CLIMATE ENVELOPE MODELING FOR Serratia

marcescens, PUTATIVE PATHOGEN OF CORAL WHITE

POX DISEASE

by

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THESIS Submitted in partial fulfillment of the requirements for the degree of

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DECLARATION

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ABBREVIATIONS

| ArcGIS | Aeronautical Reconnaissance Coverage Geographic Information System | |
|---------|---|--|
| ASCII | American Standard Code for Information Interchange | |
| AUC | Area under the Curve | |
| BCC-CSM | Beijing Climate Center Climate System Model | |
| BIOMOD | BIOdiversity modeling | |
| BRT | Boosting Regression Trees | |
| CART | Classification and Regression Trees | |
| CMFRI | Central Marine Fisheries Research Institute | |
| СТА | Classification Tree Analysis | |
| ENM | Environmental Niche Modeling | |
| FAO | Food and Agricultural Organization | |
| FDA | Factorial Discriminant Analysis | |
| GAM | Generalized Additive Models | |

| GBIF | Global Biodiversity Information Facility | |
|----------|--|--|
| GBM | Generalized Boosting Method | |
| GIS | Geographic Information System | |
| IPCC | Intergovernmental Panel on Climate Change | |
| ISMN | International Soil Moisture Network | |
| MARS | Multivariate Adaptive Regression Splines | |
| MaxEnt | Maximum Entropy Modeling | |
| ROC | Receiver Operating Characteristic Curve | |
| SEDAC | Socioeconomic Data and Applications Center | |
| SDM | Species Distribution Model | |
| SRTM-DEM | Shuttle Radar Topography Mission Digital Elevation Model | |
| SSP | Shared Socioeconomic Pathway | |
| SSS | Sea Surface Salinity | |
| WPX | White pox disease | |
| WNV | West Nile Virus | |

CHAPTER 1

INTRODUCTION

The ocean and climate are inseparable. The ocean plays a vital role in mitigating climate change because it is a crucial heat and carbon sink. The global ocean has been significantly affected by climate change and its related effects. Climate change leads to disease dynamics in the marine environment that are involved in the physical, chemical, and biological characteristics of ecosystems (Harvell *et al.*, 2002). Disease outbreaks related to mortality have resulted in widespread deterioration in marine realms. The climatic factors affect many pathogens in terrestrial and marine biota and will encourage new and altered disease dynamics (Brooks *et al.*, 2019). Such a scenario has adverse effects on the health of marine life, leading to loss of biodiversity, catalyzing population decline, and accelerated extinction (Palombo, 2021). Restoring our valuable resources will help the ocean continue to provide the services on which all life depends.

Climate-related effects lead to massive death in corals, shellfish, fish, humans, and many other vital organisms in the environment (Ricardo Cavicchioli *et al.*, 2019). Coral belongs to the class Anthozoa in the animal phylum Cnidaria, which includes sea anemones and jellyfish. The corals associated with microscopic dinoflagellate algae, zooxanthellae, reflect a close evolutionary relationship between the host and the symbiont with temporal and spatial variability. The climatic parameters which control the reef ecosystem include ocean temperature, salinity, precipitation patterns, pH, extreme storm events. Global climate change leads to a change in the physical, chemical, and biological properties of the ocean and leads to a geographical shift in suitable coral reef habitats (Freeman *et al.*, 2013).

Microorganisms play a fundamental role in the health and disease of all corals. Diseases lead to vast coral mortality. Bacterial bleaching, aspergillosis, black band, white band, white plague, white pox, yellow band, brown band, and the skeletal

eroding band are some of the coral diseases attributed to various pathogens. Disease outbreaks, high susceptibility of the host coral, and changing climatic patterns accelerate coral reef degradation (Enochs *et al.*, 2015).

White pox disease (WPX), caused by a bacterial pathogen, *Serratia marcescens*, affects the Caribbean coral *Acropora palmata* (Sutherland *et al.*, 2011). Distinct and irregularly shaped white patches of the recently exposed skeleton are the characteristics of infested corals (Wijayanti *et al.*, 2020). These patches of tissue loss can co-occur throughout the coral colony. The etiology of this disease i.e. *S. marcescens*, is the first reported marine invertebrate pathogen that is associated with the human gut. It is considered as an emerging pathogen with pathogenicity over multiple taxa across the Animal Kingdom (Mahlen *et al.*, 2011; Ferreira *et al.*, 2020). Currently, there is no information regarding the spatial distribution of this bacterium across the world. Hence, it is essential to know its global spread and the conducive environments it may occupy. A potential approach towards studying the global distribution of such pathogens is the use of predictive modeling systems (Sadeghieh *et al.*, 2020).

The species distribution model (SDM) is commonly used to estimate the potential geographic distribution of a species (Franklin *et al.*, 2013). Different kinds of SDMs are in use, such as Generalized Dissimilarity Models (GDM), Multivariate Adaptive Regression Splines (MARS), Genetic Algorithms for Rule-set Prediction (GARP), Boosted Regression Trees (BRT), Maximum Entropy Modeling (MaxEnt). The wide use of MaxEnt is because it estimates the species distribution based on presence-only data and different environmental features (Elith *et al.*, 2006). It is a suitable SDM method that can characterize the ecological distribution of various pathogens to predict high-risk areas (Alaniz *et al.*, 2017; Mwakapeje *et al.*, 2019).

Our study aims to model the most probable areas of *S. marcescens* based on its currently known occurrence, environmental and socioeconomic variables using the MaxEnt model for the first time. Climate envelope modeling of *S. marcescens*, provides information about the nature of the relationship between the putative

pathogen to influencing predictors and can enable us to understand its current extent and hotspot areas. The proximity of such hotspots towards the coral reefs will help us to understand the disease risks and further formulate guidelines to the management actions.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Coral reefs

Coral reefs are one of the most complex, species-rich, and productive marine ecosystems (Woodhead *et al.*, 2019). They cover only less than 1% of the ocean floor (Brandl and Elise, 2019). Even so, they can make a significant contribution to the economies of countries around the world (Ban *et al.*, 2014; Hoegh-Guldberg *et al.*, 2015). The richness of biodiversity and productivity provides 25% of all known marine life (Hoegh-Guldberg *et al.*, 2015). However, about 20% of the world's coral is either gone or destroyed due to natural or anthropogenic activities (Munday *et al.*, 2009). The distribution of infectious agents and climatic parameters will directly or indirectly affect the health of marine ecosystems. These changes badly affect the structural and functional framework of reef communities and vital habitats for many other reef creatures. The dynamic climate acts as a hurdle to our conservation methods, affecting every coral badly (Harris *et al.*, 2018). In recent times, the government formulated many rules and laws by prohibiting human dwellings and establishments near sea coasts for the protection and restoration of the habitat of the corals, and it also raised concerns among humans regarding the maintenance of coral ecology and reef protection (Tun *et al.*, 2005).

