry season at Escambron beach is only two-months long (February and March) and therefore, requires a different data set to achieve sufficient statistical power to determine how environmental variability influences enterococci concentrations by season.

Since the Puerto Rican economy relies mostly on tourism, proper management of Escambron Beach through targeted monitoring and early-warning systems is necessary to restrict beach advisories/closures to those that are truly necessary to protect public health [51,78]. This investigation demonstrated that satellite-derived and local environmental parameters explained enterococci variability at a tropical, Caribbean beach. The results presented will be important to future water quality investigations in the tropics, as well as to the development of the spatial and temporal components of predictive models that aim to improve forecasting and now-casting of beach water quality.

5. Conclusions

Identifying the environmental factors correlated with culturable enterococci concentrations can help to better understand their potential risk to public health in a tropical setting. This study looked into 11 years of culturable enterococci concentrations and assessed how much of its variability can be explained by environmental factors alone, where the main findings were:

- Environmental factors (i.e., direct normal irradiance (DNI), mean sea level (MSL), precipitation, turbidity, and sea surface temperature (SST)) explained 20% of the enterococci variability observed in Escambron Beach surface waters during 2005–2015.
- Identified time-lags for the different environmental factors helped better understand variability in culturable enterococci in Escambron Beach surface waters due to environmental factors.

- Increased enterococci concentrations were observed at strong positive SST and turbidity anomalies; conversely, these concentrations decreased with increased DNI and MSL anomalies in Escambron Beach surface waters.
- Specific ranges of precipitation (i.e., 481–960 mm) promoted increased enterococci concentrations, potentially due to urban run-off (e.g., resuspension of solids, soil runoff, and non-human sources), combined sewer overflow events, and/or increased leaching of septic tanks.
- The combined effects of environmental factors can help model culturable enterococci concentrations and understand ranges where these would exceed recommended 2014 US EPA BAV at Escambron Beach.
- Satellite-derived data can improve beach water quality assessments, potentially reducing in-situ sampling efforts as this data is readily available, and help identify events for early warning systems and improve beach advisory.

Supplementary Materials: The following are available online at www.mdpi.com/1660-4601/14/12/1602/s1, Table S1: Pearson's correlation coefficient to identify significant lags in enterococci concentrations in Escambron Beach surface waters with respect to the environmental parameters. Concentrations below the limit of detection were substituted by 1 MPN/CFU per 100 mL. Values are considered significant with 95% certainty ($\alpha = 0.05$). Bold values are those with the highest and significant Pearson's correlation coefficient, Table S2: Pearson's correlation coefficient to identify significant lags of enterococci concentrations in Escambron Beach surface waters with respect to the environmental parameters. Concentrations below the limit of detection were substituted by 3 MPN/CFU per 100 mL for those samples analyzed before April 2015 and 9 MPN/100 mL for samples analyzed after April 2015. Values are considered significant with 95% certainty ($\alpha = 0.05$). Bold values are those with the highest and significant Pearson's correlation coefficient, Table S3: Pearson's correlation coefficient to identify significant lags of enterococci concentrations in Escambron Beach surface waters with respect to the environmental parameters. Concentrations below the limit of detection were substituted by 2 MPN/CFU per 100 mL for those samples analyzed before April 2015 and 5 MPN/100mL for samples analyzed after April 2015. Values are considered significant with 95% certainty ($\alpha = 0.05$). Bold values are those with the highest and significant Pearson's correlation coefficient.

Acknowledgments: This investigation and A.E.L.-R. were supported by the US National Science Foundation (NSF) Partnerships for International Research (PIRE) under Grant No. 1243510 and by the US National Aeronautics and Space Administration (NASA) Headquarters under the NASA Earth and Science Fellowship Program Grant No. NNX15AN60H. A.E.L.-R. was also funded by the USF College of Marine Science Linton Tibbetts Endowed Fellowship. F.E.M.-K., D.O., and D.R. were supported by the US EPA Science to Achieve Results (STAR) grant No. 83519301. E.M.S. was supported by the USF College of Marine Science William and Elsie Knight Fellowship and US NSF grant No. OCE-1566562. Mya Breitbart (USF) and Marirosa Molina (US EPA) are recognized for their invaluable input with respect to data interpretation, as well as David Jones (USF) for his help with the multivariate and time series analyses. Thanks to Valerie J. Harwood (USF) for providing feedback for this manuscript. Additionally, we recognize Matthew E. Verbyla (École Polytechnique Fédérale de Lausanne) for his insight into left-censored data analyses, Stephenie Ayala-Flores (PREQB) for providing data and information on beach water quality monitoring in Puerto Rico, and Mark E. Luther (USF) for his insight on Caribbean surface currents.

Author Contributions: E.M.S. contributed substantially with data interpretation and manuscript preparation; D.O. and D.R.-R. contributed with obtaining satellite data and data analyses; F.E.M.-K. collected the satellite observations, edited the text, and provided extensive written comments on the manuscript; A.E.L.-R. analyzed the data, and wrote and led the manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Lugo, A.E.; González, O.M.R.; Pedraza, C.R. *The Rio Piedras Watershed and Its Surrounding Environment*; USDA Forest Service: Washington, DC, USA, 2011.
2. Diaz, M.N. *Evaluacion de la Intensidad de Lluvia y Su Efecto en la Presencia de Bacterias Fecales en Las Playas del Norte de Puerto Rico*; Ciencias en Gerencia Ambiental; Universidad Metropolitana: San Juan, Puerto Rico, 2007.
3. Soderberg, C.-A.P. Agua potable y saneamiento para comunidades aisladas: Un imperativo social y ambiental para Puerto Rico. *Rev. Int. Desastres Nat. Accid. Infraestruct. Civ.* **2008**, *8*, 1–3.
4. Quiñones, F. Impacto ambiental de pozos sépticos en puerto rico y su diseño y control. *Dimensión Revista Colegio Ingenieros Agrimensores Puerto Rico* **2012**, *1*, 16–22.

5. Garcia-Montiel, D.C.; Verdejo-Ortiz, J.C.; Santiago-Bartolomei, R.; Vila-Ruiz, C.P.; Santiago, L.; Melendez-Ackerman, E. Food sources and accessibility and waste disposal patterns across an urban tropical watershed: Implications for the flow of materials and energy. *Ecol. Soc.* **2014**, *19*, 37. [CrossRef]
6. Colford, J.M.; Wade, T.J.; Schiff, K.C.; Wright, C.C.; Griffith, J.F.; Sandhu, S.K.; Burns, S.; Sobsey, M.; Lovelace, G.; Weisberg, S.B. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* **2007**, *18*, 27–35. [CrossRef] [PubMed]
7. Pruss, A. Review of epidemiological studies on health effects from exposure to recreational water. *Int. J. Epidemiol.* **1998**, *27*, 1–9. [CrossRef] [PubMed]
8. Boehm, A.B.; Sassoubre, L.M. Enterococci as Indicators of Environmental Fecal Contamination. In *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*; Gilmore, M.S., Clewell, D.B., Ike, Y., Shankar, N., Eds.; Massachusetts Eye and Ear Infirmary: Boston, MA, USA, 2014; pp. 1–17.
9. Colford, J.M.; Schiff, K.C.; Griffith, J.F.; Yau, V.; Arnold, B.F.; Wright, C.C.; Gruber, J.S.; Wade, T.J.; Burns, S.; Hayes, J.; et al. Using rapid indicators for *Enterococcus* to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Res.* **2012**, *46*, 2176–2186. [CrossRef] [PubMed]
10. Haile, R.W.; Witte, J.S.; Gold, M.; Cressey, R.; McGee, C.; Millikan, R.C.; Glasser, A.; Harawa, N.; Ervin, C.; Harmon, P.; et al. The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology* **1999**, *10*, 355–363. [CrossRef] [PubMed]
11. Parker, J.K.; McIntyre, D.; Noble, R.T. Characterizing fecal contamination in stormwater runoff in coastal North Carolina, USA. *Water Res.* **2010**, *44*, 4186–4194. [CrossRef] [PubMed]
12. Soller, J.; Bartrand, T.; Ravenscroft, J.; Molina, M.; Whelan, G.; Schoen, M.; Ashbolt, N. Estimated human health risks from recreational exposures to stormwater runoff containing animal faecal material. *Environ. Model. Softw.* **2015**, *72*, 21–32. [CrossRef]
13. Lamparelli, C.C.; Pogreba-Brown, K.; Verhougstraete, M.; Sato, M.I.Z.; de Castro Bruni, A.; Wade, T.J.; Eisenberg, J.N. Are fecal indicator bacteria appropriate measures of recreational water risks in the tropics: A cohort study of beach goers in Brazil? *Water Res.* **2015**, *87*, 59–68. [CrossRef] [PubMed]
14. Cordero, L.; Norat, J.; Mattei, H.; Nazario, C. Seasonal variations in the risk of gastrointestinal illness on a tropical recreational beach. *J. Water Health* **2012**, *10*, 579–593. [CrossRef] [PubMed]
15. Rochelle-Newall, E.; Nguyen, T.M.H.; Le, T.P.Q.; Sengteheuanghoung, O.; Ribolzi, O. A short review of fecal indicator bacteria in tropical aquatic ecosystems: Knowledge gaps and future directions. *Front. Microbiol.* **2015**, *6*, 308. [CrossRef] [PubMed]
16. Boehm, A.B.; Ashbolt, N.J.; Colford, J.M.; Dunbar, L.E.; Fleming, L.E.; Gold, M.A.; Hansel, J.A.; Hunter, P.R.; Ichida, A.M.; McGee, C.D.; et al. A sea change ahead for recreational water quality criteria. *J. Water Health* **2009**, *7*, 9–20. [CrossRef] [PubMed]
17. Fujioka, R.S.; Solo-Gabriele, H.M.; Byappanahalli, M.N.; Kirs, M. US Recreational water quality criteria: A vision for the future. *Int. J. Environ. Res. Public Health* **2015**, *12*, 7752–7776. [CrossRef] [PubMed]
18. United States Environmental Protection Agency. *Recreational Water Quality Criteria*; US EAP: Washington, DC, USA, 2012. Available online: https://www.epa.gov/wqc/microbial-pathogenrecreational-water-quality-criteria (accessed on 17 July 2017).
19. PREQB. Beach Monitoring and Public Notification Program-Performance criteria 2016–2017. 2016. Available online: https://www2.pr.gov/agencias/jca/Documents/Monitoreo%20de%20Playas/Performance%20Criteria/BEACH_PERFORMANCE_CRITERIA_PREQB_2016-2017_final-draft.pdf (accessed on 19 July 2017).
20. United States Environmental Protection Agency. National Beach Guidance and Required Performance Criteria for Grants. 2014. Available online: https://www.epa.gov/sites/production/files/2014-07/documents/beach-guidance-final-2014.pdf (accessed on 19 July 2017).
21. United States Environmental Protection Agency. *Beaches Environmental Assessment and Coastal Health Act of 2000*; Public Law; US EAP: Washington, DC, USA, 2000; pp. 106–284.
22. PREQB. Water Quality Standards Regulation of Puerto Rico. 2010. Available online: http://www.gobierno.pr/NR/rdonlyres/5A9F2F2E-94AE-4C69-8453-CA08D616ED7D/0/Reg_Estdares_Calidad_Agua_2010.pdf (accessed on 15 September 2017).
23. Bachoon, D.S.; Markand, S.; Otero, E.; Perry, G.; Ramsubaugh, A. Assessment of non-point sources of fecal pollution in coastal waters of Puerto Rico and Trinidad. *Mar. Pollut. Bull.* **2000**, *60*, 1117–1121. [CrossRef] [PubMed]

24. Sanchez-Nazario, E.E.; Santiago-Rodriguez, T.M.; Toranzos, G.A. Prospective epidemiological pilot study on the morbidity of bathers exposed to tropical recreational waters and sand. *J. Water Health* **2014**, *12*, 220–229. [CrossRef] [PubMed]
25. Santiago-Rodriguez, T.M.; Tremblay, R.L.; Toledo-Hernandez, C.; Gonzalez-Nieves, J.E.; Ryu, H.; Domingo, J.W.S.; Toranzos, G.A. Microbial quality of tropical inland waters and effects of rainfall events. *Appl. Environ. Microbiol.* **2012**, *78*, 5160–5169. [CrossRef] [PubMed]
26. Zhang, Z.H.; Deng, Z.Q.; Rusch, K.A. Development of predictive models for determining enterococci levels at Gulf Coast beaches. *Water Res.* **2012**, *46*, 465–474. [CrossRef] [PubMed]
27. Maraccini, P.A.; Mattioli, M.C.M.; Sassoubre, L.M.; Cao, Y.P.; Griffith, J.F.; Ervin, J.S.; Van De Werfhorst, L.C.; Boehm, A.B. Solar inactivation of enterococci and *Escherichia coli* in natural waters: Effects of water absorbance and depth. *Environ. Sci. Technol.* **2016**, *50*, 5068–5076. [CrossRef] [PubMed]
28. He, L.M.; He, Z.L. Water quality prediction of marine recreational beaches receiving watershed baseflow and stormwater runoff in southern California, USA. *Water Res.* **2008**, *42*, 2563–2573. [CrossRef] [PubMed]
29. Shibata, T.; Solo-Gabriele, H.M.; Sinigalliano, C.D.; Gidley, M.L.; Plano, L.R.; Fleisher, J.M.; Wang, J.D.; Elmir, S.M.; He, G.; Wright, M.E. Evaluation of conventional and alternative monitoring methods for a recreational marine beach with nonpoint source of fecal contamination. *Environ. Sci. Technol.* **2010**, *44*, 8175–8181. [CrossRef] [PubMed]
30. Maraccini, P.A.; Ferguson, D.M.; Boehm, A.B. Diurnal variation in *Enterococcus species* composition in polluted ocean water and a potential role for the Enterococcal Carotenoid in protection against photoinactivation. *Appl. Environ. Microbiol.* **2012**, *78*, 305–310. [CrossRef] [PubMed]
31. Ortiz-Zayas, J.R.; Cuevas, E.; Mayol-Bracero, O.L.; Donoso, L.; Trebs, I.; Figueroa-Nieves, D.; McDowell, W.H. Urban influences on the nitrogen cycle in Puerto Rico. *Biogeochemistry* **2006**, *79*, 109–133. [CrossRef]
32. Puerto Rico Environmental Quality Board. *Total Maximum Daily Loads (TMDL) Rio Grande de Loiza Watershed*; Puerto Rico Environmental Quality Board: San Juan, Puerto Rico, 2007.
33. Puerto Rico Environmental Quality Board. *Carga Máxima Total Diaria de Bacterias Coliformes Fecales (TMDL) Para Unidades de Evaluación, Puerto Rico*; Departamento de Planificación y Protección Ambiental: San Juan, Puerto Rico, 2011.
34. United States Environmental Protection Agency. *Method 1600: Enterococci in Water by Membrane Filtration Using Membrane-Enterococcus Indoxyl-B-D-Glucoside Agar (mEI)*; US EPA: Washington, DC, USA, 2006.
35. Byappanahalli, M.N.; Nevers, M.B.; Korajkic, A.; Staley, Z.R.; Harwood, V.J. Enterococci in the environment. *Microbiol. Mol. Biol. Rev.* **2012**, *76*, 685–706. [CrossRef] [PubMed]
36. Chen, Z.Q.; Hu, C.M.; Muller-Karger, F. Monitoring turbidity in Tampa Bay using MODIS/Aqua 250-m imagery. *Remote Sens. Environ.* **2007**, *109*, 207–220. [CrossRef]
37. Chen, Z.Q.; Hu, C.M.; Muller-Karger, F.E.; Luther, M.E. Short-term variability of suspended sediment and phytoplankton in Tampa Bay, Florida: Observations from a coastal oceanographic tower and ocean color satellites. *Estuar. Coast. Shelf Sci.* **2010**, *89*, 62–72. [CrossRef]
38. Aurin, D.; Mannino, A.; Franz, B. Spatially resolving ocean color and sediment dispersion in river plumes, coastal systems, and continental shelf waters. *Remote Sens. Environ.* **2013**, *137*, 212–225. [CrossRef]
39. Moreno-Madrinan, M.J.; Al-Hamdan, M.Z.; Rickman, D.L.; Muller-Karger, F.E. Using the surface reflectance MODIS Terra product to estimate turbidity in Tampa Bay, Florida. *Remote Sens.* **2010**, *2*, 2713–2728. [CrossRef]
40. Schang, C.; Henry, R.; Kolotelo, P.A.; Prosser, T.; Crosbie, N.; Grant, T.; Cottam, D.; O'Brien, P.; Coutts, S.; Deletic, A.; et al. Evaluation of techniques for measuring microbial hazards in bathing waters: A comparative study. *PLoS ONE* **2016**, *11*, e0155848. [CrossRef] [PubMed]
41. Leskinen, S.D.; Harwood, V.J.; Lim, D.V. Rapid dead-end ultrafiltration concentration and biosensor detection of enterococci from beach waters of Southern California. *J. Water Health* **2016**, *7*, 674–684. [CrossRef] [PubMed]
42. Boehm, A.B. Enterococci concentrations in diverse coastal environments exhibit extreme variability. *Environ. Sci. Technol.* **2017**, *41*, 8227–8232. [CrossRef]
43. Blanchet, F.G.; Legendre, P.; Borcard, D. Forward selection of explanatory variables. *Ecology* **2008**, *89*, 2623–2632. [CrossRef] [PubMed]
44. Burnham, K.P.; Anderson, D.R. Kullback-Leibler information as a basis for strong inference in ecological studies. *Wildl. Res.* **2001**, *28*, 111–119. [CrossRef]

45. Godinez-Dominguez, E.; Freire, J. Information-theoretic approach for selection of spatial and temporal models of community organization. *Mar. Ecol. Prog. Ser.* **2003**, *253*, 17–24. [CrossRef]
46. Jones, D. *Fathom Toolbox for Matlab: Software for Multivariate Ecological and Oceanographic Data Analysis*; College of Marine Science, University of South Florida: St. Petersburg, FL, USA, 2015; Available online: http://www.marine.usf.edu/user/djones/ (accessed on 10 October 2017).
47. Herrera, A.; Suarez, P. Bacterial markers as tools for coastal water environmental quality measurement. *Interciencia* **2005**, *30*, 171–176.
48. Mote, B.L.; Turner, J.W.; Lipp, E.K. Persistence and growth of the fecal indicator bacteria enterococci in detritus and natural estuarine plankton communities. *Appl. Environ. Microbiol.* **2012**, *78*, 2569–2577. [CrossRef] [PubMed]
49. Viau, E.J.; Goodwin, K.D.; Yamahara, K.M.; Layton, B.A.; Sassoubre, L.M.; Burns, S.L.; Tong, H.I.; Wong, S.H.C.; Lu, Y.A.; Boehm, A.B. Bacterial pathogens in Hawaiian coastal streams-associations with fecal indicators, land cover, and water quality. *Water Res.* **2011**, *45*, 3279–3290. [CrossRef] [PubMed]
50. Aranda, D.; Lopez, J.V.; Solo-Gabriele, H.M.; Fleisher, J.M. Using probabilities of enterococci exceedance and logistic regression to evaluate long term weekly beach monitoring data. *J. Water Health* **2016**, *14*, 81–89. [CrossRef] [PubMed]
51. Enns, A.A.; Vogel, L.J.; Abdelzaher, A.M.; Solo-Gabriele, H.M.; Plano, L.R.W.; Gidley, M.L.; Phillips, M.C.; Klaus, J.S.; Piggot, A.M.; Feng, Z.X.; et al. Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions. *Water Res.* **2012**, *46*, 2237–2246. [CrossRef] [PubMed]
52. Feng, Z.; Reniers, A.; Haus, B.; Solo-Gabriele, H.; Fiorentino, L.; Olascoaga, M.; MacMahan, J. Modeling microbial water quality at a beach impacted by multiple non-point sources. *Coast. Eng. Proc.* **2012**, *1*, 74. [CrossRef]
53. Wright, M.E.; Abdelzaher, A.M.; Solo-Gabriele, H.M.; Elmir, S.; Fleming, L.E. The inter-tidal zone is the pathway of input of enterococci to a subtropical recreational marine beach. *Water Sci. Technol.* **2011**, *63*, 542–549. [CrossRef] [PubMed]
54. Perez-Villalona, H.; Cornwell, J.C.; Ortiz-Zayas, J.R.; Cuevas, E. Sediment denitrification and nutrient fluxes in the San José Lagoon, a tropical lagoon in the highly urbanized San Juan Bay Estuary, Puerto Rico. *Estuar. Coasts* **2015**, *38*, 2259–2278. [CrossRef]
55. Ramirez, A.; De Jesús-Crespo, R.; Martinó-Cardona, D.M.; Martínez-Rivera, N.; Burgos-Caraballo, S. Urban streams in Puerto Rico: What can we learn from the tropics? *J. N. Am. Benthol. Soc.* **2009**, *28*, 1070–1079. [CrossRef]
56. Zhang, W.W.; Wang, J.Y.; Fan, J.F.; Gao, D.L.; Ju, H.Y. Effects of rainfall on microbial water quality on Qingdao No. 1 Bathing Beach, China. *Mar. Pollut. Bull.* **2013**, *66*, 185–190. [CrossRef] [PubMed]
57. Shehane, S.D.; Harwood, V.J.; Whitlock, J.E.; Rose, J.B. The influence of rainfall on the incidence of microbial faecal indicators and the dominant sources of faecal pollution in a Florida river. *J. Appl. Microbiol.* **2005**, *98*, 1127–1136. [CrossRef] [PubMed]
58. Feng, Z.X.; Reniers, A.; Haus, B.K.; Solo-Gabriele, H.M. Modeling sediment-related enterococci loading, transport, and inactivation at an embayed nonpoint source beach. *Water Resour. Res.* **2013**, *49*, 693–712. [CrossRef]
59. Ioan, C.; Robescu, D.N. Effect of increased inflow and dilution on the activated sludge properties and viability of microbial community. *UPB Sci. Bull.* **2015**, *77*, 197–204.
60. Kleinheinz, G.T.; McDermott, C.M.; Hughes, S.; Brown, A. Effects of rainfall on *E. coli* concentrations at Door County, Wisconsin beaches. *Int. J. Microbiol.* **2009**, *2009*, 876050. [CrossRef] [PubMed]
61. Boehm, A.B.; Yamahara, K.M.; Love, D.C.; Peterson, B.M.; McNeill, K.; Nelson, K.L. Covariation and photoinactivation of traditional and novel indicator organisms and human viruses at a sewage-impacted marine beach. *Environ. Sci. Technol.* **2009**, *43*, 8046–8052. [CrossRef] [PubMed]
62. Grant, S.B.; Sanders, B.F.; Boehm, A.B.; Redman, J.A.; Kim, J.H.; Mrse, R.D.; Chu, A.K.; Gouldin, M.; McGee, C.D.; Gardiner, N.A.; et al. Generation of enterococci bacteria in a coastal saltwater marsh and its impact on surf zone water quality. *Environ. Sci. Technol.* **2001**, *35*, 2407–2416. [CrossRef] [PubMed]
63. Yamahara, K.M.; Walters, S.P.; Boehm, A.B. Growth of enterococci in unaltered, unseeded beach sands subjected to tidal wetting. *Appl. Environ. Microbiol.* **2009**, *75*, 1517–1524. [CrossRef] [PubMed]
64. Gast, R.J.; Gorrell, L.; Raubenheimer, B.; Elgar, S. Impact of erosion and accretion on the distribution of enterococci in beach sands. *Cont. Shelf Res.* **2011**, *31*, 1457–1461. [CrossRef] [PubMed]

