



MANGROVES OF LATIN AMERICA

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Universidad Espíritu Santo

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Compiler

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Prologue

Mangroveswampsareveryimportantecosystem;theirimportance is biological, economic and socio-cultural. It is an essential habitat for the juvenile stages of fish, mollusks, crustaceans, and of many other species. Mangroves maintain a trophic network with bird's nesting places and, at the same time, they are an important feeding space for other animals. They are natural and irreplaceable filters of sediments and nutrients; this makes them relevant water quality maintenance factors. Mangrove's thick vegetation and its fabulous root system avoid marine and eolic erosion. Last, mangroves are a source of food and economic maintenance for coastal groups; they also permit plentiful artisan fishing; they supply firewood, charcoal, and lumber for construction; and is source medicinal herbs. Mangroves are magic.

The information from the works that were presented in the I Mangroves Congress and that have been given you confirms the above.

Mangrove swamps survive, even though they were sacked, cut down and burned year after year.

Thanks to the dreaming and brave scientists who prepared the work you have in your hands, we now know that it is possible to restore and reforest mangroves. We also know that humans have finally learned to live in harmony with this ecosystem, but we are also warned of the dangers caused by introduced species. Last, we are also helped to understand the mangroves' socio-economic importance; and they may also be set as an example of a sustainability model.

Fernando Espinoza Fuentes PhD

Director of the Research Center

Universidad Espiritu Santo



Spatio-temporal analysis of mangroves in the Gulf of Guayaquil-Ecuador based on satellite images

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Samuel Corgne

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Introduction

Mangrove ecosystems dominate coastal wetlands in tropical and subtropical regions around the world. Biologically they are among the most important and productive ecosystems, because they provide goods and services to society (Kumar et al., 2014). Among the ecological and economic services, they provide, we can mention raw materials and food. They are important habitats and sources for other marine products (shellfish, crabs, fish, shrimp, many of the commercially important marine species spend part of their early cycle of life in these ecosystems). In addition, it contributes to the protection of coastal areas from erosion, water filtration, medicinal ingredients, tourist attraction, water purification, recreation, education and research (Kumar et al., 2014; Burgess, Li and Qin, 2015; Kuenzer et al., 2011; Blasco, Saenger and Janodet, 1996). Mangroves can filter out pollutants from the sea and sequester carbon dioxide (CO₂) released into the atmosphere due to anthropogenic activities (Heumann, 2011; Chen et al., 2013). They are also essential for maintaining beach stability and trapping sediment from river runoff.

Mangroves are one of the most vulnerable ecosystems and have decreased dramatically in the last half century (Kuenzer et al., 2011; Vo et al., 2013; World Wildlife Fund, 2017). The main threats to mangroves

are the overexploitation of its natural resources, deforestation, mine activities, pollution and industrial or urban development that extend along coastal forest areas, but especially their conversion to aquaculture (Vo et al., 2013; Hamilton and Lovette, 2015; World Wildlife Fund, 2017). Between 30 and 40 % of the total world mangrove area has been converted to aquaculture in the last 50 years (Hamilton and Lovette, 2015; World Wildlife Fund, 2017). Coastal development, which converts these forests to other land uses, has often ignored the services that mangroves can provide, leading to irreversible environmental degradation (Chen et al., 2013). International programs, such as the Ramsar Convention on Wetlands of the Kyoto Protocol, emphasize the importance of immediate protection measures and conservation activities to prevent further destruction of mangroves (Kuenzer et al., 2011).

Despite the conversion rates of mangroves into aquaculture and the fact that mangroves have a much higher carbon level per unit area than other tropical forests, their spatio-temporal evolution is not well known in Ecuador (Hamilton and Lovette, 2015). Current knowledge of the distribution, abundance and conditions of mangroves is essential for the development of management plans and programs for the conservation of these ecosystems. This study is an attempt to answer this concern by analyzing the spatial and temporal dynamics of mangrove forests in the Gulf of Guayaquil estuary, representing an emblematic site of mangrove anthropization in Ecuador.

Climate change poses a series of threats to many coastal wetlands. Mangroves can be affected by rising sea levels, rising air temperatures, and changes in the frequency and intensity of rainfall and storms. Among these threats, increased saltwater intrusion, due to sea level rise, poses one of the greatest challenges (Banerjee, Cazzolla and Mitra, 2016). Mangroves require daily flows from the ocean and fresh water for its long-term survival. The rise in sea level associated with aquatic salinization will inevitably change the hydrological regime of mangroves and change their forest ecology (Dasgupta, Sobhan and Wheeler, 2016; Banerjee, Cazzolla and Mitra, 2016).

The development of aquaculture has caused a reduction in the area of the mangrove ecosystem. Therefore, monitoring the spatial and temporal distribution of mangrove forests is essential for managing the natural resources of this ecosystem (Chen et al., 2013). The methods to determine its extent and spatial distribution have improved over time thanks to technological advances such as remote sensing, geographic positioning by satellite (GPS) and geographic information systems (GIS) (Alatorre et al., 2011). Satellite remote sensing is a powerful tool for analyzing estuaries and coastal systems and is ideal for monitoring the spatial and temporal evolution of mangrove ecosystems (Kuenzer et al., 2011; Alatorre et al., 2011; Purnamasayangasukasih et al., 2016).

Another advantage of remote sensing is that it allows a wide variety of measurements in relatively inaccessible and sensitive sites. In addition, data from multispectral sensors such as SPOT (Earth Observation System), Landsat Thematic Mapper (TM) and Enhanced Thematic Mapper Plus (ETM+), among others, provide useful sources of remote sensing data and allow discrimination of mangroves and non-mangroves (Alatorre et al., 2011). The use of remote sensors is important because the reflectance patterns of vegetation in the visible and near-infrared spectral region (NIR) provide information on vegetation cover conditions (Alatorre et al., 2011). Free images with high spatial and temporal resolution (Landsat 8 and Sentinel-2) can also be accessed to map mangrove dynamics.

Some classification methods that have been used for the study of mangroves are: (i) primary component analysis (PCA) through an unsupervised classification (Kuenzer et al., 2011; Rakotomavo and Fromard, 2010); (ii) the object classification method (Wang, Sousa and Gong, 2004; Vo et al., 2013); (iii) the object-based approach to map change in mangrove cover (Conchedda, Durieux and Mayaux, 2008); (iv) object-based image analysis and support vector machines to differentiate species associated with mangroves and mangrove species (Heumann, 2011). Finally, a maximum likelihood algorithm was used to obtain a spectral distance map of the mangrove areas (Alatorre et al., 2011; Tong et al., 2004).

In this work, we use optical remote sensing with high spatial resolution to determine the spatial and temporal evolution of mangroves within the Gulf of Guayaquil estuary, in the space of 30 years, using satellite images Landsat 4-5 (February 1985) and Landsat 8 (January 2015). We are also evaluating the potential of the Sentinel-2 satellite for better characterization of mangroves. To do this, we first detail how mangroves work in a context of global change and make a state of the art in their remote sensing monitoring. After presenting the study site and the methodology developed, we analyzed its spatial and temporal evolution over the past 30 years and evaluated the Sentinel-2 satellite, from year 2015, to optimize the characterization of mangroves. Finally we make a conclusion and some research perspectives for this work are proposed.

Study site

The mangroves in Ecuador extend along the coastal areas of the provinces of Esmeraldas, Manabí, Guayas, El Oro and the Galapagos Islands. Among them, the largest area corresponds to the Gulf of Guayaquil. The Gulf of Guayaquil is the largest gulf in the Western Coast of South America. It extent from Cabo Blanco in Peru to Punta de Santa Elena in Ecuador, covering a distance of 230 km. It is naturally divided into an outer estuary, which originates from the west side of the Puná Island, and an inner estuary, which extends from the Western Coast of the Puná island to the Northeast, entering to the Estero Salado and Guayas River systems.

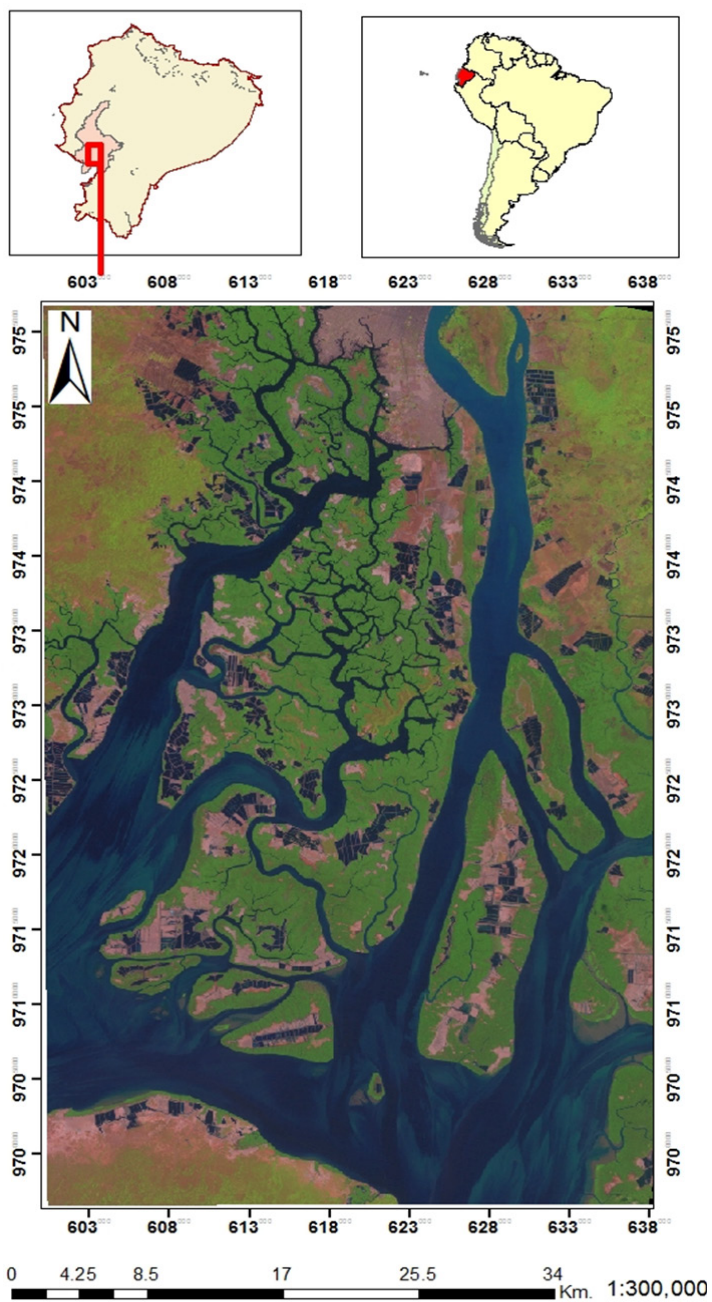


Figure 1. Location map of the study area. Source: author's own elaboration.

The Gulf of Guayaquil estuary is located in a dry tropical climate region that shows marked seasonal variations: a rainy period from December to April (791 mm/year), transition from May to June and a dry period from July to November. This climate registers precipitations of about 200 mm during the rainy season. The average air temperature in winter can exceed 31 °C and can decrease until 20 °C in summer (Vinueza et al., 2009).

The mangrove soil contains quaternary sediments, mainly clays, but there are also sands, sandstones and conglomerates. Also, soils are not well drained, and are often saturated with water the entire year (causing salinization). The sedimentary contributions of the estuary come mainly from the Daule and Babahoyo rivers, which are the two large hydrographic systems that make up the Guayas river basin. These rivers carry sediment to the plains in the inner and outer estuary of the Gulf of Guayaquil. Sediments in the Gulf bed range from sands to clay loams. Much of the sediment that reaches the estuary remains suspended in the water column; therefore, it is easily transferred from one place to another by the different currents that predominate in the area. The soils are unstable, brackish, anoxic and often acidic, which makes them extremely restrictive for any agricultural activity (Vinueza et al., 2009). The salinity of the estuary is between 24 and 29 UPS in the inner estuary and between 30 and 31 UPS in the outer estuary, during the dry season; while, in the wet season, the salinity is between 16 and 18 UPS in the inner estuary.

There are six tree species that comprise the mangrove ecosystems in the study area: *Rhizophora mangle* (Male Red Mangrove), *Rhizophora harrisonii* (Female Red Mangrove or Crawled Mangrove), *Rhizophora racemosa*, *Avicennia germinans* (Black Mangrove, Iguanero or Salty Mangrove), *Laguncularia racemosa* (White Mangrove or Manoa Mangrove) and *Conocarpus erectus* (Jelí Mangrove or Button). The *Rhizophora* genus is the dominant type on the coastal part in contact with water, since it adapts well to the environment thanks to the shape of its roots, which allow it to position itself over unconsolidated and unstable sediments (Vinueza et al., 2009).

In order to study mangrove dynamics, we used satellite imagery from Landsat 5 (TM) from February 1985 and Landsat 8 (OLI) from January 2015, as well as a Sentinel-2A image from December 2015. Open source software Quantum Gis 2.18.2 was used to download, pre-process, classify and post-process the satellite images, especially the Semi-Automatic Classification Plugin 5.3.6 developed by Congedo (2016) for Q-Gis. Images have a UTM WGS84 17S projection. The different characteristics of each satellites are summarized in Table 1, and details of the different sensor bands are given in Table 2.

Table 1. Details of satellite data used

Image	Product	Date
Landsat 5	LT50110621985038AAA08	7 February 1985
Landsat 8	LC80110622015025LGN00	25 January 2015
Sentinel-2	S2A_OPER_MSI_L1C_TL__MTI__2015 1224T202925_A002639_T17MPT	24 December 2015

Source: author's own elaboration.

Table 2. Comparison between the different sensor bands

Landsat 5			Landsat 8			Sentinel 2		
Sensor TM			Sensor OLI, TIRS (10-11)			Sensor MSI		
Band	Wavelength (μm)	Res. (m)	Banda	Wavelength (μm)	Res. (m)	Band	Wavelength (μm)	Res. (m)
			B1 Coastal	0.43-0.45	30	B1 Coastal aerosol	0.43-0.45	60
B1-Blue	0.45-0.52	30	B2-Blue	0.45-0.51	30	B2-Blue	0.45-0.52	10
B2-Green	0.52-0.60	30	B3-Green	0.53-0.59	30	B3-Green	0.54-0.57	10
B3-Red	0.63-0.69	30	B4-Red	0.64-0.67	30	B4-Red	0.65-0.68	10
						B5 Veg. Red Edge	0.69-0.71	20
						B6 Veg. Red Edge	0.73-0.74	20
						B7 Veg. Red Edge	0.77-0.79	20
B4 NIR	0.76-0.90	30				Band 8 – NIR	0.78-0.90	10
			B5 NIR	0.85-0.88	30	B8A Veg. Red Edge	0.85-0.87	20
						B9-Water Vapeur	0.93-0.95	60
			B9 Cirrus	1.36-1.38	30	B10 SWIR-Cirrus	1.36-1.39	60
B5 SWIR1	1.55-1.75	30	B6 SWIR1	1.57-1.65	30	B11 SWIR	1.56-1.65	20
B7 SWIR2	2.08-2.35	30	B7 SWIR2	2.11-2.29	30	B12 SWIR	2.10-2.28	20
			B8 Pan	0.50-0.68	15			
B6 Thermal	10.40-12.50	30	B10 TIRS1	10.60-11.19	100			
			B11 TIRS2	11.50-12.51	100			

Note: Swir = Shortwave Infrared, Nir = Near Infrared, Res = Resolution, Pan = Panchromatic. The bands used in this study are circled in red.

Source: author's own elaboration.

For the year 1985 the month of February was chosen, and the months of January and December for the year 2015, which correspond to the rainy season.



A



B



C



D

Figure 2. Main mangrove species: **A.** *Conocarpus erectus*; **B.** *Avicennia germinans*; **C.** *Laguncularia racemosa*; **D.** *Rhizophora mangle*. Source: J. Sócola.

For the present study, 30 field points were taken at different time periods between 2000 and 2009, and in different parts of the study area, depending on the accessibility to mangroves. In some places, the mangrove swamp has a large amount of unconsolidated

sediment that makes access almost impossible. At each site, the X and Y coordinates were taken with GPS, in the UTM geographic system and the WGS84 datum. Information about the height and diameter of the dominant species (*Rhizophora mangle*, *Rhizophora racemosa*, *Rhizophora harrisonii*, *Avicennia germinans*, *Laguncularia racemosa* and *Conocarpus erectus*) at each sampling location was collected (Figure 2), as well as on the different classes of vegetation cover (Table 3).

The identified classes were Mangrove, Forest (semi-deciduous), Dry Forest (deciduous), Scrub, Saline soils, Water, Aquaculture (shrimp pools), Crops and Other soils (bare soils and urban areas).

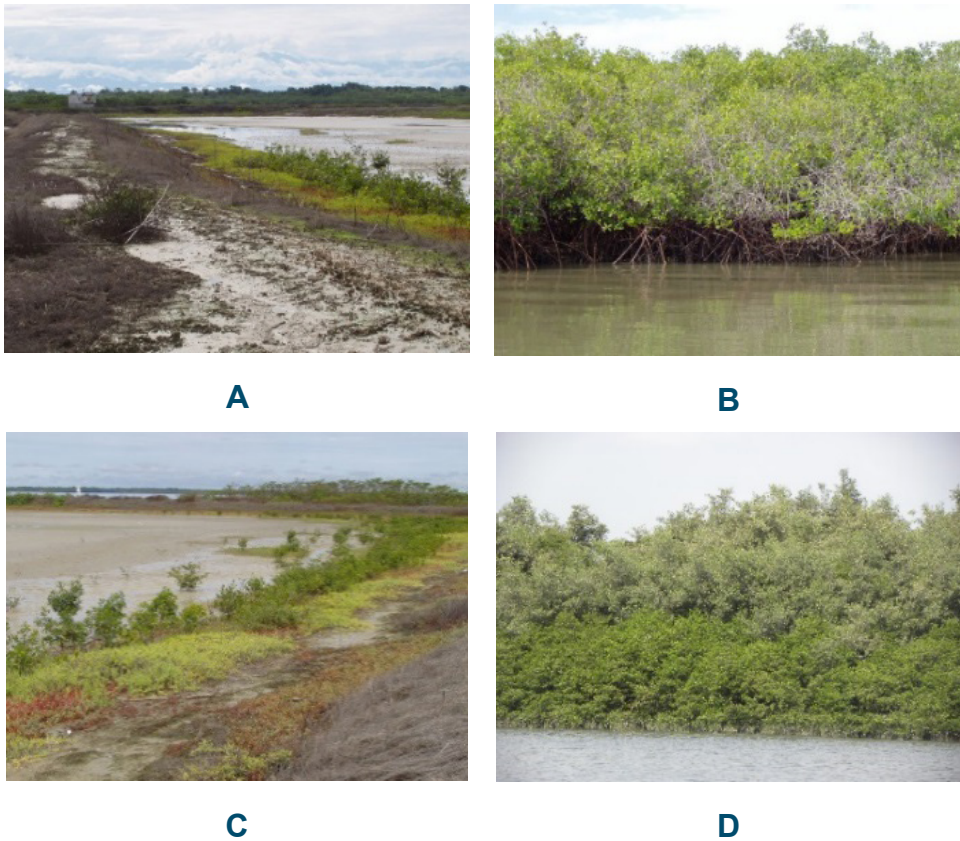


Figure 3. Soil cover in the study area: **A.** Saline soils and bare soils; **B.** Mangrove, *Rhizophora*; **C.** Aquaculture; **D.** Mangrove, *Laguncularia* and *Avicennia*. Source: J. Sócola.

For the spatio-temporal analysis between 1985 and 2015, geometric (reprojection) and atmospheric (DOS model: Dark Object Sustraction) pretreatments were performed on the Landsat bands to improve their visual quality and achieve a better interpretation of pixel reflectance values (Figure 4).

Regions of Interest (ROI's) were created with the following classes: (1) Mangrove, (2) Forest, (3) Dry forest, (4) Scrub, (5) Water, (6) Saline soils and (7) Other soils. For the year 2015 two more classes have been added: (0) Clouds and (8) Shadows. The extraction of the samples of each class is made from the Normalized Difference Vegetation Index (NDVI).