2.2 Threats

The marine ecosystem threatened by various natural and man-made stress factors is coral reefs. Nearly 40-50% of coral loss has occurred in the past 30 years (Bruno and Selig, 2007). The global decline of reef-building corals is of particular concern. In the past 50 years, catastrophic mortality of coral colonies from the Great barrier reef (De'ath *et al.*, 2009), Southeast Asia, and Indonesia (Johnstone *et al.*, 1998) was reported. These stressed reefs are exposed to a large number of microorganisms, which

results in coral mortality (Reaser *et al.*, 2000; Ritchie *et al.*, 2001). The biological and nonbiological stresses were the drivers of coral diseases (Bruno *et al.*, 2007). The first reports of coral tissue degradation appeared in the 1970s. Black band disease is the first coral disease reported in 1973, and secondly, two coral diseases were reported in 1977: white band disease of branching acroporid corals and the plague of massive and plate-forming corals (Sweet *et al.*, 2012). Over the past thirty years, the regional breakdown of coral cover occurred over the Caribbean (Gardner *et al.*, 2003) and IndoPacific region (Bruno & Selig, 2007).

Climatic variables influence the structural, functional, physiological, and ecological integrity of reef-building corals (Munday *et al.*, 2009); In addition, they affect biological connectivity in coral reef ecosystems (Munday *et al.*, 2009). The associated effects change the structure and function of marine ecosystems and directly impact vertebrates, invertebrates, and plants. Related influences will change the structural and functional integrity of marine ecosystems, and directly affect vertebrates, invertebrates and plants. Coral reefs are very vulnerable to climate change, and the effects will be far-reaching (Hoegh-Guldberg *et al.*, 1999). These reefbuilding corals are very vulnerable to climatic factors such as rising sea temperatures, changes in precipitation patterns, and ocean acidification. The amount of dissolved CO₂ increases, and the pH of the ocean decreases. Therefore, the availability of carbonate ions, which are used by calcifying organisms to form shells and skeletons, can also decrease (HoeghGuldberg *et al.*, 2007).

2.3 Coral disease as secondary stressors

Coral diseases caused the widespread degradation of coral reefs over the past five decades. It may potentially act as a bioindicator of reef health (Harvell *et al.*, 1999; Green & Bruckner, 2000). The dynamic changes in the climate can decrease the resilience of the reef and increase susceptibility to disease. Several factors may contribute to these coral disease outbreaks, including temperature stress, variations in the reef fish abundance, algal interactions, and nutrients. The critical stress factors are driven by disease outbreaks due to microorganisms, overfishing, marine pollution,

destructive fishing practices, and fluctuations in climatic patterns (Epstein *et al.*, 1998; Harvell *et al.*, 1999; Ben-Haim *et al.*, 2002).

2.4 White pox disease

A. palmata (Elkhorn coral), as a reef builder in the Caribbean, devastated by White pox disease (Patterson et al., 2002). In recent years, the health of Elkhorn coral colonies in the Florida Keys has deteriorated due to microbial fecal contamination (Holden et al., 1996). It was associated with S. marcescens (Patterson et al., 2002), a non-pathogenic saprophytic water organism. Human sewage is a source of the acroporid serratiosis pathogen. This was the first time a human origin bacteria transmitted diseases to a marine invertebrate (A. palmata) (Sutherland et al., 2011). Affected corals are characterized by irregularly shaped distinct white patches (Patterson et al., 2002). Accordingly, it causes more than 70% loss of Acroporid corals in the Florida Keys during periods of high temperature (Patterson et al., 2002). The shifts in the climatic patterns influence their pathogenicity, which showed that disease incidence and severity of white pox disease increase with rising temperatures (Harvell et al., 1999; Ritchie et al., 2006; Looney et al., 2010). Hence, the temperature has a strong influence on the growth of S. marcescens (Lin et al., 2010; Harimawan et al., 2017; Mladenovic et al., 2018).

2.5 Emerging pathogen Serratia marcescens

S. marcescens has emerged as an important healthcare-associated pathogen (Mahlen et al., 2011). Over the last four decades, it emerged as a chief causative agent of nosocomial infections such as respiratory tract infections, Urinary Tract Infections (UTI), septicemia, central nervous system infections, bloodstream infections, and surgical wound infections (Rajamohan et al., 2018; Srinivasan et al., 2016; Fedrigo et al., 2011; Guler et al., 2009). In addition, they are water and soil-borne bacteria (Liu et al., 2010; Maltezou et al., 2012; Hejazi et al., 1997). It is a ubiquitous saprophytic water organism with an innate ability to survive under different conditions (Haft et al.,

2005). It was used as a biological marker because of its easily recognized red colonies (Khanna *et al.*, 2013).

The pathogenicity over multiple taxa across the animal kingdom is contributed by its biofilm formation, motility, and biosurfactant production, prodigiosin, and nucleases (Chan *et al.*, 2013). Most of the studies related to *S. marcescens* isolated from soil specifically denote their strains (Giri *et al.*, 2004). The effect of temperature, pH, salt concentration on the biofilm formation of *S. marcescens* is explained through their different strains. Salt concentration increases with an increase in biofilm density up to a level (Burmølle *et al.*, 2006). Being an opportunistic pathogen, they become pathogenic only in susceptible hosts. Availability of biotic and abiotic variables and ecological niche models helps to estimate the potential geographic distribution of a species. Investigating its potential global extent and considerable facts on the distribution possibility, in addition to influencing environmental conditions for its growth, establishment, spread, and virulence factors, are needed. Given this lack of knowledge, we model the spatial distribution of this bacterium across the world based on its currently known occurrence, environmental and socio-economic variables.

2.6 Ecological niche modeling/ SDM methods in the marine environment

The techniques have evolved to estimate the spatial patterns of organisms based on correlations of known occurrences with environmental variables for the last twenty years (Peterson *et al.*, 2012). It helps formulate the conservation strategies and forecast possible intrusive impacts of changing climate (Lozier *et al.*, 2009; Warren *et al.*, 2008; Hijmans & Graham, 2006;). Species distribution models predict the habitat suitability of species on the basis of different environmental variables (Guillera Arroita *et al.*, 2015; Franklin *et al.*, 2013; Elith *et al.*, 2006; Elith and Leathwick, 2009).

MaxEnt is one of various SDMs. The species distribution models were constructed from the occurrence points of the species and the environmental layers using a maximum entropy algorithm. A recent study determined the ecological risk for Chagas

disease caused by *Trypanosoma cruzi* using maximum entropy algorithm. The population anticipated to be at threat for this disease (Schmunis *et al.*,1999) was calculated from the risk map produced from the distribution models and disease incidence data (Sarkar *et al.*, 2010). Another study showed that the use of niche modeling techniques helps to unveil the spatial occurrence of two tick-borne pathogens: Ehrlichia chaffeensis and Anaplasma phagocytophilum (Wimberly *et al.*, 2008). The deer tick, *Ixodes scapularis*, is the primary vector of *Borrelia burgdorferi*, the agent of Lyme disease, in North America (Dennis *et al.*, 1998; Keirans *et al.*, 1996). A spatially predictive logistic model was developed for *I. scapularis* in the United States to highlight areas of potential emerging disease risk (Brownstein *et al.*, 2003).

West Nile Virus (WNV) has emerged as a major mosquito-borne pathogen causing animal diseases around the world (Hess *et al.*, 2018). In all these cases, the spatial predictions of etiological agents were done with the help of environmental data. Hence, the geospatial environmental data sets and disease occurrence data at specific locations can be used to develop disease risk maps (Peterson *et al.*, 2012).

CHAPTER 3

MATERIAL AND METHODS

3.1 Study area

The area chosen for the present study is the Northern Indian Ocean, 65°E, 95°E, 4°N, 26°N Grid. The Indian Ocean includes nearly 20% of the water in the world (Fatima and Jamshed, 2015). The Indian Ocean is the smallest and warmest among the tropical oceans.