65. Dodds, W.K.; Gudder, D.A. The ecology of *Cladophora*. *J. Phycol.* **1992**, *28*, 415–427. [CrossRef]
66. Ishii, S.; Yan, T.; Shively, D.A.; Byappanahalli, M.N.; Whitman, R.L.; Sadowsky, M.J. *Cladophora* (Chlorophyta) spp. harbor human bacterial pathogens in nearshore water of Lake Michigan. *Appl. Environ. Microbiol.* **2006**, *72*, 4545–4553. [CrossRef] [PubMed]
67. Lamb, J.B.; van de Water, J.; Bourne, D.G.; Altier, C.; Hein, M.Y.; Fiorenza, E.A.; Abu, N.; Jompa, J.; Harvell, C.D. Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. *Science* **2017**, *355*, 731–733. [CrossRef] [PubMed]
68. Whitman, R.L.; Shively, D.A.; Pawlik, H.; Nevers, M.B.; Byappanahalli, M.N. Occurrence of *Escherichia coli* and enterococci in *Cladophora* (Chlorophyta) in nearshore water and beach sand of Lake Michigan. *Appl. Environ. Microbiol.* **2003**, *69*, 4714–4719. [CrossRef] [PubMed]
69. Serrano, E.; Moreno, B.; Solaun, M.; Aurrekoetxea, J.J.; Ibarluzea, J. The influence of environmental factors on microbiological indicators of coastal water pollution. *Water Sci. Technol.* **1998**, *38*, 195–199.
70. Soupir, M.L.; Mostaghimi, S.; Dillaha, T. Attachment of Escherichia coli and Enterococci to Particles in Runoff. *J. Environ. Qual.* **2010**, *39*, 1019–1027. [CrossRef] [PubMed]
71. Landmeyer, J.E.; Garigen, T.J. *Relation between Enterococcus Concentrations and Turbidity in Fresh and Saline Recreational Waters, Coastal Horry County, South Carolina, 2003–04*; US Geological Survey: Reston, VA, USA, 2016.
72. Phillips, L.; Liss, P. The Physical Chemistry of Air-Sea Gas Exchange. In *The Sea Surface and Global Change*; Cambrigde University Press: Cambridge, UK, 1997; pp. 207–250.
73. Byappanahalli, M.N.; Whitman, R.L.; Shively, D.A.; Nevers, M.B. Linking non-culturable (qPCR) and culturable enterococci densities with hydrometeorological conditions. *Sci. Total Environ.* **2010**, *408*, 3096–3101. [CrossRef] [PubMed]
74. Nevers, M.B.; Whitman, R.L. Nowcast modeling of *Escherichia coli* concentrations at multiple urban beaches of southern Lake Michigan. *Water Res.* **2005**, *39*, 5250–5260. [CrossRef] [PubMed]
75. Liu, L.; Phanikumar, M.S.; Molloy, S.L.; Whitman, R.L.; Shively, D.A.; Nevers, M.B.; Schwab, D.J.; Rose, J.B. Modeling the transport and inactivation of *E. coli* and enterococci in the near-shore region of Lake Michigan. *Environ. Sci. Technol.* **2006**, *40*, 5022–5028. [CrossRef] [PubMed]
76. United States Environmental Protection Agency. *Draft National Pollutant Discharge Elimination System (NPDES) Permit to Discharge into the Waters of the United States*; US EPA: Washington, DC, USA, 1994; p. 15.
77. Huang, H.; Fergen, R.E.; Proni, J.R.; Tsai, J.J. Probabilistic analysis of ocean outfall mixing zones. *J. Environ. Eng.* **1996**, *122*, 359–367. [CrossRef]
78. Isleib, R.; Thuman, A. Verification of large scale watershed modeling analysis using small subwatershed models. In Proceedings of the 2010 Watershed Management: Innovations in Watershed Management under Land Use and Climate Change, Madison, WI, USA, 23–27 August 2010; pp. 1405–1416.
79. Murphy, S.F.; Stallard, R.F. Hydrology and climate of four watersheds in eastern Puerto Rico. In *Water Quality and Landscape Processes of Four Watersheds in Eastern Puerto Rico*; U.S. Geological Survey: Reston, VA, USA, 2012; Volume 100, pp. 43–84.
80. Crowther, J.; Kay, D.; Wyer, M.D. Relationships between microbial water quality and environmental conditions in coastal recreational waters: The Fylde coast, UK. *Water Res.* **2001**, *35*, 4029–4038. [CrossRef]
81. Boehm, A.B.; Fuhrman, J.A.; Mrse, R.D.; Grant, S.B. Tiered approach for identification of a human fecal pollution source at a recreational beach: Case study at Avalon Cay, Catalina Island, California. *Environ. Sci. Technol.* **2003**, *37*, 673–680. [CrossRef] [PubMed]
82. Fleisher, J.M.; Fleming, L.E.; Solo-Gabriele, H.M.; Kish, J.K.; Sinigalliano, C.D.; Plano, L.; Elmir, S.M.; Wang, J.D.; Withum, K.; Shibata, T.; et al. The BEACHES Study: Health effects and exposures from non-point source microbial contaminants in subtropical recreational marine waters. *Int. J. Epidemiol.* **2010**, *39*, 1291–1298. [CrossRef] [PubMed]
83. Mavani, J.; Chen, L.; Joksimovic, D.; Li, S. *Development and Testing of Data Driven Nowcasting Models of Beach Water Quality*; City University of New York: New York, NY, USA, 2014; p. 318.

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Supplementary Materials: Environmental Factors Correlated with Culturable Enterococci Concentrations in Tropical Recreational Waters: A Case Study in Escambron Beach, San Juan, Puerto Rico

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Table S1. Pearson's correlation coefficient to identify significant lags in enterococci concentrations in Escambron Beach surface waters with respect to the environmental parameters. Concentrations below the limit of detection were substituted by 1 MPN/CFU per 100 mL. Values are considered significant with 95% certainty ($\alpha = 0.05$). Bold values are those with the highest and significant Pearson's correlation coefficient.

Precipitation			**Sea Surface Temperature**			**Dew Point**			**Mean Sea Level**			**Direct Normal Irradiance**			**Turbidity**		
Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value
1	0.12	0.0278	1	0.05	0.3504	1	0.15	0.0086	1	0.09	0.1064	1	−0.25	0.0004	1	0.23	0.0235
2	0.23	0.0002	2	0.07	0.2150	2	0.17	0.0042	2	0.07	0.2376	2	−0.22	0.0010	2	0.05	0.6267
3	**0.23**	**0.0002**	3	0.08	0.1604	3	0.17	0.0028	3	0.05	0.3720	3	−0.22	0.0006	3	−0.02	0.8455
4	**0.23**	**0.0004**	4	0.08	0.1688	4	0.19	0.0028	4	0.01	0.8484	4	−0.22	0.0002	4	−0.17	0.1049
5	0.22	0.0002	**5**	**0.12**	**0.0436**	5	0.20	0.0008	5	−0.04	0.4962	5	−0.22	0.0002	5	0.13	0.2510
6	0.19	0.0010	6	0.07	0.2382	6	0.20	0.0004	6	−0.11	0.0502	6	−0.20	0.0004	6	0.03	0.7851
7	0.18	0.0014	7	0.10	0.0790	**7**	**0.21**	**0.0006**	7	−0.17	0.0028	7	−0.20	0.0014	7	0.08	0.4977
8	0.17	0.0050	8	0.08	0.1796	8	0.20	0.0006	8	−0.21	0.0006	8	−0.19	0.0014	8	−0.15	0.1640
9	0.15	0.0108	9	0.06	0.2708	9	0.20	0.0010	**9**	**−0.23**	**0.0004**	9	−0.17	0.0040	9	0.11	0.3536
10	0.14	0.0154	10	0.01	0.8526	10	0.20	0.0010	10	−0.22	0.0002	10	−0.17	0.0042	10	0.00	0.9726
11	0.14	0.0140	11	0.04	0.4992	11	0.20	0.0004	11	−0.19	0.0012	11	−0.17	0.0062	11	0.16	0.1341
12	0.14	0.0158	12	0.00	0.9968	12	0.20	0.0006	12	−0.13	0.0214	12	−0.16	0.0060	12	−0.15	0.1564
13	0.14	0.0130	13	−0.03	0.6000	13	0.20	0.0008	13	−0.05	0.3908	13	−0.16	0.0068	13	−0.12	0.2779
14	0.12	0.0350	14	−0.03	0.6330	14	0.20	0.0018	14	0.00	0.9984	14	−0.15	0.0126	14	−0.15	0.1561
15	0.12	0.0376	15	−0.03	0.6562	15	0.20	0.0008	15	0.04	0.4748	15	−0.14	0.0182	15	−0.05	0.6400
16	0.11	0.0450	16	0.01	0.9226	16	0.19	0.0016	16	0.04	0.5312	16	−0.14	0.0220	16	−0.01	0.9330
17	0.12	0.0380	17	−0.02	0.7208	17	0.18	0.0026	17	0.02	0.7450	17	−0.14	0.0250	17	−0.15	0.1591
18	0.13	0.0238	18	−0.03	0.5506	18	0.18	0.0028	18	−0.03	0.6560	18	−0.13	0.0290	18	−0.08	0.5045
19	0.13	0.0188	19	0.00	0.9512	19	0.18	0.0028	19	−0.07	0.2368	19	−0.14	0.0230	19	0.07	0.5134
20	0.14	0.0178	20	0.03	0.5778	20	0.18	0.0030	20	−0.10	0.0824	20	−0.14	0.0272	20	−0.03	0.8150
21	0.12	0.0310	21	0.05	0.3852	21	0.18	0.0020	21	−0.14	0.0200	21	−0.13	0.0296	21	0.00	0.9687

Table S2. Pearson's correlation coefficient to identify significant lags of enterococci concentrations in Escambron Beach surface waters with respect to the environmental parameters. Concentrations below the limit of detection were substituted by 3 MPN/CFU per 100 mL for those samples analyzed before April 2015 and 9 MPN/ 100mL for samples analyzed after April 2015. Values are considered significant with 95% certainty ($\alpha = 0.05$). Bold values are those with the highest and significant Pearson's correlation coefficient.

Precipitation			**Sea Surface Temperature**			**Dew Point**			**Mean Sea Level**			**Direct Normal Irradiance**			**Turbidity**		
Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value
1	0.13	0.0234	1	0.07	0.2196	1	0.13	0.0234	1	0.11	0.0594	1	−0.24	0.0002	1	0.25	0.0150
2	0.23	0.0004	2	0.09	0.1252	2	0.15	0.0102	2	0.08	0.1694	2	−0.21	0.0006	2	0.04	0.7100
3	**0.23**	**0.0002**	3	0.09	0.0962	3	0.15	0.0102	3	0.06	0.2728	3	−0.20	0.0010	3	−0.03	0.7696
4	**0.22**	**0.0006**	4	0.10	0.0930	4	0.17	0.0028	4	0.02	0.7198	4	−0.21	0.0012	4	−0.19	0.0700
5	0.21	0.0004	**5**	**0.12**	**0.0428**	5	0.18	0.0018	5	−0.03	0.6370	5	−0.20	0.0008	5	0.11	0.2838
6	0.17	0.0026	6	0.08	0.1796	6	0.18	0.0022	6	−0.10	0.0812	6	−0.18	0.0030	6	−0.01	0.9500
7	0.15	0.0088	7	0.11	0.0672	**7**	**0.19**	**0.0014**	7	−0.15	0.0066	7	−0.17	0.0038	7	0.09	0.4264
8	0.15	0.0072	8	0.08	0.1782	8	0.19	0.0018	8	−0.19	0.0016	8	−0.17	0.0046	8	−0.17	0.1161
9	0.13	0.0184	9	0.06	0.3234	9	0.18	0.0022	**9**	**−0.19**	**0.0012**	9	−0.15	0.0126	9	0.10	0.3817
10	0.12	0.0290	10	0.02	0.7010	10	0.18	0.0024	10	−0.18	0.0030	10	−0.15	0.0132	10	0.00	0.9630
11	0.12	0.0356	11	0.06	0.3414	11	0.19	0.0020	11	−0.13	0.0226	11	−0.15	0.0146	11	0.15	0.1362
12	0.12	0.0488	12	0.02	0.6708	12	0.19	0.0012	12	−0.08	0.1874	12	−0.14	0.0168	12	−0.16	0.1264
13	0.12	0.0444	13	−0.01	0.8966	13	0.18	0.0018	13	0.00	0.9530	13	−0.14	0.0176	13	−0.11	0.3476
14	0.10	0.0832	14	−0.01	0.8910	14	0.18	0.0018	14	0.04	0.4280	14	−0.13	0.0308	14	−0.14	0.1859
15	0.10	0.0956	15	−0.01	0.9104	15	0.18	0.0014	15	0.08	0.1590	15	−0.12	0.0392	15	−0.05	0.6391
16	0.09	0.1126	16	0.03	0.6328	16	0.17	0.0038	16	0.07	0.2022	16	−0.12	0.0506	16	−0.01	0.9101
17	0.09	0.1040	17	0.00	0.9874	17	0.17	0.0046	17	0.05	0.3648	17	−0.12	0.0480	17	−0.17	0.1077
18	0.10	0.0724	18	−0.01	0.8112	18	0.16	0.0064	18	0.01	0.9166	18	−0.11	0.0540	18	−0.06	0.6013
19	0.10	0.0694	19	0.02	0.6892	19	0.16	0.0068	19	−0.04	0.5060	19	−0.12	0.0522	19	0.09	0.4156
20	0.10	0.0612	20	0.06	0.3450	20	0.16	0.0064	20	−0.06	0.2552	20	−0.12	0.0486	20	−0.06	0.5993
21	0.09	0.1056	21	0.09	0.1220	21	0.17	0.0044	21	−0.10	0.0886	21	−0.11	0.0588	21	−0.01	0.9104

Table S3. Pearson's correlation coefficient to identify significant lags of enterococci concentrations in Escambron Beach surface waters with respect to the environmental parameters. Concentrations below the limit of detection were substituted by 2 MPN/CFU per 100 mL for those samples analyzed before April 2015 and 5 MPN/ 100mL for samples analyzed after April 2015. Values are considered significant with 95% certainty (α = 0.05). Bold values are those with the highest and significant Pearson's correlation coefficient.

Precipitation			**Sea Surface Temperature**			**Dew Point**			**Mean Sea Level**			**Direct Normal Irradiance**			**Turbidity**		
Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value	Lag	r	*p*-value
1	0.13	0.0184	1	0.07	0.2504	1	0.15	0.0130	1	0.10	0.0714	1	−0.25	0.0002	1	0.25	0.0153
2	0.23	0.0002	2	0.09	0.1452	2	0.16	0.0070	2	0.07	0.1988	2	−0.21	0.0008	2	0.05	0.6674
3	**0.23**	**0.0002**	3	0.09	0.1226	3	0.16	0.0072	3	0.06	0.3266	3	−0.21	0.0006	3	−0.02	0.8347
4	**0.23**	**0.0006**	4	0.09	0.1046	4	0.18	0.0032	4	0.01	0.8076	4	−0.21	0.0010	4	−0.19	0.0827
5	0.21	0.0004	**5**	**0.12**	**0.0408**	5	0.20	0.0010	5	−0.04	0.5224	5	−0.21	0.0006	5	0.12	0.2557
6	0.18	0.0026	6	0.08	0.1896	6	0.20	0.0008	6	−0.11	0.0554	6	−0.19	0.0020	6	0.00	0.9898
7	0.16	0.0032	7	0.11	0.0618	**7**	**0.20**	**0.0012**	7	−0.16	0.0038	7	−0.18	0.0034	7	0.09	0.4446
8	0.16	0.0086	8	0.08	0.1856	8	0.20	0.0012	8	−0.20	0.0008	8	−0.17	0.0034	8	−0.16	0.1262
9	0.14	0.0138	9	0.06	0.2966	9	0.19	0.0014	**9**	**−0.21**	**0.0002**	9	−0.16	0.0100	9	0.10	0.3723
10	0.13	0.0218	10	0.02	0.7396	10	0.19	0.0014	10	−0.20	0.0008	10	−0.16	0.0092	10	0.01	0.9401
11	0.13	0.0228	11	0.05	0.3700	11	0.20	0.0004	11	−0.15	0.0082	11	−0.15	0.0076	11	0.16	0.1371
12	0.13	0.0236	12	0.02	0.7604	12	0.20	0.0006	12	−0.09	0.1024	12	−0.15	0.0120	12	−0.15	0.1319
13	0.13	0.0254	13	−0.01	0.8258	13	0.19	0.0010	13	−0.01	0.7968	13	−0.15	0.0124	13	−0.11	0.3280
14	0.11	0.0548	14	−0.01	0.8540	14	0.19	0.0016	14	0.03	0.6050	14	−0.14	0.0194	14	−0.15	0.1696
15	0.11	0.0618	15	−0.01	0.9078	15	0.19	0.0014	15	0.07	0.2406	15	−0.13	0.0294	15	−0.05	0.6535
16	0.10	0.0784	16	0.03	0.6704	16	0.18	0.0020	16	0.06	0.2742	16	−0.12	0.0370	16	0.00	0.9754
17	0.10	0.0670	17	0.00	0.9690	17	0.18	0.0028	17	0.04	0.4590	17	−0.12	0.0346	17	−0.17	0.1131
18	0.11	0.0428	18	−0.02	0.7438	18	0.17	0.0048	18	0.00	0.9580	18	−0.12	0.0396	18	−0.07	0.5217
19	0.12	0.0370	19	0.01	0.8076	19	0.17	0.0028	19	−0.05	0.4234	19	−0.12	0.0392	19	0.08	0.4360
20	0.12	0.0376	20	0.05	0.4334	20	0.17	0.0056	20	−0.07	0.1826	20	−0.12	0.0392	20	−0.04	0.7054
21	0.11	0.0612	21	0.08	0.1786	21	0.18	0.0038	21	−0.11	0.0548	21	−0.12	0.0478	21	−0.01	0.9278

APPENDIX C

Application of Artificial Neural Networks for dengue fever outbreak predictions in the northwest coast of Yucatan, Mexico and San Juan, Puerto Rico

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Tropical Medicine and Infectious Disease

Article

Application of Artificial Neural Networks for Dengue Fever Outbreak Predictions in the Northwest Coast of Yucatan, Mexico and San Juan, Puerto Rico

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Received: 31 October 2017; Accepted: 2 January 2018; Published: 5 January 2018

Abstract: Modelling dengue fever in endemic areas is important to mitigate and improve vector-borne disease control to reduce outbreaks. This study applied artificial neural networks (ANNs) to predict dengue fever outbreak occurrences in San Juan, Puerto Rico (USA), and in several coastal municipalities of the state of Yucatan, Mexico, based on specific thresholds. The models were trained with 19 years of dengue fever data for Puerto Rico and six years for Mexico. Environmental and demographic data included in the predictive models were sea surface temperature (SST), precipitation, air temperature (i.e., minimum, maximum, and average), humidity, previous dengue cases, and population size. Two models were applied for each study area. One predicted dengue incidence rates based on population at risk (i.e., numbers of people younger than 24 years), and the other on the size of the vulnerable population (i.e., number of people younger than five years and older than 65 years). The predictive power was above 70% for all four model runs. The ANNs were able to successfully model dengue fever outbreak occurrences in both study areas. The variables with the most influence on predicting dengue fever outbreak occurrences for San Juan, Puerto Rico, included population size, previous dengue cases, maximum air temperature, and date. In Yucatan, Mexico, the most important variables were population size, previous dengue cases, minimum air temperature, and date. These models have predictive skills and should help dengue fever mitigation and management to aid specific population segments in the Caribbean region and around the Gulf of Mexico.

Keywords: nonlinear models; *Aedes aegypti*; *Aedes albopictus*; remote sensing; early warning systems

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1. Introduction

Dengue fever is considered a global burden, with more than 500,000 cases reported annually [1–4]. This vector-borne disease is mostly transmitted by the *Aedes aegypti* mosquitoes, but can also be transmitted by *Aedes albopictus* [1,3]. *Aedes aegypti* are found in tropical/sub-tropical areas, where they

Trop. Med. Infect. Dis. **2018**, *3*, 5; doi:10.3390/tropicalmed3010005 www.mdpi.com/journal/tropicalmed

have adapted to urbanized environments. This complicates management and mitigation of the organism and the disease [2,5–7]. Countries in the Gulf of Mexico and Caribbean have adopted various methods to control spreading of the disease, including mosquito control, monitoring and early warning systems, and educating the population [8–10]. The state of Yucatan (Mexico) and the island of Puerto Rico (USA) first reported dengue fever cases in the late 1970s. In successive seasons, a cyclic occurrence and all four serotypes (i.e., DENV-1, DENV-2, DENV-3, and DENV-4) have been reported [9,11–13]. Each location reports around 10,000 cases annually [13–16].

In Yucatan, dengue cases are monitored by the National Epidemiological Surveillance System. The system issues weekly epidemiological reports that track incidence [14]. The Dengue Surveillance System from the USA Centers for Diseases Control and Prevention (CDC) and Puerto Rico's Department of Health publish similar weekly reports [15]. The phenology of dengue fever cases for both locations is similar, with cases typically increasing in August and September, and decreasing around December and January. This follows the rainy season at both locations [2,15]. In Yucatan, the magnitude of dengue fever epidemics varies, in part due to different serotypes expressing themselves in different years and population susceptibility and movement to and from affected areas [17–20]. Puerto Rico shows similar patterns [15]. Understanding factors that may lead to an epidemic and some predictive capability are important to design and implement strategies that mitigate incidence [4,21–26].

Disease occurrence models can be based on linear and nonlinear approaches that simulate complex relations between short- and long-term (climate) environmental variables and dengue fever incidence [1,27,28]. Linear models are often unable to simulate complex interactions between these factors, and powers tend to be smaller [2]. Nonlinear approaches have generally shown greater power than linear models [29]. For example, Husin et al. [30] predicted dengue fever in Malaysia using a nonlinear model, to help the government fight the disease. A similar study in Singapore used genetic algorithms and support vector machines to predict the number of dengue fever cases [31]. Studies in Thailand, Singapore, and Malaysia have also used artificial neural network (ANN) models to predict dengue fever cases, achieving accuracies greater than 80% [32–34]. A similar study in Sri Lanka with ANNs showed a lower accuracy (i.e., 60%) [35]. ANNs are attractive because they generally achieve a higher skill than other types of models [36].