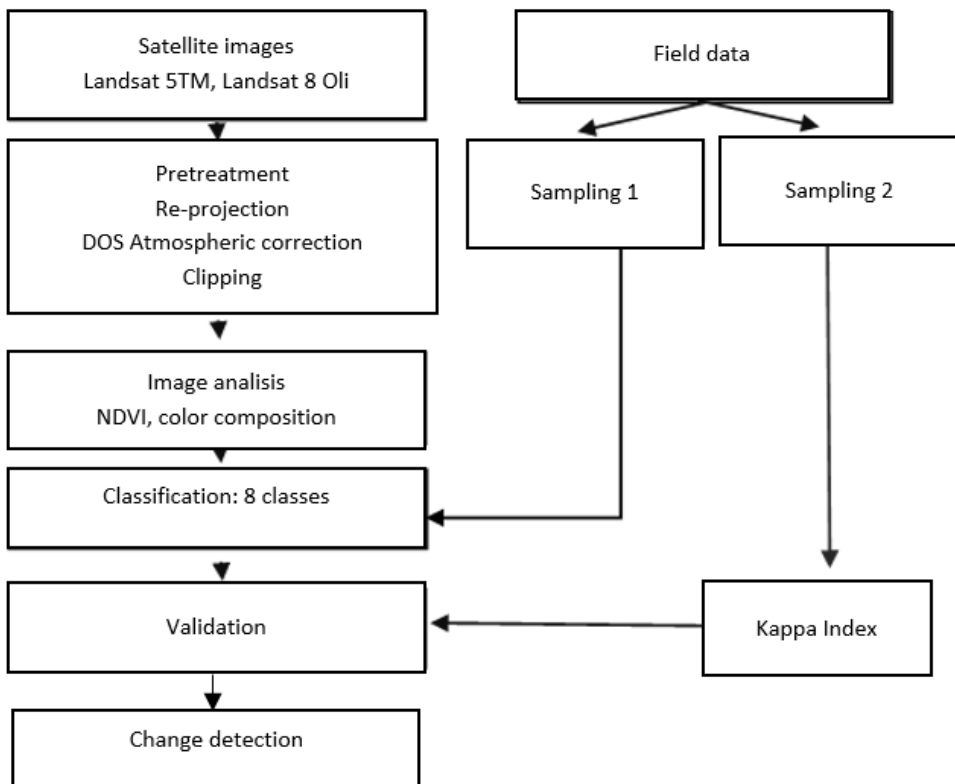


Figure 4. Classification process for analysis of changes in land use. Source: author's own elaboration.

The NDVI is a standardized differentiated vegetation index that explains the amount of vegetation present on a surface and the health status of that vegetation. This index is based on the difference between the visible red band and the near infrared band, and is expressed mathematically by the formula: $NDVI = (NIR - R) / (NIR + R)$; where NIR = Near Infra Red and R = Visible Red. Therefore, values between 0 and 1 correspond to surfaces with vegetation, and values between 0 and -1 have surfaces with little or no vegetation, such as desert or urbanized surfaces.

We opted for a supervised classification based on the maximum likelihood algorithm. Finally, we analyze the changes in land use using the “Crosstab” function. This function allows us to compare two classifications by visualizing the change in pixels from one class to another. The principle is to create a new map from two thematic maps to highlight the change in pixel mapping between two dates. Furthermore, it is possible to isolate certain classes and calculate the variation rate of the area between two years.

For the Comparison and classification with Landsat 8 and Sentinel-2A the following pre-treatments were performed: reprojection, atmospheric correction, clipping, masking improvements, DOS1 atmospheric correction and sampling. This increases the visual quality and allows a better interpretation of the reflectance values of the pixels (Figure 5).

For the 2015 image classifications, 13 classes were obtained: (1) Mangrove, (2) Forest, (3) Dry forest, (4) Scrub, (5) Estuary, (6) River, (7) Aquaculture, (8) Crops, (9) Saline soils, (10) Construction and (11) Other soils. The Clouds were given class 0 (unsorted) and (12) Shadows.

The extraction of samples of each class was performed from the NDVI value. A supervised classification based on the maximum likelihood algorithm was chosen. The same samples were used to classify Landsat 8 and Sentinel-2 images. Both classifications were compared using validations and Kappa index values.

Finally, the intersection of the two classifications was made. In order to do this procedure the classification obtained from the Sentinel-2 had to be resized to a spatial resolution of 30 m.

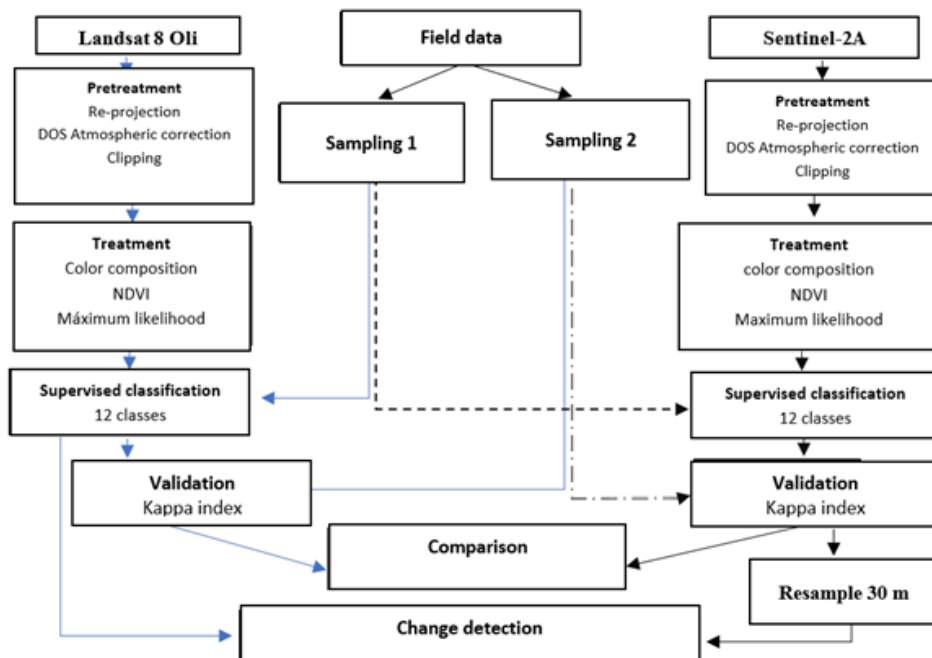


Figure 5. Landsat 8 and Sentinel-2 image processing process. Source: author's own elaboration.

Land Use Change between 1985 and 2015

In the 1985 images, seven classes were identified according to the different spectral signatures. One of the main problems encountered in classifying the Landsat 5 image of that year was the spectral proximity between bare soil, inactive shrimp pools and urban areas, so these three characteristics were included in a single class called "Other soils." A similar problem was observed with the water bodies, since the water from the Guayas River, the estuarine water and

the water from the shrimp pools also showed very similar spectral signatures; therefore, these three classes were grouped into a single class called “Water.”

According to the results shown in Table 3, the mangrove area is 56,735 hectares and mainly occupies the central part of the study area. The exploitation of mangroves for the construction of shrimp pools began in 1969, therefore, by 1985, aquaculture activity already has an important role, however, many shrimp pools are inactive. Shrimp farms are found in the central zones of mangrove ecosystems, in the area corresponding to natural Saline soils. The existing saline soils have an area of 7190.91 ha. The area occupied by active aquaculture is 7873.94 ha. The active aquaculture area in 1985 was obtained by subtracting the value of water (river and estuary) from the total value of the class “Water” (Figure 6). The urban area located in the northern part, corresponding to the city of Guayaquil, has an area of 4371.94 ha.

Table 3. Area in ha and % for 1985

		1985	
Classe		Superficie	
		(Ha)	(%)
0	Unclassified	1263.96	0.5
1	Mangrove	56735.01	24.5
2	Forest	7147.17	3.1
3	Dry Forest	16265.52	7.0
4	Scrub	25695.45	11.1
5	Water	85595.94	36.9
6	Saline soils	7190.91	3.1
7	Other soils	31904.46	13.8
8	Shadow		

Source: author's own elaboration.

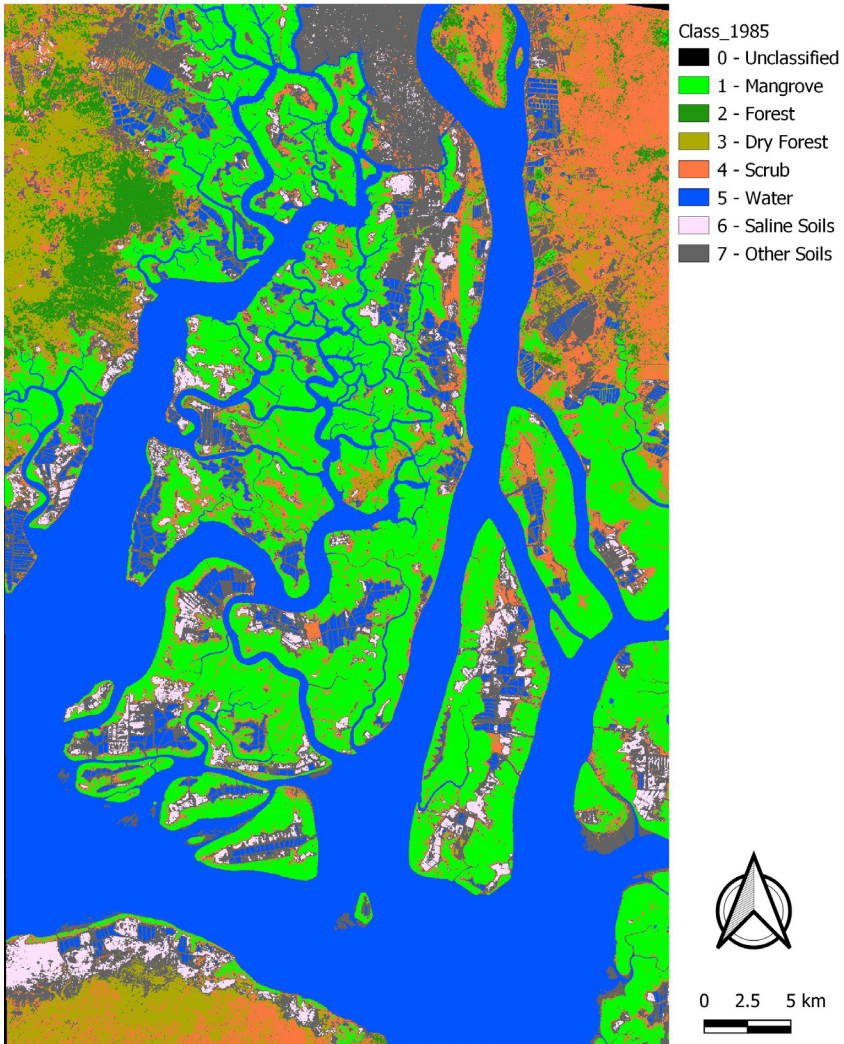


Figure 6. Landsat 5 image classification map, 1985. Source: author's own elaboration.

To validate the classification of the image of the year 1985, an error matrix was generated, where the classes were compared with the classes in Sample 2 (validated with field data). The precision of the classification was evaluated by calculating the Kappa index that reached 0.89, which indicates a correct level of classification (Table 4).

Table 4. Values of the Kappa index obtained for the Landsat 5 image, 1985

	Producer Accuracy (%)	User Accuracy (%)	Kappa Hat
Mangrove	97.92	97.68	0.97
Forest	97.88	89.46	0.87
Dry Forest	73.14	97.10	0.97
Scrub	89.60	78.67	0.76
Water	99.40	100.00	1.00
Saline soils	54.55	95.56	0.95
Other soils	97.07	84.63	0.82
Kappa Hat Classification			0.89

Source: author's own elaboration.

In the classification of the Landsat 8 image from 2015, one of the main problems was the presence of clouds and shadows. It was necessary to add an additional class (8), which corresponds to the “Shadows,” while the clouds were classified with a value of 0, which creates a mask for the classification. According to this, the mangrove area is 51623.82 ha, the saline soils have an area of 1 203.75 ha (Table 5). Aquaculture intensifies in 16,141 ha. There are more active shrimp farms compared to 1985 (Figure 7). The Kappa index obtained has a value of 0.87, confirming that our classification is correct (Table 6).

Table 5. Area in ha and % for 2015

	Classe	Superficie	
		(Ha)	(%)
1	Mangrove	51623.82	22.3
2	Forest	8493.75	3.7
3	Dry Forest	4887.45	2.1
4	Scrub	11293.2	4.9

	Classe		Superficie
5	Water	101737.35	43.9
6	Saline soils	1203.75	0.5
7	Other soils	43905.96	18.9
8	Shadow	2516.67	1.1

Source: author's own elaboration.

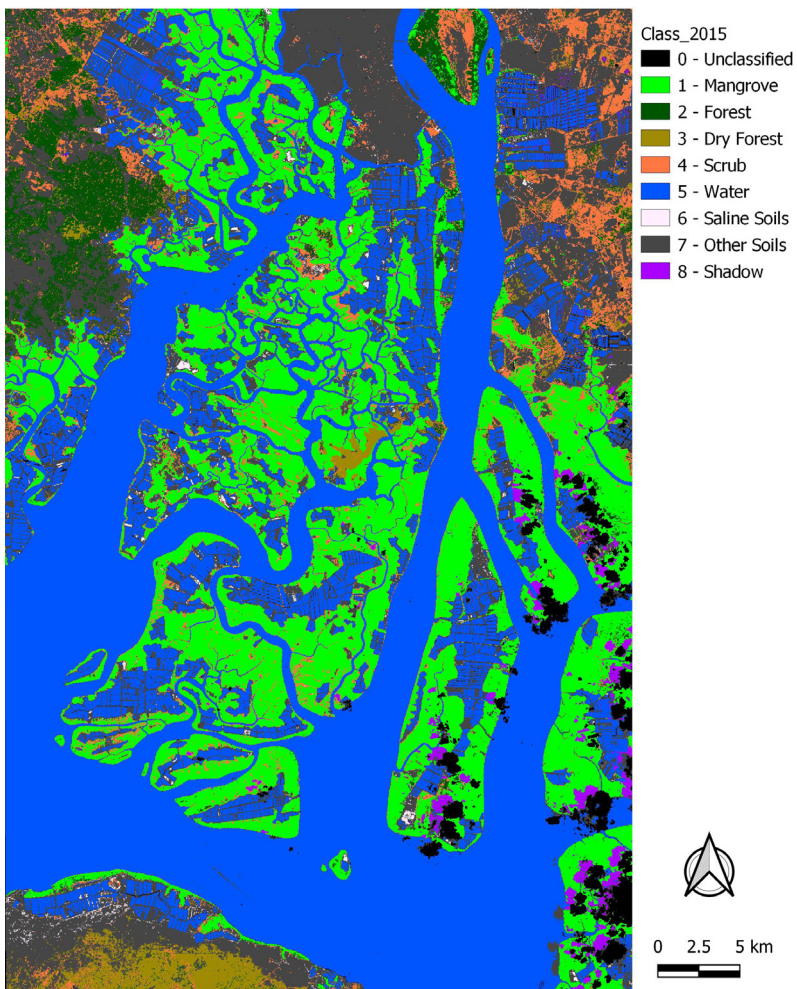


Figure 7. Landsat 8 image classification map, 2015. Source: author's own elaboration.

Table 6. Kappa index values obtained for the Landsat 8 image, 2015

	Producer Accuracy [%]	User Accuracy [%]	Kappa Hat
Mangrove	95.55	94.29	0.93
Forest	53.14	70.46	0.69
Dry Forest	79.86	92.28	0.90
Scrub	74.63	57.12	0.55
Water	98.62	99.86	1.00
Saline soils	79.43	94.96	0.95
Other soils	98.22	77.16	0.74
Shadow	95.34	85.71	0.86
Kappa Hat Classification			0.87

Source: author's own elaboration.

The analysis of changes in land use between 1985 and 2015 (Table 7) shows that there is a decrease in the area of mangroves, dry forests, bushes and saline soils. However, there has been a sharp increase in aquaculture, which has developed more intensively. The class “Other floor” has also increased. Aquaculture expansion increased by 16141.11 ha (18 %). Saline soils, known as natural salt flats, are wetlands within the mangrove and are considered part of the mangrove. This is the most affected class, since it decreased by 83.26 % (Table 7). Dry forest has decreased in favor of urbanized areas such as Chongón in the West; in addition, agricultural activities have developed considerably in the area. The bushes have decreased in favor of the growth of the city of Durán, the conversion to agricultural areas and the transformation into shrimp pools.

Table 7. Area of each class in hectares and percentage (%) for each year and percentage of change in land use (%)

	Class	1985		2015		Change (%)
		Surface		Surface		
		(ha)	(%)	(ha)	(%)	
0	Unclassified	1263.96	0.5	6136.47	2.6	385.5
1	Mangrove	56735.01	24.5	51623.82	22.3	-9.0
2	Forest	7147.17	3.1	8493.75	3.7	18.8
3	Dry Forest	16265.52	7.0	4887.45	2.1	-70.0
4	Scrub	25695.45	11.1	11293.2	4.9	-56.0
5	Water	85595.94	36.9	101737.35	43.9	18.9
6	Saline soils	7190.91	3.1	1203.75	0.5	-83.3
7	Other soils	31904.46	13.8	43905.96	18.9	37.6
8	Shadow			2516.67	1.1	

Source: author's own elaboration.

In 2015, six classes changed their water cover which now corresponds to aquaculture (Figure 8): “Other soils” (10555.74 ha) mainly due to the year 1985, inactive shrimp pools were included in this class; “Saline soils” (3792.96 ha) because the vegetation present is of the shrub type, which represents an easy change, mainly of the species of white mangrove (*Laguncularia racemosa*) and a black mangrove (*Avicennia germinans*) of adults and young; “Scrub” (3270.24 ha) because, in the northeast part of the study area, were covered with shrubs and herbaceous plants, which have been displaced by aquaculture areas; “Mangrove” (1977.93 ha) lost part of its surface for the construction of shrimp pools; “Dry Forest” (1140.57 ha), it has been transformed into aquaculture zones mainly in the Northwest part of the study area.

The “Forest” (189.9 ha) located mainly in the Northwest zone has become to aquaculture, and can also be attributed to a

misclassification, since for the 1985 classification, some patches are observed in mangroves (islands). In 2015, most aquaculture areas are operational and new areas for aquaculture have been built.

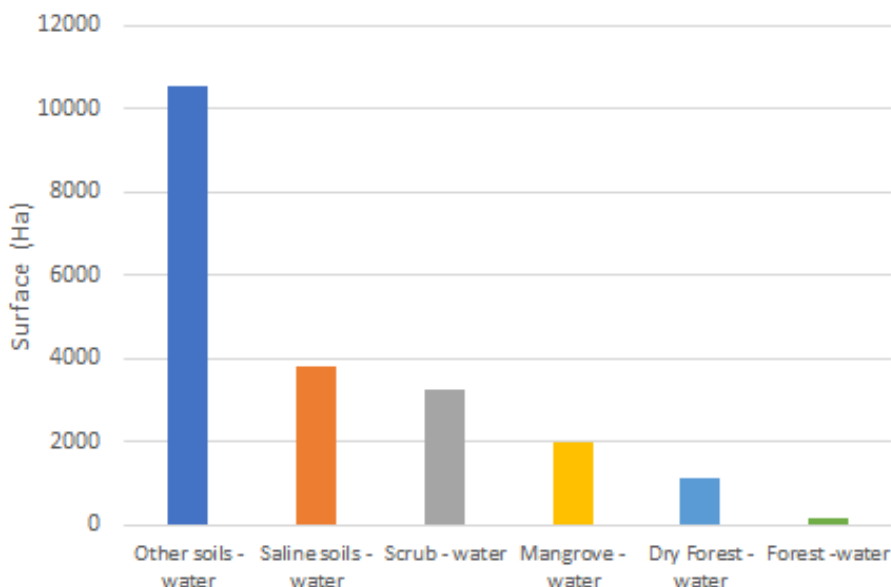


Figure 8. Conversion of classes to water (Aquaculture) during the period 1985-2015. Source: author's own elaboration.

The loss of mangroves occurred in the 30-year period is 9668.43 ha. The main cause was due to the conversion of “Mangrove” to “Other soils” (Figure 9), due to the increase of urban areas, as a result of the population growth in the city of Guayaquil, which increased by 652.16 ha. The change from “Mangrove” to “Scrub” is mainly due to the cutting of mangroves. The conversion of “Mangrove” to “Forest” and “Dry forest” could be a consequence of the cutting of mangroves and the subsequent growth of typical forest vegetation, and also to a possible error in the classification, since in the classification of the 2015 the class “Forest” has a Kappa index of 0.69.