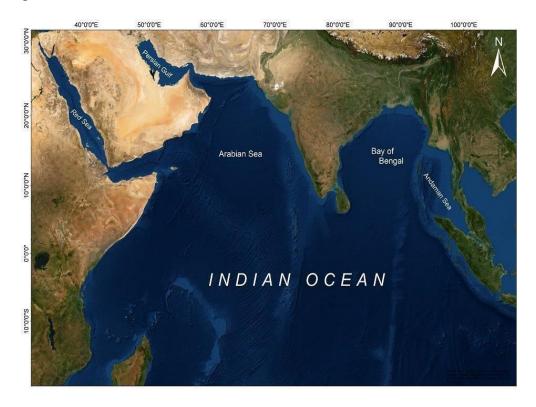


Figure 1 Study area – Northern Indian Ocean

3.2 Species occurrence data and climatic predictors

We collected the occurrence points of *S. marcescens* from the global biodiversity information facility (Ueda *et al.*, 2020). A total of 363 occurrence points were

identified and included in this study to model the current and future distribution of the species. Elevation and Climatic data, including 19 bioclimatic variables, were downloaded from the WorldClim database (http://www.worldclim.org/), available at approximately 1 km2 (30 arcseconds) spatial resolution. Shuttle Radar Topography Mission Digital Elevation Model (SRTM-DEM) was used to derive Slope data. Harmonized World Soil Database of FAO soil portal (Fischer *et al.*, 2008) was used to obtain the soil parameters such as nutrient availability, salt concentration, and soil type. Soil Respiration Database (SRDB) was used to obtain the data on soil respiration (Bond-Lamberty and Thomson, 2014). The Millennium Ecosystem Assessment (SEDAC, 2005) was used for gathering the land cover data. Data on soil moisture was downloaded from the International Soil Moisture Network (Dorigo *et al.*, 2011). Global livestock population density (Gilbert *et al.*, 2010) was used to access the livestock population data. We also included human population density information in our study, as it may interfere with the distribution of *S. marcescens*; this data was retrieved from the Socioeconomic Data and Applications Center (Gridded Population of the World, version 4.0, CIESIN, 2018). We collected 29 predictor variables for *S. marcescens* distribution and suitability analysis.

3.3 Modeling of the *S. marcescens* distribution

The predictive modeling of the *S. marcescens* has been studied, which helps to determine the suitable sites where the species can grow successfully. The model was constructed using the MaxEnt software version 3.4.1 (Phillips *et al.*, 2008). This model uses a presence-only technique (Franklin *et al.*, 2013) to estimate the geographic information regarding species based on different environmental factors. It is widely used because it unveils the species suitability based on statistical techniques (Elith *et al.*, 2006).

The occurrence points of *S. marcescens* were used as the input dependent variable in the model. All the directory layers were converted to ASCII (American Standard Code for Information Interchange) raster grid format. Model evaluation was done using the ENMeval package in R (Muscarella *et al.*, 2014), and it offers suitable regularization

multiplier and feature class combinations in addition to being taken into consideration as a building tool of model settings. It uses five attributes (linear, product, quadratic, hinge, and threshold) that frame the potential geographical distribution of habitat or a species. Out of the 363-occurrence data of *S. marcescens* within the study area, the training used 75% of occurrence data and 25% for testing the model. Using the R 4.0.3 (R core team, 2020) software, we evaluated the normality of the data with the ShapiroWilk test; then, we determined the level of correlation between pairs of variables in the presence points. The variables selected according to their correlation index had to be low (less than \pm 0.8). The model was generated using the subsampling technique with 15 replicates and 1000 iterations each, including 18 selected variables. The final model employed linear, product, quadratic, hinge, and threshold feature types. Potential distribution maps were built by interpolating the occurrence data and environmental variables.

3.4 Model evaluation and validation

Maxent provides output such as the AUC values, the response curve, and tables of percentage contribution towards environment variables. The final output was the average of all replicas with better prediction. The accuracy or performance of models can be viewed in the form of a graph of average omission and predicted area, as well as a graph of sensitivity and specificity (Phillips *et al.*, 2006). The graph of average omission and predicted area will show the accuracy of the model, whereas the graph of sensitivity and specificity demonstrated the results of an evaluation of the model (Lobo *et al.*, 2008). The Area Under Curve (AUC) value was used to indicate the model performance and measured its discrimination capacity (Phillips *et al.*, 2006). AUC values range from 0 to 1. An AUC value of 0.5 or less than 0.5 was used to indicate the less predictive model, while a value closer to one was used as a better predictive model as per the previous recommendations (Fielding and Bell, 1997).

Furthermore, a jackknife test was employed to quantify the significance of the bioclimatic variables by measuring the percentage contribution of each environmental variable and in order to understand evaluate the importance of environmental factors

in affecting the geographical distribution of species (Guillera-Arroita *et al.*, 2011). Finally, the potential distribution map for *S. marcescens* with percentage values ranging from 0 to 100 was generated. The values representing the suitable habitats of *S. marcescens* were further categorized (Figure 8) as follows; rare (0-1%), very low (1–20%), low (20–40%), moderate (40–75%), and high (75 –100%). Area calculation for the identified classes was carried out in the ArcGIS environment. Besides, to estimate the potential transmission risk among humans, we used a geographic information system (GIS) to associate the probability of the current occurrence of the pathogen with the world exposed population. So, both of these grids were multiplied using the Raster Calculator tool of ArcGIS v 10.1. The spatial quantification of the world population exposed to *S. marcescens* infection can be measured using spatial analyst tools in ArcGIS.

3.5 Prediction of Future Distribution

The study used projection grids of human population and bioclimatic predictors (19) for the years 2040-2060 and 2080-2100 based on the Shared Socioeconomic Pathways (SSPs): SSP1 and SSP5. The SSPs differ in terms of the socio-economic challenges they present for climate change mitigation and adaptation and describe a number of plausible trends in the evolution of societies in the 21st century. SSP1 stands for "sustainability" and SSP5 stands for "fossil fuel intensive" development.

The study used an ensemble forecasting approach (Araújo & New, 2007) to predict future habitat suitability under climate change scenarios. BIOMOD (BIOdiversity modeling, Thuiller *et al.*, 2009) enables the species distributions using R software (4.0.3; R Core Development Team) with several techniques (Zhang *et al.*, 2019). Hence, the future distribution of *S. marcescens* was modeled with the help of the biomod2 package (Thuiller *et al.*, 2016) in R. Furthermore, it enables testing models with a wide range of approaches, project species distributions into different environmental conditions and dispersal functions. It also assesses the species temporal turnover, plots species response curves, and tests the strength of species interactions with predictor variables. As in an ensemble framework, we used regression methods

such as generalized linear models (GLM); machine-learning methods maximum entropy (MaxEnt), surface range envelop (SRE), generalized boosting method (GBM/BRT), random forest (RF), and two classification methods; classification tree analysis (CTA) and factorial discriminant analysis (FDA).

3.6 Estimating the Disease risk of corals

A. palmata is a major reef-building coral in the Caribbean, providing essential habitat for multiple reef organisms (Sutherland et al., 2004; Patterson et al., 2002). It is characterized by parallel, obliquely inclined, very thick, tapered branches, thus its common name, Elkhorn coral. White pox disease exclusively affects A. palmata (Sutherland et al., 2004), caused by S. marcescens (Patterson et al., 2002).