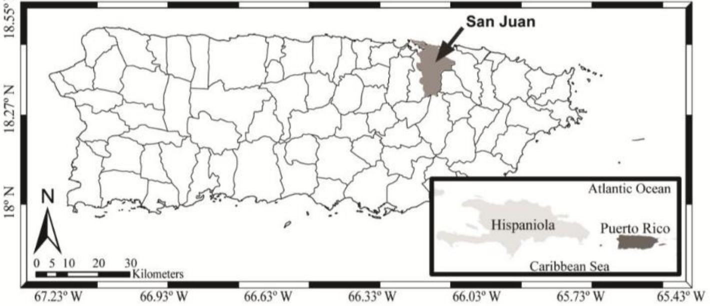
Artificial neural networks use combinations of predictor variables (e.g., environmental factors) to simulate relationships with target variables (e.g., dengue fever outbreak occurrences). These models can be adapted to assimilate data, and this helps improve the functional relationships between climatic factors and dengue fever outbreak occurrences. In our present study, we applied ANNs trained with genetic algorithms to predict dengue fever outbreak occurrences in the state of Yucatan, Mexico, and in San Juan, Puerto Rico. We identified environmental factors that are important in driving dengue fever outbreak occurrences at these different locations. The candidate variables were air surface temperature, sea surface temperature (SST), humidity, precipitation, previous dengue cases, and human population size. Previous dengue cases are defined as those cases that occurred weeks before an outbreak [37]. The model was used to detect occasions when the number of cases would go above a threshold and lead to potential outbreaks. These models were also used to predict dengue fever outbreak occurrences based on population at risk (i.e., younger than 24 years) and vulnerable population (i.e., younger than five years and older than 65 years) [1,38–40]. Our results help us to understand dengue fever dynamics and improve epidemiological surveillance and early warning for specific population segments in the Caribbean and Gulf of Mexico.

2. Materials and Methods

2.1. Study Area

The study areas were the municipality of San Juan in Puerto Rico, USA (17.92° N–18.52° N, 65.62° W–67.28° W), and the northwest coast of the state of Yucatan, Mexico (19.55° N–21.63° N, 87.53° W–90.40° W; Figure 1). San Juan has an average precipitation of ~1800 mm per year, with annual average air surface temperatures of 24–29 °C [41]. Municipalities included for Yucatan State were Chicxulub Pueblo, Dzemul, Hunucma, Ixil, Progreso, Telchac Pueblo, Telchac Puerto, Ucu, and Merida. Yucatan shows higher precipitation between July and October (400–700 mm). The dry season occurs between March and June (0–50 mm). Air temperatures generally range from 36 to 40 °C during the dry season and 30–35 °C during the rainy season [42,43].

A) Map depicting study area: San Juan, Puerto Rico.



B) Map depicting study area: Northwest coast, Yucatan, Mexico.

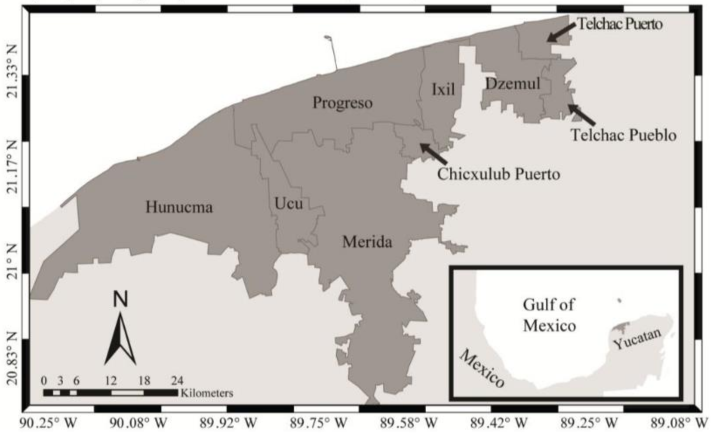


Figure 1. Map of locations of study areas. Maps depicting locations of the study areas in: (**A**) the municipality of San Juan, Puerto Rico; and (**B**) northwest coast of the Yucatan Peninsula, Mexico.

2.2. Data Sources

2.2.1. Dengue Fever Cases and Demographic Data

Confirmed daily dengue fever data for the northwest coast of the Yucatan Peninsula (n = 12,448 daily cases) were obtained from the Universidad Autonoma de Yucatan (2007–2012). Data of confirmed dengue fever cases for San Juan (n = 5678 daily cases) were provided by the Dengue Branch of the

USA CDC located in Puerto Rico and the Puerto Rico Department of Health, through the University of Puerto Rico, Medical Science Campus. The data included the number of cases per day from 1994 to 2012. The daily incidence data were used to calculate weekly dengue cases for both locations. These were converted to incidence rates (i.e., number of cases per 100,000 inhabitants) using data from Puerto Rico's Census for San Juan, and using the National Institute of Statistics and Geography for Mexico. Total weekly observations for Puerto Rico were 986, and 310 for Mexico. These cases were further divided into population younger than 24 years, and population younger than five years and older than 65 years old. Total daily cases for Puerto Rico younger than 24 were $n = 3466$; Puerto Rico younger than five and older than 65 years were $n = 736$; Mexico younger than 24 were $n = 7908$; and Mexico younger than five and older than 65 years were $n = 735$.

2.2.2. Environmental Data for San Juan and Yucatan State

Precipitation and minimum and maximum air surface temperatures were obtained from the USA National Oceanic and Atmospheric Administration's (NOAA) National Centers for Environmental Information (1994–2012) for San Juan. Data for Yucatan from 2007 to 2012 were obtained from the National Water Commission of the state of Yucatan. Weekly means were calculated for these datasets. SST data for both Puerto Rico and Mexico were obtained from the NOAA Advanced Very High Resolution Radiometer (AVHRR; 1 km spatial resolution) satellite-based sensor. SST data were extracted from 1994 to 2012 (Puerto Rico) and 2007 to 2012 (Mexico) using the average of three 3- by 3-pixel boxes located immediately offshore (i.e., coastal area) of the areas of study. These covered a total of 9 km^2 along the coast. We used Interactive Data Language (IDL; v. 7.2) to extract data.

2.2.3. Data Input and Organization

Weekly dengue incidence rates were log-transformed and used as inputs for the predictive model. Environmental variables used to predict dengue fever in Mexico were: humidity, cumulative one-week precipitation, SST, population size, previous dengue incidence, minimum air temperature, and date. For Puerto Rico, the data included: cumulative four-week precipitation, SST, population size, previous dengue incidence, minimum and maximum air temperature, and date. Significant four-week and one-week cumulative lags for precipitation were identified for Mexico and Puerto Rico, respectively, using Pearson's correlation analyses [2,43]. Selected predictors were based on previous work done in the study areas that identified these factors as significantly correlated with dengue fever cases [2,15,44].

The models were configured to predict dengue fever outbreak occurrences of population at risk and of the most vulnerable population segments. Mendez-Lazaro et al. [15] identified population at risk in Puerto Rico as people younger than 24 years, and more specifically the most vulnerable to be people younger than five and older than 65 years. These age groups were also used for Mexico, based on dengue fever cases data and previous studies [19,45–47]. The weekly data were thus partitioned as: Puerto Rico: population at risk $n = 986$ weekly cases; vulnerable population: $n = 986$ weekly cases. Population at risk in Mexico: $n = 310$ weekly cases; vulnerable population: $n = 310$ weekly cases. These numbers were similar for both population segments per location as data showed dengue fever cases for all age-groups.

2.3. Artificial Neural Network Model Setup

2.3.1. Training and Validation

This study implemented the ANN used for the Radar Pluvial flooding Identification for Drainage System (RAPIDS), which was developed to predict flooding in sewer systems by the University of Exeter and was modified to predict dengue fever outbreak occurrences [48,49]. The approach normalizes data (e.g., natural log transformation) to make the weight values more sensitive to changes. The ANNs models used a nondominated sorting genetic algorithm II (NSGA-II) for optimization and training purposes [50,51]; results were validated using the leave-one-out-cross-validation (LOOCV) approach [51,52]. Our objective was to reduce false positives (FP) and false negatives (FN). A weighting factor ('a') was used within the model to provide a higher relative importance of false positive ratios (FPR) over false negative ratios (FNR) [53]. This value minimized the number of incorrectly predicted passes (i.e., values below thresholds). Different a-factor values were tested as part of the testing process, and a = 3 was used for Puerto Rico and Mexico. By using this value, we weighted health risks as three times more important.

The model runs were evaluated for accuracy and predictive power. An accuracy band was calculated based on the percentage of true positives (TP) and true negatives (*TN*) compared to false positives and false negatives (*FP* and *FN*) relative to actual observations. These values change throughout the stages of the models and final values were an overall average based on a confusion matrix (or error matrix). The power of the model to predict these outcomes was based on an F-measure (*FM*; Equation (1)), which provided the importance of *FP* over *FN* using the weighting factor (a) discussed above; these values ranged from 0 to 1. The area under the receiving operating characteristic (ROC) curve, or AuC, was also used to test model power, and this was based on TPR and TNR [51]. The ROC curve helped establish the optimal trade-off between FPR and FNR (i.e., FPR = 1 − TPR) [51].

$$FM = \frac{(1+a)TN}{(1+a)TN + aFP + FN)} \tag{1}$$

In addition to accuracy and model power, the strength of the relationships and relevant influence (i.e., excitatory or inhibitory) between predictors (i.e., environmental factors) and target variable (i.e., dengue fever outbreak occurrences) was assessed. The model weighted in environmental factors and identified those that had the most influence to predict dengue fever outbreak occurrences. The neural pathway strength feature selection (NPSFS) method was used to identify the most relevant inputs, by creating an ensemble of ANNs and comparing the similarities of the weight results (i.e., pathway strength) for the model inputs [51]. Those inputs with the most similarity of pathway strengths (i.e., the strength of relationship) across the whole ensemble of ANNs were selected as the most relevant [51]. First, weights and biases were calculated to understand the relationship between inputs (combined environmental factors) and outputs (dengue fever outbreak occurrences) through NPSFS [51,54,55]. The ANN models processed data values unidirectional from inputs towards outputs, creating these sets of weights and biases [51]. The weights were calculated for the hidden layers (W_1) and for the output layer (W_2; Figure 2). The neural pathway strengths (W_0) of each input were calculated after the completion of the training using NSGA-II, employing the NPSFS methodology (i.e., matrix math of the ANNs hidden layer weights matrix (W_1) and ANN output layer weights vector (W_2)) to identify the most influential factors [47]. Each of the members of the ensemble is trained on a similar but different subset of the full training data set. As a result, the weights obtained in each ANN will have different values. The net effect of each input was analyzed by NPSFS, by multiplying W_1 to W_2, as shown above. Therefore, for a single output ANN, the result is a vector that specifies the combined pathway strength of each input on the output (neglecting non-linearity of activation functions). These weights were then combined through the combined neural pathway strength analysis (CNPSA) by looking at the spread of the pathway strengths for each input, where we identified if the majority of these treated the given input in the same sense, excitatory or inhibitory [54,55]. If so, we say that the input is relevant to predict potential dengue fever outbreak occurrences.

These weights were optimized using the crossover and mutation rates, incorporated by NSGA-II, during the training period [48]. The NSGA-II crossover and mutation rate factors helped differentiate each new weight generation from the parent weight generation for predictive purposes [51]. Different crossover and mutation rates were tested iteratively. The optimal values to predict dengue fever outbreak occurrences were a 0.1 crossover rate and 0.2 mutation rate for both locations. The minimization of cost (i.e., cost function) was based on false positive rates and false negative rates. These were derived by the ROC using the minimum Euclidean distance to the ideal true positive ratio equal to one [56]. These were used for optimization by NSGA-II to assess the quality of solutions [51,56].

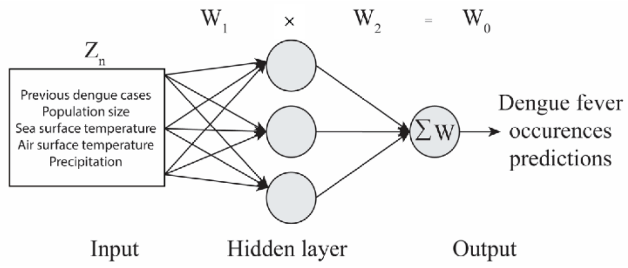


Figure 2. Artificial neural networks (ANNs) schematic. Example of an ANN schematic showing the input layer, hidden layer, and output layer. ANNs calculate weights from variables (Z_n) in the input to hidden layer (W_1). The hidden layer then combines these weights and calculates a new set of weights (W_2). The neural pathway strengths (W_0) are then calculated, with the equation above the schematic ($W_1 \times W_2 = W_0$), to obtain the strength of influence to predict outcomes, following Duncan et al. [51] and Duncan [56].

Training, validation, and testing of dengue fever outbreak occurrences was achieved by dividing the data from Puerto Rico and Mexico into different epochs (i.e., years). Model sample sizes were identified as follows: Puerto Rico less than 24 years (N_{PR24}), Puerto Rico younger than five and older than 65 years ($N_{PR5\text{-}65}$), Mexico younger than 24 (N_{MX24}), and Mexico younger than five and older than 65 years ($N_{MX5\text{-}65}$). For Puerto Rico, the first ten epochs of data were divided into eight for training and two for validation. The validations were done every fifth epoch. The following seven epochs had six for training and one for validating. Validation was done every third epoch. The last two epochs of data were used for testing. Therefore, we had 14 epochs for training (N_{PR24} = 1950 daily cases; $N_{PR5\text{-}65}$ = 437 daily cases), three epochs for validating (N_{PR24} = 609 daily cases; $N_{PR5\text{-}65}$ = 140 daily cases), and two epochs for testing (N_{PR24} = 907 daily cases; $N_{PR5\text{-}65}$ = 159 daily cases), for a total of 19 epochs.

Due to the shorter time series, some of the years/epochs for the Yucatan dataset were divided into two (i.e., 26 weeks/epochs each), totaling 12 epochs. The first ten epochs were divided into eight training epochs and two validating epochs; this was done by training three consecutive epochs and using the fourth as validation. The last two of those ten epochs were used as training, and epochs 11 and 12 were testing epochs. There was a total of six epochs for training (N_{MX24} = 2169 daily cases; $N_{MX5\text{-}65}$ = 187 daily cases), two epochs for validating (N_{MX24} = 1643 daily cases; $N_{MX5\text{-}65}$ = 89 daily cases), and two epochs for testing (N_{MX24} = 4096 daily cases; $N_{MX5\text{-}65}$ = 459 daily cases). These divisions ensured that data for training and validation were different from those used for the final testing of predictions.

2.3.2. Thresholds to Identify and Predict Potential Dengue Fever Outbreaks

Specific thresholds were identified for Mexico and Puerto Rico to predict potential outbreaks based on dengue fever outbreak occurrences. The number of cases was divided into three periods for a full year (52 epidemiological weeks): pre-epidemic (weeks 10–20); epidemic (weeks 21–40); and post-epidemic (weeks 41–49) for Puerto Rico, based on the work of Mendez-Lazaro et al. [15]. This same distribution was used for Mexico, based on weekly dengue cases data from 2007 to 2012 (Figure 3) [2]. The periods were identified based on the distribution of cases, where the pre- and post-epidemic periods showed a slow increase and decrease in number of cases, respectively, and the epidemic period was when the majority of the cases were observed. The average number of cases during those periods was then calculated.

To provide an epidemic threshold for the model, the 75th percentile of all weekly cases per year was calculated for each period and age-distributions. The 75th percentile for the threshold has been used and identified by previous studies as the cutoff for threshold selection regarding infectious diseases [57–59].

Dengue fever epidemics were identified as three or more suspected cases (per 1000 individuals) for two consecutive weeks [15]. These threshold numbers represent the number of cases per week. The following thresholds were obtained and used in the models. For Puerto Rico, population younger than 24 years: 2 (pre-epidemic), 6 (epidemic), and 5 (post-epidemic); and population younger than five and older than 65 years: 1 (pre-epidemic), 2 (epidemic), 1 (post-epidemic). For Mexico: population younger than 24 years: 2 (pre-epidemic), 33 (epidemic), and 13 (post-epidemic); and for population younger than five and older than 65 years: 1 (pre-epidemic), 6 (epidemic), and 4 (post-epidemic). The models compared the observed and predicted values to each of these thresholds to identify them as a 'no outbreak period' (i.e., value below threshold) or a 'potential outbreak period' (i.e., value above thresholds). These models were run as categorical models; therefore, the results were interpreted based on the influence, and magnitude, of inputs to predict a potential outbreak based on the specific thresholds mentioned above. Therefore, the results are shown as inputs having an (inhibitory or excitatory) influence on outputs in terms of outputs crossing the set thresholds.

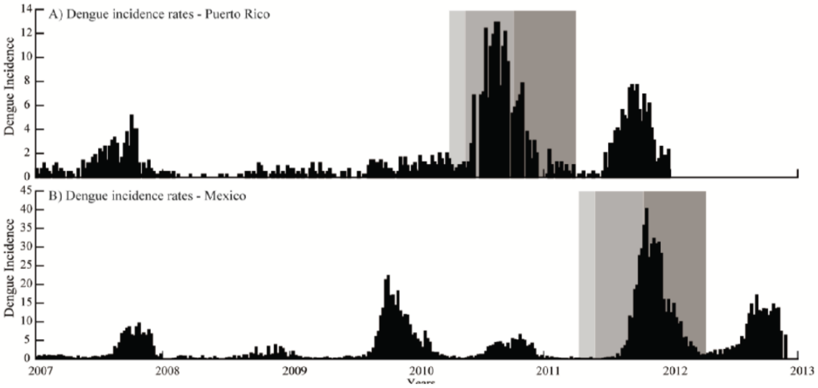


Figure 3. Dengue fever incidence rates distributions for San Juan, Puerto Rico and the northwest region of Yucatan, Mexico. Dengue incidence rates per 100,000 inhabitants from 2007 to 2012 for (**A**) San Juan, Puerto Rico; and (**B**) northwest coast of Yucatan, Mexico. Data only includes these years to show patterns and epidemic years, which for both locations were 2007 and 2011. The three shaded gray boxes represent the pre-epidemic (weeks 10–20), epidemic (weeks 21–40), and post-epidemic (weeks 41–49) periods.

3. Results

3.1. Model Accuracy

The accuracies of the predictions for both locations were similar, with predictions for the 'no outbreak periods' having a higher accuracy compared to predictions for the 'potential outbreak periods'. Model accuracy bands for San Juan, Puerto Rico were 47% for the population at risk model (i.e., younger than 24 years), and 58% for the vulnerable population model (i.e., younger than five and older than 65 years). More specifically, the 47% accuracy band for the population at risk model represented a prediction of 19% of the 'potential outbreak periods' and 28% of the 'no outbreak periods'. The model representing the most vulnerable population, with an accuracy band of 58%, represented a prediction of 17% of the 'potential outbreak periods' and 41% of the 'no outbreaks periods'. These models showed a higher accuracy band for periods of 'no outbreaks' compared to 'potential outbreak periods'.

A similar pattern was observed for models in Mexico. The population at risk model in Mexico showed an accuracy of 51%, which represented an accurate prediction of 12% of 'potential outbreaks period' and 39% of predicted 'no outbreaks periods'. The most vulnerable population model showed a 66% accuracy band, representing 5% of predicted 'potential outbreak periods' and 61% of 'no outbreak periods'. In both instances, the four models showed a higher accuracy for the 'no outbreak period' compared to 'potential outbreak period', and the accuracies were higher for the most vulnerable population models compared to the population at risk models.

3.2. Evaluation of ANNs Model Predictive Power Based on F-Measure and ROC Curve

The overall power for the four model runs was above 70%. This was based on ROC curves and FM. ROC curves were calculated for all four model runs (Figure 4A–D) using the weights (W_0) calculated by the ANN ensembles. The population at risk model for San Juan showed an FM of 0.97 and an AuC of 0.91. The most vulnerable population model showed an FM of 0.71 and an AuC of 0.81. Overall, these models showed a predictive power greater than 90% and 70% for San Juan. Conversely, the population at risk model showed a higher predictive power compared to the most vulnerable population model.

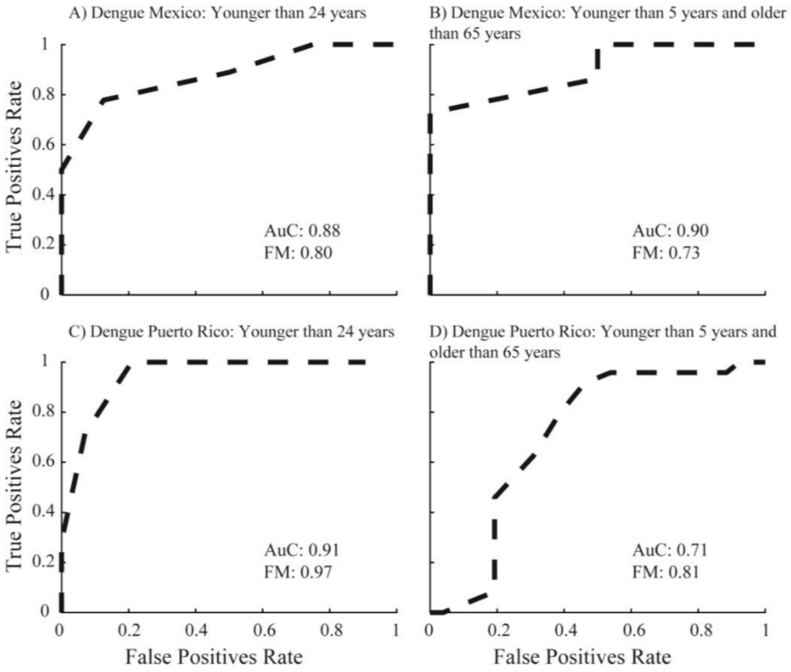


Figure 4. Performance of model to predict dengue fever outbreak occurrences. Graphs show the receiver operating characteristic (ROC) curve results of the four artificial neural network models for San Juan and Mexico. Dashed lines are the calculated true positive ratios (TPR) and false positive ratios (FPR) for all four models. The areas under the curves (AuC) were calculated based on TPR/FPR ratios and F-measures (FM) describe the importance of false positives over false negatives. These AuC and FM values show models' power [55]. AuC and FM were calculated for: (**A**) population younger than 24 years, Mexico; (**B**) population younger than five years and older than 65 years, Mexico; (**C**) population younger than 24 years, Puerto Rico; and (**D**) population younger than five years and older than 65 years, Puerto Rico.

Model runs for Yucatan showed similar results as those for San Juan, with the overall power above 70 percent. The population at risk model showed an FM of 0.80 and an AuC of 0.88. The most vulnerable population model showed an FM of 0.73 and AuC of 0.90. However, the overall power for the population at risk model was higher and numbers were closer compared to the power obtained by the most vulnerable population model; thus, these results are consistent with those observed in Puerto Rico. A series of baseline multiple linear regression (MLR) models were also evaluated. The results showed that ANN models had a higher statistical power compared to MLR models using the same data approach (Supplementary Materials, Tables S1–S4).

3.3. Environmental Factors Relevant for Dengue Fever Outbreak Occurrences Predictions

The models result showed that both environmental factors and demographic variables had an important influence on dengue fever predictions, based on weights calculated through the NPSFS method. These weights (W_0) represented the strength of the relationship between inputs and outputs, and those variables identified as the most influential had values different than zero. Therefore, those parameters with weights equal to zero were those having no influence on predicting dengue fever outbreak occurrences for Mexico and Puerto Rico. Furthermore, these environmental factors were identified as having an excitatory (represented by positive weights) or inhibitory (represented by negative weights) influence. For example, Figure 5 shows previous dengue cases as having an inhibitory influence on predicting dengue fever outbreak occurrences. This means that, overall, previous dengue cases inhibit dengue fever outbreak occurrences to cross the set thresholds (i.e., pass). Similarly, population size shows an excitatory influence, meaning that this variable stimulates dengue fever outbreak occurrences to cross the outbreak threshold (i.e., fail).