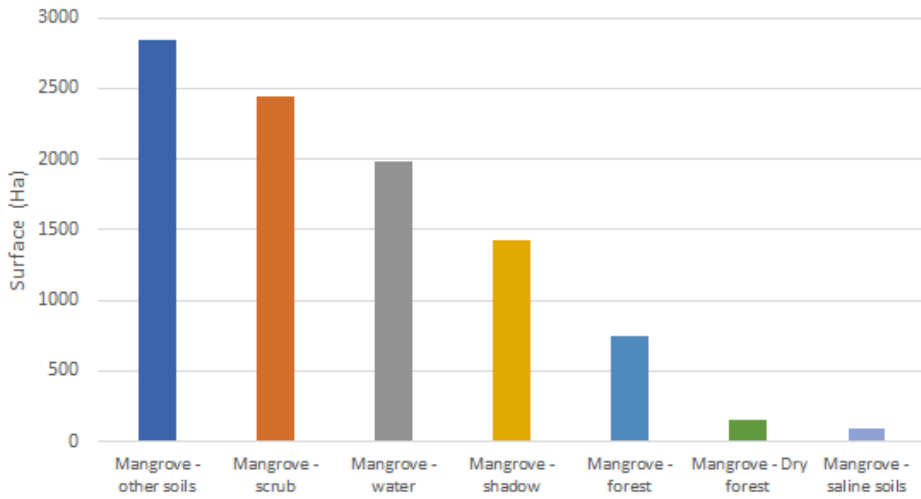


Figure 9. Conversion of the Mangrove class to other classes, during the period 1985-2015.
Source: author's own elaboration.

One of the main problems when comparing Sentinel-2 and Landsat 8 was the presence of clouds and shadows in both images. Despite this, the Sentinel-2 image allowed us to distinguish 12 classes that were also used in the sampling for the classifications in the Landsat 8 image (Figures 10, 11)

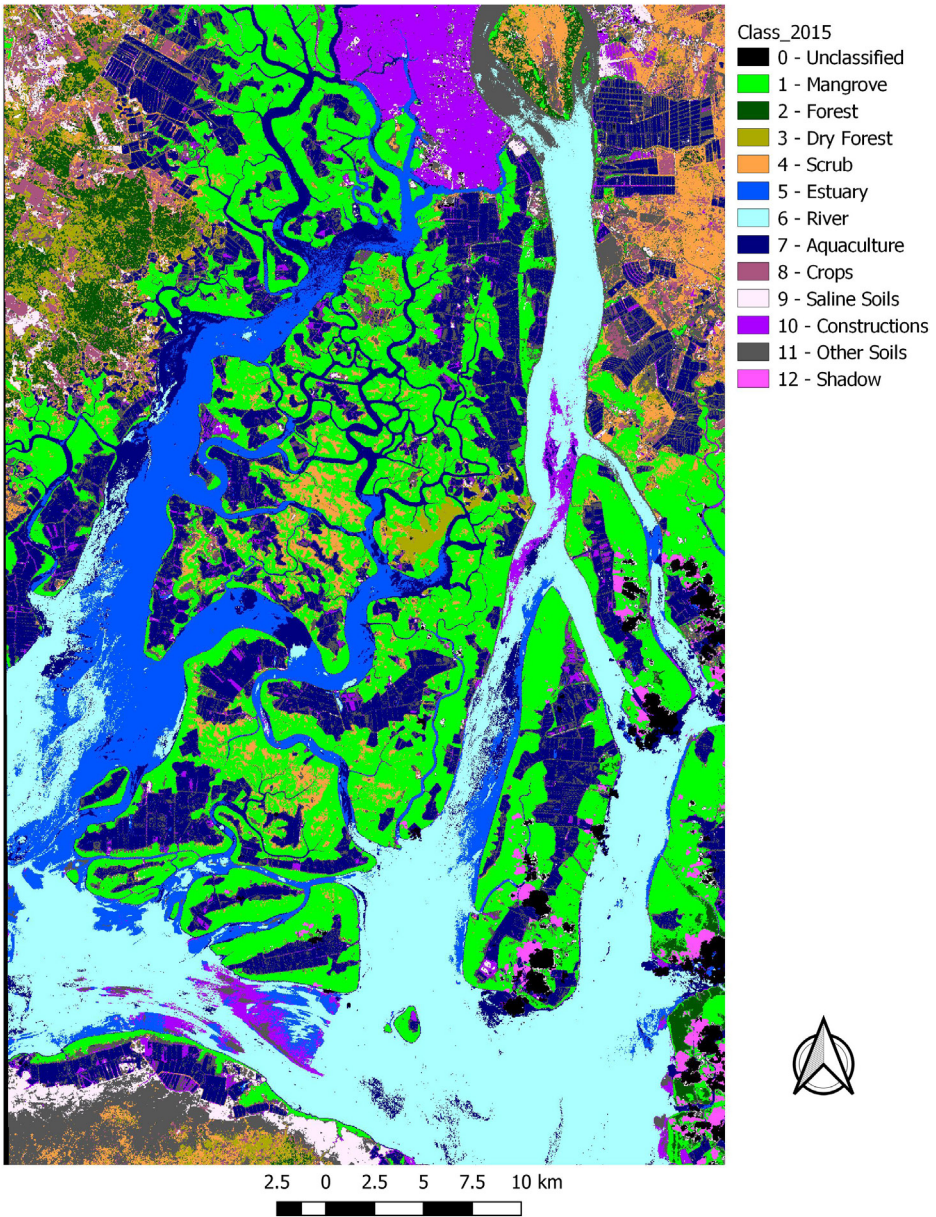


Figure 10. Landsat 8, 2015 images classification map. Source: author's own elaboration.

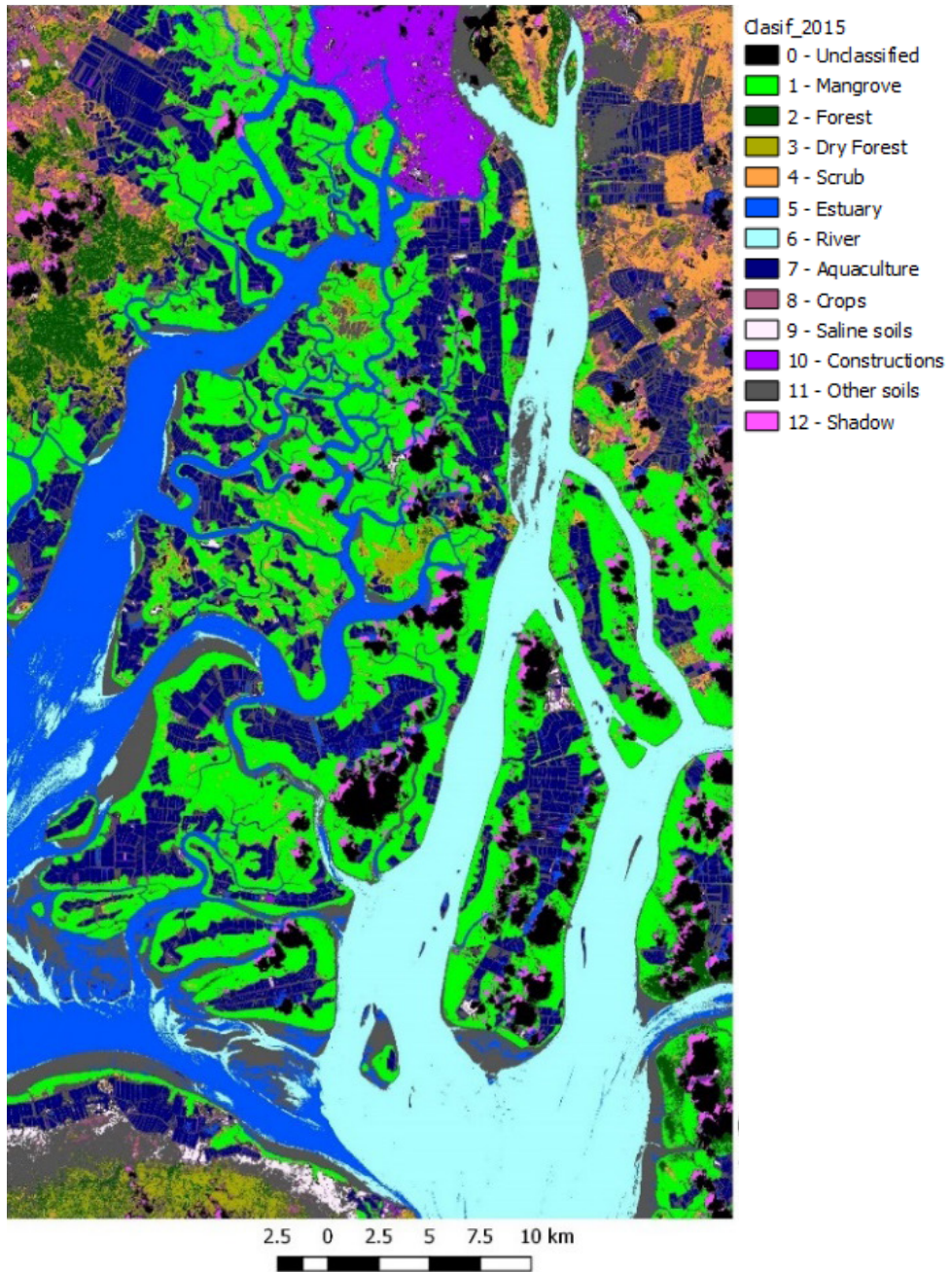


Figure 11. Sentinel-2, 2015 images classification map. Source: author’s own elaboration.

The validation of the Landsat 8 image classification has a Kappa index of 0.66, indicating an erroneous classification (Table 8).

Table 8. Values of the Kappa index obtained for the Landsat 8 image, 2015

	Producer Accuracy [%]	User Accuracy [%]	Kappa Hat
1 Mangrove	94.75	92.11	0.91
2 Forest	75.17	86.56	0.85
3 Dry Forest	88.98	90.83	0.90
4 Scrub	63.04	60.84	0.57
5 Estuary	0.00	0.00	-0.09
6 River	54.01	45.11	0.39
7 Aquaculture	77.80	45.54	0.42
8 Crops	93.12	67.61	0.65
9 Saline soils	80.08	86.39	0.86
10 Construction	98.18	93.36	0.93
11 Other soils	32.10	44.64	0.40
12 Shadow	83.98	99.73	1.00
Kappa Hat Classification			0.67

Source: author's own elaboration.

The Sentinel-2 image classification validation has a Kappa index of 0.88, which corresponds to a good classification (Table 9).

Table 9. Kappa index values for the Sentinel-2 image, 2015

	Producer Accuracy [%]	User Accuracy [%]	Kappa Hat
1 Mangrove	99.19	94.90	0.94
2 Forest	76.86	85.85	0.84
3 Dry Forest	80.92	89.32	0.88
4 Scrub	90.90	83.97	0.82

	Producer Accuracy [%]	User Accuracy [%]	Kappa Hat
5 Estuary	62.37	99.23	0.99
6 River	98.51	88.13	0.87
7 Aquaculture	92.05	96.64	0.96
8 Crops	97.38	90.54	0.90
9 Saline soils	89.37	98.58	0.98
10 Construction	98.61	98.24	0.98
11 Other soils	95.65	76.33	0.74
12 Shadow	91.75	83.48	0.83
Kappa Hat Classification			0.88

Source: author's own elaboration.

In the analysis of changes between Landsat 8-2015 and Sentinel-2 2015 images, we noted that the Sentinel-2 image has many unclassified pixels due to the presence of many clouds, compared to Landsat 8 image with fewer clouds. The largest number of clouds in the Sentinel-2 image is in the Eastern part of the study area, mainly covering mangroves. Therefore, based on the results, the Landsat-8 image has a larger area of mangrove, aquaculture, saline soils, buildings, and scrub (Figure 12).

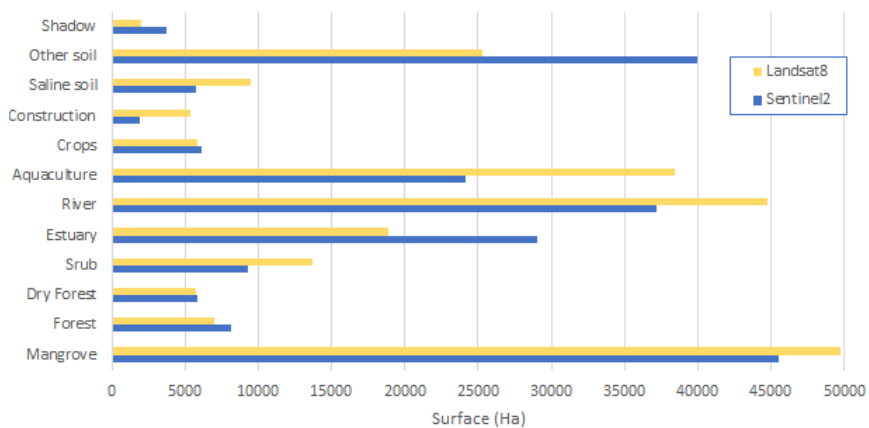


Figure 12. Comparison of Sentinel-2 and Landsat 8 classifications. Source: author's own elaboration.

The classes that have the greatest similarity between the two images are “River” (82 %), “Mangrove” (78.8 %), “Construction” (75 %) and “Aquaculture” (71 %). While those with the least similarity are “Other soils” (25.2 %), “Crops” (26.5 %) and “Dry forest” (26.5 %) (Figure 13).

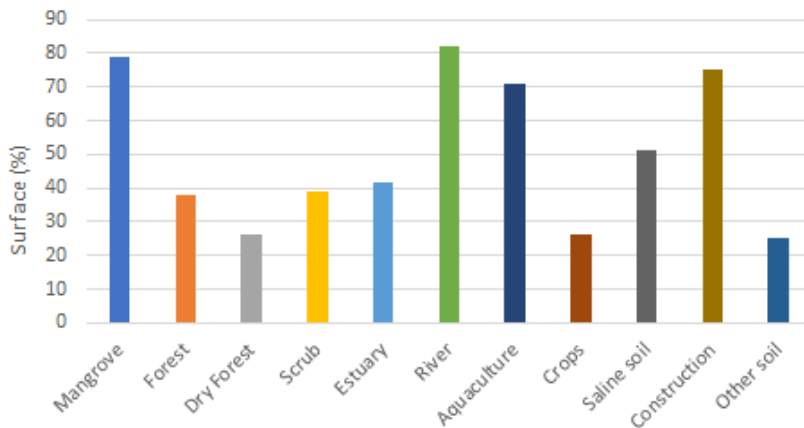


Figure 13. Similarity (%) of each class between Landsat 8 and Sentinel-2 images. Source: author’s own elaboration.

For analysis of the change in land use between 1985 and 2015, based on the Landsat image classifications of 1985 and 2015, we have seen many changes in 30 years. The change in land use in the inner estuary region of the Gulf of Guayaquil has been highlighted by the decrease in the size of the “Mangrove” 9 %, “Forest” and “Dry Forest” classes 70 %, “Scrub” 56 % and “Saline Soils” 83 %, while “Aquaculture” increased 18.9 % and the “Other Soils” class, which includes bare soils and urbanized surfaces, increased 37.6 %. The main impact of the expansion of aquaculture and the growth of urban areas mainly affected mangroves and saline soils.

When comparing Landsat-8 and Sentinel-2 images, we are faced with the presence of clouds and shadows that modify the results. For

example, in the Sentinel-2 image, 15000 hectares have 0 values (not classified due to cloud or shadow), which has detracted from the comparison. Contrasting the Sentinel-2 and Landsat-8 images, it can be concluded that the spectral proximity between different mangrove species is not separable with Landsat-8 unlike Sentinel-2, and there are significant differences in the precision of the classification between the two sensors. This is reflected in the Kappa indices of 0.66 for Landsat-8 and 0.88 for Sentinel-2. This difference is the result of the lowest spatial resolution in Landsat-8 (30 meters) respect to Sentinel-2 (10 meters), and the difference in Landsat-8 spectral resolution (11 spectral bands) respect to Sentinel-2 (13 spectral bands), mainly those bands in the near infrared zone (0.7 μm).

This allows us to have better results in the study of vegetation due to the rapid change in the chlorophyll reflection factor. This offers many research opportunities for the future, mainly in the management and conservation of endangered ecosystems such as mangroves. The main benefits of carrying out this type of study are that they could collect information on the entire mangrove surface, since in some areas it is difficult to access the mangrove, saving time and effort. Information from a large area can also be collected quickly.

One of the problems encountered in conducting this study was the difficulty of obtaining cloud-free images taken at the same time. In fact, despite the corrections and treatments made with the images, there is a range of error in the classification due to the presence of clouds and their shadows on the vegetation. Another difficulty was to spectrally separate the different types of water surfaces and have an absolute value of the aquaculture area.

Conclusion

The results for the detection of species are encouraging, in fact, the precision achieved by the classification made with the sentinel-2 image allows us to consider this platform and this methodology to be applied to another sector. This study is an important tool for

mangrove management in Ecuador and can serve as a basis for spectral separation of mangrove species, and thus determine the spatial distribution of species using Sentinel-2 digital images. The methodology is now established for future sampling campaigns.

Through this study, the importance of the use of remote sensing and Geographic Information Systems for a rapid, economic and timely evaluation of the ecosystems affected by the exploitation of resources and urban expansion was observed. Using the classification of high-resolution images, valuable information has been obtained that contributes to estimating the changes in the mangroves and the surrounding ecosystems. The same information opens the possibility of studying the vulnerability of mangrove swamps to climate change.

During the course of this study, some lines of research and development emerged. Firstly, improvements in methods for controlling mangroves, and secondly, the applicability of these methods in other types of research, such as prospecting for carbon stocks in mangroves, agriculture and other ecosystems.

The methods developed in this work are applicable to other data sets and transferable to other studies, such as those spatial systems designed for monitoring over time. Ultimately, the widespread use of these methods in many of the plant ecosystems could offer new decision-making opportunities for monitoring and managing resources.

The maximum likelihood method can be used to process images acquired at high spatial resolution with other sensors in different areas. The implementation of this method is simple and fast. However, achieving the desired objectives requires the availability of masks in the cloud.

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Application of vegetation indices in the multitemporal analysis of changes in protected area coverage

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Introduction

Salamanca Island Parkway (VPIS) is referred to by the System of Natural National Parks of Colombia as a collection of islands formed by the accumulation of sediments from the mouth of the Magdalena river at the bottom of an old bay. These islands are connected to each other by small channels and they establish a barrier that separates the Great Swamp of Santa Marta from the Caribbean ocean. VPIS was declared as a Natural National Park in 1964 and before its categorization as a natural park it had suffered many environmental conflicts such as fires, exploitation of resources, illegal occupations, etc. One of the gravest conflicts, which left great impacts on the ecosystem, was the construction of the road which connects the cities Barranquilla and Santa Marta called the trans-Caribbean road. This finished between 1955 and 1956. This design and planning included a system of bridges referred to as “box-culverts” and connections with sewage that permit the continuous flow of water between the mangroves located at a side of the road. However, these links are not located in established sites, the installed quantities were not sufficient to supply the necessities of the ecosystem, and the direct pipes between the ocean and the Great Swamp of Santa Marta were closed. This affected the natural drainage of sediment and, in many sectors toward the central part of the park, prevented the flow of freshwater, hereby augmenting salinity and

promoting mortality among large extensions of mangroves, especially *Rhizophora mangle*, between the years 1976 and 1978 (Sánchez, 1988).

With the objective of evaluating the changes that occurred in the vegetative coverage of this protected area, a timeline was constructed with the most important and impactful events for this ecosystem since the 1940 until today. Additionally, by measuring aerial photographs, Landsat images, and the application of 7 vegetation indices in the program QGIS, a multitemporal analysis observing the behavior of these covers and their impacts on the ecosystem was created. The vegetation index that showed the behavior of these coverages in the greatest details was NDVI (Normalized Difference Vegetation Index), revealing that the deterioration was not constant throughout the whole area but that its recovery was seen to be affected by the obstruction of the natural freshwater pipes, leading to the proposal of projects to help such restoration manifest more efficiently. The development of these GIS tools in conjunction with the satellite imagery showed to be a great alternative for observing the deterioration and/or progress of these zones, which are in some cases difficult to monitor and with which help managers take appropriate action or improve their strategies to help restore the valuable protected area.

Mangroves

Mangroves pertain to tropical zones and are ecosystems composed of diverse communities of trees and shrubs, which develop in areas that are regularly flooded by tides and in turn present the capacity of developing in saltwater (Ramírez & Segovia, 2003). They are considered one of the five most productive ecological entities in the world because they provide a great quantity of goods and services; in addition, to the abundance of species of flora and fauna that are housed in their trunks, roots, and sediment (mud). The Ministry of the Environment and Sustainable Development affirms that Colombia has a mangrove extension of approximately 285,049 ha, distributed in 90,160 and 194,880 ha in the littoral zones of the Caribbean and Pacific, respectively.