The occurrence records of *A. palmata* were collected from the Global Biodiversity Information Facility (GBIF, 2021) during May 2021. A total of 445 valid records from distinct locations were used to model the current distribution of *A. palmata*. The Biooracle v2.0 was used for obtaining the environmental predictors (Assis *et al.*, 2017). The study used 17 potential predictors that are capable of influencing the occurrence of *A. palmata*. These include Mean Sea Surface Temperature, Minimum Sea Surface Temperature, Maximum Sea Surface Temperature, Mean Sea Surface Salinity (SSS),

Minimum Sea Surface Salinity, Maximum Sea Surface Salinity, Chlorophyll-a,

Phytoplankton, Primary productivity, Mean Current Velocity, Maximum Current Velocity, Minimum Current Velocity, Phosphate, Nitrate, Iron, Silicate and Dissolved oxygen. The spatial modeling of *A. palmata* was done with the help of the biomod2 package in R software. Geographic Information System (GIS) is a tool for visualizing and analyzing the entire process of epidemiological management and analysis. It is possible to access the spatial relations between the disease and other information such as distance of the possible pollution sources, vectors, and the presence of clusters, through the GIS tools (Cromley *et al.*, 2019). So, this valuable information contributes to the designing of epidemiological analysis.

CHAPTER 4

RESULTS

4.1 Serratia marcescens

- 4.1.1 Prediction of the current distribution
- 4.1.1.1 The Model Performance and variable contributions

The model performance is assessed by using the average test AUC value for 15 replicates was 0.918 with a standard deviation of 0.028. The sensitivity *vs.* 1specificity graph shows the area under the Receiver Operating Characteristic (ROC) curve or AUC. AUC is a common metric for assessing the predictive ability and hence utility of a habitat suitability model. A close relationship between the presence data and the prediction results is shown by the average omission and predicted area of the model (*Figure 3*). The red line shows the average value of the AUC, and the blue line shows the average value of the standard deviation. The closer the red line to the left (values approaching 1), the smaller the standard deviation value, and the performance of the model can be shown in *Figure 2*.

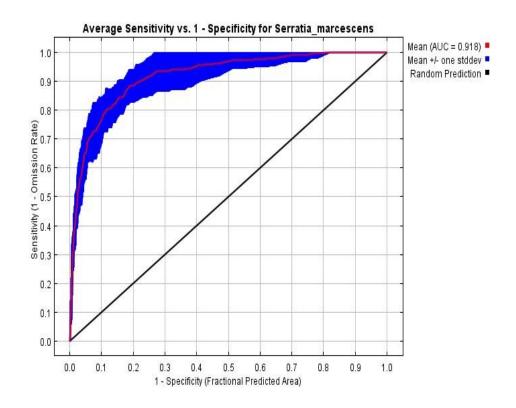


Figure 2 Results of the area under the receiver operating characteristics curve (AUC) analysis

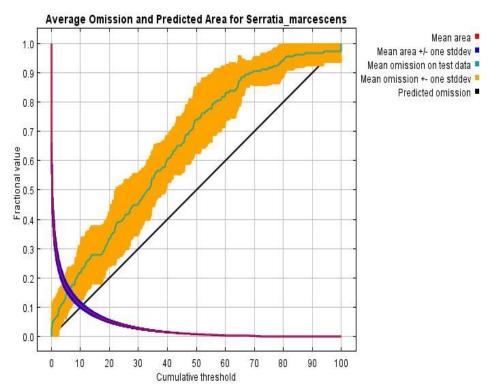


Figure 3 Omission rates versus predicted area for S. marcescens

The Red line indicates the mean area, the black line indicates the predicted omission rate, and the light blue line indicates the omission rates of the model training samples. In the model, the lines of omission from the training data were close to predicted omission rates.

4.1.1.2 Contribution of predictor variables

| Sn | Variable | Percent Contribution | Permutation importance |
|----|------------------|-------------------------|------------------------|
| 1 | Human Population | 43.6 | 28.8 |
| | Density | | |
| 2 | Bio 1 | 9.8 | 9.7 |
| 3 | Land cover | 6.7 | 1.2 |
| 4 | Bio 14 | 6.5 | 6.9 |
| 5 | Bio 4 | 5.7 | 13.3 |
| 6 | Bio 12 | 5 | 1.3 |
| 7 | Livestock | 4.6 | 5.8 |
| 8 | Soil type | 4.3 | 4.7 |
| 9 | Soil respiration | 3.5 | 7.1 |
| 10 | Bio 2 | 2.4 | 2.8 |
| 11 | Bio 18 | 1.4 | 0.8 |
| 12 | Soil moisture | 1.3 | 2.4 |
| 13 | Nut | 1.2 | 3.3 |
| 14 | Slope | 1.1 | 1.1 |
| 15 | Elevation | 1 | 2.5 |
| 16 | Bio 15 | 0.9 | 1.5 |
| 17 | Bio 5 | 0.8 | 1.2 |
| 18 | Salt | 0.2 | 5.5 |

Table 1 Percent contribution and permutation importance of all environmental variables to the model for S. marcescens

MaxEnt output gives the relative contribution of each predictor variable, as shown in Table 1. Our analysis showed that the occurrence of the *S. marcescens* is mainly associated with human population density. Among all the variables, the human population and mean annual temperature were the major biotic and abiotic contributors that influenced *S. marcescens* distribution, respectively (Table 1). Further, five variables viz., annual mean temperature (bio1), land cover, precipitation of driest month (bio14), temperature seasonality (bio4), and annual precipitation (bio12) are found to be significant contributors. The individual contribution of the parameters was more compared to their performance when considered together.

4.1.1.3 Jackknife analysis of AUC for the *S. marcescens* using all the variables

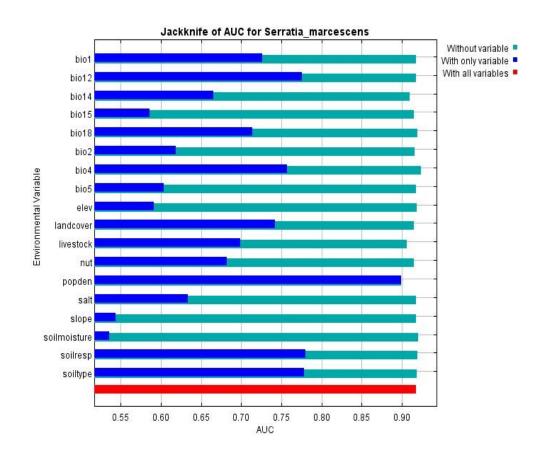


Figure 4 Jackknife analysis of AUC for the S. marcescens using all the variables

The Jackknife of AUC for *S. marcescens* shows environmental variables with the highest gain when used in isolation is human population density itself. The Jackknife of regularized training graphs indicates each variable's gain when used in isolation.