Weights with the most overall influence identified by ANNs in Mexico for the population at risk model were previous dengue cases, minimum air temperature, population size, cumulative one-week lag precipitation, and SST (Figure 5A). However, for the most vulnerable population model, previous dengue cases and population size were the most influential, and other factors only slightly influenced dengue fever outbreak occurrences (Figure 5B). The model runs for Puerto Rico showed that the most influential parameters overall for dengue fever were previous dengue cases, population size, date, and maximum air temperature for the population at risk model (Figure 5C). The model for the most vulnerable population showed population size, previous dengue cases, and maximum temperature as the most influential factors (Figure 5D). The models for Puerto Rico showed a wider spread of weights compared to those for Mexico. Overall, all four model runs had previous dengue fever and population size as the two variables with the most influence on predicting dengue fever in both study areas.

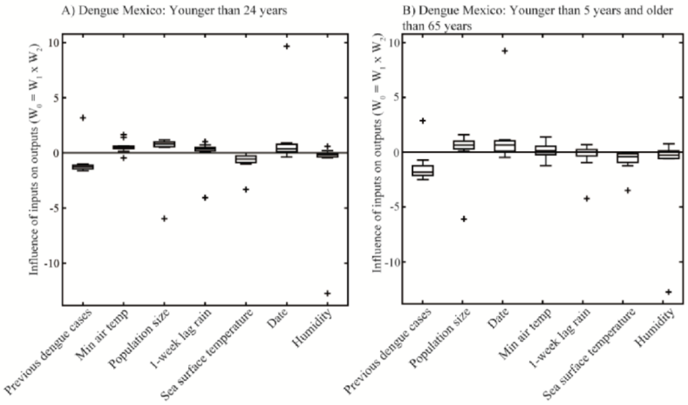


Figure 5. *Cont.*

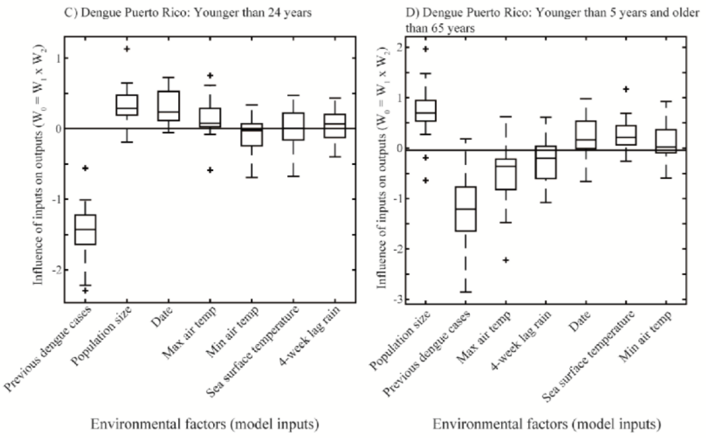


Figure 5. Distribution of environmental factors weights in the ANNs to predict dengue fever outbreak occurrences in Mexico and Puerto Rico. The box and whiskers plots show the influence of each environmental factor on dengue fever outbreak occurrences predictions based on weights distributions (W_0) calculated by the ANNs' models. Boxes show the distribution of weights, lines inside the boxes are the mean values of weights. Zero lines represent no relevance for predicting dengue fever outbreak occurrences. These weights are shown in order of importance for: (**A**) population younger than 24 years, Mexico; (**B**) population younger than five years and older than 65 years, Mexico; (**C**) population younger than 24 years, Puerto Rico; and (**D**) population younger than five years and older than 65 years, Puerto Rico.

4. Discussion

4.1. Most Relevant Environmental and Social Factors Influencing Dengue Fever Outbreak Occurrences

Dengue fever cases are influenced by a series of environmental and demographic parameters [60–62]. Precipitation, air temperatures, and social factors (i.e., previous dengue cases and population size) were the most influential to predict dengue fever outbreak occurrences in both the Yucatan and San Juan. These factors influence dengue fever elsewhere [2,15,63]. Precipitation and temperatures (i.e., maximum and minimum temperatures) are related to the timing of the mosquitoes' development and virus replication. Dengue fever outbreak occurrences follow precipitation patterns as these provide breeding sites. Warmer temperatures also reduce the developing time of mosquitoes and help increase their densities [3,64,65].

The most influential inputs for predicting dengue fever were those related to demographic changes. Overall, the most relevant factors in predicting dengue fever outbreak occurrences in Yucatan and San Juan were population size, previous dengue incidence, and air temperatures. Changes in population is a key factor influencing virus spreading and further peaks in cases [17,19]. These factors have been demonstrated as drivers in other regions [2,15,44,63]. The relationship between population changes and an increase in dengue fever cases can be due to more people being susceptible to dengue fever (e.g., had never been exposed to the virus; [13,66]).

The second demographic parameter identified as most influential was 'previous dengue cases'. While this can be affected by changes in population size, this factor could be lower because of increased immunity with time [67,68]. Nevertheless, decreased immunity due to other factors like hygiene and age, as well as shifts in serotypes, can lead to peaks in dengue fever outbreak occurrences due to secondary infections [69,70].

Air temperatures showed a significant influence on predicting dengue fever outbreak occurrences in both study areas. An increase in minimum air temperature can promote mosquito development [2,3], but as it becomes warmer than normal, their development slows down or is inhibited [68]. Increased air temperature is highly correlated with increased mosquito bites due to the animal's energy demands, leading to higher probabilities of humans becoming infected [71]. Only the model for population at risk for Yucatan showed SST as slightly influential on predicting dengue fever outbreak occurrences, so this factor was not very important relative to those mentioned above for these coastal regions. Lastly, date was also identified as a significant factor by the models. This is related to the cyclic pattern that dengue fever cases show across the Americas. Clearly, there is a correlation and lack of independence between date and seasonality of precipitation patterns [72–75].

4.2. ANNs Model Performance on Predicting Dengue Fever in Mexico and Puerto Rico

The overall power of 70% was obtained through the combination of these variables. This builds upon previous work, where ANN modeling power ranged from 60 to 80% [32–35]. These power results were also higher compared to multiple linear regression models. By modeling two population segments, those considered vulnerable and those considered at risk, these models were able to identify specific variables that were most influential to understand dengue dynamics depending on susceptibility. The main differences seen in power and accuracy among these models could be due to those younger than 24 years old being at risk due to social mobility (e.g., work, school, home), and those considered most vulnerable could be due to biological conditions such as leakage of plasma (e.g., those less than five years old having a lower threshold for fluid escape from intravascular to extravascular space; [76]) and chronic degenerative diseases (i.e., greater than 65 years old; [77]). Therefore, models identifying demographics as the most influential could be due to: (1) primary infections based on exposure (i.e., population susceptibility; [78]); (2) secondary infections [79]; (3) geographic locations (e.g., houses without protection, such as windows and doors screens; [2,80]; and (4) public services (e.g., trash pick-up, drainage cleaning; [79,80]). Similarly, simultaneous circulation of more than one serotype can increase peaks in cases [19,77,79].

The power calculated by the ANN models, through AuC and FM, accounted for the ratios of true positives and true negatives compared to false positives and false negatives, as shown in Equation (1), and was improved by the weighting factor. On the other hand, the accuracy band only looked at the predicted values individually compared to the original values, where percentages might be affected by the number of pass/fail in the original observations. Therefore, we see a difference between accuracy and power due to the methodology, where the models' powers were based on the rate and capability of them to predict potential dengue fever outbreaks. Dividing the data into three periods to predict dengue fever outbreak occurrences (i.e., pre-epidemic, epidemic, and post-epidemic) could have also influenced the outcomes of the models. The magnitudes of cases during these periods changed annually. These differences across years are influenced by those factors discussed above (e.g., social factors and epidemiology). Thresholds used for this study were identified using data available for Puerto Rico and Mexico during those specific timeframes. Including longer, or shorter, datasets can influence these thresholds depending on dengue fever dynamics, as these tend to be location specific [81,82].

4.3. Study Limitations and Future Work

There were many factors that were not considered in defining the predictive capacity of the ANN models. This includes population susceptibility to dengue fever (e.g., serotypes, population movement). To be able to accurately identify risk and vulnerability to dengue fever outbreak occurrences, the serotype(s) circulating must be known [13,22,83–85]. When there is a shift in serotypes, those who had not been previously exposed to the new one become more vulnerable, leading to an increased incidence of dengue fever [66,67]. This is also observed in the different peaks per years, where environmental variables are not the only factors influencing these strengths, thus affecting the accuracy of these models.

Changes in population segments considered at risk and vulnerable may also lead to differences. People that had never been exposed to dengue fever can be part of one of these two population segments, and this was not considered due to data limitations. The thresholds used for the three periods that the data were divided into could have affected the prediction capability of the models and future studies might need to consider different thresholds according to vulnerable population, populations at risks, and geographic locations, as well as circulating serotypes. Lastly, Puerto Rico had 19 years of data, whereas Mexico had six years; however, both study areas provided equivalent results. Future studies should consider expanding these time series to better understand temporal and spatial differences across dengue fever endemic areas, as well as applying ANNs in this area to predict coming seasons.

5. Conclusions

Modelling dengue fever using environmental and demographic factors with a nonlinear neural network approach can help predict dengue fever incidence rates in Mexico and Puerto Rico with a power greater than 70 percent. Four model runs, two for population at risk and two most vulnerable population in Yucatan, Mexico and San Juan, Puerto Rico, identified precipitation, population size, air temperature, previous dengue cases, and date as the most influential factors that predict dengue fever outbreak occurrences. Demographic factors, including population size and previous dengue cases, were of most importance. Understanding behavior of the population and education programs can help improve the effectiveness of early warning systems in this region and mitigate the disease. Further studies are needed to incorporate vector and dengue fever virus dynamics into models, as these can help improve the skill of simulations and understand similar diseases that depend on climate and environmental changes.

Supplementary Materials: The following are available online at http://www.mdpi.com/2414-6366/3/1/5/s1, Table S1. Mexico multiple linear regression model for population younger than 24 years old. Bold values are significant with 95% certainty ($\alpha = 0.05$). Parametric and non-parametric *p*-values are included. Power of these models (r^2) are shown above the table, Table S2. Mexico multiple linear regression model for population younger than five and older than 65 years old. Bold values are significant with 95% certainty ($\alpha = 0.05$). Parametric and non-parametric *p*-values are included. Power of these models (r^2) are shown above the table, Table S3. Puerto Rico multiple linear regression model for population younger than 24 years old. Bold values are significant with 95% certainty ($\alpha = 0.05$). Parametric and non-parametric *p*-values are included. Power of these models (r^2) are shown above the table, Table S4. Puerto Rico multiple linear regression model for population younger than five and older than 65 years old. Bold values are significant with 95% certainty ($\alpha = 0.05$). Parametric and non-parametric *p*-values are included. Power of these models (r^2) are shown above of the table.

Acknowledgments: We would like to thank the teams from the University of Puerto Rico-Medical Campus, Universidad Autonoma of Yucatan, and Centre for Water Systems for their help and input during this work. We would also like to thank the IMaRS team for their input and help in manuscript revisions. This material is based upon work supported by the National Science Foundation Partnerships for International Research (PIRE) under Grant No. 1243510, by NASA Headquarters under the NASA Earth and Science Fellowship Program Grant No. NNX15AN60H, and the USF College of Marine Science Linton Tibbetts Endowed Fellowship.

Author Contributions: A.P.D. and D.A.S. developed and tested the ANNs predictive model, and contributed with edits for the manuscript; P.A.M.-L., J.E.G.-R., S.G.-C., and J.F.-A. collected and provided dengue fever data for Yucatan, Mexico; F.E.M.-K. collected the satellite observations, edited the text, and provided extensive written comments on the manuscript; A.E.L.-R. analyzed the data, and wrote and led the manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Bhatt, S.; Gething, P.W.; Brady, O.J.; Messina, J.P.; Farlow, A.W.; Moyes, C.L.; Drake, J.M.; Brownstein, J.S.; Hoen, A.G.; Sankoh, O.; et al. The global distribution and burden of dengue. *Nature* **2013**, *496*, 504–507. [CrossRef] [PubMed]

2. Laureano-Rosario, A.E.; Garcia-Rejon, J.E.; Gomez-Carro, S.; Farfan-Ale, J.A.; Muller-Karger, F.E. Modelling dengue fever risk in the State of Yucatan, Mexico using regional-scale satellite-derived sea surface temperature. *Acta Trop.* **2017**, *172*, 50–57. [CrossRef] [PubMed]
3. Murray, N.E.A.; Quam, M.B.; Wilder-Smith, A. Epidemiology of dengue: Past, present and future prospects. *Clin. Epidemiol.* **2013**, *5*, 299–309. [CrossRef] [PubMed]
4. Shepard, D.S.; Coudeville, L.; Halasa, Y.A.; Zambrano, B.; Dayan, G.H. Economic impact of dengue illness in the Americas. *Am. J. Trop. Med. Hyg.* **2011**, *84*, 200–207. [CrossRef] [PubMed]
5. Cheong, W.H. Preferred *Aedes aegypti* larval habitats in urban areas. *Bull. WHO* **1967**, *36*, 586. [PubMed]
6. Gubler, D.J. Dengue and dengue hemorrhagic fever. *Clin. Microbiol. Rev.* **1998**, *11*, 480–496. [PubMed]
7. Gratz, N.G. Emergency control of *Aedes aegypti* as a disease vector in urban areas. *J. Am. Mosq. Control Assoc.* **1991**, *7*, 353–365. [PubMed]
8. Perez-Guerra, C.L.; Halasa, Y.A.; Rivera, R.; Peña, M.; Ramirez, V.; Cano, M.P.; Shepard, D.S. Economic cost of dengue public prevention activities in Puerto Rico. *Dengue Bull.* **2010**, *34*, 13–23.
9. Dick, O.B.; San Martin, J.L.; Montoya, R.H.; del Diego, J.; Zambrano, B.; Dayan, G.H. The history of dengue outbreaks in the Americas. *Am. J. Trop. Med. Hyg.* **2012**, *87*, 584–593. [CrossRef] [PubMed]
10. Manrique-Saide, P.; Che-Mendoza, A.; Barrera-Perez, M.; Guillermo-May, G.; Herrera-Bojorquez, J.; Dzul-Manzanilla, F.; Gutierrez-Castro, C.; Lenhart, A.; Vazquez-Prokopec, G.; Sommerfeld, J.; et al. Use of insecticide-treated house screens to reduce infestations of dengue virus vectors, Mexico. *Emerg. Infect. Dis.* **2015**, *21*, 308–311. [CrossRef] [PubMed]
11. Diaz-Quijano, F.A.; Waldman, E.A. Factors associated with dengue mortality in Latin America and the Caribbean, 1995–2009: An ecological study. *Am. J. Trop. Med. Hyg.* **2012**, *86*, 328–334. [CrossRef] [PubMed]
12. Stramer, S.L.; Linnen, J.M.; Carrick, J.M.; Foster, G.A.; Krysztof, D.E.; Zou, S.M.; Dodd, R.Y.; Tirado-Marrero, L.M.; Hunsperger, E.; Santiago, G.A.; et al. Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. *Transfusion* **2012**, *52*, 1657–1666. [CrossRef] [PubMed]
13. Dantes, H.G.; Farfan-Ale, J.A.; Sarti, E. Epidemiological trends of dengue disease in Mexico (2000–2011): A systematic literature search and analysis. *PLoS Negl. Trop. Dis.* **2014**, *8*, e3158. [CrossRef] [PubMed]
14. De Salud, S. Panoramas Históricos Epidemiológicos de Dengue. Dirección General de Epidemiología: Secretaría de Salud, 2016. Available online: http://www.epidemiologia.salud.gob.mx/dgae/panodengue/historicos_dengue.html (accessed on 10 July 2017).
15. Mendez-Lazaro, P.; Muller-Karger, F.E.; Otis, D.; McCarthy, M.J.; Pena-Orellana, M. Assessing climate variability effects on dengue incidence in San Juan, Puerto Rico. *Int. J. Environ. Res. Public Health* **2014**, *11*, 9409–9428. [CrossRef] [PubMed]
16. Sharp, T.M.; Hunsperger, E.; Santiago, G.A.; Munoz-Jordan, J.L.; Santiago, L.M.; Rivera, A.; Rodríguez-Acosta, R.L.; Feliciano, L.G.; Margolis, H.S.; Tomashek, K.M. Virus-specific differences in rates of disease during the 2010 dengue epidemic in Puerto Rico. *PLoS Negl. Trop. Dis.* **2013**, *7*. [CrossRef] [PubMed]
17. Cavalcanti, L.P.; Viler, D.; Souza-Santos, R.; Teixeira, M.G. Change in age pattern of persons with dengue, northeastern Brazil. *Emerg. Infect. Dis.* **2011**, *17*, 132–134. [CrossRef] [PubMed]
18. Garcia-Rivera, E.J.; Rigau-Perez, J.G. Dengue severity in the elderly in Puerto Rico. *Rev. Panam. Salud Pública* **2003**, *13*, 362–368. [CrossRef] [PubMed]
19. Guzman, M.G.; Kouri, G.; Bravo, J.; Valdes, L.; Vazquez, S.; Halstead, S.B. Effect of age on outcome of secondary dengue 2 infections. *Int. J. Infect. Dis.* **2002**, *6*, 118–124. [CrossRef]
20. Garcia-Rejon, J.E.; Lorono-Pino, M.A.; Farfan-Ale, J.A.; Flores-Flores, L.F.; Lopez-Uribe, M.P.; Najera-Vazquez, M.D.; Nuñez-Ayala, G.; Beaty, B.J.; Eisen, L. Mosquito infestation and dengue virus infection in *Aedes aegypti* females in schools in Merida, Mexico. *Am. J. Trop. Med. Hyg.* **2011**, *84*, 489–496. [CrossRef] [PubMed]
21. Halasa, Y.A.; Shepard, D.S.; Zeng, W. Economic cost of dengue in Puerto Rico. *Am. J. Trop. Med. Hyg.* **2012**, *86*, 745–752. [CrossRef] [PubMed]
22. Hladish, T.J.; Pearson, C.A.B.; Chao, D.L.; Rojas, D.P.; Recchia, G.L.; Gomez-Dantes, H.; Halloran, M.E.; Pulliam, J.R.C.; Longini, I.M. Projected impact of dengue vaccination in Yucatan, Mexico. *PLoS Negl. Trop. Dis.* **2016**, *10*. [CrossRef] [PubMed]
23. Deming, R.; Manrique-Saide, P.; Barreiro, A.M.; Cardena, E.U.K.; Che-Mendoza, A.; Jones, B.; Liebman, K.; Vizcaino, L.; Vazquez-Prokopec, G.; Lenhart, A. Spatial variation of insecticide resistance in the dengue vector *Aedes aegypti* presents unique vector control challenges. *Parasites Vectors* **2016**, *9*. [CrossRef] [PubMed]

24. Gubler, D.J. The global threat of emergent/re-emergent vector-borne diseases. In *Vector Biology, Ecology and Control*; Springer: Dordrecht, The Netherlands, 2010; pp. 39–62.
25. Dobson, A. Climate variability, global change, immunity, and the dynamics of infectious diseases. *Ecology* **2009**, *90*, 920–927. [CrossRef] [PubMed]
26. Tabachnick, W.J. Challenges in predicting climate and environmental effects on vector-borne disease episystems in a changing world. *J. Exp. Biol.* **2010**, *213*, 946–954. [CrossRef] [PubMed]
27. Racloz, V.; Ramsey, R.; Tong, S.L.; Hu, W.B. Surveillance of dengue fever virus: A review of epidemiological models and early warning systems. *PLoS Negl. Trop. Dis.* **2012**, *6*. [CrossRef] [PubMed]
28. Medeiros, L.C.D.; Castilho, C.A.R.; Braga, C.; de Souza, W.V.; Regis, L.; Monteiro, A.M.V. Modeling the dynamic transmission of dengue fever: Investigating disease persistence. *PLoS Negl. Trop. Dis.* **2011**, *5*. [CrossRef] [PubMed]
29. Parham, P.E.; Michael, E. Modeling the effects of weather and climate change on malaria transmission. *Environ. Health Perspect.* **2010**, *118*, 620–626. [CrossRef] [PubMed]
30. Husin, N.A.; Salim, N.; Ahmad, A.R. Modeling of dengue outbreak prediction in Malaysia: A comparison of neural network and nonlinear regression model. In Proceedings of the International Symposium on Information Technology, Kuala Lumpur, Malaysia, 26–28 August 2008; Volumes 1–4, pp. 1796–1799.
31. Wu, Y.; Lee, G.; Fu, X.J.; Hung, T. Detect climatic factors contributing to dengue outbreak based on wavelet, support vector machines and genetic algorithm. In Proceedings of the World Congress on Engineering 2008, London, UK, 2–4 July 2008; Volume 1, pp. 303–307.
32. Rachata, N.; Charoenkwan, P.; Yooyativong, T.; Chamnongthai, K.; Lursinsap, C.; Higuchi, K. Automatic prediction system of dengue haemorrhagic-fever outbreak risk by using entropy and artificial neural network. In Proceedings of the International Symposium on Communications and Information Technologies, Vientiane, Laos, 21–23 October 2008.
33. Aburas, H.M.; Cetiner, B.G.; Sari, M. Dengue confirmed-cases prediction: A neural network model. *Expert Syst. Appl.* **2010**, *37*, 4256–4260. [CrossRef]
34. Hwang, S.; Clarite, D.S.; Elijorde, F.I.; Gerardo, B.D.; Byun, Y. A web-based analysis for dengue tracking and prediction using artificial neural network. In *Advanced Science and Technology Letters*; Science & Engineering Research Support Society: Sandy Bay, TAS, Australia, 2016; Volume 122, pp. 160–164.
35. Nishanthi, P.; Perera, A.; Wijekoon, H. Prediction of dengue outbreaks in Sri Lanka using artificial neural networks. *Int. J. Comput. Appl.* **2014**, *101*, 1–5.
36. Lee, K.Y.; Chung, N.; Hwang, S. Application of an artificial neural network (ANN) model for predicting mosquito abundances in urban areas. *Ecol. Inform.* **2016**, *36*, 172–180. [CrossRef]
37. Focks, D.A.; Barrera, R. (Eds.) *Dengue Transmission Dynamics: Assessment and Implications for Control. Special Programme for Research and Training in Tropical Diseases*; World Health Organization: Geneva, Switzerland, 2006.
38. Halstead, S.B. Pathogenesis of dengue—Challenges to molecular biology. *Science* **1988**, *239*, 476–481. [CrossRef] [PubMed]
39. Monath, T.P. Yellow fever and dengue—The interactions of virus, vector and host in the re-emergence of epidemic disease. *Semin. Virol.* **1994**, *5*, 133–145. [CrossRef]
40. Almeida, M.C.D.; Caiaffa, W.T.; Assuncao, R.M.; Proietti, F.A. Spatial vulnerability to dengue in a Brazilian urban area during a 7-year surveillance. *J. Urban Health Bull. N. Y. Acad. Med.* **2007**, *84*, 334–345. [CrossRef] [PubMed]
41. Murphy, D.J.; Hall, M.H.; Hall, C.A.S.; Heisler, G.M.; Stehman, S.V.; Anselmi-Molina, C. The relationship between land cover and the urban heat island in northeastern Puerto Rico. *Int. J. Climatol.* **2011**, *31*, 1222–1239. [CrossRef]
42. Herrera Silveira, J.A. Spatial heterogeneity and seasonal patterns in a tropical coastal lagoon. *J. Coast. Res.* **1994**, *10*, 738–746.
43. Gonzalez, F.U.T.; Ilveira, J.; Aguirre-Macedo, M.L. Water quality variability and eutrophic trends in karstic tropical coastal lagoons of the Yucatan Peninsula. *Estuar. Coast. Shelf Sci.* **2008**, *76*, 418–430. [CrossRef]
44. Colon-Gonzalez, F.J.; Lake, I.R.; Bentham, G. Climate variability and dengue fever in warm and humid Mexico. *Am. J. Trop. Med. Hyg.* **2011**, *84*, 757–763. [CrossRef] [PubMed]
45. Thai, K.T.D.; Nishiura, H.; Hoang, P.L.; Tran, N.T.T.; Phan, G.T.; Le, H.Q.; Tran, B.Q.; van Nguyen, N.; de Vries, P.J. Age-specificity of clinical dengue during primary and secondary infections. *PLoS Negl. Trop. Dis.* **2011**, *5*, e1180. [CrossRef] [PubMed]