The environmental services that mangrove ecosystems offer are of great relevance owing to the diverse action categories in which they are manifested. As for protection of the coasts, according to the WCMC (World Conservation Monitoring Centre), they can absorb up to 70 % of the energy of waves and winds; in turn, they protect the soil by trapping sediments on in their roots and preventing it from being dragged by the water of the ocean (Miththapala, 2008). This diminishes the impact that sediment transport has on coral reefs. Seen from the other sphere, mangrove ecosystems are important agents against global warming. For example, they capture and fix the CO₂ that, according to sources collected by Eong (1993), are approximately 25.5 million tons per year; and, at a more local context, thanks to their high rates of evapotranspiration, regulate the microclimate, sending humidity to the atmosphere, hereby diminishing temperatures in the zone.

For protection, management, and zonation of these ecosystems, the Colombian government established the Resolutions 1602 of 1995 and 020 of 1996. The first of these dictates the means of guaranteeing the sustainability of mangroves in Colombia through control and vigilance by the Autonomous Regional Corporation, support from the institute of marine and coastal research (INVEMAR), and the continuation and evaluation of its activities by the Ministry of the Environment and Sustainable Development; the second clarifies the erroneous interpretation that the Resolution 1602 of 1995 sought to ban all types of activities in the mangrove zone, contradicting the principal objective of the Ministry of the Environment, which is to bring an element of human sustainable development, adding to the allowed use of these areas (León & Puerto, 2017).

Salamanca Island Parkway

VPIS is a protected area pertaining to the System of Natural National Parks of Colombia, located between the municipalities of Pueblo Viejo and Sitio Nuevo in the department of Magdalena and whose geographic coordinates are 11°07'19"N 74°20'34"O and 10°53'07"N 74°51'00"O respectively (Balaguera & González, 2010).

VPIS consists of an extension of 56,200 ha and comprising part of the ecosystem of the lake complex that is the Great Swamp of Santa Marta. As a Biosphere Reserve and Ramsar Site, it is an important element for sustainable development at the international level (PNNC, 2017).

The Great Swamp of Santa Marta is limited by the Caribbean Sea, the new Clarín drainage, and the Magdalena river in sector in the North, East, South, and West directions, respectively. The climate of the zone counts on average temperatures of 27 °C, a maximum precipitation of 100 mm in October, from 400 mm at its Eastern extreme and 760 mm in the Western sector. Evapotranspiration is 1400 mm, indicating that the park has an arid climate with a water deficit. The zone presents matured brown soils above profiles with large quantities of clay, which are affected by groundwater with high concentrations of leaks, as well as the soils in the beaches and dunes, which are sandy. Those that are rich in organic material are presented in the mangrove zones and the terraces of Magdalena river (Sánchez, 1988).

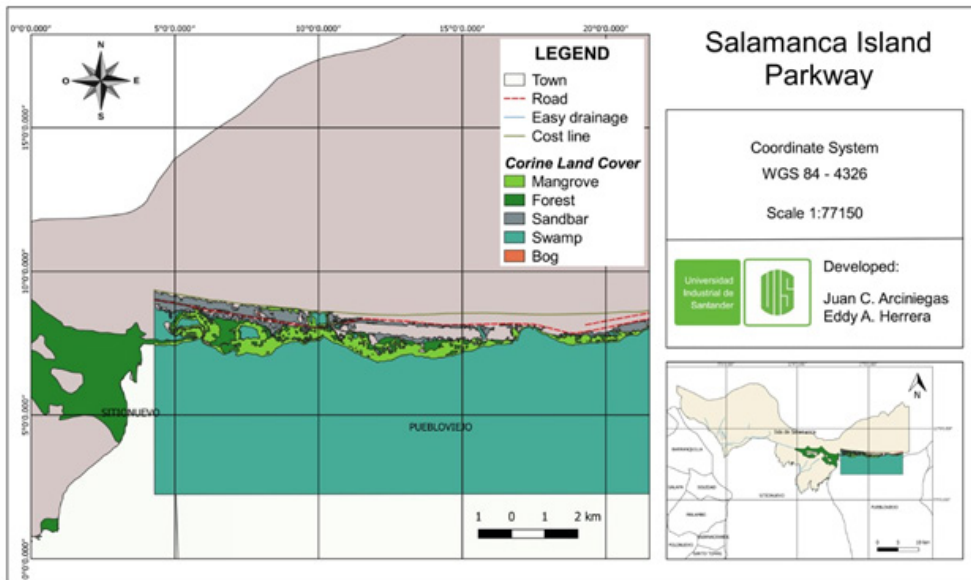


Figure 1. Map of Salamanca Island Parkway. Source: author's own elaboration.

The principal vegetative associations present in the park are: the mangrove forests composed of the species *Avicennia germinans* (back mangrove), *Laguncularia racemosa* (bobo mangrove), and *Conocarpus erectus* (Zaragoza mangrove), among others. They are located in the Eastern and Western sectors near the tunnels, swamps, and freshwater marshes; xerophytic forests located in the Northeast park of the island integrates them with species such as *Thespesia populnea* (clemón) and *Prosopis juliflora* (Trupillo), among others. In the banks and tributaries of the Magdalena river, there are mixed or riparian forests (PNNC, 2017).

PNNC (2017) announces that the faunal composition of the island comprises a great biodiversity of species such as crustaceans, mollusks, reptiles, amphibians, and mammals that use this ecosystem as their site for resting, feeding, and shelter. These results in fish and bird groups, some of which are year-round and others those are migratory.

Satellite Imagery and GIS Tools

In 1972 the ERTS-1, later called Landsat 1, the first satellite designed for the observation of high-resolution land coverage, was launched into space. This allowed for a great advancement in the analysis and evaluation of percent land coverage. Throughout the years, sensors were added to the satellites. These sensors add new information, such as vegetation coverage and its seasonal variation, dynamics of cloudiness, ocean surface temperatures, and large-scale events such as desert expansions, polar melting, and tropical deforestation, among others. This increases the knowledge of these phenomena and generates alternatives to endure each one of them.

Multitemporal analysis is one of the types of studies that employ satellite imagery to evaluate the changes occurring in land coverage. This is achieved by comparing various images or maps in different dates from the same place, splitting the selection and interpretation of the images according to the classification of observed coverages, and finally executing an analysis of the increase or decrease in these covers in their respective zone.

Vegetation Indices

A useful instrument for the development of these studies is vegetation indices, parameters calculated to establish perturbations to vegetative coverage according to the soil, atmospheric conditions, and spectral characterization. To obtain these parameters, free software was employed. This included QGIS, ArcGIS, gvSIG, GRASS and GIS because of their ease of use and the higher independence regarding informative licensing for their development and functioning.

Table 1. Vegetation indices

Group		Index	Characteristics
1	Background soil	NDBI	Suspicious to differentiate the soil from vegetation.
		OSAVI	Reduces the effect of the soil.
		LAI	Receptive to the reflection of the soil in the background.
2	Mid-infrared Energy Interaction with Water in Spongy Mesophyll Cells	NDWI	Sensitive to the quantity of water present in the vegetation.
		NDOO	Depends on the changes in the biomass and the hydrological stress of the vegetation.
3	Visible Light Interaction with Pigments in Palisade Mesophyll Cells	GARI	Depends on a wide range of chlorophyll concentrations.
4	Near-Infrared Energy Interaction with Spongy Mesophyll Cells	NDVI	Susceptible to the state of the chlorophyll and spongy cell contents.

Source: Abd and Smith (2017).

Since the selected dates previously covered a large time period, there is a change in the sensor of the Landsat images every two dates, such that it is necessary to pay attention to the spectral bands of each

group of images to correctly determine the vegetation indices. In Tables 2, 3, 4, and 5, the spectral bands are shown in accordance with each Landsat satellite employed in the study.

Table 2. Landsat 1-5 spectral band images

Band	Color	Wave Longitude
4	Green	0.5-0.6 μm
5	Red	0.6-0.7 μm
6	NIR 1	0.7-0.8 μm
7	NIR 2	0.8-1.1 μm

Source: author's own elaboration.

Table 3. Landsat 4 spectral band images

Band	Color	Wave Longitude
1	Blue	0.45-0.52 μm
2	Green	0.52-0.60 μm
3	Red	0.63-0.69 μm
4	NIR	0.76-0.90 μm
5	SWIR 1	1.55-1.75 μm
6	TIR	10.40-12.50 μm
7	SWIR 2	2.08-2 μm

Source: author's own elaboration.

Table 4. Landsat 7 spectral band images

Band	Color	Wave Longitude
1	Blue	0.45-0.52 μm
2	Green	0.52-0.60 μm
3	Red	0.63-0.69 μm

Band	Color	Wave Longitude
4	NIR	0.77-0.90 μm
5	SWIR	1.55-1.75 μm
6	Thermal infrared	10.40-12.50 μm
7	SWIR	2.08-2.35 μm
8	Pachromatic	0.52-0.90 μm

Source: author's own elaboration.

Table 5. Landsat 8 spectral band images

Band	Color	Wave Longitude
1	Coastal aerosol	0.43-0.45 μm
2	Blue	0.450-0.51 μm
3	Green	0.53-0.59 μm
4	Red	0.64-0.67 μm
5	NIR	0.85-0.88 μm
6	SWIR 1	1.57-1.65 μm
7	SWIR 2	2.11-2.29 μm
8	Panchromatic	0.50-0.68 μm
9	Cirus	1.36-1.38 μm
10	TIRS 1	10.6-11.19 μm
11	TIRS 2	11.5-12.51 μm

Source: author's own elaboration.

Normalized Difference Water Index (NDBI)

At first NDBI was designed with the objective of tracking the development and distribution of urban areas; however, it has been demonstrated to be very useful for isolating dry soil from soil with

vegetation through the MidIR bands (band 6 in Landsat 8) and NIR highly reflected by dry soil, in the following edition (Abd and Smith, 2017):

$$\text{NDBI} = \frac{\text{MidIRBand6} - \text{NIR}}{\text{MidIRBand6} + \text{NIR}}$$

Optimized Soil Adjusted Vegetation Index (OSAVI)

The OSAVI uses the red bands and near-infrared (NIR) to diminish the influence that the reflectance of the soil possesses over the variations of chlorophyll content in a community or ecosystem. Abd and Smith (2017) created the following equation for its measurement:

$$\text{OSAVI} = \frac{1.5 * (\text{NIR} - \text{RED})}{(\text{NIR} + \text{RED} + 0.16)}$$

Leaf Area Index (LAI)

The LAI determines the proportion of direct solar radiation that enters the canopy of a stand or forest related to the density of the current of light under the coverage (L_c) and the density of the current of light outside the coverage (L_o), such as is observed in the equation designed by English, Wilkinson and Baker (1997), where the zenith angle of the sun (θ) and the coefficient of the extinction of the canopy light (K) ($K = 0.525$ for mangrove stands) are considered (Green et al., 2000):

$$\text{LAI} = \frac{\log \log e \left(\frac{L_c}{L_o} \right)}{K} * \cos \theta$$

Abd and Smith (2017) creates two steps for the calculation of this index, the first of these found the enhanced vegetation index

(EVI) which is sensible in regions with high biomass, diminishing the influence of the soil and the atmosphere above the vegetation. It is determined by the following formula:

$$EVI = 2.5 * \frac{(NIR - RED)}{(NIR + 6 * RED - 7.5 * BLUE + 1)}$$

After this, late-based determination is made by the following equation devised by Boegh et al. (2002):

$$LAI = 3.618 * EVI - 0.118 > 0$$

Normalized Difference Water Index (NDWI)

The NDWI is called as such because it perceives the changes of water content and vegetation coverage (Gao, 1996). The reflection of the water in the green band and NIR allows the maximization and reduction, respectively. Also, it is used in conjunction with the normalized difference vegetation index (NDVI), which makes it more efficient to distinguish bodies of water from wet lands and vegetation. NDWI is calculated in the following manner (Abd and Smith, 2017):

$$NDWI = \frac{GREEN - NIR}{GREEN + NIR}$$

Normalized Difference Infrared Index (NDII)

Sriwongsitanon et al. (2016) describes NDII as the most effective to determine the magnitude of water accumulation in the radicular zone when there is a deficit of humidity. Because of this, it is a great

indicator for the valuation of drought. NDII was designed with the objective to measure humidity content of vegetation and achieve the categorization of dry soil with bodies of water. Its measurement uses bands 5 and 6 of Landsat 8 (Abd and Smith, 2017):

Normalized Difference Vegetation Index (NDVI)

The NDVI is named as such because it evaluates the energy that the different elements of the terrestrial coverage absorbs and emits, revealing data about the quantity and vitality of vegetation. Its values oscillate between -1 and +1, showing the bodies of water and naked soils with values less than 0.1 and values higher for communities with higher levels of photosynthesis (Meneses, 2012). Abd and Smith (2017) present a formula for its estimation, dividing the difference between near-infrared (NIR) and red light (Red) between the sum of the same elements, as follows:

$$\text{NDVI} = \frac{\text{NIR} - \text{Red}}{\text{NIR} + \text{Red}}$$

The number of hectares pertaining to each coverage was determined through the vectorization of the NDVI from 2000 to 2019 because the shape used for the five ranges was determined to cover the values of this index. This analysis established the land coverage through the CORINE Land Cover methodology adapted for Colombia.

The Case of Salamanca Island Parkway

Following the dates of the most important events that were crucial in the history and development of VPIS in its ecological aspect, changes that occurred in the present coverage of this ecosystem, and the vitality of these changes, were able to be detected with respect to the group of images of the vegetation indices.

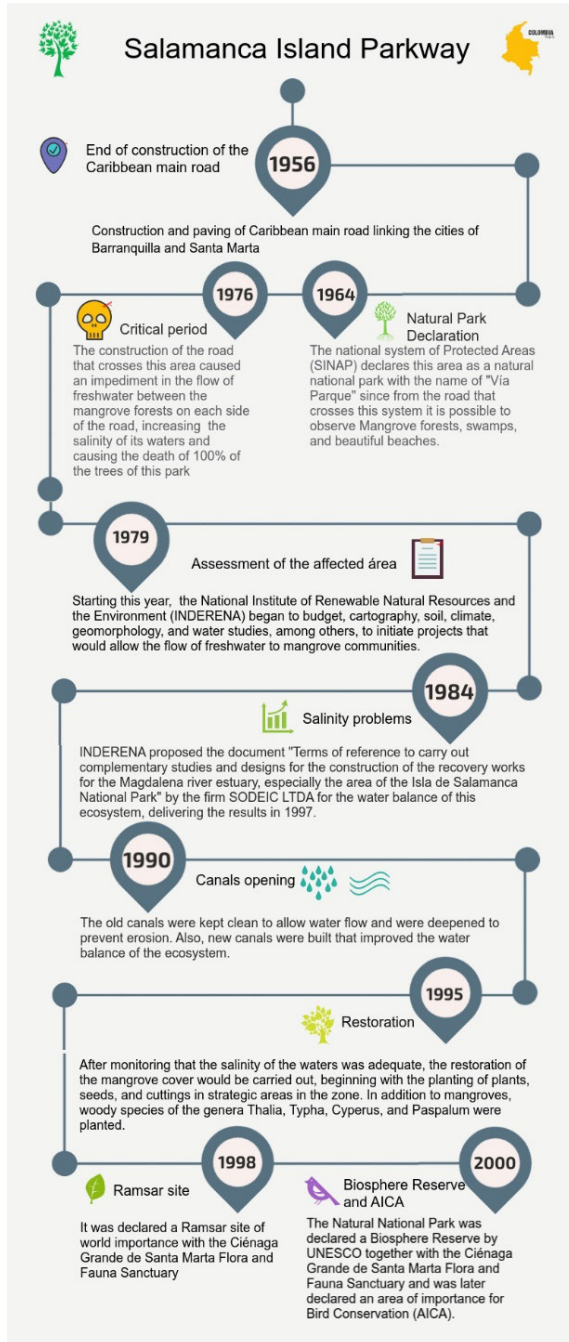


Figure 2. Timeline of Salamanca Island Parkway. Source: VPIS.

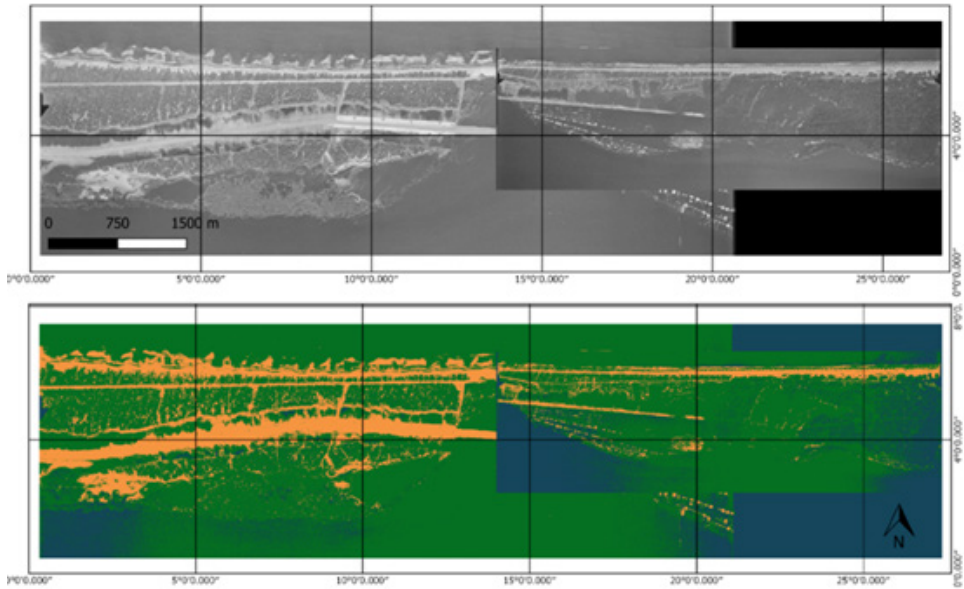


Figure 3. Initial State Map of Salamanca Island Parkway. Source: VPIS.

Through aerial photographs from 1938, it can be observed that three coverages were able to increase in Figure 3 in three different colors. The green color selects the areas with abundant, dense vegetation, light orange determines bare soils and/or those with little vegetation, and blue grouped areas without data that did not pertain to the extension of the protected area. This, shown atypical values between the second and third color due to the similar ranges that presented the pixels of both coverages.

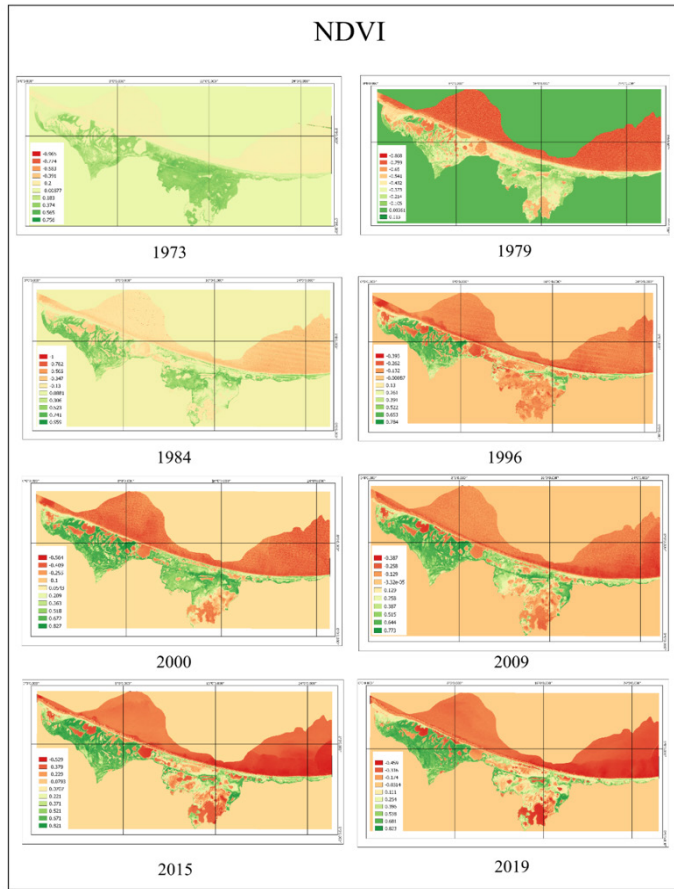


Figure 4. NDVI. Source: author's own elaboration.