4.1.1.4 Response curves

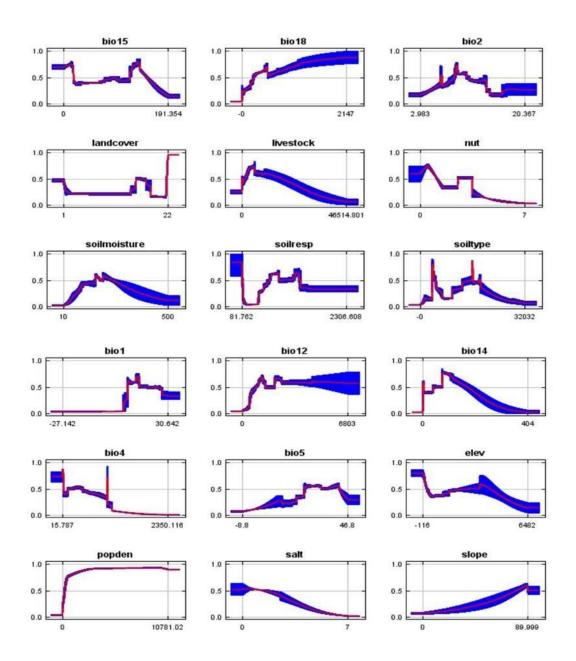


Figure 5 The response curves for the S. marcescens model

It shows the change in predicted probability when the corresponding variable is used in isolation and averaged for 15 replicates. These plots demonstrate the dependence of predicted suitability on the selected variables and the dependencies induced by correlations between each variable and other variable.

4.1.1.5 Prediction of the current distribution of the *S. marcescens*

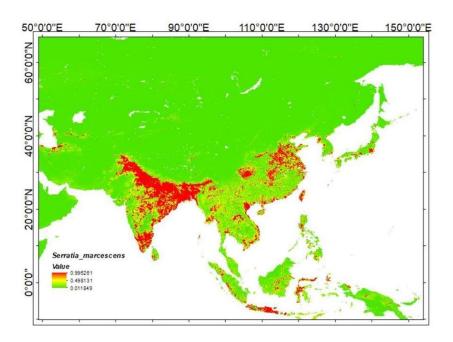


Figure 6 The predicted distribution of S. marcescens

4.1.1.6 Global distribution of *S. marcescens*

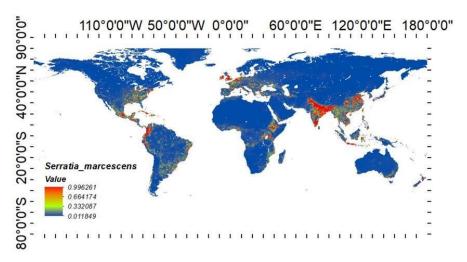


Figure 7 Global distribution model of S. marcescens

4.1.1.7 Suitability model of an opportunistic pathogen, *S. marcescens*

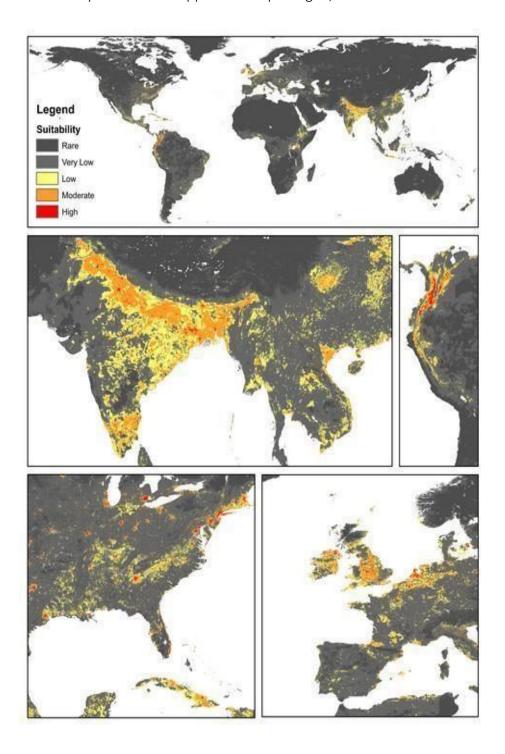


Figure 8 Global distribution pattern of S. marcescens based on suitability

The distribution map of *S. marcescens* illustrates several potential sites of *S. marcescens* along the tropical and subtropical regions of the world. The predicted current distribution is mainly concentrated at the Indian side of the Himalayan ranges, islands of Indonesia and Hanoi Province of Vietnam in Asia; Ecuador, Columbia in South America; Mexico, Belize, Nicaragua, Panama in North America; Northern

Europe, South Australia, and North New Zealand with a probability greater than 75%. The arid areas such as central and north Asia, North America, Sahara, and Antarctica show minor occurrences.

4.1.2 The Future distribution of *S. marcescens* under different Climate Scenarios

4.1.2.1 Future distribution of *S. marcescens* under SSP1 and SSP5 for the years 2040-2060 and 2080-2100

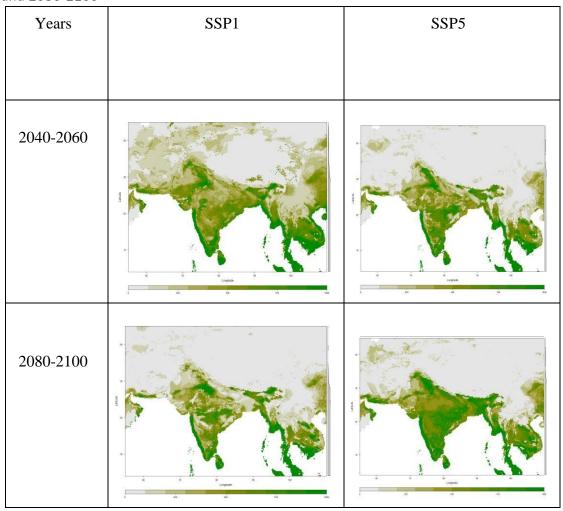


Table 2 Prediction of future habitat suitability of S. marcescens under SSP1 and SSP5 for the years 2040-2060 and 2080-2100

4.2 Acropora palmata

4.2.1 Prediction of the current distribution

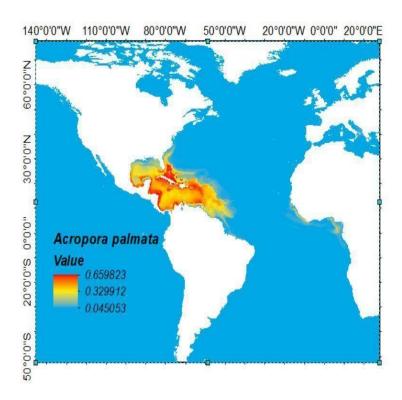


Figure 9 Prediction of the current distribution of A. palmata

CHAPTER 5

DISCUSSION

Infectious diseases caused by *S. marcescens* over multiple taxa have reached dire conditions, and it has a great potential to impact human health, global economies, and biodiversity. It has been concluded from extensive literature and field surveys that the species has an innate ability to survive under various conditions (Haft *et al.*, 2005). Investigating its potential global extent needs knowledge about the occurrence status and the governing factors of pathogens essential for the proper allocation of resources, preventive and control measures against the disease. Accordingly, the present study modeled the climatic envelope of the *S. marcescens* for the current years and identified the potential areas of its distribution based on its currently known occurrence, environmental and socioeconomic variables using MaxEnt (Tarkesh and Jetschke, 2012).