46. Hammond, S.N.; Balmaseda, A.; Perez, L.; Tellez, Y.; Saborio, S.I.; Mercado, J.C.; Videa, E.; Rodriguez, Y.; Pérez, M.A.; Cuadra, R.; et al. Differences in dengue severity in infants, children, and adults in a 3-year hospital-based study in Nicaragua. *Am. J. Trop. Med. Hyg.* **2005**, *73*, 1063–1070. [PubMed]
47. Rigau-Perez, J.G.; Vorndam, A.V.; Clark, G.G. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994–1995. *Am. J. Trop. Med. Hyg.* **2001**, *64*, 67–74. [CrossRef] [PubMed]
48. Duncan, A.; Chen, A.S.; Keedwell, E.; Djordjevic, S.; Savic, D. Urban flood prediction in real-time from weather radar and rainfall data using artificial neural networks. In Proceedings of the Weather Radar and Hydrology International Symposium, Exeter, UK, 18–21 April 2011.
49. Duncan, A.; Chen, A.S.; Keedwell, E.; Djordjevic, S.; Savic, D. RAPIDS: Early warning system for urban flooding and water quality hazards. In Proceedings of the Machine Learning in Water Systems Symposium: Part of AISB Annual Convention 2013, Exeter, UK, 3–5 April 2013.
50. Deb, K.; Pratap, A.; Agarwal, S.; Meyarivan, T. A fast and elitist multiobjective genetic algorithm: NSGA-II. *IEEE Trans. Evol. Comput.* **2002**, *6*, 182–197. [CrossRef]
51. Duncan, A.P. The Analysis and Application of Artificial Neural Networks for Early Warning Systems in Hydrology and the Environment. Ph.D. Thesis, University of Exeter, Exeter, UK, 2014.
52. Cawley, G.C.; Talbot, N.L.C. Efficient leave-one-out cross-validation of kernel Fisher discriminant classifiers. *Pattern Recognit.* **2003**, *36*, 2585–2592. [CrossRef]
53. Stidson, R.T.; Gray, C.A.; McPhail, C.D. Development and use of modelling techniques for real-time bathing water quality predictions. *Water Environ. J.* **2012**, *26*, 7–18. [CrossRef]
54. Basheer, I.A.; Hajmeer, M. Artificial neural networks: Fundamentals, computing, design, and application. *J. Microbiol. Methods* **2000**, *43*, 3–31. [CrossRef]
55. Haykin, S. *Neural Networks: A Comprehensive Foundation*, 2nd ed.; Pearson Education Inc.: New Delhi, India, 1999.
56. Duncan, A.; Tyrrell, D.; Smart, N.; Keedwell, E.; Djordjevic, S.; Savic, D. Comparison of machine learning classifier models for bathing water quality exceedances in UK. In Proceedings of the 35th IAHR World Congress, Chengdu, China, 8–13 September 2013.
57. Teklehaimanot, H.D.; Schwartz, J.; Teklehaimanot, A.; Lipsitch, M. Alert threshold algorithms and malaria epidemic detection. *Emerg. Infect. Dis.* **2004**, *10*, 1220–1226. [CrossRef] [PubMed]
58. Althouse, B.M.; Yng Ng, Y.; Cummings, D.A. Prediction of dengue incidence using search query surveillance. *PLoS Negl. Trp. Dis.* **2011**, *5*, e1258. [CrossRef] [PubMed]
59. Ocampo, C.B.; Mina, N.J.; Carabali, M.; Alexander, N.; Osorio, L. Reduction in dengue cases observed during mass control of *Aedes* (*Stegomyia*) in street catch basins in an endemic urban area in Colombia. *Acta Trop.* **2014**, *132*, 15–22. [CrossRef] [PubMed]
60. Hanley, J.A.; McNeil, B.J. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* **1982**, *143*, 29–36. [CrossRef] [PubMed]
61. Brady, O.J.; Johansson, M.A.; Guerra, C.A.; Bhatt, S.; Golding, N.; Pigott, D.M.; Delatte, H.; Grech, M.G.; Leisnham, P.T.; de Freitas, R.M.; et al. Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings. *Parasites Vectors* **2013**, *6*, 12. [CrossRef] [PubMed]
62. Johansson, M.A.; Dominici, F.; Glass, G.E. Local and global effects of climate on dengue transmission in Puerto Rico. *PLoS Negl. Trop. Dis.* **2009**, *3*. [CrossRef] [PubMed]
63. Colon-Gonzalez, F.J.; Fezzi, C.; Lake, I.R.; Hunter, P.R. The effects of weather and climate change on dengue. *PLoS Negl. Trop. Dis.* **2013**, *7*, e2503. [CrossRef] [PubMed]
64. Ten Bosch, Q.A.; Singh, B.K.; Hassan, M.R.A.; Chadee, D.D.; Michael, E. The role of serotype interactions and seasonality in dengue model selection and control: Insights from a pattern matching approach. *PLoS Negl. Trop. Dis.* **2016**, *10*. [CrossRef] [PubMed]
65. Chan, M.; Johansson, M.A. The incubation periods of dengue viruses. *PLoS ONE* **2012**, *7*, e50972. [CrossRef] [PubMed]
66. Eisen, L.; Garcia-Rejon, J.E.; Gomez-Carro, S.; Vazquez, M.D.N.; Keefe, T.J.; Beaty, B.J.; Loroño-Pino, M.A. Temporal correlations between mosquito-based dengue virus surveillance measures or indoor mosquito abundance and dengue case numbers in Merida City, Mexico. *J. Med. Entomol.* **2014**, *51*, 885–890. [CrossRef] [PubMed]
67. Guabiraba, R.; Ryffel, B. Dengue virus infection: Current concepts in immune mechanisms and lessons from murine models. *Immunology* **2014**, *141*, 143–156. [CrossRef] [PubMed]

68. Liu-Helmersson, J.; Stenlund, H.; Wilder-Smith, A.; Rocklov, J. Vectorial capacity of *Aedes aegypti*: Effects of temperature and implications for global dengue epidemic potential. *PLoS ONE* **2014**, *9*, e89783. [CrossRef] [PubMed]
69. Gubler, D.J.; Clark, G.G. Dengue/dengue hemorrhagic fever—The emergences of a global health problem. *Emerg. Infect. Dis.* **1995**, *1*, 55–57. [CrossRef] [PubMed]
70. Rothman, A.L. Dengue: Defining protective versus pathologic immunity. *J. Clin. Investig.* **2004**, *113*, 946–951. [CrossRef] [PubMed]
71. Paaijmans, K.P.; Cator, L.J.; Thomas, M.B. Temperature-dependent pre-bloodmeal period and temperature-driven asynchrony between parasite development and mosquito biting rate reduce malaria transmission intensity. *PLoS ONE* **2013**, *8*, e55777. [CrossRef] [PubMed]
72. Hu, W.B.; Clements, A.; Williams, G.; Tong, S.L. Dengue fever and El Niño/southern oscillation in Queensland, Australia: A time series predictive model. *Occup. Environ. Med.* **2010**, *67*, 307–311. [CrossRef] [PubMed]
73. Johansson, M.A.; Cummings, D.A.T.; Glass, G.E. Multiyear climate variability and dengue-El Niño southern oscillation, weather, and dengue incidence in Puerto Rico, Mexico, and Thailand: A longitudinal data analysis. *PLoS Med.* **2009**, *6*, e1000168. [CrossRef] [PubMed]
74. Patz, J.A.; Campbell-Lendrum, D.; Holloway, T.; Foley, J.A. Impact of regional climate change on human health. *Nature* **2005**, *438*, 310–317. [CrossRef] [PubMed]
75. Ramadona, A.L.; Lazuardi, L.; Hii, Y.L.; Holmner, A.; Kusnanto, H.; Rocklov, J. Prediction of dengue outbreaks based on disease surveillance and meteorological data. *PLoS ONE* **2016**, *11*, e0152688. [CrossRef] [PubMed]
76. Amancio, F.F.; Ferraz, M.L.; Almeida, M.C.D.; Pessanha, J.E.M.; Iani, F.C.M.; Fraga, G.L.; Lambertucci, J.R.; Carneiro, M. Dengue virus serotype 4 in a highly susceptible population in southeast Brazil. *J. Infect. Public Health* **2014**, *7*, 547–552. [CrossRef] [PubMed]
77. Briseno-Garcia, B.; Gomez-Dantes, H.; ArgottRamirez, E.; Montesano, R.; Vazquez-Martinez, A.L.; Ibanez-Bernal, S.; Madrigal-Ayala, G.; Ruíz-Matus, C.; Flisser, A.; Tapia-Conyer, R. Potential risk for dengue hemorrhagic fever: The isolation of serotype dengue-3 in Mexico. *Emerg. Infect. Dis.* **1996**, *2*, 133–135. [CrossRef] [PubMed]
78. Vázquez-Pichardo, M.; Rosales-Jiménez, C.; Núñez-León, A.; Rivera-Osorio, P.; Cruz-Hernández, S.D.L.; Ruiz-López, A.; González–Mateos, S.; López–Martínez, I.; Rodríguez–Martínez, J.C.; López–Gatell, H.; et al. Serotipos de dengue en México durante 2009 y 2010. *Bol. Médico Hosp. Infant. Mex.* **2011**, *68*, 103–110.
79. Gomez-Carro, S.; Méndez-Domínguez, N.; Mendez-Galván, J. Dengue seropositivity in a randomly selected sample from Yucatan analyzed in the context of dengue cases reported between 1996 and 2006. *J. Epidemiol. Res.* **2016**, *3*, 23. [CrossRef]
80. Garcia-Rejon, J.; Lorono-Pino, M.A.; Farfan-Ale, J.A.; Flores-Flores, L.; Rosado-Paredes, E.D.; Rivero-Cardenas, N.; Najera-Vazquez, R.; Gomez-Carro, S.; Lira-Zumbardo, V.; Gonzalez-Martinez, P.; et al. Dengue virus-infected *Aedes aegypti* in the home environment. *Am. J. Trop. Med. Hyg.* **2008**, *79*, 940–950. [PubMed]
81. Badurdeen, S.; Valladares, D.B.; Farrar, J.; Gozzer, E.; Kroeger, A.; Kuswara, N.; Ranzinger, S.R.; Tinh, H.T.; Leite, P.; Mahendradhata, Y.; et al. Sharing experiences: towards an evidence-based model of dengue surveillance and outbreak response in Latin America and Asia. *BMC Public Health* **2013**, *13*. [CrossRef] [PubMed]
82. Runge-Ranzinger, S.; McCall, P.J.; Kroeger, A.; Horstick, O. Dengue disease surveillance: An updated systematic literature review. *Trop. Med. Int. Health* **2014**, *19*, 1116–1160. [CrossRef] [PubMed]
83. Martinez Torres, E. Dengue. *Estud. Avançados* **2008**, *22*, 33–52. [CrossRef]
84. Guzman, M.G.; Brathwaite, O.; Enria, D.; Hunsperger, E.; Kouri, G.; Montoya, R.; San Martín, J.L. Dengue in the American Region: An update. *J. Clin. Virol.* **2013**, *27*, 1–13. [CrossRef]
85. Guzman, M.G.; Kouri, G. Dengue: An update. *Lancet Infect. Dis.* **2002**, *2*, 33–42. [CrossRef]

Application of Artificial Neural Networks for Dengue Fever Outbreak Predictions in the Northwest Coast of Yucatan, Mexico and San Juan, Puerto Rico

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Table S1. Mexico multiple linear regression model for population younger than 24 years old. Bold values are significant with 95% certainty ($\alpha = 0.05$). Parametric and non-parametric p-values are included. Power of these models (r^2) are shown above the table.

Mexico younger than 24 years (**$r^2 = 0.85$**)

Variable	b	t-stat	Parametric p-value	Non- parametric p-value
Date	-327.54	-1.39	0.166	0.102
Humidity	1.02	3.01	**0.003**	**0.002**
SST	1.11	1.51	0.132	0.114
Precipitation 1-week lag	0.07	3.55	**0.000**	**0.002**
Population	-30.08	-1.75	0.081	**0.018**
Minimum air temperature	1.16	3.67	**0.000**	**0.002**
Previous dengue cases	5.46	24.97	**0.000**	**0.002**

Table S2. Mexico multiple linear regression model for population younger than 5 and older than 65 years old. Bold values are significant with 95% certainty ($\alpha = 0.05$). Parametric and non-parametric p-values are included. Power of these models (r^2) are shown above the table.

Mexico younger than 5 and older than 65 years (**$r^2 = 0.84$**)

Variable	b	t-stat	Parametric p-value	Non-parametric p-value
Date	-79.89	-0.43	0.668	0.654
Humidity	0.44	1.64	0.103	0.220
SST	0.19	0.33	0.743	0.612
Precipitation 1-week lag	0.04	2.97	**0.003**	**0.040**
Population	-11.35	-0.84	0.404	0.364
Minimum air temperature	0.58	2.33	**0.021**	**0.038**
Previous dengue cases	10.37	26.36	**0.000**	**0.002**

Table S3. Puerto Rico multiple linear regression model for population younger than 24 years old. Bold values are significant with 95% certainty ($\alpha = 0.05$). Parametric and non-parametric p-values are included. Power of these models (r^2) are shown above the table.

Puerto Rico younger than 24 years (r^2 **= 0.74**)

Variable	b	t-stat	Parametric p-value	Non-parametric p-value
Date	-3.62	-2.95	**0.003**	**0.002**
SST	0.07	1.92	0.055	**0.046**
Precipitation	0.00	-1.13	0.258	0.282
Population	-0.19	-6.34	**0.000**	**0.002**
Minimum air temperature	0.03	0.84	0.400	0.370
Maximum air temperature	-0.03	-0.80	0.425	0.394
Previous dengue cases	0.05	41.94	**0.000**	**0.002**

Table S4. Puerto Rico multiple linear regression model for population younger than 5 and older than 65 years old. Bold values are significant with 95% certainty ($\alpha = 0.05$). Parametric and non-parametric p-values are included. Power of these models (r^2) are shown above the table.

Puerto Rico younger than 5 and older than 65 years (r^2 **= 0.33**)

Variable	b	t-stat	Parametric p-value	Non-parametric p-value
Date	-42.24	-2.21	**0.027**	**0.030**
SST	0.05	0.09	0.928	0.976
Precipitation	0.01	0.42	0.678	0.654
Population	-1.60	-3.37	**0.001**	**0.002**
Minimum air temperature	1.70	2.88	**0.004**	**0.012**
Maximum air temperature	-0.19	-0.31	0.756	0.810
Previous dengue cases	0.38	16.04	**0.000**	**0.002**

APPENDIX D

Artificial Neural Networks better predict exceedances of recreational water quality criteria at Escambron Beach, San Juan, Puerto Rico

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Journal of Water and Health

Artificial Neural Network better predict exceedances of recreational water quality criteria at Escambron Beach, San Juan, Puerto Rico

--Manuscript Draft--

Manuscript Number:	JWH-D-18-00116
Full Title:	Artificial Neural Network better predict exceedances of recreational water quality criteria at Escambron Beach, San Juan, Puerto Rico
Article Type:	Research Paper
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Abstract:	Predicting recreational water quality is key to protecting public health from exposure to wastewater-associated pathogens. It is not feasible to monitor recreational waters for all pathogens; therefore, monitoring programs use fecal indicator bacteria (FIB), such as enterococci, to identify wastewater pollution. Artificial Neural Networks (ANNs) were used to predict when culturable enterococci concentrations exceeded the U.S. Environmental Protection Agency (U.S. EPA) Recreational Water Quality Criteria (RWQC) at Escambron Beach, San Juan, Puerto Rico. Ten years of culturable enterococci data were analyzed together with satellite-derived sea surface temperature (SST), direct normal irradiance (DNI), turbidity, and dew point, along with local observations of precipitation and mean sea level (MSL). The factors identified as the most relevant for enterococci exceedance predictions based on the U.S. EPA RWQC were DNI, turbidity, cumulative 48 h precipitation, MSL, and SST; they predicted culturable enterococci exceedances with an accuracy of 75% and power greater than 60% based on the Receiving Operating Characteristic curve and F-Measure metrics. Results show the applicability of remote sensing data and ANNs to predict recreational water quality at Escambron Beach. Future work should incorporate local sanitary survey data in order to better predict risky recreational water conditions and protect human health.

Artificial Neural Network better predict exceedances of recreational water quality criteria at Escambron Beach, San Juan, Puerto Rico

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Abstract

Predicting recreational water quality is key to protecting public health from exposure to wastewater-associated pathogens. It is not feasible to monitor recreational waters for all pathogens; therefore, monitoring programs use fecal indicator bacteria (FIB), such as enterococci, to identify wastewater pollution. Artificial Neural Networks (ANNs) were used to predict when culturable enterococci concentrations exceeded the U.S. Environmental Protection Agency (U.S. EPA) Recreational Water Quality Criteria (RWQC) at Escambron Beach, San Juan, Puerto Rico. Ten years of culturable enterococci data were analyzed together with satellite-derived sea surface temperature (SST), direct normal irradiance (DNI), turbidity, and dew point, along with local observations of precipitation and mean sea level (MSL). The factors identified as the most relevant for enterococci exceedance predictions based on the U.S. EPA RWQC were DNI, turbidity, cumulative 48 h precipitation, MSL, and SST; they predicted culturable enterococci exceedances with an accuracy of 75% and power greater than 60% based on the Receiving Operating Characteristic curve and F-Measure metrics. Results show the applicability of remote sensing data and ANNs to predict recreational water quality at Escambron Beach.

Future work should incorporate local sanitary survey data in order to better predict risky recreational water conditions and protect human health.

Keywords

Bathing water quality; enterococci; machine learning; remote sensing; water quality prediction

Introduction

Recreational water quality monitoring programs exist worldwide to protect humans from potential exposure to pathogens and are based upon fecal indicator bacteria (FIB; Colford *et al.* 2007). The FIB monitored varies across latitudes and water types, where *Escherichia coli*, fecal coliforms, and *Enterococcus* spp. are most common (Colford *et al.* 2007; U.S. EPA 2012), and can be correlated with illness in areas with known fecal contamination sources at temperate latitudes (*e.g.*, ~ 33.4ºN-37.8ºN; Colford *et al.* 2012; Boehm & Sassoubre 2014). In the U.S., the Environmental Protection Agency (U.S. EPA) monitors *Enterococcus* spp. in recreational marine waters. Based on the 2012 Recreational Water Quality Criteria (RWQC), enterococci cannot exceed the geometric mean of 35 colony forming units (CFU) per 100 mL, which represents 36 illnesses per 1000 primary contact recreators (U.S. EPA 2012). This value was then modified to 70 CFU/100 mL based on the Beach Action Value (BAV), recommended by the U.S. EPA National Beach Guidance and Required Performance Criteria for Grants (U.S. EPA 2014). These guidelines were adopted by the Puerto Rico Environmental Quality Board (PREQB), where they monitor recreational water quality around the island of Puerto Rico biweekly in accordance with the 2000 U.S. Beaches Environmental Assessment and Coastal Health Act (U.S. EPA 2000; Cordero *et al.* 2012) and other water quality standards of Puerto Rico (PREQB 2010).

Escambron Beach is located in San Juan, Puerto Rico and is one of the most visited beaches in the region. Escambron Beach is within the Rio Piedras watershed (Diaz 2007; Lugo *et al.* 2011) and has a stormwater drainage outfall (18.46ºN, 66.09ºW), which discharges rainwater, agricultural runoff, and other greywaters (Diaz 2007). This FIB point source and the Bayamon Regional and Puerto Nuevo Regional wastewater treatment plant (WWTP; primary wastewater treatment) ocean outfall, located 5 km offshore, are the most prominent point sources of fecal pollution at the beach (Ortiz-Zayas *et al.* 2006). The nearby Rio Grande de Loiza river mouth also discharges human and non-human fecal pollution to the coastline (Quiñones 2012; Garcia-Montiel *et al.* 2014). In addition to the impact of known fecal pollution sources, culturable enterococci concentrations are influenced by environmental factors (Sanchez-Nazario *et al.* 2014; Laureano-Rosario *et al.* 2017). Such factors include: precipitation through increased runoff (Cordero *et al.* 2012); solar radiance bacterial inactivation (Maraccini *et al.* 2012; 2016); turbidity being a source of FIB or protecting them from ultraviolet (UV) light (He & He 2008; Shibata *et al.* 2010); and the resuspension of FIB in sediment reservoirs through increased winds and waves (Byappanahalli *et al.* 2012; Feng *et al.* 2013).

Predicting when FIB exceed water quality criteria has been a management goal and researchers have approached this using a variety of mathematical methods (*e.g.*, linear and nonlinear statistical modelling). Some studies have applied linear models to understand FIB relationships with environmental factors; however, these complex interactions may not be adequately characterized by linear models, which typically describe less than 50% of the variability (Gonzalez & Noble 2014; Laureano-Rosario *et al.* 2017). Furthermore, previous modelling efforts lacked infrastructure and human activities data (*i.e.*, land use); consequently, they did not accurately predict FIB concentrations (Rochelle-Newall *et al.* 2015). FIB vary depending on location, sources, and environmental factors; thus, a nonlinear approach is more appropriate due to FIB complexity and relationship with multiple parameters. Thus, nonlinear modelling is essential to understanding the complex relationships between environmental variations and FIB.

Studies using nonlinear methods, mostly based on machine learning, focused on relationships between FIB and environmental factors to predict recreational water quality (He & He 2008). These studies used different methods, such as artificial neural networks (ANNs), decision trees, and Monte Carlo approaches to predict recreational water quality in both marine and freshwaters (Jiang *et al.* 2013). These models take into account non-continuous relationships by creating a nonlinear combination of predictors to assess their relationship with FIB. For example, He & He (2008) applied ANNs and predicted FIB in recreational waters based on specific environmental factors (*e.g.*, turbidity, pH, temperature). Similarly, Chebud *et al.* (2012) applied ANNs and forecasted water quality parameters (*e.g.*, chlorophyll-a, turbidity, total phosphorus) in Florida, US. Our study helps fill research gaps in the Caribbean for recreational water quality predictions using ANNs in the context of environmental variability.