The NDVI shows coverages lacking vegetation with values lower than 1 and those with high photosynthesis with values close to 1. It can be appreciated that in 1938 the protected area presented a great extension and good state of mangrove coverage, as well as that of other vegetation types, because of the low anthropogenic intervention that existed. The values for this year were between -0.391 and -0.2 (red tones) for soils without vegetation and ranged between 0.183 and 0.756 (green tones) for soils with vegetation (Figure 3). It continued in this manner until 1973, almost 20 years later at the end of the construction of trans-Caribbean road (Figure 4).

In 1979 the representative values of photosynthetic activity notably decreased to the range between 0.00361 and 0.0113, hereby showing the consequences of the crisis of the loss of almost 100 % of the mangrove canopy, caused by the obstruction of freshwater, which began in 1976 and climaxed in 1978.

Despite the problems of salinity that the park was presented in 1984, there still existed small stretches of vigorous vegetation with a value of 0.741, distributed throughout the park but most concentrated in the western and southeastern zones (the limits of CGSM). The prevailing ranges between -0.13 and 0.306 indicate little photosynthetic activity.

In 1996, six years after the reestablishment of freshwater flow to the affected communities and one year of mangrove restoration, a large frequency of vegetation in the range between 0.391 and 0.653 appeared. Small, dispersed stains with a high photosynthetic activity (a value of 0.784), allows the deduction that the vegetative coverage of the park was undergoing a strong recovery even though it was still presenting a moderate extension in the range between -0.132 and -0.393. These values close to zero, allow the interpretation that the zones were not completely devoid of vegetation and are now not very worrying.

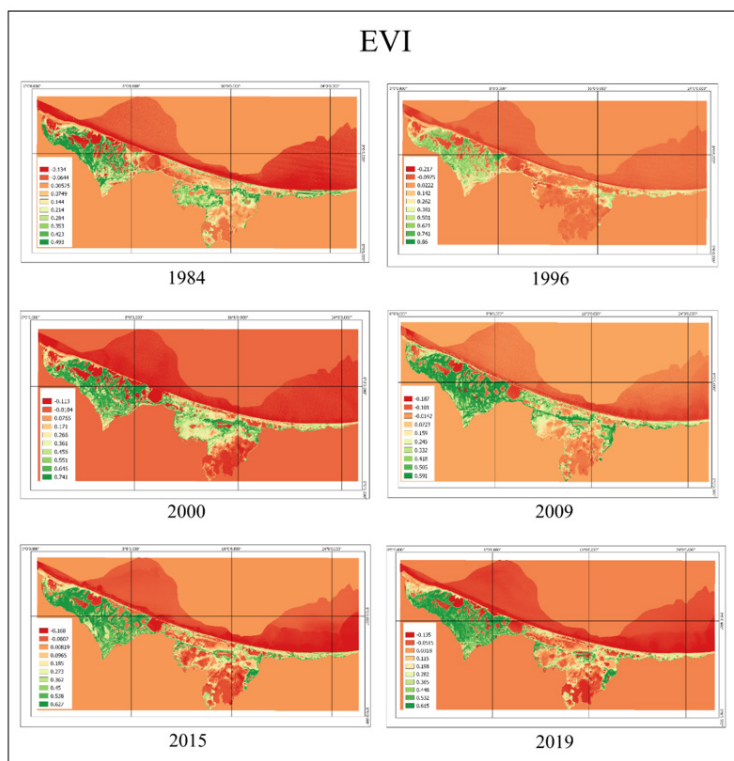


Figure 5. EVI. Source: author's own elaboration.

From 2000, the great majority of vegetative coverage showed extension and vitality, with ranges approximately between 0.38 and 0.83, remaining consistent until today but presenting low values in 2009 (0.258 to 0.73) because of the consequences that brought the phenomenon of El Niño in the Caribbean zone and at the national level. These values are supported by the results produced in the EVI, where it was noticed that there was a behavior similar to NDVI with a range between 0.36 and 0.62 for the vegetation and a decrease in 2009 (Figure 5).

The data produced by OSAVI in 1979 (Figure 6) show a greater frequency of values that signal a soil coverage, with a maximum value of -0.722 and a lower range for the vegetation (between 0.0277 and

0.121). Since 2000 there was an observed increase of the maximum value for the vegetation from 0.96 that constantly continuing until today, in this way showing a correlation with the aforementioned NDVI values.

NDWI describes the movement of the bodies of fresh and salt water in the park with green zones, with a great extension of this element until 1973 (Figure 7) and a decrease in the affected zone through the trans-Caribbean road until 1996, where there a constant increase in the expansion of this element was noted until today.

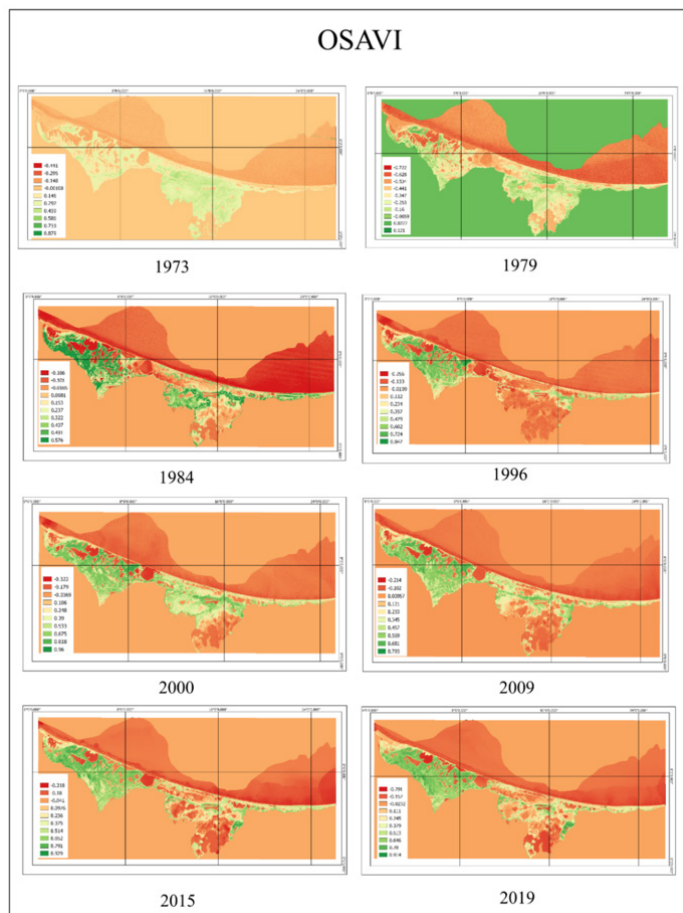


Figure 6. OSAVI. Source: author's own elaboration.

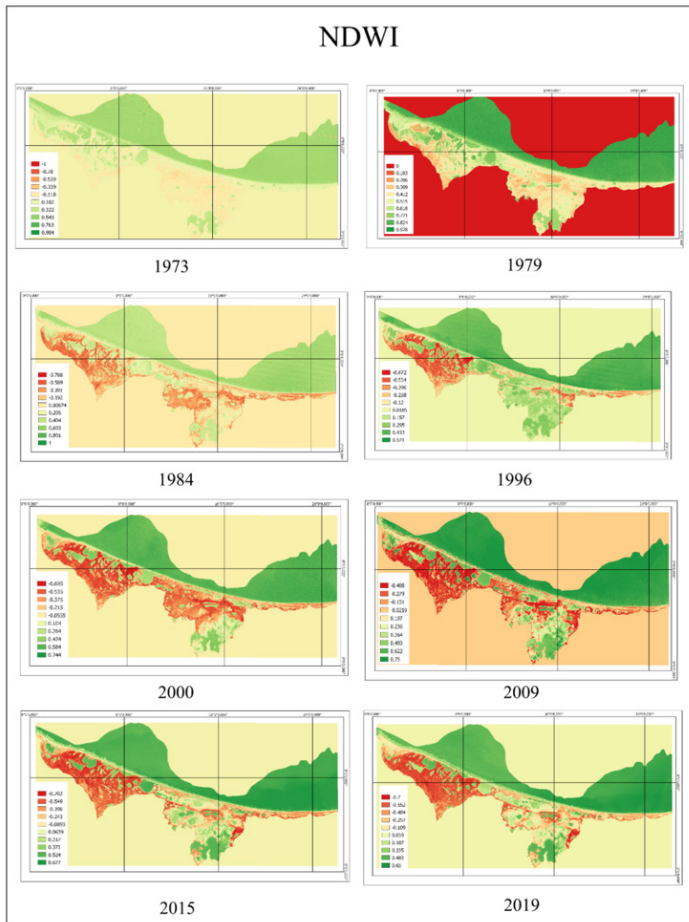


Figure 7. NDWI. Source: author's own elaboration.

NDII and NDBI show the actual state of the coverages of the natural park, differentiating the soil with red tones from the bodies of water in green colors (NDII) and showing an inverse relationship in the colors characterizing the soil and the vegetation with green and reds, respectively (NDBI). With these indices, it can be observed that the presence of arid soils is much reduced and the largest part is found at the border of the road which crosses through the park. The concentrations of relative humidity present between the canopy and its vegetative coverage are consistent in the whole protected area.

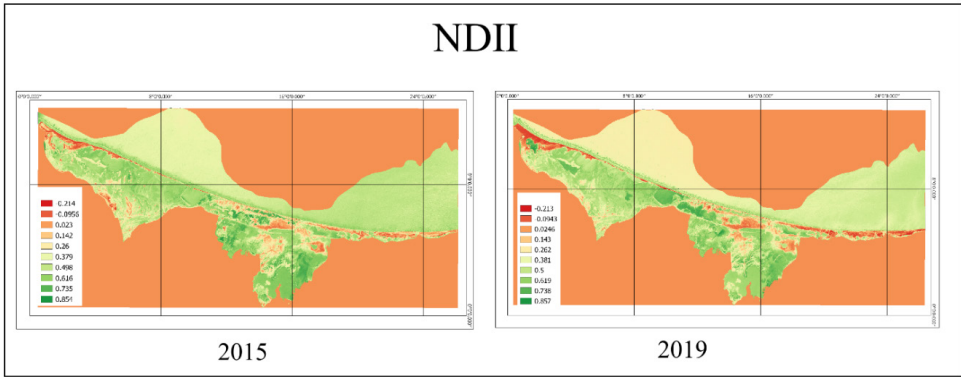


Figure 8. NDII. Source: author's own elaboration.

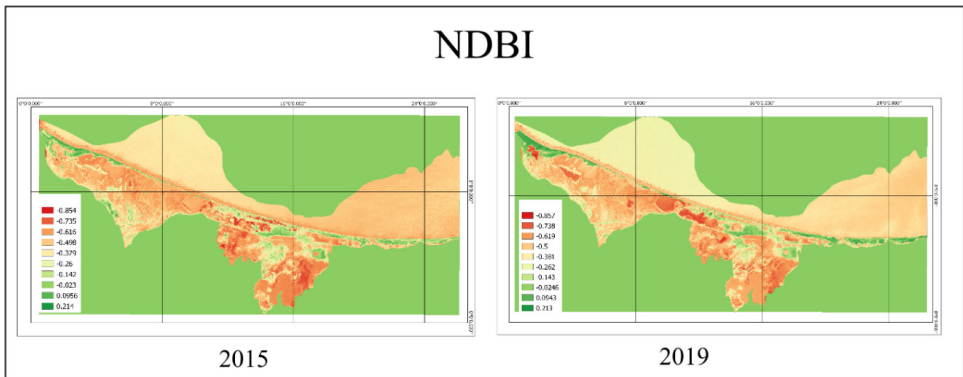


Figure 9. NDBI. Source: author's own elaboration.

To measure and analyze the capacity of light capture that enters the canopy and hereby estimate the capacity that the ecosystem has to regenerate and offer an opportunity for the seedling and sapling stages to grow and generate new shoots, LAI was employed. There was an increased production of photosynthesis of both mangrove forest and other vegetation types (Figure 10), which showed a great likeness to the distribution of high photosynthetic activity in the park represented by the NDVI. This revealed great improvements in the

distribution of present vegetative coverages and in turn demonstrated that it is an ecosystem with the capacity to recover and establish itself as a healthy community in constant growth.

Finally, the ranges were classified in five classes produced by NDVI. A vegetative coverage following the CORINE Land Cover (Figure 12) was assigned to each of these.

Table 6. Salamanca Island Parkway coverages

Class	Range	VPIS Coverage
1	≤ -0.583	Natural sandy areas
2	-0.582 a -0.2	Naked and degraded land
3	-0.19 a 0.183	Fragmented forest with secondary vegetation
4	0.184 a 0.565	Tall, open, flooded forest
5	≥ 0.566	Tall, dense mangrove

Source: author's own elaboration.

Since the values provided by NDVI for each year presented significant variations, it was necessary to unify it in five ranges, with the goal of achieving a correct comparison of the behavior of the coverages of VPIS. Keeping in mind that the specifications of the vegetation index that signals areas devoid of vegetation with values close to -1 and areas with vigorous vegetation as close to 1, the most appropriate ranges for representing the present vegetation in the natural park were chosen. These were determined in accordance with those observed in the aerial photographs, vegetation indices, secondary information, and announcements, according to the CORINE Land Cover methodology.

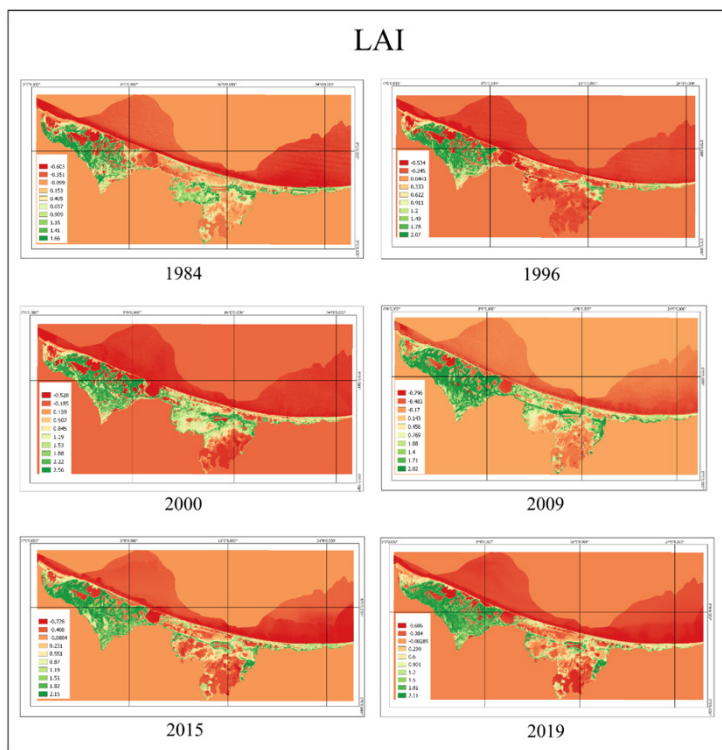


Figure 10. LAI. Source: author's own elaboration.

Table 7. Areas of the coverage of Salamanca Island Park

VPIS Coverage	2000 (ha)	2009 (ha)	2015 (ha)	2019 (ha)
Natural sandy areas	2,583.38	2,092.891	31,726.5	34,563.8
Naked and degraded lands	32,588.744	13,164.834	34,241.893	31,978.531
Fragmented forest with secondary vegetation	5,208.577	24,020.057	6,409.429	8,455.092
Tall, open, flooded forest	8,610.254	8,726.707	6,623.754	6,579.129
Tall, dense mangrove	9,534.087	8,195.512	8,607.659	8,841.610
Total	56,200 ha			

Source: author's own elaboration.

Therefore, the values smaller than or equal to -0.583 were referred to as naturally sandy zones. These are comprised of sandy and stony soils and of those which make part of the littoral beaches, river beaches, banks the sand of rivers, fields, and dunes. Their maximum areas were presented in 2009 with 2,092.891 ha and diminished by more than 80 % to today with 345,638 ha. The coverage assigned in the range from -0.582 to -0.2 represents terrain devoid of or with little vegetation because of anthropogenic or natural processes. This is referred to in the CORINE Land Cover key as naked and degraded lands; its coverage experienced a reduction of its area by 619,213, with 2009 being the lowest point at 13,164.834 ha. Fragmented forests, with secondary vegetation, was the coverage determined in the range from -0.19 to 0.183 because it comprises natural forests in recovery that maintain their original structure. These showed an increase in area from 3,246.515 ha. For Class 4, with a range between 0.184 and 0.565, the coverage of the tall, open, flooded forest was established. The canopy of this class comprises between 30 and 70 % of the total area, with individuals greater than 15 m in height located next to bodies of water and revealing a constant depreciation in its area of 2,031.125 ha. Lastly, the a range greater than 0.566 was designated as a tall, dense mangrove forest that presents a canopy of greater than 70 % of the total area and its individuals, which are taller than 15 m. These forests are found close to the bodies of water, showing a small area of 692,477 ha.

However, although the area that is almost devoid of vegetation represents the greatest area in the park, the area covered by vegetation represents 42.48 % of its total area and shows a growing behavior, allowing the inferences that the mangrove vegetation restoration process had positive results and that the ecosystem recovered its dynamic of growth and development (Table 7).

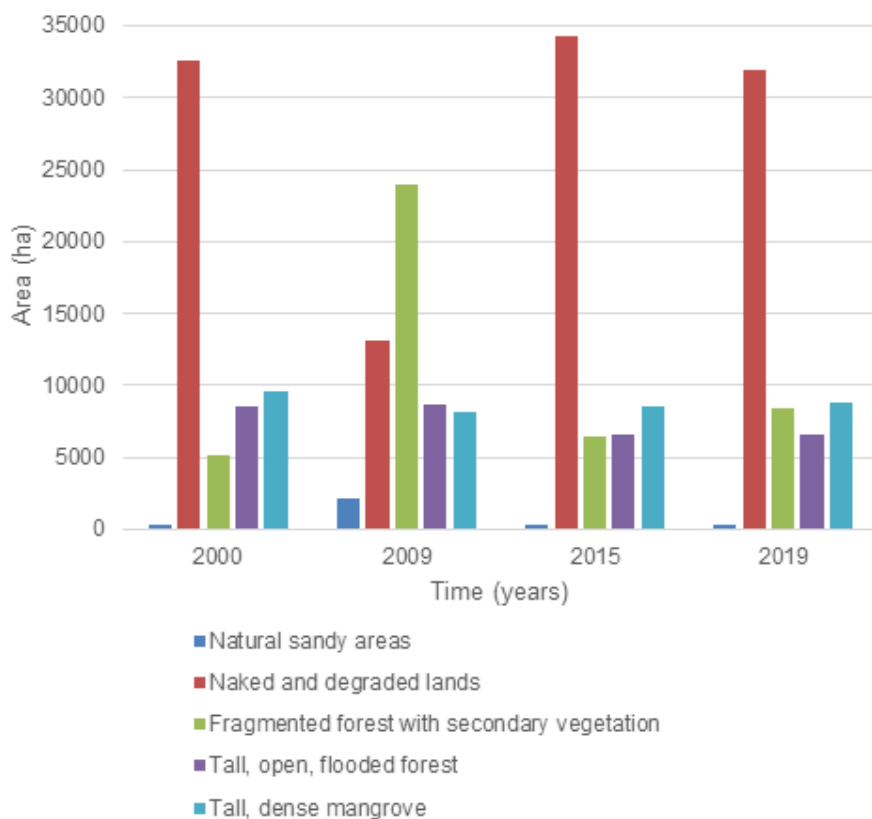


Figure 11. Behavior of the coverage of VPIS in the period of 2000-2019. Source: author's own elaboration.

The drastic change that is presented in 2009 with the increase in the area of fragmented forest with secondary vegetation can be interpreted as an effect of El Niño and La Niña phenomena. These presented succeeding hydrological deficits, prolonged flooding, and the loss and/or accumulation of these zones, as reflected in the 24,020 ha of the first year and only 5,208 ha in the second.