A total of biotic and abiotic factors from multiple sources were used for the present study. Historical reports suggested that the survival ability of *S. marcescens* is influenced by diverse biotic and abiotic factors (Haft *et al.*, 2005). The model tried using various settings in MaxEnt software by changing feature types, no of iterations, and regularization multiplier. To minimize difficulties caused by multicollinearity, we excluded one predictor from a set of highly correlated (≥0.80) predictors. Hence, Karl Pearson's correlation coefficients between these 29 predictors were calculated, and a set of 18 variables (two biotic and sixteen abiotic) was selected as input independent variables in the final model. The 18 variables such as annual mean temperature (Bio 1), mean diurnal range (Bio 2), temperature seasonality (Bio 4), a maximum temperature of the warmest month (Bio 5), annual precipitation (Bio 12), precipitation of the driest month (Bio 14), precipitation seasonality (Bio 15), precipitation of the warmest quarter (Bio 18), elevation, slope, nutrient availability, excess salts, land cover, soil respiration, soil type, soil moisture, human population density and animal population density used in the final species distribution analysis. 75% of data were

used for training, and 25% were used as test data to evaluate models. The final model employed attributes such as linear, product, quadratic, hinge, and threshold. ENMeval package in R frames the model settings, and it was allowed to run 15 times repeatedly.

The information regarding the prediction of species suitability is obtained from the external output such as the AUC values, the response curve, and the table of percentage contribution towards environment variables. These predictor variables are based on the rankings of its contribution and its importance accessed via jackknife analysis. The accurate measurement of the final output is analyzed through AUC values of the receiver operator characteristic (ROC) plot analysis within MaxEnt (Phillips and Dudík, 2008). The evaluation index, such as training gain, test gain, and AUC, was contained in the Jackknife test on models. The AUC value close to 1 implies the best predictive model. The relationship between the probability of *S. marcescens* with environment variables can be seen in the response curve.

The final model of the present study had an AUC value of 0.918 ± 0.028 , implying that the model had high predictability. The high predictive capacity of this model is interpreted based on the AUC value. Our analysis showed that the occurrence of the *S. marcescens* is mainly associated with human population density. Among all the variables, the human population and mean annual temperature (Bio1) were the major biotic and abiotic contributors that influenced *S. marcescens* distribution, respectively (*Table 1*). Further, five variables viz., land cover, precipitation of driest month (bio14), temperature seasonality (bio4), and annual precipitation (bio12) were found to be other significant contributors shown by the percentage contribution table (*Table 1*). The Jackknife test further demonstrated that annual temperature, annual precipitation, precipitation of warmest month, temperature seasonality, land cover, population density, soil respiration, and soil type were the most significant variables having scores of > 0.7. The environmental variable with the highest gain is human population density. The species response to the influential factors located above 0.5 in the response curve is considered to be nonlinear. The response of the human population

density factor was above 0.5 and was a powerful predictor of species suitability. Its pattern shows a sharp increase and then follows a steady path.

The spatial model of *S. marcescens* was successfully predicted through the present study. The resultant map could classify the whole world into five categories based on the probable distribution of S. marcescens as unsuitable, extremely low suitable, low suitable, moderately suitable, and highly suitable. This criterion supports the study conducted by Lugito et al. (2016) that S. marcescens infections varied among different geographic areas of the world. The transmission risk is not homogeneous throughout the tropics. The predicted distribution showed higher proportions over Himalayan ranges, islands of Indonesia, and Hanoi Province of Vietnam in Asia. The Indian side of the Himalayan ranges has one of the fertile regions in the world supplied by the Gangetic and Brahmaputra Rivers and their tributaries. The fertile soil, presence of many rivers, favorable climate, and the availability of flat terrain have made this region one of the most densely populated areas of the world (Ray et al., 2014). The population density may be the reason for the observed higher suitability of S. marcescens in the thickly populated regions of the world. In parallel to our findings, Lugito et al. (2016) suggested that S. marcescens infections vary among different geographic areas of the world. Bhattarai et al. (2021) observed that its occurrence frequency is higher in North America and Europe than in other parts. Besides, its infections have been reported from Middle Eastern countries (Israel, Iran), Mexico, South and East Asia, and certain North African countries due to the subtropical climatic patterns. The least occurrence of this microbe was found in Central and North Asia, north of North America, and Sahara. Therefore, socioeconomic conditions and climatic patterns may be related to the occurrence of this pathogen. According to the MaxEnt results, the human population density is a powerful predictor of habitat suitability.

Model output has shown human population density as the most prominent contributor to the occurrence of *S. marcescens*. There is evidence that these bacteria are frequently seen in the human and animal gut (Escribano *et al.*, 2019). The results can be presumed that the areas with higher human or livestock density will have a reasonable occurrence probability of these bacteria. The probability of occurrence of these bacteria explains

the higher suitability of habitats in the thickly populated regions in the world, such as Asian countries. Our results signify that the regions with higher population density identified by the modeling have greater chances of various disease outbreaks mediated by this opportunistic pathogen.

Based on area calculation, the total area of high occurrence (probability >75%) was 7,86,039 km², occupying around 0.52% of the world's land area. The moderately suitable sites (probability ranging from 40 to 75%) were mainly distributed along the northwest of Europe and southeast coast of Australia, certain parts of the Indian side of the Himalayas, and northern Africa occupying a total area of 18,90,964 km². Altogether, total suitable sites, including highly and moderately suitable, were found to occupy an area of 26,77,003 km², accounting for 17.9% of the total global land area.

The total area of highly suitable sites in different continents viz., Asia, North America, South America, Europe, Africa and Australia were estimated as 3,30,257 km², 1,46,023 km², 1,29,900 km², 1,24,280 km², 34,541 km², 21,038 km² respectively. In short, the order of different continents in the probability of having incidences of *S. marcescens* related infections was found as Asia > North America > South America > Europe > Africa > Australia.

According to the spatial quantification of the world human population with potential transmission risk, the zones of medium and above transmission risk represent 0.20% of the global human population. Around 0.15%, the world population has a moderate risk of *S. marcescens* infection. The 0.05% population is at high risk. About 1.22 billion people, representing moderate transmission risk due to *S. marcescens*. The world population under medium and above risk levels includes 1.61 billion people. Estimating human transmission risk helps to monitor the frequency of infestation and formulate the guidelines for management actions.

Temperature-based predictors such as annual mean temperature, temperature seasonality contributed 15.5%, and precipitation-based variables contributed 11.5%. The environmental factors might have a pivotal role in producing various pigments by

S. marcescens (Hardjito et al., 2002; Mahlen et al., 2011). Notably, temperature affects

the pathogenicity and virulence of *S. marcescens* (Mladenovic *et al.*, 2018). Previous reports were consistent with these findings; high temperatures can reduce the virulence and infectivity of *S. marcescens* (Lin *et al.*, 2010). Hence, significant temperature variation restricts suitable climatic space for the pathogen.