Since ANNs do not assume functional relationships between environmental factors and FIB and are self-driven data-adaptive methods, they can identify nonlinear, functional relationships between FIB and environmental factors. Forecasting recreational water quality can greatly improve the management of recreational waters as managers are able to overcome the time-lag associated with routine beach water quality monitoring (Enns *et al.* 2012; Thoe *et al.* 2014).

Even though ANNs have been widely applied to predict bathing water quality throughout the world, our study expands on this by using long-term ocean color satellite-derived data together with *in situ* bacterial sampling in Puerto Rico. This study implemented an ANNs approach, based upon ten years of culturable enterococci concentration data together with *in situ* and satellite-derived environmental data, to predict recreational water quality at Escambron Beach, San Juan, Puerto Rico. More specifically, the model was developed using satellite-derived direct normal irradiance (DNI), turbidity, sea surface temperature (SST), and dew point with local observations of mean sea level (MSL), and cumulative precipitation from 24 h up to 120 h. The objectives of this study were: 1) to identify the most relevant environmental factors to predict culturable enterococci RWQC exceedances at Escambron Beach from 2005–2014; 2) to show the applicability of nonlinear modelling for early warning system based on ANNs; and 3) show the benefit of incorporating remotely sensed data.

The results of this study can help understand the complex relationship between environmental factors and FIB in the Caribbean, with the aim of predicting exceedances and helping with management and mitigation of recreational water quality standards.

Materials and methods

Escambron Beach, San Juan, Puerto Rico

This study took place at Escambron Beach (Figure 1), one of the most popular beaches of San Juan, Puerto Rico (18.47ºN, 66.08ºW). This beach has a year-long swimming season. The municipality of San Juan (17.92ºN–18.52ºN, 65.62ºW–67.28ºW) has a tropical climate. In San Juan, the annual average precipitation is ~1800 mm and average air surface temperatures range between 24–29 ºC. The study area is potentially influenced by the following sources of fecal pollution: stormwater outfall (Diaz 2007), the Rio Grande de Loiza river (Ortiz-Zayas *et al.* 2006; PREQB 2007), San Juan Bay Estuary (Perez-Villalona *et al.* 2015), and the Bayamon and Puerto Nuevo Regional WWTP ocean outfall (Ortiz-Zayas *et al.* 2006; PREQB 2007; 2011).

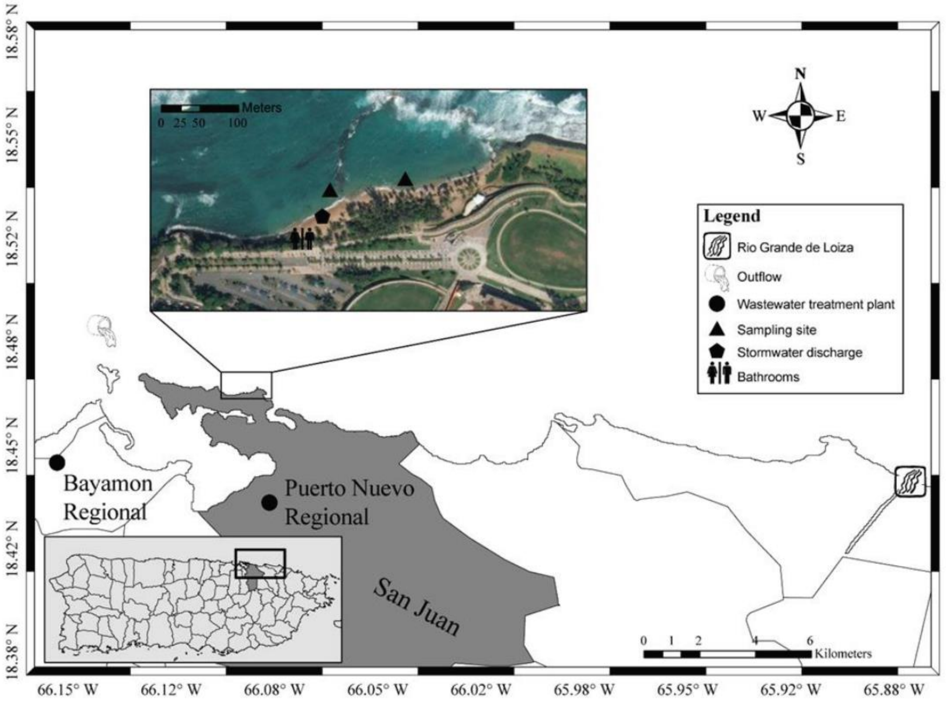

Figure 1. Escambron Beach is located in San Juan, Puerto Rico. The inset map shows a stormwater outfall (pentagon), which is located on the beach, bathrooms (bathroom symbol), and sampling sites (triangles). The Rio Grande de Loiza river mouth (river symbol) discharges 22 km east of the beach. The combined ocean outfall, discharging primary-treated domestic wastewater (outfall symbol), is 5 km from the study site and discharges at 40 m depth.

Culturable enterococci data

Culturable enterococci data for Escambron Beach were downloaded from the U.S. EPA Storage and Retrieval data warehouse (2005–2012). Data were for two sites separated by a distance of ~100 m. This dataset was extended from 2012 to 2014 with data provided by

PREQB; thus, a total of ten years of data were used (*n* = 273 observations for both sites combined). The culturable enterococci data were generated by the PREQB using U.S. EPA method 1600 and had a detection limit of 4 CFU/100 mL. All enterococci concentrations described as below the limit of detection were substituted by the next highest concentration (*e.g.*, 3 CFU/100 mL; Laureano-Rosario *et al.* 2017). Biweekly (*i.e.*, every other week) geometric means, commonly used due to bacterial variability, were then calculated combining the concentrations from both sampling sites. These geometric means were used in all further analyses.

Satellite-derived and *in situ* environmental data

Daily precipitation data were obtained from the U.S. National Oceanic and Atmospheric Administration (NOAA) National Center for Environmental Information from 2005–2014. DNI and dew point were obtained from the U.S. National Solar Radiation Database (2005–2014; 30-min temporal resolution and 4 km spatial resolution). MSL was obtained from the University of Hawaii Sea Level Center from 2005–2014. Day- and night-time SST were obtained from the U.S. NOAA Advanced Very High Resolution Radiometer (1 km spatial resolution) from 2005–2014. Data were extracted using the average of three 3- x 3-pixel boxes, for the north coast of San Juan, Puerto Rico. Interactive Data Language (IDL; v. 7.2) was used to extract data. Remote sensing reflectance at 645 nm (R_{rs} 645; Chen *et al.* 2007) was used as a proxy for turbidity from the NASA Moderate Resolution Imaging Spectroradiometer (MODIS-Terra; 250 m spatial resolution). Data were extracted using MATLAB (v. 2014b; The MathWorks Inc., Natick, MA, 2000); the average of two 3- x 3-pixel boxes was used for turbidity for this coastal region. The environmental variables included in the model to predict culturable enterococci exceedances were: MSL, cumulative precipitation for 24 h, 48 h, 72 h, 96 h, and 120 h, SST, DNI, dew point, and turbidity. These variables were normalized for predictive purposes.

Artificial Neural Network model setup

Training, validation, and testing

The Radar Pluvial flooding Identification for Drainage System (RAPIDS) approach was implemented, which is based on ANNs (Duncan *et al.* 2011, 2013), to train, validate, and test the Escambron Beach recreational water quality model. This model was also used to predict bathing water quality in previous studies in the United Kingdom (Duncan 2014). The ANNs model used the non-dominated sorting genetic algorithm II (NSGA-II; Deb *et al.* 2002) for optimizing and training. Data were subsequently validated using the Leave-One-Out-Cross-Validation (LOOCV) approach. The model included a weighting factor (a), which minimized the number of incorrectly predicted passes (*i.e.*, values below thresholds; false positive ratios [FPR]) (Stidson *et al.* 2012). During the testing stage, a series of weighting factors (a) were tested, where the best and the one used for culturable enterococci exceedance predictions at Escambron Beach was a = 3, weighting health risks three times more important. Accuracy was determined by the accuracy band, which was calculated using the percentages of true positive rates (TPR) and true negative rates (TNR) compared to FPR and FNR. We used the F-measure (FM; Eq. 1) as one of the power metrics, providing the importance of false positives (FP) over false negatives (FN) through the weighting factor (a). By emphasizing the importance of FP, we reduce model misclassifications that could lead to potential health risks (i.e., saying that is safe to swim when bacterial concentrations are indeed higher). The second power metric used was the area under the Receiving Operating Characteristic (ROC) curve or AuC; this curve was based on the ratios of true positives (TP) and true negatives (TN) (Hanley & McNeil 1982; Duncan *et al.* 2013;

Duncan 2014). The AuC helped establish the ideal trade-off between FPR and FNR (*i.e.*, FPR = 1 - TPR).

$$FM = \frac{(1+a)TN}{(1+a)TN+aFP+FN} \qquad \text{(Equation 1)}$$

Culturable enterococci concentration was used as the target variable. Environmental variables (inputs) were weighted in to identify those that were the most relevant for predicting culturable enterococci exceedances. A total of ten years (*i.e.*, epochs) were included in the model. Data from 2012 and 2013 were pooled due to missing dates. The Neural Pathway Strength Feature Selection (NPSFS) method helped identify the most relevant inputs, through an ensemble of ANNs and comparing the similarities of the weight results (*i.e.*, pathway strength) for the model inputs (*i.e.*, environmental factors). Inputs with the most similarity of pathway strengths for the whole ensemble of ANNs were selected as the most relevant (Duncan *et al.* 2013; Duncan 2014). The strength of the relationship as well as their relevancy (*i.e.*, excitatory or inhibitory) to predict enterococci exceedances were identified.

ANNs calculate weights and biases to understand strengths and relationships between inputs and outputs (Basheer & Hajmeer 2000; Duncan *et al.* 2013). ANN weights were calculated for the hidden layers (W_1) and for the output layer (W_2; Figure 2). The final weights (W_0) were calculated through matrix math of the ANNs hidden layer weights matrix and ANNs output layer weights vector (*i.e.*, $W_1 \cdot W_2 = W_0$; Duncan *et al.* 2013; Duncan 2014). These final weights values were used to identify the most relevant parameters through NPSFS to predict culturable enterococci concentration exceedances. Members of the ensemble were trained on a similar but different subset of the full training data set. Therefore, weights obtained in each ANN had different values. For a single output ANN, the result was a vector that specified the combined pathway strength of each input on the output. Combined Neural Pathway Strength Analysis (CNPSA) was used to identify if the relationships were excitatory or inhibitory (Basheer & Hajmeer 2000). In that case, the input is considered relevant to predict enterococci exceedances.

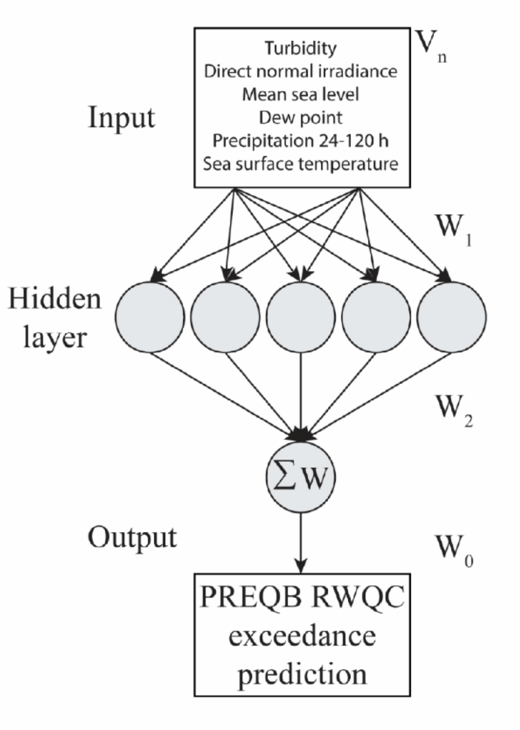

Figure 2. The Artificial Neural Networks model schematic for predicting Puerto Rico Environmental Quality Board Recreational Water Quality Criteria (PREQB RWQC) exceedances at Escambron Beach, San Juan, Puerto Rico.

Crossover and mutation rates, incorporated by NSGA-II during the training period, were used to optimize weights. These crossover and mutation rate factors differentiated new weights generations from the parent generation (Duncan 2014). Different crossover and mutation rate input values were tested; however, the crossover rate used was 0.2 and the mutation rate was 0.1. These input rates were used as they provided the best optimization results during the predictions of exceedances. Model's cost function used false positive rates and false negative rates. We used the minimum Euclidean distance to an ideal true positive ratio equal to one; these distances were derived from the ROC and used for optimization by NSGA-II to assess the quality of solutions (Duncan *et al.* 2013; Duncan 2014). Data were divided into ten epochs to ensure that the data used for training and validation were different than the data used for testing predictions as follows: epochs 1-3, 5, and 7 for training (*n* = 152); epochs 4, 6 and 8 for validation (*n* = 66); and epochs 9-10 for testing (*n* = 55).

Threshold selection for culturable enterococci exceedance predictions

To predict when enterococci exceeded the PREQB RWQC for safe recreation, the threshold selected for this study was the geometric mean concentration of 70 CFU/100 mL (PREQB 2016). This concentration is the BAV recommended by the U.S. EPA to ensure no more than 36 illnesses per 1000 recreators and was adopted by the PREQB in 2015 (U.S. EPA 2014; PREQB 2016). The model compared the observed and predicted enterococci concentrations to this BAV threshold and identified them as "safe for swimming" (*i.e.*, below threshold) and "potentially unsafe for swimming" (*i.e.*, above BAV threshold). Results showed the influence, and magnitude, of inputs to predict enterococci exceedances based on the specific thresholds mentioned above. These are shown as inputs having an inhibitory or excitatory influence on outputs regarding outputs crossing the set thresholds. Based on the 70 CFU/100 mL, we had a total of 238 passes and 35 fails in the original data.

Results

ANN model evaluation for accuracy and predictive power

The model predicted culturable enterococci exceedances with an accuracy band of 76% for Escambron Beach during 2005–2014. This accuracy represented how many correct versus incorrect predictions were obtained compared to observed values. More specifically, the 76% accuracy band was composed of 4% accurately predicted fails and 72% accurately predicted passes. Overall, the model accurately predicted culturable enterococci exceedances based on the PREQB RWQC for safe recreation, with a significant power greater than 60%, where the FM was 0.61 and the AuC was 0.74 (Figure 3).

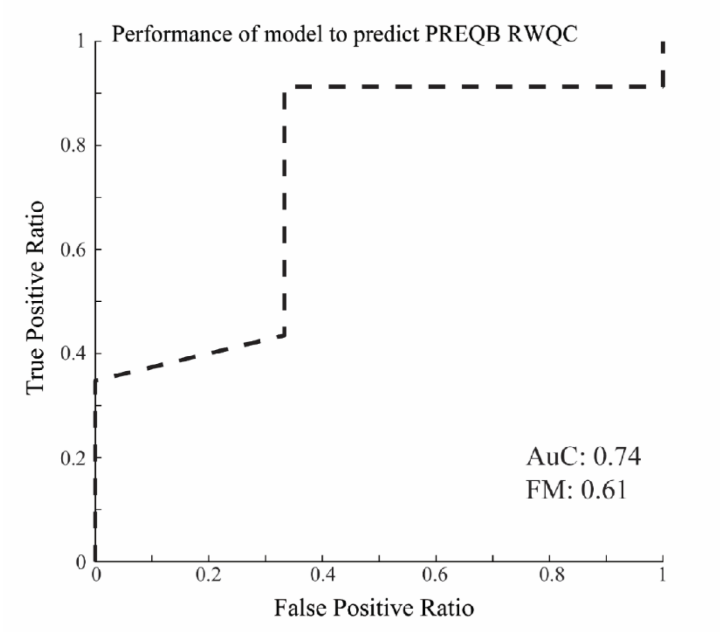

Figure 3. Receiving Operating Characteristic curve for Puerto Rico Environmental Quality Board Recreational Water Quality Criteria (PREQ RWQC) exceedance predictions at Escambron Beach, San Juan, Puerto Rico depicts the performance of the model. The dashed line depicts the calculated true positive ratios (TPR) and false positive ratios (FPR). The area under the curve (AuC) was calculated based on TPR/FPR ratios. The F-measure (FM) describes the power of model and importance of false positives over false negatives (Stidson 2012).

Relevant environmental factors for culturable enterococci concentration predictions

The most relevant parameters to predict culturable enterococci concentrations at Escambron Beach from 2005–2014 were DNI, turbidity, 48 h cumulative precipitation, MSL, and SST (Figure 4). Only MSL and DNI showed an excitatory relationship; whereas, turbidity, 48 h cumulative precipitation, and SST showed an inhibitory relationship. The most relevant variables were DNI and turbidity, where DNI showed a smaller spread of weights compared to turbidity. These environmental factors were identified as showing either an excitatory (positive weights) or inhibitory (negative weights) influence. For example, DNI had an excitatory influence on predicting enterococci exceedances (Figure 4), which represented an overall stimulus of DNI on culturable enterococci concentrations to cross the BAV threshold (*i.e.*, fail); this is likely due to lower values of DNI across the entire time series. On the other hand, turbidity showed an inhibitory influence, meaning that it inhibits culturable enterococci concentrations to cross the BAV threshold (*i.e.*, pass). Lastly, six variables (*i.e.*, cumulative 24 h precipitation, cumulative 96 h precipitation, date, cumulative 120 h precipitation, and dew point), which crossed the zero line of the box and whisker plots, were not considered relevant to predict culturable enterococci exceedances at Escambron Beach surface waters.

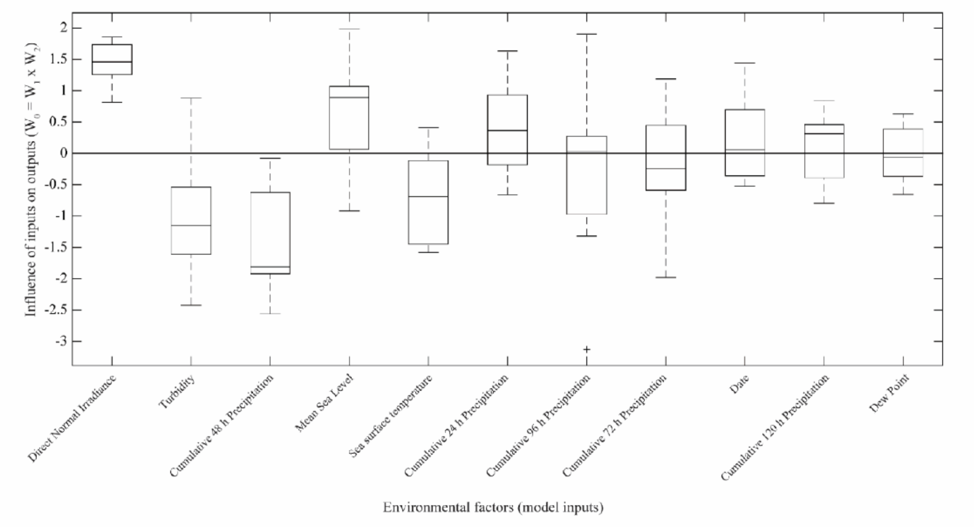

Figure 4. Distribution of environmental variable weights in the ANNs model to predict Recreational Water Quality Criteria exceedances at Escambron Beach. The box and whisker plots show the distribution of weights from the ANNs model for each environmental factor: Direct normal irradiance, turbidity, cumulative 48 h precipitation, mean sea level, sea surface temperature, cumulative 24 h precipitation, cumulative 96 h precipitation, cumulative 72 h precipitation, cumulative 120 h precipitation, and dew point. Boxes are distributions of weights, lines inside boxes are mean values of the weights. The zero line represents no relevance for predicting outputs. Relevance for predictions was based on weight values calculated for predictions (W_0), multiplying those weights from the hidden layer (W_1) by weights from output layer (W_2). These W_0 weights represent the strength of the influence of input on output. Environmental factors are in order of importance for predictions, such that the most relevant variables are listed on the left.

Discussion

This study investigated the use of a nonlinear model to predict exceedance of the PREQB RWQC for safe recreation at Escambron Beach, San Juan, Puerto Rico. The most relevant variables in this model were DNI, turbidity, cumulative 48 h precipitation, MSL, and SST. These results showed that accurately predicting culturable enterococci exceedances, based on the 2014 BAV value, at Escambron Beach can be achieved using the aforementioned environmental variables. Notwithstanding, this model could make improved predictions by including a larger data set and geo-referenced sanitation infrastructure data.

ANN model success for predicting exceedance of the PREQB RWQC

The ANN modelling described in this study showed the importance of identifying how environmental conditions can influence culturable enterococci concentration, as well as the complexity of these relationships between FIB and environmental factors. The use of ANNs to model culturable enterococci concentrations at Escambron Beach provided an accuracy band of 76% for exceedances, with greater than 60% model power, which is higher than previous models using linear approaches (*e.g.*, Laureano-Rosario *et al.* 2017), and similar to those using ANNs for FIB predictions (*e.g.*, He & He 2008; Chebud *et al.* 2012). Modelling enterococci exceedance at Escambron Beach was achieved by using the U.S. EPA and PREQB BAV (70 CFU/100 mL) as the model threshold concentration. By using this threshold, the model identified 35 occasions in which enterococci concentrations exceeded the BAV (*i.e.*, model fails) in the original data and these events were then used for predictive purposes. AuC and FM provided model's power and accounted for the ratios of true positives and true negatives. The accuracy band accounted for predicted values individually compared to the original values. These percentages might be affected by the number of passes (*n* = 238) and fails (*n* = 35) in the original observations. Therefore, the differences between accuracy and power are due to the methods used when predicting enterococci exceedances.

Model improvement

Despite the high model power observed, future studies could improve upon the model created in this study by considering FIB watershed sources and longshore currents sources. For example, it is likely that failing sanitation infrastructure (*e.g.*, leaky sewer pipes and septic systems) influenced FIB at Escambron Beach (Naidoo and Olaniran 2014). Additionally, nearby river, WWTP, as well as stormwater discharges could be a potential source of FIB throughout the year at various levels and future studies should take them into account. Lastly, climatic conditions vary annually, and this natural variability can affect enterococci predictions over time.

The presence of enterococci in beach sands and vegetation (*e.g.*, seagrass, green alga; Whitman *et al*. 2003; Sanchez-Nazario *et al.* 2014; Halliday *et al.* 2015) should also be considered to understand how these non-fecal sources influence enterococci water concentrations (Feng *et al.* 2012, 2013). Thus, predictive models can likely be improved by the inclusion of these data. Furthermore, there is also the need to identify other factors that might be of importance (*e.g.*, through microbial source tracking, different fecal indicators, infrastructure data), to better predict these exceedances, identify when those are related to human fecal contamination versus non-human fecal contamination versus non-fecal contamination, and protect public health.