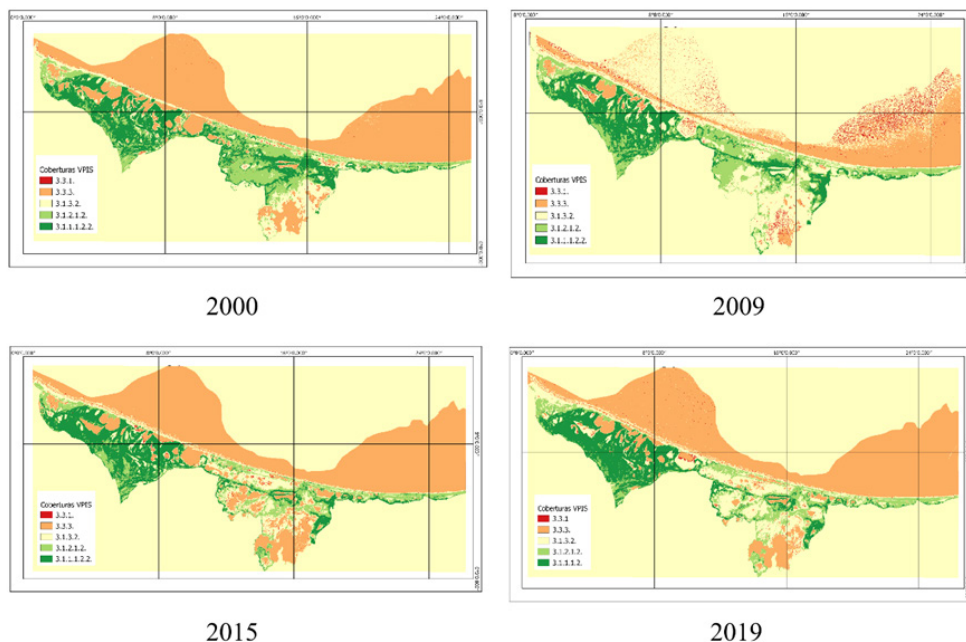


Figure 12. Coverages of VPIS. Source: author's own elaboration.

Díaz (2018) completed a multitemporal analysis in 2018 to observe the transformation of the vegetation coverages affected by the Ciénaga-Barranquilla stretch of the trans-Caribbean road through the use of aerial photographs (1953, 1966) and satellite images (1973, 1985, 1996, 2007, 2016). This was accomplished through combinations of bands (natural color and False color RGB 532) in order to observe the structures and coverages that are found in the zone with detail.

With the help of the NDVI analysis, principal components, and the Tasseled cap transformation (TTC) obtained similar results to this study, indicating that the deterioration of vegetative coverage was scarce in 1973. Additionally, a strong distinction between the bodies of water and the vegetation was able to be accomplished. The vegetation only presented a by hydrological stress until 185 and deteriorated until 2007 where a process of recovery and vitality began that lasted until 2016.

With regard to the little differentiation of the bare soils between 1996 and 2007, it concluded that it ought to be because the soil was covered by the shadow of the existing mangrove forest canopy and that the deterioration varied according to the distance at which vegetation was found from bodies of water.

Bayona (2016) represented the behavior of mangrove ecosystems located near the Mallorquin Swamp in the department of the Atlantic over the last 30 years using satellite imagery and completing an analysis with NDVI and the RGB 453 color, affirming that it is one of the best combinations that visually interpretes mangrove coverage. This analysis identifies mangroves with dark orange tones, the rest of the vegetation with clear orange, and the rocky outcrops in green. They classified 10 categories of coverage, finding that there was the largest presence of pastures and cultivations in 1991 and that these were found on the left zone and on the other side of the open forests, mangroves, and swamps. For 2001 the coverage changed, the pastures and cultivations decreased in extension and there was an increase in the open forests and mangroves. Both the investigation in question and the one previously explained provide evidence of the occurred ecosystem changes through the application of NDVI, highlighting their recovery during the last years of analysis in which the extent of intervened areas were noticeably reduced by anthropogenic activities and in turn increased the mangrove and forest areas of coverage.

Conclusions

Taking into account the results of the vegetation indices in terms of the changes occurred in the coverage of VPIS by the influence of the trans-Caribbean road, it was discovered that the loss and recovery of vegetation was best represented by NDVI now that there is a clear distinction between the areas that present vegetation and is supported by NDWI and OSAVI to identify the bodies of water of the soils devoid of vegetation.

In this way it is concluded that the loss of coverage in the protected area did not present a consistent behavior. At some points in time the same ecosystem was slowly recovering and the help of the Natural National Park was significant in promoting the regeneration and growth of these communities. However, the current difficulty of constant blockages in the canals between the fresh and saltwater cause other problems such as fires, overexploitation of animal species, expansion projects of roads and port areas, and scares resources for restoration and protection projects (PNNC, 2015). This puts a check on this unique and important habitat by fostering a new crisis. As such, it is necessary to have more control of the pertinent entities that carry out activities in the park and promote projects that promote projects that solve the most crucial setbacks and prioritize the proper development of this ecosystem.

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Mangrove restoration is more than reforestation

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Introduction

Since mangrove ecosystems are located in tropical and subtropical coastlines, they are considered among the most valuable ecosystems globally. They provide a number of ecosystem services such as protection from natural disasters (typhoons, hurricanes, and tsunamis), nutrient sequestration, and a high availability of organic material (Walters et al., 2008; Polidoro et al., 2010; Alongi, 2011; Duke et al., 2007). With respect to carbon (C), they are able to sequester up to 1023 Mg C ha⁻¹, representing two to three times more than terrestrial forests (Adame et al., 2013; Donato et al., 2011; Kauffman et al., 2011). In addition, they are the economic foundations of some coastal areas that provide at least 1.6 billion USD per year in ecosystem services at the global scale (Costanza et al., 1997; Field et al., 1998; Polidoro et al., 2010).

Despite their importance, these ecosystems have been reduced by 35 % of their total area between 1980 and 2005, surpassing the destruction percentages of both other tropical forests and coral reefs (Valiela, Bowen and York, 2001). Therefore, there is an urgent need to take into consideration restoration methods to restore these ecosystems. Although mangrove restoration projects have increased globally over the past few years, the success of these efforts remains unknown. It has been observed that the lack of success of

restoration projects owed to: insufficient information, inadequate restoration methods, a failure to involve local communities, and not following experiences already written in the literature. It has been noted that the principal restoration action that is carried out on a global level is reforestation, demonstrating that mangrove restoration maintains a focus on trees that does not include ecosystem nor multidisciplinary perspectives.

Strategies, actions, and costs of restoration

With the goal of knowing the strategies, actions, and costs of restoration projects at the global level, this literature review considers scientific articles, reports, and theses, from 1980 to 2015. Discreet information was extracted from these documents in Table 1, which includes details about actions, methods, and evaluation. Measurements of the structural and functional components, as well as the costs of American restoration projects, were extracted in the ecological category.

Table 1. Characteristics used to classify the registered documents from the literature

Classification characteristics	
Type of document	Type of document (reviews, projects, thesis, grey literature), year of publication
Characteristics of the restoration project	Country, continent, type of degradation, methods used for restoration
Evaluation/ monitoring methodology	Restoration years, use of reference site, evaluated attributes (hydrology, macro/ micro invertebrates), plants (structure/ function), biochemical processes
Costs	Total cost of the restoration projects

Source: author's own elaboration.

With the objective to know the time required to determine the success of the restoration, only documents that contain data of the restored and the reference sites were considered. From the obtained data, the rate of response was calculated with the formula from Hedges, Gurevitch and Curtis (1999), which indicates that a value closer to zero is more similar to the reference site. With respect to the costs, these were standardized to 2015 values.

The restoration projects had been carried out in 43 countries, with Asia containing the highest number of documents (143) of any continent and China the highest number of any country. In America (North America, Central America, South America, and the Caribbean), Mexico presents the highest number of documents, followed by the United States. Despite the fact that Africa is the continent with the second greatest extension of mangroves, this fact is not reflected in the number of documents. Furthermore, Oceania registered the lowest number of projects (11).

However, 95% of the documents do not include a social, economic, and ecological component. The restoration action that has been considered for the past 50 years until 2015 is reforestation following afforestation (planting mangroves in zones where previously they did not exist or where there is no knowledge of their prior existence). Since 2011, restoration projects have also included topographic modification, with the goal of cushioning the level of inundation in flood zones (Figure 1).



Figure 1. Restoration projects in the zone. Source: author's own elaboration.

The recuperation of the different components, such as biochemical processes, to the conditions of the reference sites tends to stabilize over the years. While biological structure is the most evaluated component, the macro and micro invertebrates in these ecosystems are the least commonly evaluated. The hydrological component becomes closer to the reference conditions two years after the execution of the restoration actions. It was observed that the communities of macro invertebrates and vertebrates (abundance, density, species richness) exhibits a relatively high value in the restored sites compares to those of the reference immediately (1 year) after the restoration actions. With respect to the structural variables of the restored mangrove individuals (litter-fall, root productivity, photosynthesis), it is observed that the structural characteristics are similar to the reference site 10 years after the restoration action and progressively increase until 15 years. At this time the average observations indicate that the structural values of the restored mangrove plants and the reference are similar, although there is a large amount of spread in the data (variability from one to another). Additionally, the trend cannot be distinguished because there is only information from sites 45 years before restoration. Even though the physio-chemical variables of the interstitial water (salinity, pH, redox potential) are important variables for these ecosystems, they are rarely measured, which is reflected in the low quantity of registered data (330).

Regarding the costs of restoration projects, there is a great variability between the cost and the restored hectares. It is worth mentioning that the results integrate the total costs of the restoration projects that include labor wages, restoration actions (reforestation and/or hydrological rehabilitation), construction of nurseries in some cases, etc. Data were only obtained from projects conducted in Asia and America (North America and South America). The cost of restoration actions in America varies between 100\$ and 176,000 by ha^{-1} . This variability between the restoration costs is related to factors such as the cost of labor and the type of restoration action.

The results presented here demonstrate that restoration in mangrove ecosystems, as well as the reporting of data about restoration results, has notably increased during the recent decades. Even though there is an increase in projects, these cannot be considered strategies that involve a social, economic, and ecological component. The planting or reforestation of mangroves is considered the principal restoration action that was executed until 2015 (Field, 1996; Ellison, 2000; Alongi, 2002). This action has been conducted at the global scale, but has led to the creation of monocultures that use few species. For example, *Rhizophora apiculata*, *Rhizophora mucronata* and *Sonneratia apetala* are principally used in Asia, whereas *Rhizophora mangle* and *Avicennia germinans* have been widely used in America (Tomlinson, 1999; Ellison, 2000; Mckee and Faulkner, 2000; Alongi, 2002). Other frequent restoration actions consist of topographic modification and the construction of barriers to cushion the force of wave action, as well as opening canals.

As more plantings take place, the evaluation of success of restoration is based on measurements of structural variables such as height and density (Bosire et al., 2008; Walters, 2000). In contrast, the results of this review indicate that, in general, to achieve values similar to reference sites, the time that ought to transpire from the time of the restoration action, with respect to the structural and functional characteristics of the mangroves, is from 10 to 15 years. This demonstrates a wide variety in recovery responses, such that detailed future studies testing the factors that impose this variability would be interesting.

The biological component referring to the macro invertebrates/vertebrates presents a positive response a year after completing the restoration actions. This represents a different behavior from other restored wetlands, in which vertebrates only achieve or succeed the reference site after five years, whereas macro-invertebrates are not similar to the reference site even after 20 years (Moreno-Mateos et al., 2012).

The physiochemical component of the interstitial water is rarely measured in restoration projects, but our results indicate that, similarly with the hydro-period, this factor responds quickly after restoration actions. Regarding the restoration cost of mangroves, there is a logical tendency to augment the costs according to the size of the restored area. This is seen most clearly in the cases of America, where other authors mention that mangrove restoration presents a cost between 1,000\$ and 100,000 by ha⁻¹ (Bayraktarov et al., 2016), and others range from 3,000\$ and 510,000 by ha⁻¹ (Spurgeon, 1998). This variability in costs depends on many components, such as the cost of labor in each country, as well as the type of restoration action, because reforestation projects that include hydrological rehabilitation are more expensive.

Mangrove ecosystem restoration requires the integration of distinct stakeholders (government institutions, NGO, civil society, academics, and funders). Additionally, this must be executed at different scales. Nevertheless, mangrove restoration efforts at the global level offer opportunities to address scientific questions of specific lines of investigation at the population, community, and ecosystem scales. There continues to exist important holes in the information in mangrove restoration projects, such as: (i) information about the trajectory of mangrove recovery at the regional or landscape scale, (ii) details of the secondary succession process beyond the recovery of the tree community, (iii) evaluation of restoration as a means of climate change mitigation, (iv) integrated indicators of restoration and monitoring over time, and (v) detailed social and economic evaluations.

Conclusions

Even though many mangrove restoration projects have been executed at the global level, the success of restoration remains unknown, owing to the lack of evaluation and dissemination of information. Mangrove restoration projects are seen as planting. This, the most commonly executed restoration action in the world, has demonstrated that achieving similar values to the reference sites,

with respect to structural characteristics, requires 10 to 15. However, there are other components, such as biodiversity of the site, that respond with less time to restoration actions.

The least evaluated component of mangrove restoration projects is hydrology, and this is one of the most important because it influences processes that range from the growth of an individual plant to the development of a biological community and the landscape aspects of a mangrove forest. Restoration projects generally do not take an ecosystem perspective, due to the fact that they are viewed as mere plantings. This activity, which is more expensive than other restoration actions, does not consider the regulatory factors of environmental conditions, hence the habitual failure of mangrove restoration.

The little or nonexistent success of restoration projects is due to: insufficient information about the causes of death or degradation of mangroves, inadequate methodology of restoration actions, lack of involvement of local communities, and failure to follow the experiences already written in the literature. Therefore, the actors that work in ecological restoration of mangroves ought to base projects in the ecology of ecosystem restoration, hereby understanding and applying basic concepts that permit the evaluation of patterns and processes of restored mangroves.

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IV

Evaluation of the damage caused by *Coccotrypes rhizophorae* (Coleoptera: Curculionidae: Scolytinae) in propagulos of *Rhizophora* genus in the Gulf of Guayaquil

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Introduction

Mangroves grow in the coastal zones of tropical and subtropical regions, and are restricted to the edges of sandy or clay-like sediment or bays, lagoons, ocean canals, estuaries, bars of sand or mud, and marshes, such they cover a width from a few meters to hundreds of hectares (Flores-Verdugo et al., 2003). This ecosystem is considered to be the highest primary producer, surpassing rainforests and exceeding the productivity of the ocean by a factor of 20 (Flores-Verdugo, 1989). Mangroves perform a fundamental role in the carbon cycle by sequestering CO₂ and serving as a source of ocean carbon, as well as helping to protect the coasts from storm surges, tsunamis, and hurricanes. At the socioeconomic level, they provide communities with wood and other resources (Uribe & Urrego, 2009). Moreover, larvae, fish, and crustaceans use mangroves as habitat for their protection and food (Díaz, 2011).

In spite of their importance at the global level, mangroves have been progressively disappearing due to a variety of causes. According to the FAO (2007), mangroves covered a global area of approximately

18.8 million hectares in 1980, which had been reduced to 15.5 million hectares by 2005. This loss of 3.6 million hectares is equivalent to a 20 % loss in the full extent of these ecosystems. Statistics indicate that mangroves are disappearing at rates of 1-2 % annually, a speed equal to or greater than the extinction of coral reefs and tropical forests (Uribe & Urrego, 2009).

Human activities generate threats and dangers to these ecosystems (Yáñez, Twiley & Lara, 1998) and include the following: road construction, human settlements, fragmentation and elimination of forests, felling or extraction of wood and increased allocation of land for cultivation and ranching. Consequently, mangroves are restricted to a thin strip by the ocean, which eliminates organic material and augments salinity (Cruz & Pérez, 2017). In order to reverse the damage caused to the mangroves at the global level, it is necessary to utilize distinct methods such as restoration, rehabilitation, and reforestation; as such, González-Maya et al. (2012) cites that the implementation of ecological restoration projects with a foundation in reforestation programs in tropical countries has been the most applied method in Latin America. Nevertheless, the realized scope of these achievements remains unknown. The natural regeneration of mangroves is fundamental, granting that distinct species persist for a long time. This continuation and resistance depends on the production and dispersal of the propagules for the successful establishment of plants. However, mangroves are frequently attacked by harmful predators such as insects in their developmental stage (Hoyos, Urrego & Lema, 2012), thus hindering restoration efforts.

One of the insect parasites that infect the propagules of the genera *Rhizophora* is *Coccotrypes rhizophorae*, pertaining to the order Cleópters, family Curculionidae, and subfamily Scolytinae. This infestation is especially prominent when the plants suffer environmental stress due to anthropogenic activities, as well as environmental factors such as inundation or drought (Menéndez, Guzmán & Núñez, 2006). The first studies of this insect come from a case in Cuba in 1972, where two species that attacked *Rhizophora mangle*

where identified: *C. rhizophorae* and *Pityophthorus* sp. In Mexico, insect capture was executed in the mangroves and the presence of *C. rhizophorae* was detected (Gerónimo et al., 2015). Recently, in Tumulco, Veracruz, a number of insect-bored propagules were identified and damage in the basal, medial, and apical areas was observed, also due to climatic conditions (Martínez-Zacarías et al., 2017).

Mangroves in Ecuador share their Pacific coast location with Colombia and Northern Peru, and are composed of 13 species, including: true mangroves (5), minor mangroves (2), and facultative mangroves (6) (Cornejo, 2014). Between 1968 and 2006 Ecuador lost 55,738.77 hectares of mangrove (27 %), but there was an extension to 148,230.23 mangroves since 2006 (Cornejo, 2014; Molina-Moreira, Lavayen-Tamayo & Fabara-Suárez, 2015). The Ministry of the Environment in 2018 registered 161,835.03 hectares, such that Ecuador is one of the countries where mangroves have been recovering because of ecological restoration processes. Mangroves are considered a priority ecosystem for conservation in Ecuador. Ecuador is one of the 130 countries in the Ramsar Convention, which is the principal means of cooperation that supports the protection and adaptation of wetlands (Astrálaga, 2006). The Organic Law of the Conservation and Restoration of Mangroves Ecosystems was implemented in 2011. In terms of strategies, since 1999, the Program of Coastal Resource Management has implemented concessions for ancestral users of mangroves to ensure their sustainable management (Freile, 2010). About 68,000 hectares of mangrove have been granted to the benefit of 7000 users. Based on a diagnosis of existing mangroves, ecological restoration has been implemented with five mangrove species since 2000 in Parque Histórico Guayaquil; there was a 17,000 m² extension of *R. mangle* and *R. harrisonii*, the presence of *C. rhizophorae* has been observed, and an evaluation of the harm cause by insects to the propagules was recommended (Molina et al., 2000; Molina & Molina, 2005; Mendoza & Molina-Moreira, 2015; Cusme & Molina-Moreira, 2018).

One of the problems posed to the restoration of mangroves in Ecuador is the lack of knowledge about the harm that *C. rhizophorae*

is causing to the direct sowing of propagules and in the mangrove nurseries of *Rhizophora*. Thus, this investigation presents an “Evaluation of the harm caused by *C. rhizophorae* in propagules collected in the Gulf of Guayaquil.” This study will determine the percentage of infestation, identify the most affected area of the propagule, and estimate at what period of development the insect most impacts the propagules. The results of these objectives will offer guidelines for establishing better methodologies for propagule selection to ensure that mangrove restoration programs are able to achieve the highest rates of mangrove survival and viability.

Study Areas

The study was developed in four mangrove areas in the Gulf of Guayaquil: Parque Histórico Guayaquil, Mangrove Wildlife Refuge El Morro, Mangrove Faunistic Production Reserve El Salado (Puerto Hondo and Malecón Estero Salado), and Puente Zig-Zag.

Parque Histórico Guayaquil

Parque Histórico Guayaquil is a cultural, educational, touristic, and recreational area that has been open to the public since 1999 under the administration of the Central Bank of Ecuador. Subsequently, in 2016, the management was overtaken by the Public Sector Inmobiliary Management Service (Mora & Molina-Moreira, 2017). It contains diverse areas, including: the Wildlife Zone, the Architectural Urban Zone, and the Traditional Zone. This park exhibits the most representative ecosystems of the Ecuadorian coast. For example, it contains mangroves and their diverse species: *R. mangle*, *R. harrisonii*, *Laguncularia racemosa*, *Avicennia germinans*, and *Conocarpus erectus* (Cusme & Molina-Moreira, 2018). The Wildlife Zone in Parque Histórico Guayaquil contains 17000 m² of mangroves (Molina & Molina, 2005).

Mangrove Wildlife Refuge El Morro

The Official Record 180 of September 28th, 2018, declared the establishment of the Mangrove Wildlife Reserve El Morro. This reserve is 10,130.16 ha and is composed of: mangrove forests, lowlands, estuaries, and natural channels. It is located in the enclosure El Morro, of the El Morro parish in Guayas province. It is one of the most visited Protected Areas in the Guayas province, owing to its tourism activities: trails as well as sightings of dolphins, birds, pink herons, oysters, crabs, and a variety of shellfish. Additionally, it is important to highlight that the primary industry of the surrounding area is shrimping (Medina, 2017).