Spatial-temporal distribution, sensitivity, and vulnerability to future climatic conditions were analyzed based on climate scenarios through an ensemble forecasting approach. The future distribution maps of S. marcescens showed higher proportions occur over the Southeast Asian region. Based on MaxEnt, the modeling of the current investigation implies the influence of biotic and abiotic variables for the occurrence of microbes. The prevalence of this species in future scenarios needs to be examined. Because climate change due to natural or anthropogenic impacts might alter the overall sensitivity of pathogens to the environment in the future. Temperature and precipitation-based predictors mainly influence the current occurrence. Considering the previous studies showed that S. marcescens ability to produce virulence factors over a wide temperature range is a reflection of infestation in both vertebrate and invertebrate animals. The pivotal role of various climatic variables on the growth of this pathogen was identified by Mahlen et al. (2011). In parallel with our observation, previous laboratory experiments on Serratia species showed the virulence properties to infect a host and, in some cases, showed temperature-dependent responses (Petersen et al., 2012). Another fact is that wetting activities of S. marcescens on different surfaces in nature have been investigated in historical studies (Matsuyama et al., 2010). Water is a major contributor to microbes. Most of the bacteria require a moderately moist surface to grow and reproduce. The growth source of S. marcescens is environmental dust, moist surfaces, and fomites, according to Mahlen et al. (2011). Hence, it may explain that the moist surface can stimulate or sedate the pathogenicity in S. marcescens. Abiotic factors influence the current spatial epidemiology of S. marcescens, contributing 27% according to the MaxEnt model. The precise information on the influence of environmental variables on the growth of this bacterial spread is hitherto unclarified. According to the United States Environmental Protection Agency, Future climate change causes a warmer atmosphere, a warmer and more acidic ocean, higher sea levels, and heavy precipitation events. The production of

virulence-associated substances by *S. marcescens* depends upon environmental conditions based on previous experiments. These premises suggest that bioclimatic variables may enhance the probability for increased *S. marcescens* associated infections under climate change scenarios, and profound evidence can be seen in the future.

The current global risk map shows that the Indian side of the Himalayas, the Indonesian islands, and the Asian provinces of Hanoi, Vietnam; South American countries; Belgium, the Netherlands, and the United Kingdom in Europe; Mexico, Belize, Nicaragua, Panama; Victoria in South Australia and some parts of Africa have a 75% probability. Human population density is the main factor behind the existence of this bacteria based on predictive models. A spectacular current abundance can be observed in Asian countries. According to the 2020 World Population Analysis, more than half of the world's population lives in Asia, around 3.7 billion, only a fifth of the world's land surface. From this presumption, the presence of fertile soil, extensive river systems, an abundance of water resources, favorable climate, and availability of flat terrain have made it one of the most densely populated areas of the world (Ray *et al.*, 2014), which might be the reason for the higher probable distribution in the Asian regions.

Future risk map of *S. marcescens* using projection grids of climatic predictors and human population clearly shows the probability of higher occurrences along the Asian countries especially in Indian regions. Table 2 shows that more probability of occurrence along India are noticed under the 2100 SSP5 scenario. Considering 2020 World Population statistics, the world population will grow from 7.8 billion in 2020 to 9.9 billion in 2050. Consequently, the future trend shows rapid population growth in many countries in Asia, making it the most vulnerable area for *S. marcescens* affected by socio-economic factors in the future. These findings suggest that the invasion of *S. marcescens* may affect global society, demographics, and economics over the next century.

Acroporid corals exhibited white pox disease caused by S. marcescens (William et al., 2005; Sutherland et al., 2011). The spatial modeling of A. palmata is highly influenced by a diverse array of marine layers. A total of seventeen marine environmental variables were obtained from the Bio-oracle v2.0 (Assis et al., 2017) used for the prediction of current coral occurrence. The current spatial distribution of A. palmata towards various environmental parameters was modeled with the aid of the biomod2 package in R. It employed seven models such as generalized linear models (GLM); machine-learning methods maximum entropy (MaxEnt), surface range envelop (SRE), generalized boosting method (GBM/BRT), random forest (RF) and two classification methods classification tree analysis (CTA) and factorial discriminant analysis (FDA). The resulting distribution pattern in the Bahamas and the Caribbean (Figure 9). The marine layers that influence the spatial occurrence of A. palmata were extracted from this model. In this case, coral distribution models indicate that nutrients like phosphate, iron, silicates, dissolved oxygen, and mean temperature hold the place of contributors. It was reinforced by Ames et al. (2016); it shows that most Acropora reef species are affected by various factors such as nutrients, temperature, light availability, wave movement, water depth. The mean temperature has a profound impact on the physiology of elkhorn coral, as demonstrated by Randall et al. (2009).

Since the late 1990s, a white pox disease has affected the health of *A. palmata* in the Florida Keys and other parts of the Caribbean (Joyner *et al.*, 2014). *S. marcescens* mainly exist in the mucus layer of *Acropora* sp. Human pathogens spread diseases to marine invertebrates through land-based sources, chemicals, wastewater, and sewage effluent. According to NOAA Fisheries, the habitat suitability of the threatened Elkhorn coral *A. palmata* is observed from south Florida south to the north coast of Venezuela, and its native population is notable primarily in the Bahamas and the Caribbean. Similar observations are found through spatial modeling of the widespread and conspicuous coral reef builder *A. palmata* in the Gulf of Mexico, Belize, El Salvador, Honduras, Nicaragua, and Panama, in the north of Central and South America. The global distribution of etiological agent *S. marcescens* was marked in northeast parts of India and Southeast Asian regions; Ecuador and Columbia in South

America; Belgium, Netherlands, and the UK in Europe; Mexico, Belize, Nicaragua, Panama; Victoria province in South Australia and Kenya, Uganda and Nigeria in Africa. Therefore, the habitat modeling of *A. palmata* and *S. marcescens* presents their occupancy and the nature of the spread of putative pathogens into the coral species via sewage, chemicals, nutrients, and agricultural runoff. The loss of Caribbean corals is linked to declining water quality, fecal contaminants, chemicals, the release of landbased pollutants such as significant changes in the drainage basins due to agriculture, deforestation, and urbanization into adjacent reef systems, bleaching associated with heat stress, sewage, an infestation of microorganisms, and diseases. It could be one of the reasons for the severe occurrence of acroporid serratiosis in the coral reefs of the Caribbean.

Previous reports showing white pox disease incidence of Acroporid corals increase with rising temperatures (Harvell *et al.*, 1999; Ritchie *et al.*, 2006; Looney *et al.*, 2010). Therefore, suitable bioclimatic space for this pathogen may be changed by the significant variation in temperature, which further raises the infectious diseases of marine invertebrates in future scenarios. The nature of the future appearance of *A. palmata* is unknown due to the unavailability of SSP data. However, the current modeling of *S. marcescens* and *A. palmata* presents a habitat pattern near Caribbean waters. It gives information about the proximity of reefs and pathogen appearance.

The profound influence of temperature on the growth of *S. marcescens* (Lin *et al.*, 2010; Harimawan *et al.*, 2017; Mladenovic *et al.*, 2018) has been confirmed by early laboratory experiments. Consistent with previous reports, the incidence and severity of white pox disease of corals rising with rising temperatures (Harvell *et al.*, 1999; Ritchie *et al.*, 2006; Looney *et al.*, 2010). The infectivity in Acroporid corals due to this microbe via human fecal contamination of near-shore and off-shore waters. In addition, with changing climate scenarios, rising temperatures are exacerbating infectious diseases of marine invertebrates. Hence, the formulation of the coastal water resources helps to prevent the future spread of this opportunistic bacteria.