Most relevant environmental factors influencing Escambron Beach water quality

Culturable enterococci concentration variability in coastal areas is influenced by fecal pollution sources, secondary, extraintestinal reservoirs, as well as by environmental factors (Viau *et al.* 2011). The current study accounted for specific environmental factors, such as DNI, turbidity, precipitation, MSL, SST, and dew point. These environmental factors have been shown to influence culturable enterococci concentrations, and other FIB, in temperate and tropical environments as well as marine and freshwaters (Enns *et al.* 2012; Lamparelli *et al.* 2015; Aranda *et al.* 2016).

As regards to environmental variables, precipitation most often explains the majority FIB variability observed (He & He 2008; Feng *et al.* 2013; Laureano-Rosario *et al.* 2017); however, this study identified DNI as the most relevant environmental variable (Maraccini *et al.*, 2012; 2016). The three most influential variables predicting PREQB RWQC exceedance were DNI, turbidity, and 48 h cumulative precipitation. DNI was the most important environmental variable to consider for PREQB RWQC exceedance predictions, likely due to bacterial inactivation (Maraccini *et al.* 2012, 2016). Since Escambron Beach is located in a tropical setting, it is no surprise that sunlight is one of the most influential environmental factors (Rochelle-Newall *et al.* 2015). Exposure to UV light results in bacterial inactivation, and consequently, a decrease in bacterial concentrations (Byappanahalli *et al.* 2012; Walters *et al.* 2014). The next most influential predictive environmental variable was turbidity, which has been documented to protect bacteria from UV light exposure. Turbidity is also associated with increased FIB when precipitation facilitates runoff into coastal waters (Halliday *et al.* 2015; Aragones *et al.* 2016). Thus, the combined turbidity and DNI effects on enterococci concentrations could be the reason why these were identified as the most relevant parameters to predict culturable enterococci concentration exceedances.

The third most relevant parameter that predicted culturable enterococci exceedance at Escambron Beach was 48 h cumulative precipitation. He & He (2008) also identified 24–48 h of cumulative precipitation as significantly correlated with FIB at Torrey Pines State Beach and San Elijo State Beach, San Diego County, California, US. Rainfall is known to increase FIB concentrations due to runoff (Colford *et al.* 2012), inadequately treated wastewater effluents (*e.g.*, septic seepage; Naidoo & Olaniran 2014), and combined sewer-stormwater systems (He & He 2008). Since a nonlinear modelling approach was used in this study, the previously identified holistic influence of the aforementioned environmental conditions was able to be incorporated into the model and improved predictions were generated (Noble *et al.* 2004).

The least two relevant environmental variables associated with PREQB RWQC exceedance predictions at Escambron Beach were MSL and SST. Previously at other beaches in Florida and California U.S., increased MSL was associated with lower culturable enterococci concentrations due to dilution and decreased MSL was associated with higher concentrations due to backwashing of waves and increased discharge into the coastal areas (Maraccini *et al.* 2012; Feng *et al.* 2016). However, Escambron Beach is a low-wave action beach, with a minimal tidal range; thus, MSL is not expected to strongly influence enterococci concentrations. In terms of SST anomalies, warmer waters have been documented to increase bacterial replication (Byappanahalli *et al.* 2012) and consequently, SST warm-anomalies have been shown to be related to increased culturable enterococci concentrations in tropical settings (Pachepsky *et al.* 2014; Laureano-Rosario *et al.* 2017). Even though SST was not the most influential environmental variable identified by the model, it still provided information to predict PREQB RWQC exceedances.

Conclusion

This work shows that nonlinear models help to predict water quality with relatively good accuracy (76%). Data availability is an important aspect, especially the information regarding coastal water quality and both anthropogenic and environmental factors, due to their influence on FIB variability and phenology. Thus, collection of data and water quality monitoring programs are important to better understand FIB variability. Through modelling culturable enterococci concentration exceedances, this study found:

- The most relevant parameters to predict culturable enterococci surface water concentrations at Escambron Beach from 2005–2014 were DNI, turbidity, cumulative 48 h precipitation, MSL, and SST.
- ANNs were able to predict enterococci concentration exceedances at Escambron Beach with an accuracy of 76% and a power greater than 60%, which is higher than most statistical linear models.
- Among the environmental variables evaluated, DNI, turbidity, and 48 h cumulative precipitation showed the highest influence on predicting culturable enterococci concentrations at Escambron Beach, which represent their holistic influence on enterococci concentrations FIB.
- Only DNI and MSL showed a positive influence, whereas turbidity, 48 h cumulative precipitation, and SST showed an inhibitory (negative) influence on predicting culturable enterococci concentrations at Escambron Beach.
- Model predictive power may be improved by including sanitary survey data (*e.g.*, septic system density), as well as other data describing enterococci sources, such as algal and seagrass coverage, and stormwater and river discharges.

Acknowledgments

A.E.L.R. was supported by the U.S. National Science Foundation (NSF) Partnerships for International Research (PIRE) under Grant No. 1243510 and by the U.S. National Aeronautics and Space Administration (NASA) Headquarters under the NASA Earth and Science Fellowship Program Grant No. NNX15AN60H. A.E.L.R. was also funded by the USF College of Marine Science Linton Tibbetts Endowed Fellowship. F.M.K. was supported by the U.S. EPA Science to Achieve Results (STAR) grant No. 83519301. E.M.S. was supported by U.S. NSF grant OCE-1566562. We would like to thank the teams from the Universidad Autonoma of Yucatan and Centre for Water Systems for their help and input for this work. We would also like to thank the IMaRS team for their input and help in manuscript revisions.

References

Aragones, L., Lopez, I., Palazon, A., Lopez-Ubeda, R. & Garcia, C. 2016 Evaluation of the quality of coastal bathing waters in Spain through fecal bacteria *Escherichia coli* and *Enterococcus*. *Sci. Total Environ.* **566**, 288-297.

Aranda, D., Lopez, J.V., Solo-Gabriele, H.M. & Fleisher, J.M. 2016 Using probabilities of enterococci exceedance and logistic regression to evaluate long term weekly beach monitoring data. *J. Water Health* **14**(1), 81-89.

Basheer, I.A. & Hajmeer, M. 2000 Artificial neural networks: fundamentals, computing, design, and application. *J. Microbiol. Methods* **43**(1), 3-31.

Boehm, A.B. & Sassoubre, L.M. 2014 Enterococci as indicators of environmental fecal contamination. In Enterococci: *From Commensals to Leading Causes of Drug Resistant Infection*; Gilmore, M.S., Clewell, D.B., Ike, Y., Shankar, N., Eds.; Massachusetts Eye and Ear Infirmary: Boston, MA, USA. pp. 1–17.

Byappanahalli, M.N., Nevers, M.B., Korajkic, A., Staley, Z.R. & Harwood, V.J. 2012 Enterococci in the environment. Microbiol. *Mol. Biol. Rev.* **76**(4), 685-706.

Chebud, Y., Naja, G.M., Rivero, R.G. & Melesse, A.M. 2012 Water quality monitoring using remote sensing and an Artificial Neural Network. *Water Air Soil Pollut* **223**(8), 4875-4887.

Chen, Z.Q., Hu, C.M. & Muller-Karger, F. 2007 Monitoring turbidity in Tampa Bay using MODIS/Aqua 250-m imagery. *Remote Sens. Environ.* **109**(2), 207-220.

Colford, J.M., Schiff, K.C., Griffith, J.F., Yau, V., Arnold, B.F., Wright, C.C., Gruber, J.S., Wade, T.J., Burns, S., Hayes, J., McGee, C., Gold, M., Cao, Y.P., Noble, R.T., Haugland, R. & Weisberg, S.B. 2012 Using rapid indicators for *Enterococcus* to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Res.* **46**(7), 2176-2186.

Colford, J.M., Wade, T.J., Schiff, K.C., Wright, C.C., Griffith, J.F., Sandhu, S.K., Burns, S., Sobsey, M., Lovelace, G. & Weisberg, S.B. 2007 Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* **18**(1), 27-35.

Cordero, L., Norat, J., Mattei, H. & Nazario, C. 2012 Seasonal variations in the risk of gastrointestinal illness on a tropical recreational beach. *J. Water Health* **10**(4), 579-593.

Deb, K., Pratap, A., Agarwal, S. & Meyarivan, T. 2002 A fast and elitist multiobjective genetic algorithm: NSGA-II. *IEEE Transactions on Evolutionary Computation* **6**(2), 182-197.

Diaz, M.N. 2007 Evaluacion de la intensidad de lluvia y su efecto en la presencia de bacterias fecales en las playas del norte de Puerto Rico. Masters thesis, Universidad Metropolitana: San Juan, Puerto Rico.

Duncan, A., Chen, A.S., Keedwell, E., Djordjevic, S. & Savic, D. 2011 Urban flood prediction in real-time from weather radar and rainfall data using artificial neural networks. In Proceedings of theWeather Radar and Hydrology International Symposium, Exeter, UK, 18-21 April.

Duncan, A., Chen, A.S., Keedwell, E., Djordjevic, S. & Savic, D. 2013 RAPIDS: early warning system for urban flooding and water quality hazards. In Proceedings of the Machine Learning in Water Systems Symposium: Part of AISB Annual Convention 2013, Exeter, UK, 3-5 April.

Duncan, A.P. 2014 The Analysis and Application of Artificial Neural Networks for Early Warning Systems in Hydrology and the Environment. Ph.D. Thesis, University of Exeter, Exeter, UK.

Enns, A.A., Vogel, L.J., Abdelzaher, A.M., Solo-Gabriele, H.M., Plano, L.R.W., Gidley, M.L., Phillips, M.C., Klaus, J.S., Piggot, A.M., Feng, Z.X., Reniers, A., Haus, B.K., Elmir, S.M., Zhang, Y.F., Jimenez, N.H., Abdel-Mottaleb, N., Schoor, M.E., Brown, A., Khan, S.Q., Dameron, A.S., Salazar, N.C. & Fleming, L.E. 2012 Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions. *Water Res.* **46**(7), 2237-2246.

Feng, Z., Reniers, A., Haus, B., Solo-Gabriele, H., Fiorentino, L., Olascoaga, M. & MacMahan, J. 2012 Modeling microbial water quality at a beach impacted by multiple non-point sources. *Coastal Engineering Proceedings* **1**(33), 74.

Feng, Z.X., Reniers, A., Haus, B.K. & Solo-Gabriele, H.M. 2013 Modeling sediment-related enterococci loading, transport, and inactivation at an embayed nonpoint source beach. *Water Resour. Res.* **49**(2), 693-712.

Feng, Z.X., Reniers, A., Haus, B.K., Solo-Gabriele, H.M. & Kelly, E.A. 2016 Wave energy level and geographic setting correlate with Florida beach water quality. *Mar. Pollut. Bull.* **104**(1-2), 54-60.

Gonzalez, R.A. & Noble, R.T. 2014 Comparisons of statistical models to predict fecal indicator bacteria concentrations enumerated by qPCR- and culture-based methods. *Water Res.* **48**, 296-305.

Halliday, E., Ralston, D.K. & Gast, R.J. 2015 Contribution of sand-associated enterococci to dry weather water quality. *Environ. Sci. Technol.* **49**(1), 451-458.

He, L.M. & He, Z.L. 2008 Water quality prediction of marine recreational beaches receiving watershed baseflow and stormwater runoff in southern California, USA. *Water Res.* **42**(10-11), 2563-2573.

Jiang, Y.C., Nan, Z.R. & Yang, S.C. 2013 Risk assessment of water quality using Monte Carlo simulation and artificial neural network method. *J. Environ. Manage.* **122**, 130-136.

Lamparelli, C.C., Pogreba-Brown, K., Verhougstraete, M., Sato, M.I.Z., de Castro Bruni, A., Wade, T.J. & Eisenberg, J.N. 2015 Are fecal indicator bacteria appropriate measures of recreational water risks in the tropics: A cohort study of beach goers in Brazil? *Water Res.* **87**, 59-68.

Laureano-Rosario, A.E., Symonds, E.M., Rueda, D., Otis, D. & Muller-Karger, F.E. 2017 Environmental factors correlated with culturable enterococci concentrations in tropical recreational waters: A case study in Escambron Beach, San Juan, Puerto Rico. *Int. J. Environ. Res. Publ. Health* **14**(12), 1602.

Lugo, A.E., González, O.M.R. & Pedraza, C.R. 2011 *The Rio Piedras Watershed and Its Surrounding Environment*; USDA Forest Service: Washington, DC, USA.

Maraccini, P.A., Ferguson, D.M. & Boehm, A.B. 2012 Diurnal variation in *Enterococcus* species composition in polluted ocean water and a potential role for the enterococcal carotenoid in protection against photoinactivation. *Appl. Environ. Microbiol.* **78**(2), 305-310.

Maraccini, P.A., Mattioli, M.C.M., Sassoubre, L.M., Cao, Y.P., Griffith, J.F., Ervin, J.S., Van De Werfhorst, L.C. & Boehm, A.B. 2016 Solar inactivation of enterococci and *Escherichia coli* in natural waters: Effects of water absorbance and depth. *Environ. Sci. Technol.* **50**(10), 5068-5076.

Naidoo, S. & Olaniran, A.O. 2014 Treated wastewater effluent as a source of microbial pollution of surface water resources. *Int. J. Environ. Res. Public Health* **11**(1), 249-270.

Noble, R.T., Lee, I.M. & Schiff, K.C. 2004 Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. *J. Appl. Microbiol.* **96**(3), 464-472.

Ortiz-Zayas, J.R., Cuevas, E., Mayol-Bracero, O.L., Donoso, L., Trebs, I., Figueroa-Nieves, D. & McDowell, W.H. (2006) Urban influences on the nitrogen cycle in Puerto Rico. *Biogeochemistry* **79**(1-2), 109-133.

Pachepsky, Y.A., Blaustein, R.A., Whelan, G. & Shelton, D.R. 2014 Comparing temperature effects on *Escherichia coli*, *Salmonella*, and *Enterococcus* survival in surface waters. *Lett. Appl. Microbiol.* **59**(3), 278-283.

Perez-Villalona, H., Cornwell, J.C., Ortiz-Zayas, J.R. & Cuevas, E. 2015 Sediment denitrification and nutrient fluxes in the San José Lagoon, a tropical lagoon in the highly urbanized San Juan Bay Estuary, Puerto Rico. *Estuar Coasts* **38**(6), 2259-2278.

Puerto Rico Environmental Quality Board (PREQB). 2007 Total maximum daily loads (TMDL) Rio Grande de Loiza watershed. Junta de Calidad Ambiental, San Juan, Puerto Rico. p. 281.

Puerto Rico Environmental Quality Board (PREQB). 2010 Water quality standards regulation of Puerto Rico. Available online: http://www.gobierno.pr/NR/rdonlyres/5A9F2F2E-94AE-4C69-8453 CA08D616ED7D/0/Reg_Estdares_Calidad_Agua_2010.pdf (accessed on 30 September 2017).

Puerto Rico Environmental Quality Board (PREQB). 2011 Total Maximum Daily Loads (TMDL) of fecal coliform for evaluation units, Puerto Rico. Division de Planes y Proyectos Especiales, Area de Evaluacion y Planificacion Estrategica. Junta de Calidad Ambiental, San Juan, Puerto Rico. p. 164.

Puerto Rico Environmental Quality Board (PREQB). 2016 Beach Monitoring and Public Notification Program-Performance criteria 2016-2017. Available online: https://www2.pr.gov/agencias/jca/Documents/Monitoreo%20de%20Playas/Performance%20Criteria/BEACH_PERFORMANCE_CRITERIA_PREQB_2016-2017_final-draft.pdf (accessed on 31 July 2017).

Rochelle-Newall, E., Nguyen, T.M.H., Le, T.P.Q., Sengteheuanghoung, O. & Ribolzi, O. 2015 A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. *Front. Microbiol.* **6**, 308.

Sanchez-Nazario, E.E., Santiago-Rodriguez, T.M. & Toranzos, G.A. 2014 Prospective epidemiological pilot study on the morbidity of bathers exposed to tropical recreational waters and sand. *J. Water Health* **12**(2), 220-229.

Shibata, T., Solo-Gabriele, H.M., Sinigalliano, C.D., Gidley, M.L., Plano, L.R., Fleisher, J.M., Wang, J.D., Elmir, S.M., He, G. & Wright, M.E. 2010 Evaluation of conventional and alternative monitoring methods for a recreational marine beach with nonpoint source of fecal contamination. *Environ. Sci. Technol.* **44**(21), 8175-8181.

Stidson, R.T., Gray, C.A. & McPhail, C.D. 2012 Development and use of modelling techniques for real-time bathing water quality predictions. *Water Environ J* **26**(1), 7-18.

Thoe, W., Gold, M., Griesbach, A., Grimmer, M., Taggart, M.L. & Boehm, A.B. 2014 Predicting water quality at Santa Monica Beach: Evaluation of five different models for public notification of unsafe swimming conditions. *Water Res.* **67**, 105-117.

United States Environmental Protection Agency (U.S. EPA). 2000 Beaches Environmental Assessment and Coastal Health Act of 2000; Public Law: Washington, DC, USA, 2000; pp. 106–284.

United States Environmental Protection Agency (U.S. EPA). 2012 *Recreational Water Quality Criteria*; US EPA:Washington, DC, USA. Available online: https://www.epa.gov/wqc/microbial-pathogenrecreational-water-qualitycriteria (accessed on 30 July 2017).

United States Environmental Protection Agency (U.S. EPA). 2014 National Beach Guidance and Required Performance Criteria for Grants. Available online: https://www.epa.gov/sites/production/files/2014-07/documents/beach guidance-final-2014.pdf (accessed on 30 July 2017).

Viau, E.J., Goodwin, K.D., Yamahara, K.M., Layton, B.A., Sassoubre, L.M., Burns, S.L., Tong, H.I., Wong, S.H.C., Lu, Y.A. & Boehm, A.B. 2011 Bacterial pathogens in Hawaiian coastal streams-Associations with fecal indicators, land cover, and water quality. *Water Res.* **45**(11), 3279-3290.

Walters, E., Graml, M., Behle, C., Muller, E. & Horn, H. 2014 Influence of particle association and suspended solids on UV Inactivation of fecal indicator bacteria in an urban river. *Water Air Soil Pollut* **225**(1), 1822.

Whitman, R.L., Shively, D.A., Pawlik, H., Nevers, M.B. & Byappanahalli, M.N. 2003 Occurrence of *Escherichia coli* and enterococci in *Cladophora* (*Chlorophyta*) in nearshore water and beach sand of Lake Michigan. *Appl. Environ. Microbiol.* **69**(8), 4714-4719.

APPENDIX E

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Authors contributions

Appendix A: Modelling dengue fever risk in the State of Yucatan, Mexico using regional-scale satellite-derived sea surface temperature.

AE Laureano-Rosario: provided leadership, collected data, analyzed data, made graphs and figures, and wrote the initial manuscript.

JE Garcia-Rejon, S Gomez-Carro, and JA Farfan-Ale: collected and provided dengue fever data from Yucatan, Mexico (2007–2012), provided intellectual guidance and edited the manuscript.

FE Muller-Karger: provided intellectual guidance, edited and provided extensive written comments on the manuscript.

Appendix B: Environmental factors correlated with culturable enterococci concentrations in tropical recreational waters: A case study in Escambron Beach, San Juan, Puerto Rico.

AE Laureano-Rosario: analyzed the data, and wrote and led the manuscript.

EM Symonds: contributed substantially with data interpretation and manuscript preparation.

D Rueda-Roa, D Otis, and FE Muller-Karger: collected the satellite observations, edited the text, and provided extensive written comments on the manuscript.

Appendix C: Application of Artificial Neural Networks for dengue fever predictions in the northwest coast of Yucatan, Mexico and San Juan, Puerto Rico.

AE Laureano-Rosario: analyzed the data, and wrote and led the manuscript.

AP Duncan, DA Savic: developed and tested the ANNs predictive model, and contributed with edits for the manuscript.

PA Mendez-Lazaro, JE Garcia-Rejon, S Gomez-Carro, JA Farfan-Ale: collected and provided dengue fever data for Yucatan, Mexico.

FE Muller-Karger: collected the satellite observations, edited the text and provided extensive written comments on the manuscript.

Appendix D: Artificial Neural Network better predict exceedances of recreational water quality criteria at Escambron Beach, San Juan, Puerto Rico.

AE Laureano-Rosario: analyzed the data, and wrote and led the manuscript.

AP Duncan, DA Savic: developed and tested the ANNs predictive model, and contributed with edits for the manuscript.

EM Symonds: contributed substantially with data interpretation and manuscript preparation.

FE Muller-Karger: collected the satellite observations, edited the text and provided extensive written comments on the manuscript.