The Mangrove Faunistic Production Reserve El Salado

The Mangrove Faunistic Production Reserve El Salado, categorized as such since November 15th 2002 by the Ministry Accord No. 142, is located in the Northeast of the estuary of the Gulf of Guayaquil. In 2007 the mangroves of Puerto Hondo were added and subsequently included in the national System of Protected Areas. This constituted 5,309 ha composed of salt beds, remnants of tropical dry forest, diverse estuaries, and a strip of mangroves, the ecosystem that predominates and covers 75.4 % of the total surface area. Many of these ecosystems have been recuperated by interventions on behalf of the state port authorities. Additionally, there is a high index of flora and fauna that are directly related to the mangrove forests and that provide the community with many benefits: oxygen, forests, flood control, and natural lungs for Guayaquil and Durán, among others (Quinteros, Castro & León, 2017). The mangrove forests can be entered though many access points along *Estero Salado*. This study selected two access points: Puerto Hondo and Malecón del Estero Salado.

Puerto Hondo

Urban growth has undoubtedly replaced mangrove systems in Guayaquil. Because of this, the remaining mangroves in this zone were protected by the creation of this reserve. These mangroves are not only habitat for native flora and fauna, but also provide landscape, aesthetic, and recreational benefits for this zone.

Malecón del Estero Salado (Puente Zig-Zag)

The rate of contamination has increased because of the disequilibrium that exists between uncontrolled population growth, industrial growth, entertainment locations in the zone, and other factors which generate a grave problem for the sector (Mariscal et al., 2018). The Malecón del Salado is justly named due to its location along an estuary of the same name. It possesses an extension of about 400 meters and is considered an urban regeneration park in the city. The area has lookouts to the estuary and the city, contains a suspended pedestrian point that is 55 m in length, contains exuberant flora and fauna, and features plazas that pays homage to notable Guayaquileños.

Study Species

Rhizophora genus

Rhizophora, or red mangrove, is a genus composed of three species and a hybrid: *R. mangle*, *R. racemosa*, and *R. harrisonii*. Of these species, the most representative of the *Rhizophora* genus is *R. mangle* (Duke and Allen, 2006), a shrub or tree with astringent bark and the capacity to surpass a height of 10 m. It has opposite leaves that are approximately 5-15 cm long and that are coriaceous, elliptical, obtuse, containing elongated stipules, interpetiolar, deciduous, and having petioles of 0.5-1.5 cm long; it also forms an impenetrable thicket due to its numerous roots (Regalado, Sánchez & Mancebo, 2016). The red mangrove grows best in muddy, shallow sediment with continuous

tidal influence: it is salty and associated with abundant fresh water drainage and rain from 800 to 1000 mm per year, temperatures that oscillate between 21 and 30 °C, and sensitivity to frost. Mangrove sediment generally presents a high pH with an elevated carbon-nitrogen relationship, as well as a high presence of sulfur, nitrogen, and phosphorus, among other nutrients. With respect to its reproduction, its viviparity causes reproduction by propagules that grow in a period from 3 to 6 months before falling from the tree, with an appearance of elongated stems with variable size and weight. These seedlings are transported by ocean currents and later establish when the primary roots drop anchor, in approximately two weeks (Jiménez, 1985).

Coccotrypes rhizophorae

C. rhizophorae is a curculionid that acts as an obligate parasite, and its presence depends on environmental factors such as humidity, light, and salinity. It causes harm that includes propagule and seedling mortality, which can affect ecosystem resilience (Woodruff, 1970). Studies indicate that this beetle originated in Indonesia and was introduced to the American continent, especially Florida and the Galapagos Islands, by ocean currents (Wood, 1982). This caused severe mortality in these ecosystems. This insect has infested mangroves in different countries such as: Mexico, United States, Costa Rica, Peru, and Ecuador (Sousa, Quek and Mitchell, 2003); however, investigations that evaluate the status of the development of *C. rhizophorae* in the propagules of *R. mangle* are scarce.

Species in the genus *Rhizophora* are already susceptible to propagule depreciation by lepidopteron and coleopteran arthropods that can reduce the quality and number of the seedlings in their establishment phase (Cannicci et al., 2008). The insects of the family Curculionidae are characterized by small species (maximum 5 mm) located in branches, trunks, roots, propagules, and seedlings, where the female is situated in the middle of an orifice; here, she constructs galleries that destroy the material that is fundamental for propagule

development, establishment, and survival (Wood, Stevens & Lezama, 1991; Proffitt and Devlin, 2005).

Subsequently, the eggs are deposited individually or in small groups to later pass through the distinct biological stages of larva, pupa, and adult. This process generally takes 20-90 days and finishes when the female moves to new material. According to their biology, the majority of species of the family Curculionidae are able to tolerate temperatures from 10-38 °C (Wood, 1982).

Rhizophora Propagule Collection

For the sample to be representative, 200 propagules were collected every 15 days from July to October. As such, 600 propagules were collected in each area (n = 4), with a total of 2400 propagules. It ought to be emphasized that the collection of propagules was conducted randomly in the selected areas. Once the propagules were collected, they were placed in bags and immediately transported to the laboratory for same-day evaluation.

Determination of healthy vs. infected propagules

Using stylus knives, a perpendicular cut was executed along the propagules (n = 2400) to allow for internal analysis. Once opened, the percentage of infestation was determined based on the size of the breeding chambers. Scales with distinct percentages (0 %, 25 %, 50 %, 75 %, and 100 %) were established to enable the comparison of infection between propagules (Figure 1).



Figure 1. The percentage of propagule infestation of the genus *Rhizophora*. Source: author's own elaboration.

Analysis of the zone inside the infected propagules

Once the percentage of infestation was identified, it was critical to know the propagule zone with the highest level of infection (n = 2400). To do this, a method developed by Martínez-Zacarías et al. (2017) was employed, whereby distinct study areas are established inside the propagule: the apical zone, the medial zone, the radical zone, and their possible combinations (apical-radical, apical-medial, and medial-radical) (Figure 2).



Figure 2. Identification of affected areas in the propagules: apical zone, medial zone, and radical zone. Source: author's own elaboration.

Characterization of the biological state of C. rhizophorae

After the identification of the affected area, the biological state was determined (Figure 3) in each of the propagules (n = 2400) to determine at what stage *C. rhizophorae* is found inside the propagules.



Figure 3. Development stages of *C. rhizophorae*: **A.** Egg, **B.** Larva, **C.** Pupa, **D.** Adult. Source: author's own elaboration.

Analysis of data

Identification of the percentage of infestation

The results demonstrate that during the months of July to October, corresponding to the dry season, the percentage of *C. rhizophorae* infestation in *Rhizophora* propagules in Parque Histórico Guayaquil was low, with 425 healthy propagules, representing 71 % of the sample in the area ($n = 600$). Next was Puerto El Morro, with 374 healthy propagules (62 %), while Puerto Hondo presented the highest infestation rate with 240 healthy propagules (40 %). With regards to the percentage of the area infested in the propagule in the four sites, few propagules presented a 100 % area of infestation, as 25 % infestation dominated (Figure 4).

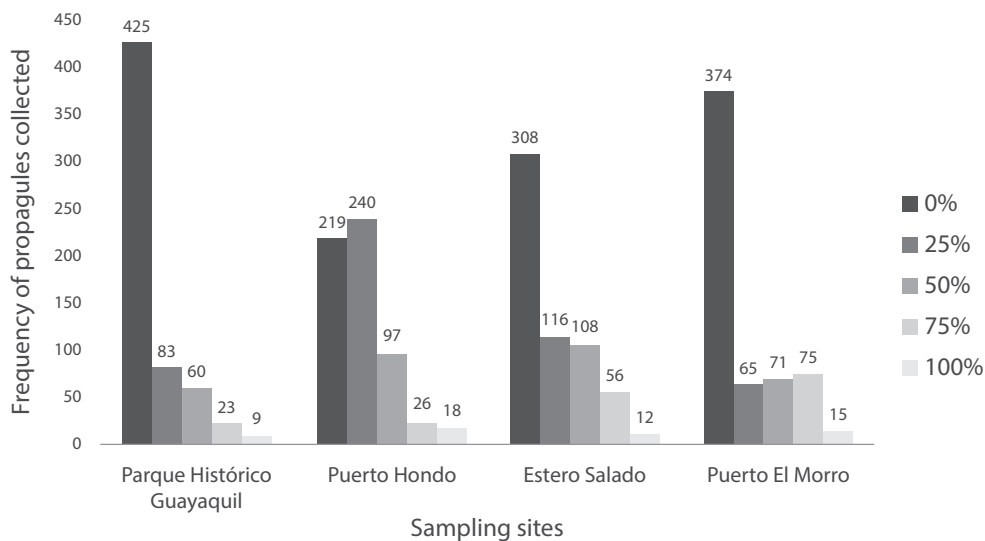


Figure 4. The infestation percentage with the scale of: 0, 25, 50, 75, and 100 % in Parque Histórico Guayaquil, Puerto Hondo, Estero Salado, and Puerto El Morro. Source: author's own elaboration.

Identification of the most infected area of the propagule

The results show that, in all of the study sites, the radical zone prevailed, with a total of 898 propagules ($n = 2400$) having a presence of *C. rhizophorae*. This was equivalent to 37.41 % of the total sample. The apical zone presented the lowest rate of infection, with 120 propagules, corresponding to 5 % of the sample. Only 40 propagules from the total sample ($n = 2400$) were affected in all of their areas: apical, medial, and radical (Figure 5).

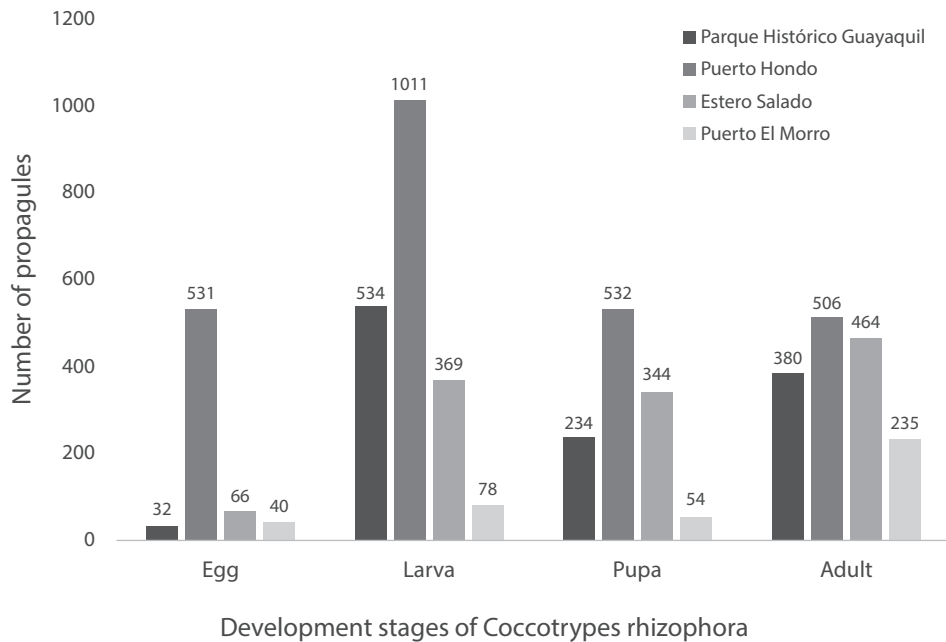


Figure 5. Area of propagule infestation by *C. rhizophorae* in the four study sites. Source: author's own elaboration.

Evaluation of the developmental stage of C. rhizophorae in the propagules

In the majority of study sites, the species *C. rhizophorae* was found in the larval stage of development, with a total of 1992 larval individuals. The majority (1011 individuals) were from Puerto Hondo. However, in Puerto El Morro, the most relevant stage was that of adult, with 235 located in the propagules from this site. On the other hand, there was a significant difference with respect to the number of eggs; Puerto Hondo presented the largest number with 531 in comparison with other areas such as Parque Histórico Guayaquil, where only 32 eggs were found (Figure 6).

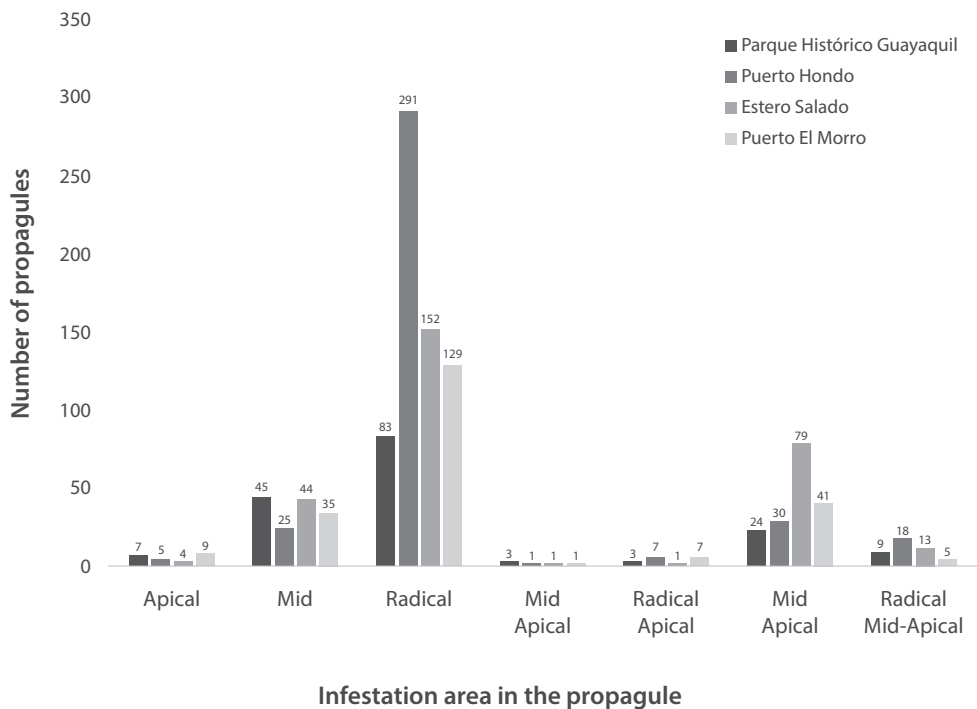


Figure 6. Developmental stages of *C. rhizophorae* that infested *Rhizophora* propagules in the four study zones. Source: author's own elaboration.

This study identifies that *C. rhizophorae* caused damage to propagules in the genus *Rhizophora* in distinct areas of the Gulf of Guayaquil. Moreover, it was observed that the majority of the drilled propagules were harmed in the radical area, with 25% infestation, and by a parasite in its larval stage. At the international level, *C. rhizophorae* has been observed in mangroves in different tropical countries in American and Asia. It is recognized as an obligate parasite of *R. mangle* (Martínez-Zacarías et al., 2017). In Ecuador, it has been reported in the Galapagos island Isabela (Bright and Peck, 1998), as well as in Parque Histórico Guayaquil (Molina et al., 2000; Mendoza & Molina-Moreira, 2015).

Relationship of the state of mangroves with the percentage infestation

Of the four sites evaluated in this study, the propagules collected in Puerto Hondo represented the highest infestation of *C. rhizophorae*, differing from Parque Histórico Guayaquil, which was the area with the lowest rate of infestation between July and October 2018. These results are also similar to those encountered in the study conducted in Mexico during the same months which correspond to the dry period, where drilled propagules were found with great frequency (Martínez-Zacarías et al., 2017). However, these results differ from a study in Cuba where the highest infection rates occurred in the wet season (Menéndez, Guzmán & Núñez, 2006). Also, it can be inferred that the infestation of *C. rhizophorae* is lowest among mangroves with the highest state of conservation. As such, mangroves that have lost zonation and are pressured by sedimentation and contamination are more harmed. Lower infestation rates are observed among more highly conserved mangroves such as Puerto El Morro, a Wildlife refuge in the National System of Protected Areas in Ecuador, and Parque Histórico Guayaquil, which is a younger mangrove forest, restored since 2000, where the majority of individuals have an average age of 18.

Relationship between the percentage of infestation and the drilled zone of the Propagule

The radical zone had the highest rate of infestation. This result coincides with Martínez-Zacarías et al. (2017), who reported a higher frequency of structural damage generated by *C. rhizophorae* in the basal or radical part of the propagule. This could be because the radical zone presents a greater thickness than the medial or apical area, so the female colonizer has more space to establish her galleries and living chambers for the development and livelihood of her larvae.

Another reason could be related to the mimicry, owing to the coloration of the radical zone, which is similar to the brown color of

the female of *C. rhizophorae*. This could help with defense against predators, since in the brown area at the entrance of the orifice in which the female is found could be easily confused with the propagules' lenticile tracks.

Relationship of the number of individuals in each stage of biological development of C. rhizophorae for each study area

The larval stage comprised the majority of individuals in Puerto Hondo and Parque Histórico, whereas the adult stage was predominant in Puerto El Morro and Estero Salado. There was not a significant relationship between the number of individuals in each stage in the study areas, which could be because the developmental stage depends on the environmental conditions of each area; the literature states that the larval stage can last from 12 days to two years before undergoing a state change, depending on the distinct species in the Curculionidae family (Wood, 1982). The relevance of this result, which is reported for the first time in Ecuador and at an international level, is related to the survival or propagules for mangrove restoration. Because the damage is greater in the larval stage but lesser in the egg and adult stages, there could be better control during the stage of development in a nursery.

Mutualistic relationships with fungus

Although there have been mutualistic relationships with fungi in certain genera the family Curculionidae, not all Curculionidae have an affinity for fungi (Wood, 1982). In this study, the presence of the fungus *Penicillium sp* (Eurotiales, Trichocomaceae) was observed, but this was exclusive to *C. rhizophorae*, so it is not a mutualistic relation.

Future recommendations

The execution of studies about the biological cycle of *C. rhizophorae* will enable an understanding of their relationship as an obligate parasite with *Rhizophora*. This will also help ascertain whether they can be used as a bioindicator for the state of mangroves.

It is important to execute the propagule evaluation throughout the entire year to compare the dry and wet seasons because the environmental conditions are distinct.

With regards to monitoring propagules in nurseries already established in their muddy substrate, it is recommended to implement sowing methods that grow propagules in water. This will reduce *C. rhizophorae* infestation because the infested propagule will develop better if it is submerged in water. Infestation occurs with higher frequency in the radical zone, and the immersion favors growth such that, when there is a presence of *C. rhizophorae*, the adults that leave the propagule then fall to the water and die. Consequently, they are not able to infect other propagules because typically the adult that leaves is a female in a gestation period.

Conclusions

This study, carried out during four months of the dry period in four sites in the Gulf of Guayaquil, verified the damage caused by *C. rhizophorae* in *Rhizophora* propagulos. However, it will be important to execute the evaluation during the entire year in order to determine in which season the infestation is lower. This would allow restoration projects to take advantage of the greater quantity of healthy propagules to be used in mangrove restoration projects. Moreover, environmental parameters such as pH, salinity, temperature, and humidity should be recorded to relate them to the percentage of damage.

It was evidenced that mangroves of better conservation status presented higher quantities of healthy propagules, whereas

mangroves with greater sedimentation had higher levels of infestation. This physical aspect of the soil can therefore be considered as a measurement parameter that ought to be related to the damage caused by *C. rhizophorae*.

Very few propagules were 100 % affected; the majority were 25 % affected and in the radical zone, which is the thickest area of the propagule and provides better conditions for the insect for the construction of their chambers and galleries. Therefore, submerging propagules as a control measure to reduce the incidence of damage should be evaluated.

Regarding the prevalence of the different biological stages of the insect, the larval state causes the most damage due to the fact that the larvae are those which consume the propagule tissues more than the adults. As is seen in this study, Puerto Hondo was the site with the greatest quantity of larvae and it presents the greatest damage.

This study reports the first evaluation of the infestation of *C. rhizophorae* in the mangroves of the Gulf of Guayaquil. It contributes to the knowledge of this obligatory parasite of the genus *Rhizophora* to improve propagule management techniques in nurseries. This assures a better survival in mangrove restoration projects. *C. rhizophorae* can be used as a bioindicator in mangrove conservation.

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V

Comparison of the harm caused by *Coccotrypes rhizophorae* (Coleoptera: Curculionidae: Scolytinae) in *Rhizophora* propagules in the Galapagos and Santa Elena

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Introduction

Globally, mangroves constitute the greatest part of the coastline. Their distribution is in tropical and sub-tropical zones with conditions of elevated salinity, intense tides, aggressive winds, extreme temperatures, and soils rich with organic materials that pertain to complex anaerobic systems. However, by living in conditions at the limit of their tolerances, mangroves can be particularly sensitive to changes or disturbances like those induced by human activities (Kathiresan and Bingham, 2001). Felling and overexploitation are the principal causes of the loss of mangrove ecosystems, which are commercialized for construction. For this reason, there have been changes in salinity, physiochemical properties of the soil, sedimentation, flooding, humidity, and lighting, among others, the stress of which render mangroves susceptible to diseases or infestations (Alongi, 2002; Bright and Peck, 1998; Menéndez, Guzmán & Núñez, 2006; Sousa, Quek and Mitchell, 2003).