The policy decision-makers design suitable preventive measures, especially in the predicted high-risk areas of this pathogen, based on the categorization of risk areas (Mwakapeje *et al.*, 2019). The spatial assessment of *S. marcescens* based on biotic and abiotic variables leads to risk maps. There was a major limitation due to the scattered information both on the spatial occurrence of this bacteria worldwide and on the detailed environmental predictors influencing the occurrence points. The results unveil the importance of quality assurance of coastal and marine waters in areas where the risk of *S. marcescens* has been identified to increase in order to protect the health and biodiversity of coral reef ecosystems.

In conclusion, the study forms the first global risk assessment of *S. marcescens*, an emerging human and coral pathogen, warranting the enhancement of epidemiological surveillance systems in the identified risk regions. The analysis showed that around 18.42% of the total global land area is found to have a high or moderately high risk for the pathogen. Further, the order of different continents in terms of current *S. marcescens* related infections was found as Asia > North America > South America > Europe > Africa > Australia. Human population density and temperature as the major influencing factors on the spatial epidemiology of *S. marcescens*. The study also needs additional physical, chemical, biological factors that significantly affect pathogen transmission. Generally, the risk areas identified in this study and the estimation of biological and non-biological factors help to take adequate precautions against the future invasion of this emerging pathogen.

CHAPTER 6

SUMMARY

Global climate change creates novel challenges for coastal and marine ecosystems. Significant variation in the climatic factors contributes to disease dynamics in the marine environment. It was documented in corals, shellfish, and finfish. For the last ten years, it is causing widespread mortality among reef-building corals. The increase is related to deteriorating water quality associated with human-made pollutants and climate change impacts. These factors may allow for the proliferation and colonization of microbes. The onset of most diseases is likely a response to multiple factors.

One of the coral diseases is White pox disease. It causes irregular white patches on the coral *A. palmata* due to disease outbreaks by *S. marcescens*. The source of this pathogen is mostly terrestrial where it is found in the guts of animals, and soil. It is an emerging pathogen for human health, causing frequent hospital infections with 0 to 45% mortality rates. Elkhorn coral (*A. palmata*) is an essential reef-building coral in the Caribbean. The precise information regarding the *S. marcescens* across the world is currently absent. This study forms the first assessment of the global distribution modeling of this critical human and coral pathogen. For analyzing the spatial occurrence of *S. marcescens*, the maximum entropy model, which is a prominent species distribution modeling tool, was used.

The occurrence points of *S. marcescens* from the GBIF, and the influencing variables acquired from the WorldClim, SEDAC, SRDB, Harmonized World Soil Database of FAO soil portal. A total of 29 biotic and abiotic factors were used in the study. Using the R 4.0.3 (R core team, 2020) software, we evaluated the normality of the data with the Shapiro-Wilk test; then, we determined the level of correlation between pairs of variables in the presence points. The variables were selected according to their correlation index and had to be low ($<\pm0.8$). Among the total 29 predictor variables, 18 variables viz., annual mean temperature, mean diurnal range, temperature

seasonality, the maximum temperature of the warmest month, annual precipitation, precipitation of the driest month, precipitation seasonality, precipitation of the warmest quarter, elevation, slope, nutrient availability, excess salts, land cover, soil respiration, soil type, soil moisture, human population density and animal population density were selected as independent variables. Only these eighteen variables were used in the final species distribution analysis. The potential areas of *S. marcescens* mapped using maximum entropy model with MaxEnt Software v.3.4.1). The model was generated using the subsampling technique with 15 replicates and 1000 iterations each, including 18 selected variables.

An AUC value for the predicted model was 0.918, which implied high predictability. Human population density showed the highest contribution of 43.6%, averaged over all replicates. Further, five variables viz; annual temperature (bio1), land cover, precipitation of driest month (bio14), temperature seasonality (bio4), and annual precipitation (bio12) were found to be other influencing predictors based on percentage contribution analysis. Human population density and temperature were the most influencing factors on S. marcescens distribution. The distribution map of S. marcescens implied its occurrence along the tropical and subtropical regions of the world. The entire world was then classified into five categories based on the risk level. The risk model indicates that highly suitable areas with greater than 75% of probability of occurrence along the Indian side of the Himalayan ranges, islands of Indonesia and Hanoi Province of Vietnam in Asia; Ecuador, Columbia in South America; Mexico, Belize, Nicaragua, Panama; Belgium, Netherlands, Denmark, and the UK; Victoria province in South Australia and Kenya, Uganda and Nigeria in Africa. The less occurrence was observed over the arid areas such as central and north Asia, North of North America, and Sahara. The probability of having incidences of S. marcescens related infections was in the order of Asia > North America > South America > Europe > Africa > Australia.

The prediction of future habitat suitability under changing climate scenarios was made with the help of the biomod2 package in R software using an ensemble

forecasting approach. The future distribution maps of *S. marcescens* unveil excess abundance that can be observed in Asian countries due to socio-economic and bioclimatic factors. Due to the impact of climate change and socio-economic factors, *S. marcescens* distribution can be seen along the study area in future scenarios. It also causes white pox disease of many critically endangered and vulnerable coral species, *A. palmata*. The prediction of *A. palmata* with regards to various marine environmental parameters was modeled. The study of spatial analysis of threatened coral, *A. palmata*, and opportunistic pathogen, *S. marcescens* offers new insights on the disease outbreaks and risk maps, which can be used to plan strategic and specific control measures against future expansion.

CHAPTER 7

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CLIMATE ENVELOPE MODELING FOR Serratia marcescens, PUTATIVE PATHOGEN OF CORAL WHITE

POX DISEASE

by

MS. SHANA S.S

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THESIS

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ABSTRACT

The global ocean is severely affected by climate change and its associated effects. The climatic fluctuations change the physical, chemical, and biological properties of the oceans and lead to a geographical change in suitable coral reef habitats, one of the most productive, biodiverse, and productive marine ecosystems. To study the precise information about disease outbreaks of coral reefs, knowledge of the current distribution of the etiological agents is necessary. This study aims to assess the risk of an emerging pathogen Serratia marcescens, the etiology of white pox disease affecting Acropora palmata. The required species and environmental data were taken from different open-source databases. The prepared habitat suitability model showed the higher occurrence of the pathogen along the Indian side of the Himalayan ranges, islands of Indonesia and Hanoi Province of Vietnam in Asia; Ecuador and Columbia in South America; Belgium, Netherlands, and the UK in Europe; Mexico, Belize, Nicaragua, Panama; South Australia, and North New Zealand with a probability greater than 75%. The highly suitable and moderately suitable areas of S. marcescens occupied around 0.52% and 17.9% of the total global land area. The prevalence of S. marcescens in the future was predicted based on the projection grids of the human population and bioclimatic variables using IPCC shared socio-economic scenarios, SSP1 and SSP5 for the period of 2040-60 and 2080-2100. The spatial distribution modeling of the endangered coral, A. palmata, was constructed by ensemble modeling using the biomod2 package of R software. The predicted current distribution of A. palmata vis-a-vis the predicted distribution of its pathogen S. marcescens gives an idea about the reef which are more in the proximity of the pathogen occurrence. The high probability of S. marcescens occurrence near the Caribbean waters (Florida, Bahamas, Cuba, etc.) throws light into the vulnerability of A. palmata in the Caribbean reefs. The knowledge of additional small-scale epidemiological studies in the identified areas, the inclusion of more variables such as the number of people with weakened immune functions, the abundance of host coral species, the land-based pollution to the sea, etc. are warranted in the future and will help to improve the disease prediction and to make a better decision for decreasing the disease incidences.