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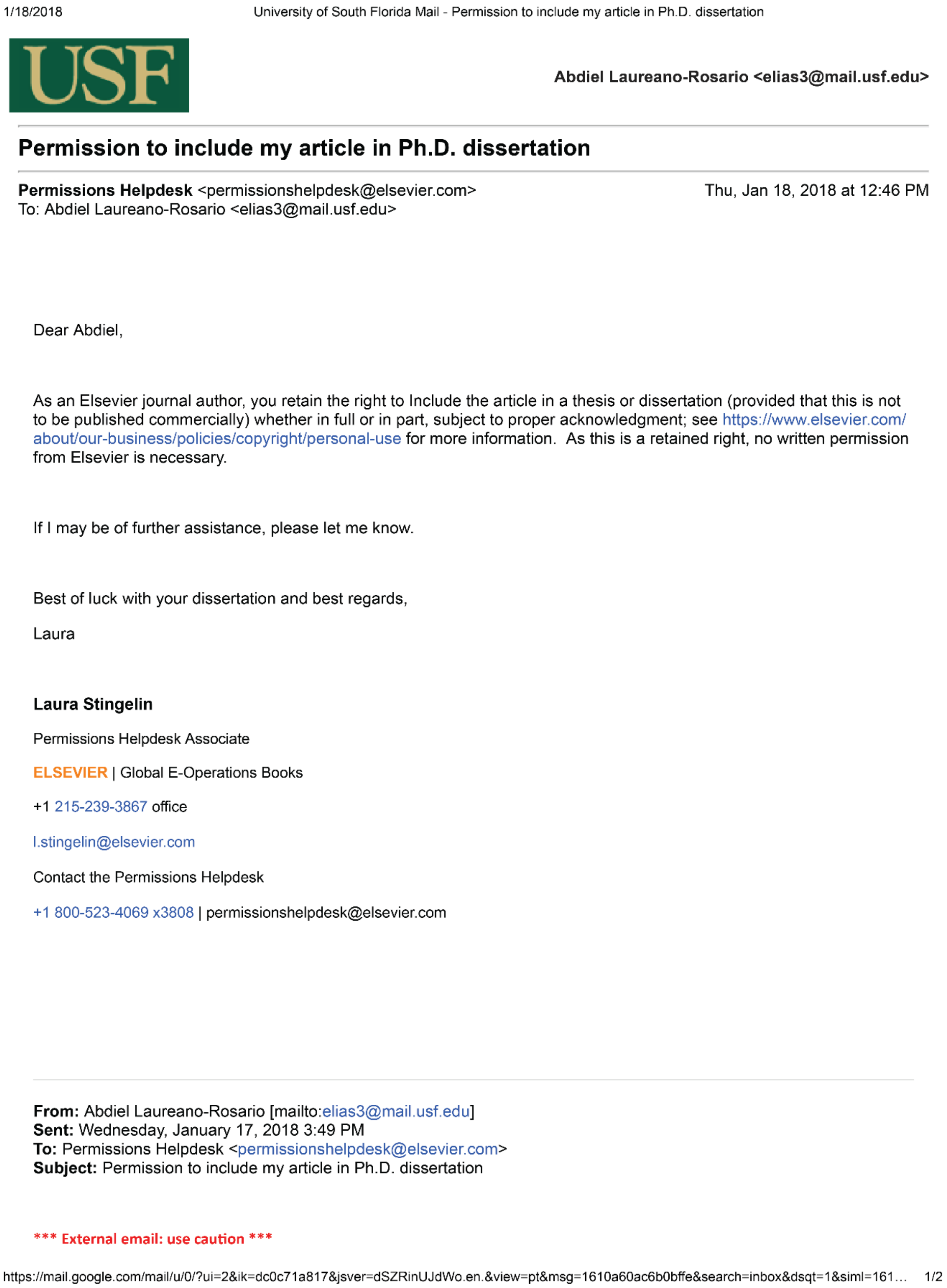

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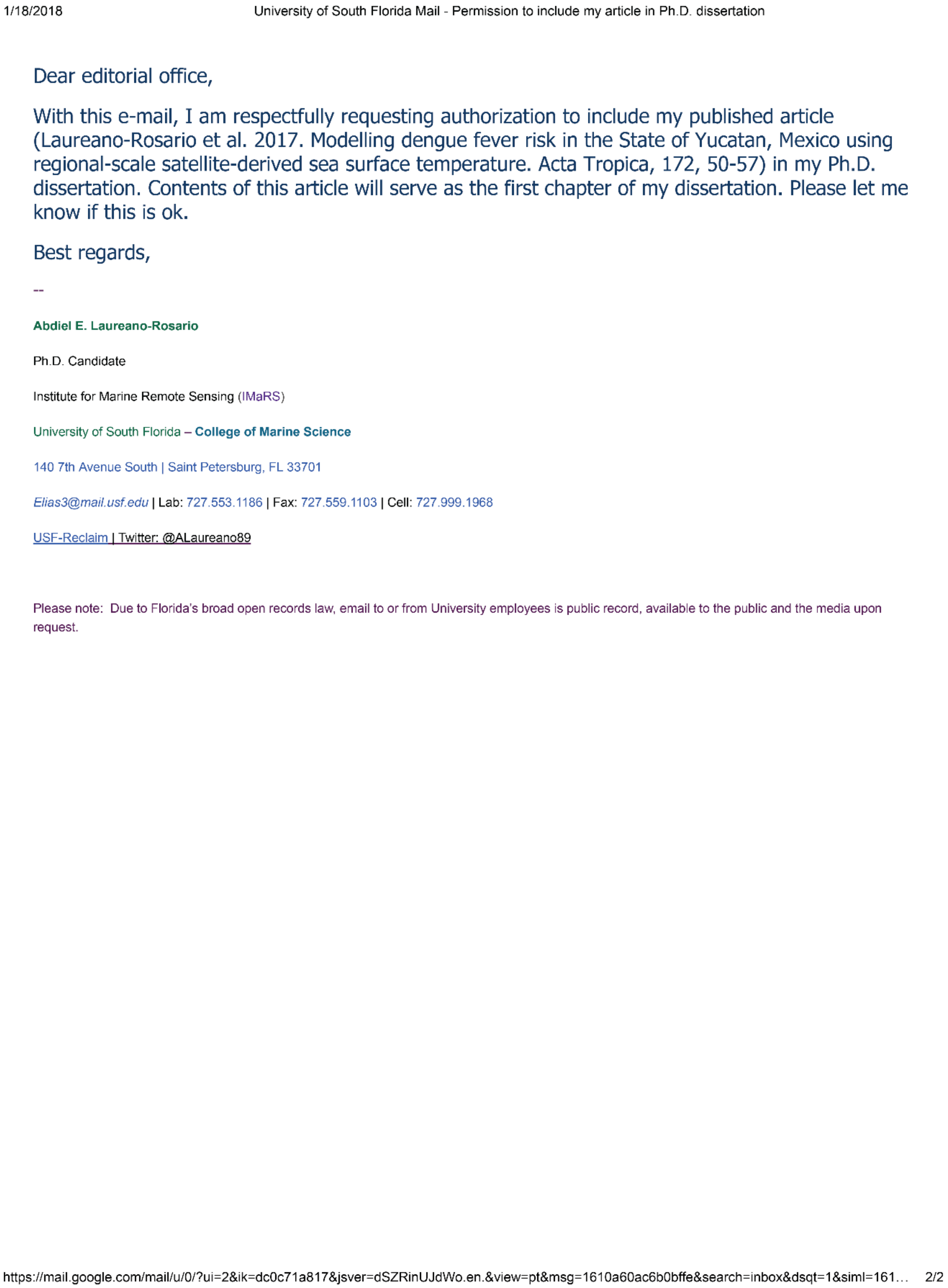

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Abdiel E. Laureano-Rosario

Ph.D. Candidate

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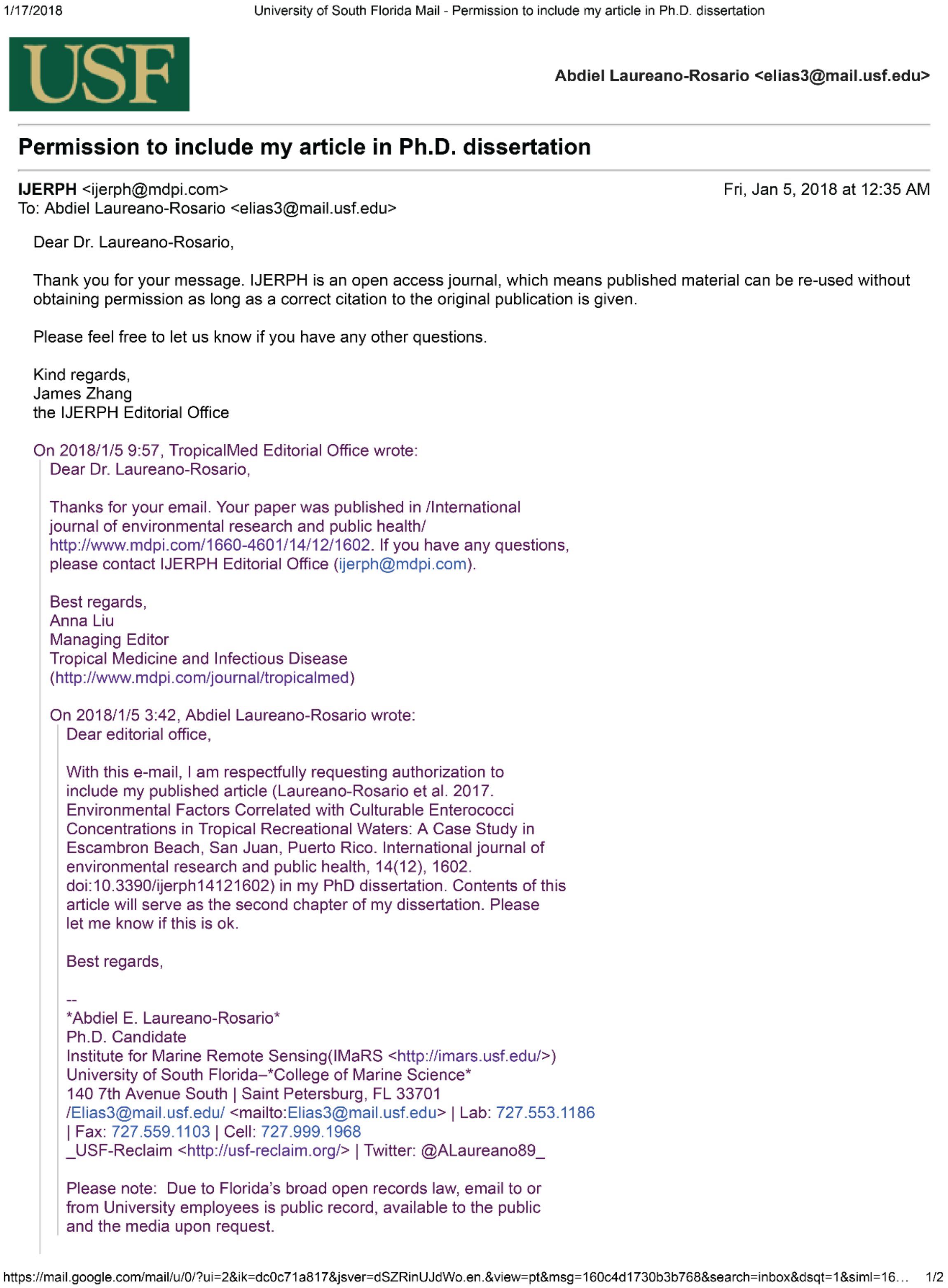

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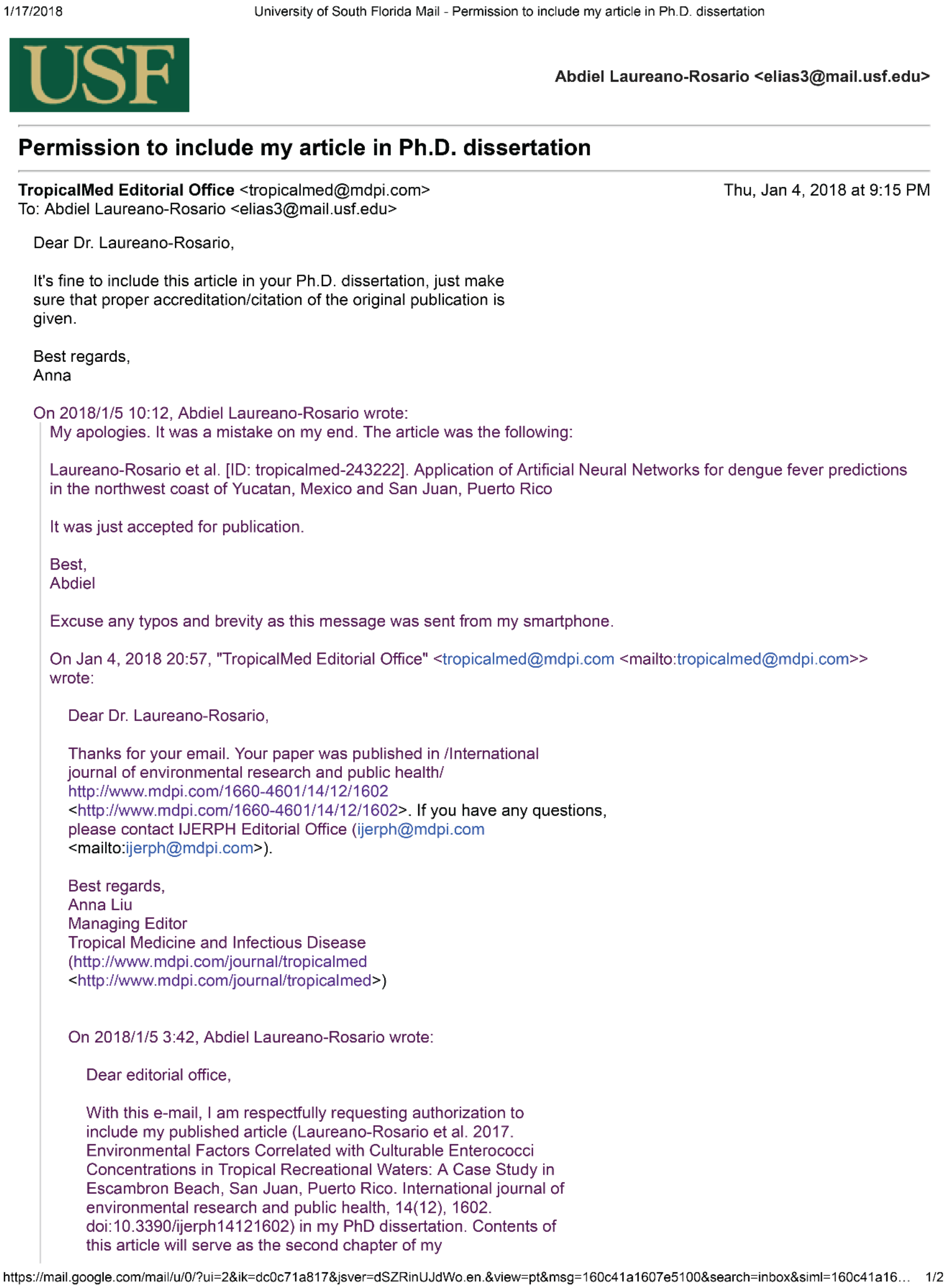

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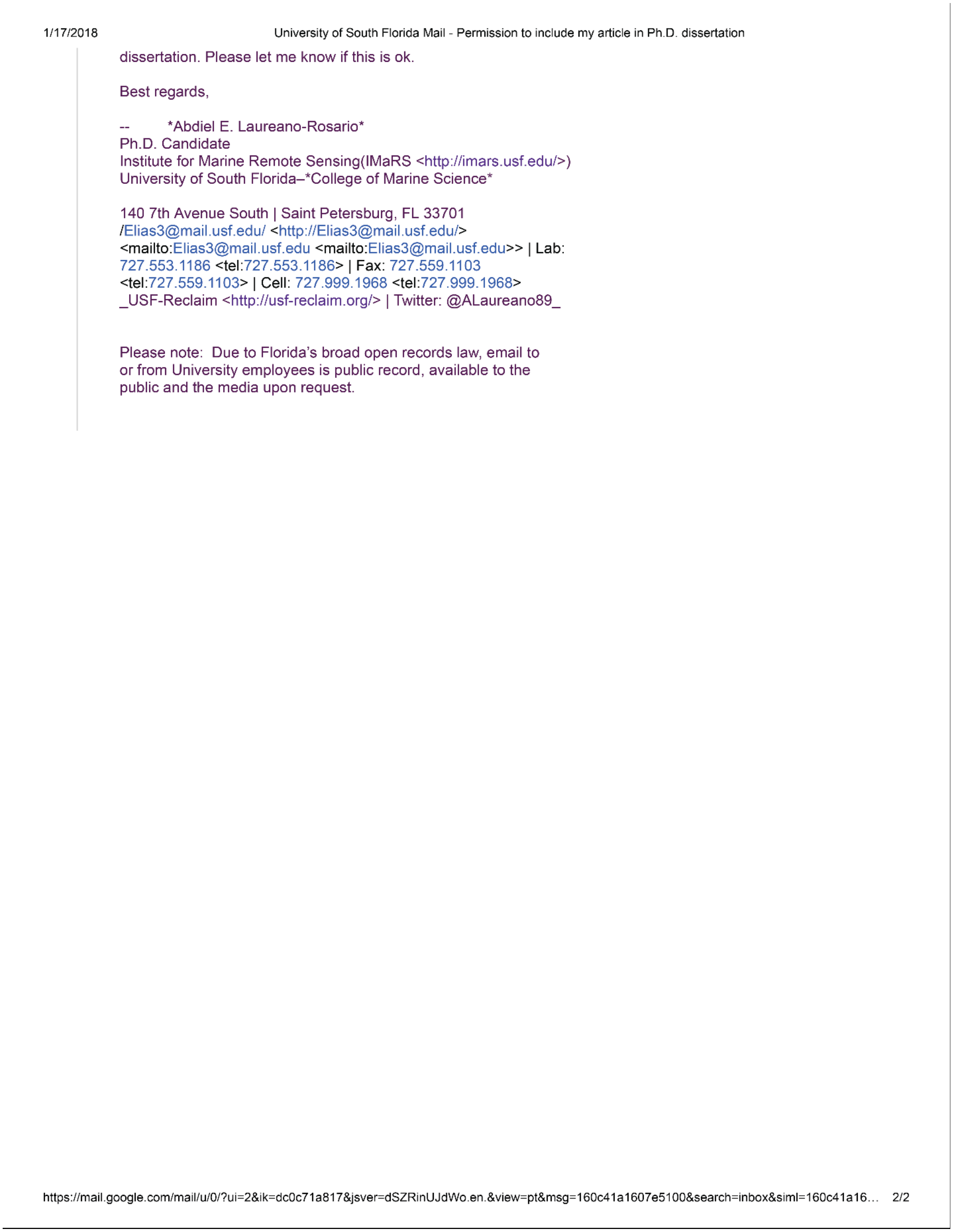

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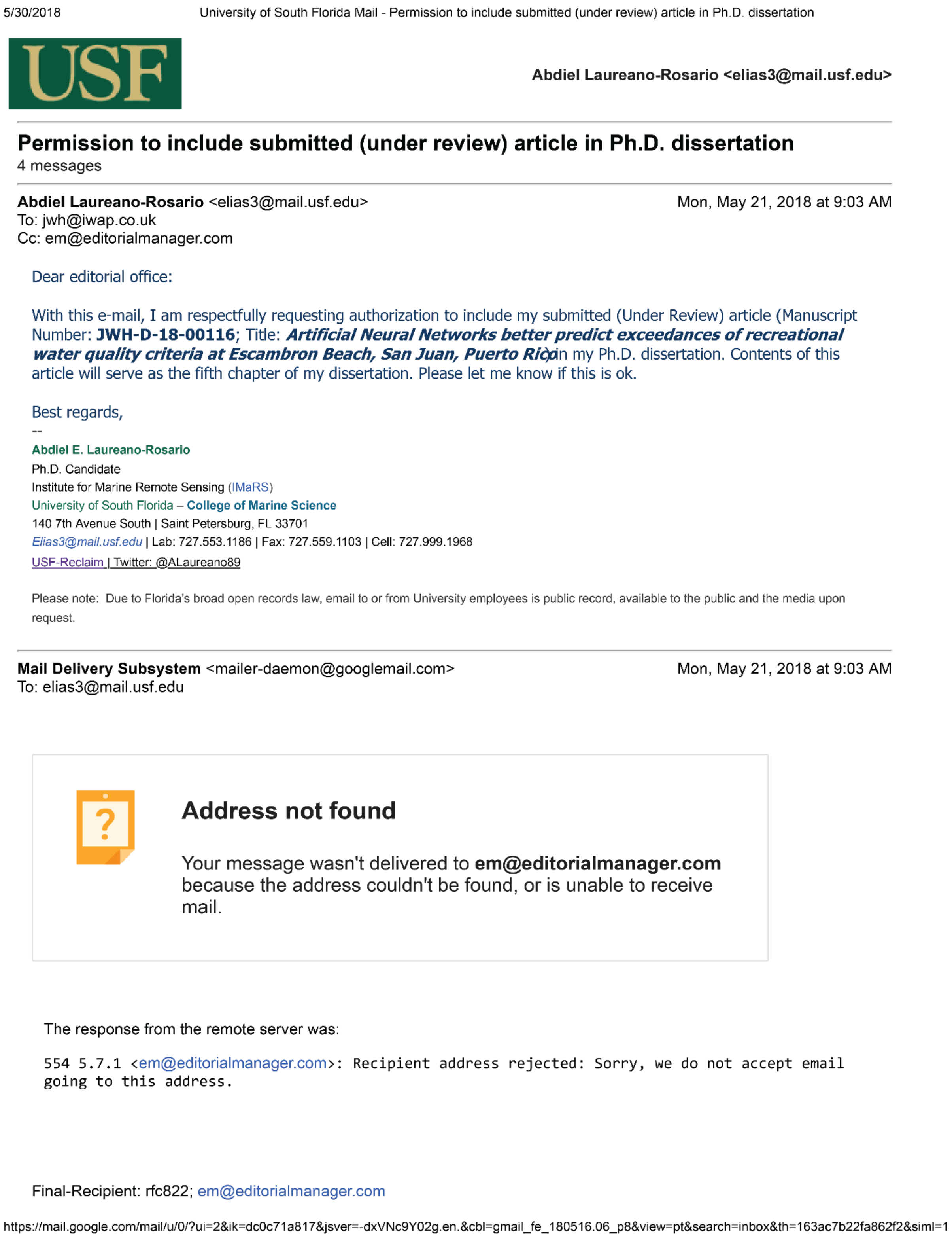

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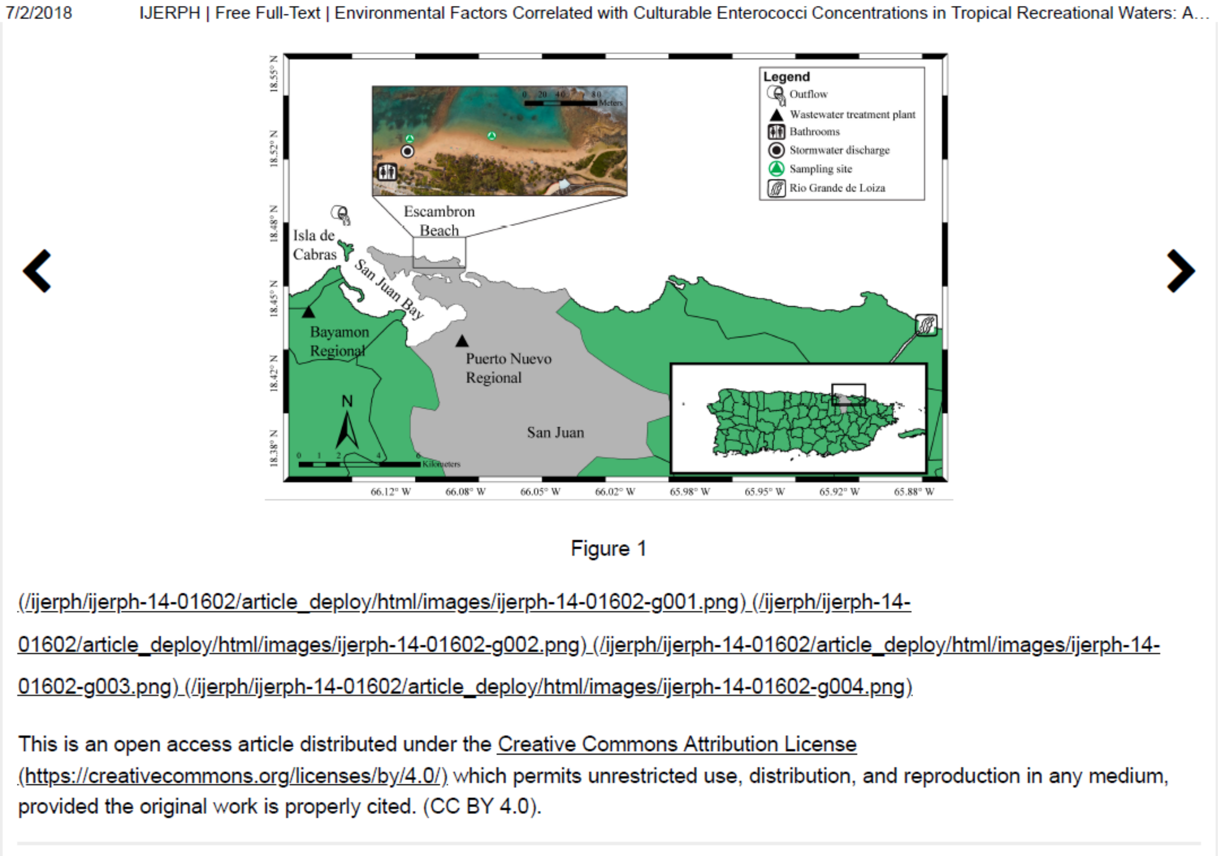

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Figure 1

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