Mangroves are associated with various organisms, characterized by different types of relationships. Bacteria play a fundamental role in mangrove ecosystems, where they control the chemical environment. For example, sulfate-fixing bacteria are primary decomposers in the anoxic mangrove sediments (Sherman, Fahey and Howarth, 1998). On the other hand, mangroves are home to a group of fungi called “Mongolian fungi,” which are of vital importance for nutrient cycling in these habitats (Kohlmeyer, Bebout and Vlkmann-Kohlmeyer, 1995). Additionally, insects constitute a large part of the animals found in mangroves, although many of them are only seasonal visitors (Kathiresan and Bingham, 2001). Many of the arthropods avoid conditions of high sunlight, elevated temperature, and dehydration, which prompt them to hide themselves in tunnels excavated by moths or beetles in the mangroves. These tunnels are a home to more than 70 species of worms, spiders, mites, moths, cockroaches, termites, and scorpions (Feller and Mathis, 1997; Rützler and Feller, 1996). There is a record of insects that attack propagules of the genus *Rhizophora*. This includes *Penicillium sp* (Eurotiales, Trichocomaceae) and *Acalles sablensis* (Coleoptera, Curculionidae) (Arias de López & Molina-Moreira, 2019; Beever, Simberloff and King, 1979; Bouillon et al., 2002; Cannicci et al., 2008; Chamorro-Florescano et al., 2014; Feller and Mathis, 1997; Martínez-Zacarías et al., 2017; Smith, 1987).

C. rhizophorae (family Curculionidae, subfamily Scolytinae) acts as an obligate parasite in the propagules of the genus *Rhizophora*. The species pertaining to the genus *Rhizophora* are *R. mangle*, *R. racemosa*, and *R. harrisonii* (natural hybrid). These three species are considered to be true mangroves, and they are trees that have the capacity to surpass 10 meters in height (Cornejo, 2014; Duke and Allen, 2006). It has been found that *C. rhizophorae* grows in abundance when the ecosystems are stressed by various environmental factors, such as light. Specifically, the family Curculionidae is associated with threats presented to forest plantations. They are characteristically species smaller than 5 mm, which utilize branches, runks, roots, and plants of some species for their development. The *C. rhizophorae* infestation process initiates when the female mate excavates a den in the surface

of the roots, seeds, or trunks, and it is where she deposits her eggs. Afterwards, the larvae excavate through the tunnel and mature, and the females search for a different host (Sousa, Quek and Mitchell, 2003).

Normally these species are distributed in tropical rainforests in Asia and Africa (Jordal et al., 2002). However, *C. rhizophorae* has already been found in different places such as Florida, Belize, Panama, Ecuador, and the Galapagos Islands (Sousa, Quek and Mitchell, 2003). In Ecuador, *C. rhizophorae* infestations have been reported in the Guayas province (Arias de López & Molina-Moreira, 2019; Cassinelli, 2018) and in the Galapagos Islands. However, there are infestations in mangroves throughout the Ecuadoran coastline. With regards to Ecuador, there is a record of mangrove species. They are distributed in Esmeraldas, Manabí, Santa Elena, Guayas, and El Oro, with an extent of 161,853 hectares. However, mangrove coverage has diminished, owing to the strong incidence of human activities. To promote mangrove conservation in Ecuador, it is critical to know more about *C. rhizophorae* infestations in these ecosystems.

In the Galapagos Islands, the first capture of the species *C. rhizophorae* was in 1985 in Isabela island; it has later been reported in Santa Cruz island, Puerto Ayora (Bright and Peck, 1998). Due to the rigorous protection of the archipelago since 1959, mangrove ecosystems in these islands are found in a state that is similar to the original, unlike the mangroves on continental Ecuador, which are found in a state of high degradation. In the Galapagos Islands there are no rivers or other freshwater sources that lead to the ocean. Despite this, these coastal mangroves have adapted to tolerate high levels of salinity. Four species of mangroves exist in the archipelago: white mangrove (*Laguncularia racemosa*), red mangrove (*Rhizophora mangle*), button mangrove (*Conocarpus erectus*) and black mangrove (*Avicennia germinans*) (Tapia, 2004). However, there are two species present in the Galapagos that are still not reported in the literature. These are *Annona glabra* and *Talipariti tiliaceus* (synonym: *Hibiscus tiliaceus*), which are included under Ecuador's classification of facultative mangroves (Atkinson, Guezou & Jaramillo, 2017; Cornejo, 2014; Fundación Charles Darwin, 2019).

There are also *Coccotrypes* infestations in the province of Santa Elena. In this province, the presence of abundant mangroves was reported in the wetlands of Chanduy, Río Grande, and Río Javita. However, these have already disappeared (except for Chanduy which has a small remnant of the original extent). Currently, the mangroves are reduced to tens of hectares located in the communes of Olón, Oloncito, Colochne (Jambelí), San José, El Palmar, Malgaralto, Valdivia, and a small patch in the Punta Carnero estuary (Ceme & Zambrano, 2018). The species registered in Santa Elena are: *R. mangle*, *Avicennia germinans*, *Laguncularia racemosa*, and *Conocarpus erectus*.

The meager presence of mangroves is attributed to the combination of anthropogenic intervention and geological events that contribute to the decline of the population. Additionally, natural phenomena such as El Niño provoke sedimentation, which in turn causes mangrove mortality. Among the anthropogenic activities that affect the mangroves in the Santa Elena peninsula include the intense felling for wood extraction, which diminished the population from hundreds of hectares to less than 100 ha. Also, the intense shrimping activities in the Colonch and Chanduy and the expansion of filling in Malgaralto, Montañita, and Olón are examples of said activities (Ceme & Zambrano, 2018; Ferdon, 1981; Stothert, Piperno and Andres, 2003). *C. rhizophorae* infestations are also a grave problem for mangrove forests.

Studies indicate that the level of infestation of propagules with *C. rhizophorae* is seen to be related to the quantity of human intervention in the mangroves. This includes the sedimentation produced by said activities, the size of the mangrove population, the quantity of available nutrients in the sediment, and the size of the seeds (Onuf, Teal and Valiela, 1977; Sousa, Kennedy and Mitchell, 2003). However, information about *C. rhizophorae* infestation in the Galapagos and Santa Elena is lacking.

The objective of this study is to evaluate the harm caused by *C. rhizophorae* in the propagules of the species *R. mangle* in the Galapagos Islands and in the Santa Elena province.

Characteristics of the Study Site

The Galapagos Islands is located 972 km from the Ecuadorian coast and has a surface area of 8010 km². It is comprised of five principal islands, eight secondary islands, five tertiary islands, and more than 40 islets and rocks (Cuvi & Georgii, 2013). The present study was developed in the four inhabited islands in the Galapagos: Santa Cruz, Isabela, San Cristóbal and Floreana (Figure 1).

Santa Cruz island is one of the largest islands in the archipelago; it contains a surface area of 985.6 km², a maximum altitude of 864 m.a.s.l., and a total population of 15393 people. There is a total of 338.9 hectares of mangrove coverage reported (Granda León & Chóez Salazar, 2013; Moity, Delgado and Salinas-de-León, 2019). Three sampling sites were established on Santa Cruz island: Tortuga Bay beach (latitude: 0°45'49.9998"; length: -90°20'25.0002"), Las Ninfas lagoon (latitude: 0°44'52.0008"; length: -90°18'59.0004"), and Los Alemanes beach (latitude: 0°45'10.0002"; length: -90°18'41.0004").

Isabela is the largest island in the archipelago, having a surface area of 4588 km², occupying 60 % of the total surface area, and with a total population of 2256 people. A total coverage of 2904 ha of mangrove forest was reported on the island (Granda León & Chóez Salazar, 2013; Moity, Delgado and Salinas-de-León, 2019). Three sampling sites were established on Isabela island: Concha Perla (latitude: 0°57'43.7502"; length: -90°57'26.8308"), the lagoon of flamingos (latitude: 0°57'15.3036"; length: -90°58'16.827"), and the coasts of the bay (latitude: 0°57'45"; length: -90°57'33.9984").

Puerto Baquerizo Moreno, on San Cristobal Island, is the capital of the Galapagos province. It has a total surface area of 558.09 km² and has a total population of 7330 people. The island features total mangrove coverage of 118.1 hectares (Granda León & Chóez Salazar, 2013; Moity, Delgado and Salinas-de-León, 2019). Three study sites were established on San Cristobal island: Manglecito (latitude: 0°49'33.999"; length: -89°32'26.9988"), La Tortuga (latitude: 0°43'3.8994"; length:

-89°23'23.9388"), and the coasts of Malecón (latitude: 0°54'11.0016"; length: -89°36'44.9994").

Floreana island is found on the south of the archipelago and has an area of 172,5 km², with a maximum height of 640 m.a.s.l. and with a total population of 145 people. The total mangrove coverage on the island is 15.4 hectares (Granda León & Chóez Salazar, 2013; Moity, Delgado and Salinas-de-León, 2019). Three study sites were established on Floreana island: Punta Cormorán (latitude: -1°13'30"; length: -90°25'59.9988"), the viewpoint at Baronessa (latitude: -1°13'45.9978"; length: -90°26'32.9994"), and the coasts of the bay (latitude: -1°16'50.0016"; length: -90°29'21.0006").

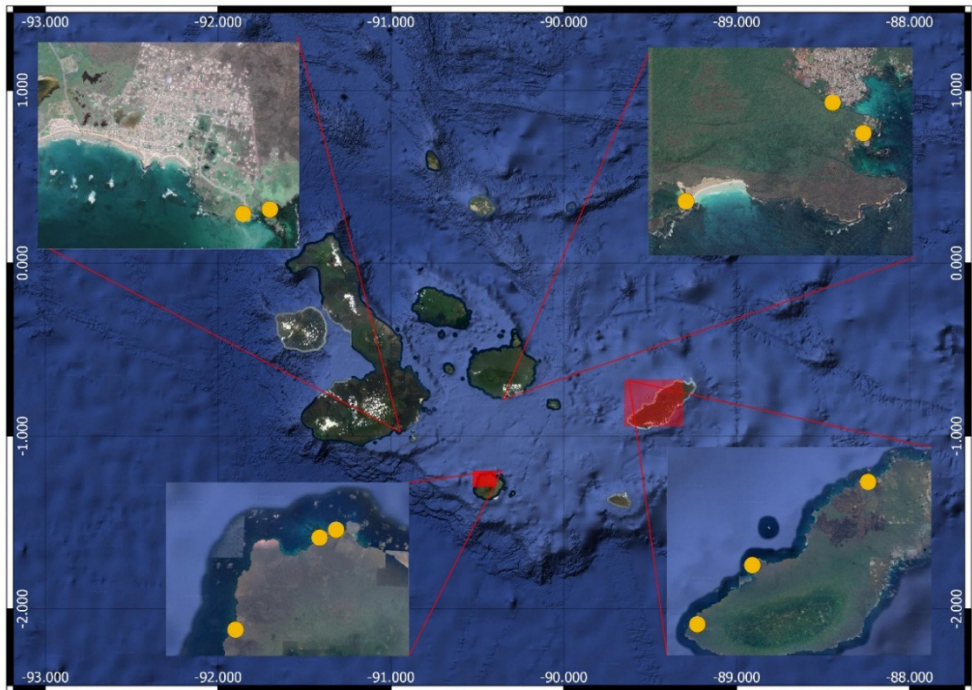


Figure 1. Map of the Galapagos islands and sampling sites in the four populated islands.
Source: author's own elaboration.

The average altitude of Santa Elena province is 540 m.a.s.l., with a maximum of 810 m.a.s.l., and a minimum of 0 m.a.s.l.; the temperature ranges between a minimum of 16-24 °C and a maximum of 25-32 °C. There are two distinct seasons: dry (May- December) and wet (the end of December-April). The average precipitation level is 66 mm per year. However, in El Niño years the precipitation can reach up to 2800 mm per year. The ecosystems present on Santa Elena include: coastal marine, mangrove, dry-deciduous forest, and tropical rainforest. The study was carried out in three remaining mangrove areas in Santa Elena: Punta Carnero estuary, El Palmar estuary, and Manglaralto estuary (Figure 2).

Punta Carnero (latitude: -2°17'28"; length: -80°54'32") is a beach located on a beach in the Salinas parish. The average annual precipitation ranges from 125 to 150 mm, with temperatures between 21 and 30 °C.

El Palmar commune (latitude: -2°1'9"; length: 80°44'13") is situated in the North of Santa Elena province, 40 km from La Libertad parish. It has approximately 1000 families, most of who live off of fishing. It features a flat topography and 36 ha of mangroves (Ceme & Zambrano, 2018; González & González, 2015; Reyes & Panchana, 2009).

The Manglaralto parish (latitude: 1°50'49"; length: 80°44'47") is located in the North of Santa Elena province, its area is 497.2 km², and it has 29,512 inhabitants.

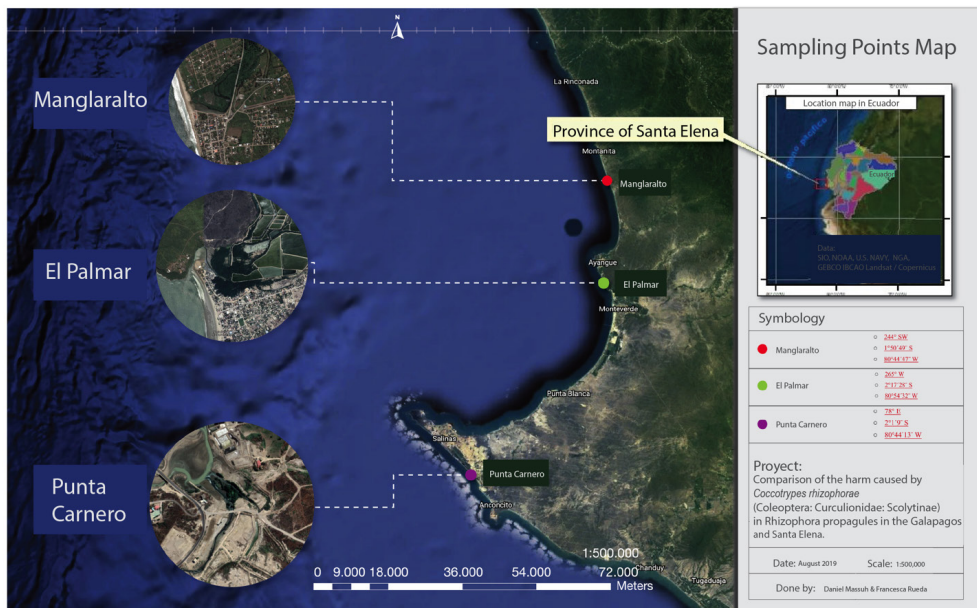


Figure 2. Map of the sampling sites in Santa Elena province. Source: author's own elaboration.

Strategies to compare the damage caused by *C. rhizophorae* in propagules in the genus *Rhizophora* in the Galapagos and Santa Elena

The collection of Rhizophora propagules: a total of 600 propagules were collected in the Galapagos. Collection was carried out during July and August 2019, keeping in mind both mangroves altered by human activities and pristine mangroves. In Santa Elena, 100 propagules were collected in the three study sites in the estuaries of Punta Carnero, El Palmar, and Manglaralto, with three replicates for each month in August, September, and October 2019, with a total of 9000 propagules of *Rhizophora*.

Differentiation of the healthy vs. infested propagules: to determine the infested propagules, they were evaluated the same day as collection, with the naked eye and a microscope. The infested

propagules were selected and a vertical cut was made to observe the percentage of damage caused by the *C. rhizophorae* infestation. Photographs were taken so that the individuals could be identified using taxonomic keys. The infestation percentage of the propagule was estimated with a predetermined scale: 0 %, 25 %, 50 %, 75 %, and 100 % (Arias de López & Molina-Moreira, 2019) (Figure 3).

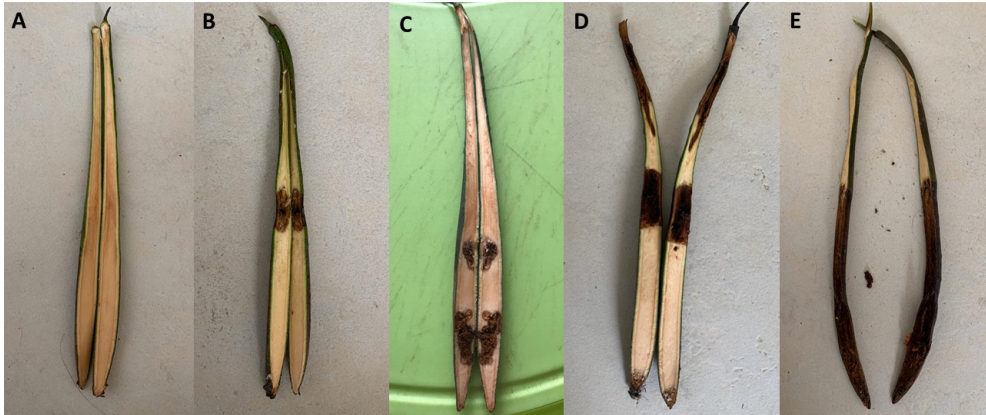


Figure 3. Infestation percentage in *R. mangle* propagules: **A.** 0 %, **B.** 25 %, **C.** 50 %, **D.** 75 %, **E.** 100 %. Source: author's own elaboration.

Quantification of the biological stages of C. rhizophorae: to determine the quantity of *C. rhizophorae* individuals in each biological stage in each propagule, the number of eggs, larvae, pupae, and adults was counted through a microscope (Arias de López & Molina-Moreira, 2019) (Figure 4).

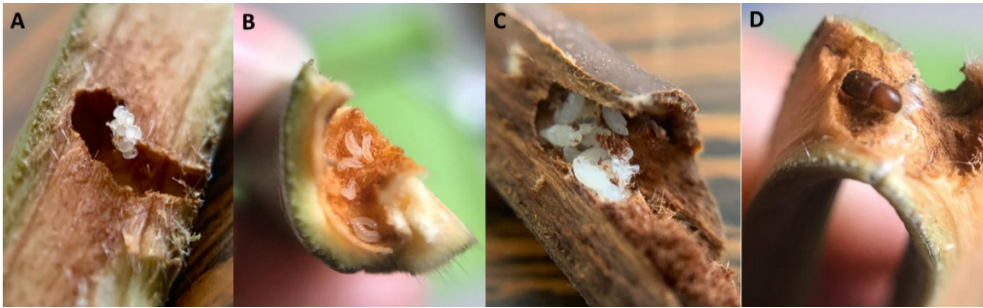


Figure 4. Biological stages of *C. rhizophorae*: **A.** eggs, **B.** larvae, **C.** pupae, **D.** adults. Source: author's own elaboration.

C. rhizophorae is considered an obligate parasite of the genus *Rhizophora*, and it has been recorded in various countries in America and Asia. These insects help to mold the structure of mangroves and impact the wildlife. As such, Sousa, Kennedy and Mitchell (2003) observed that, in the three mangrove genera (*Rhizophora*, *Avicennia*, and *Laguncularia*), the propagules which were most damaged or consumed by insects had the lowest germination rates. Also, the propagules that were the least damaged were able to germinate, but more slowly compared to the healthy propagules of the same size. In turn, Onuf, Teal and Valiela (1977) also found that the infestations of *Rhizophora* propagules by *C. rhizophorae* impeded establishment and growth. Because of this, it is highly important to know the infestation characteristics of *C. rhizophorae* in mangroves.

Determination of the percent damage

In the Galapagos Islands, the percentage of damage caused by *C. rhizophorae* in *R. mangle* propagules was 25 % of the total sample (n = 600), but there were differences between the islands (Figure 5). On Isabela island, there were 104 infested propagules, which represents 69.33 % of the sample of this island (n = 150). On Santa Cruz island, 46 propagules (30.66 % of the total sample) were infested (n = 150). On San Cristobal and Floreana islands, there was 0

% infestation (n = 150). Referring to the infestation percentage of the individual propagules, 25 % damage prevailed with 103 propagules (17.16 % of the total sample n = 600). 36 propagules (6 % of the total sample n = 600) were evaluated at 50 %.

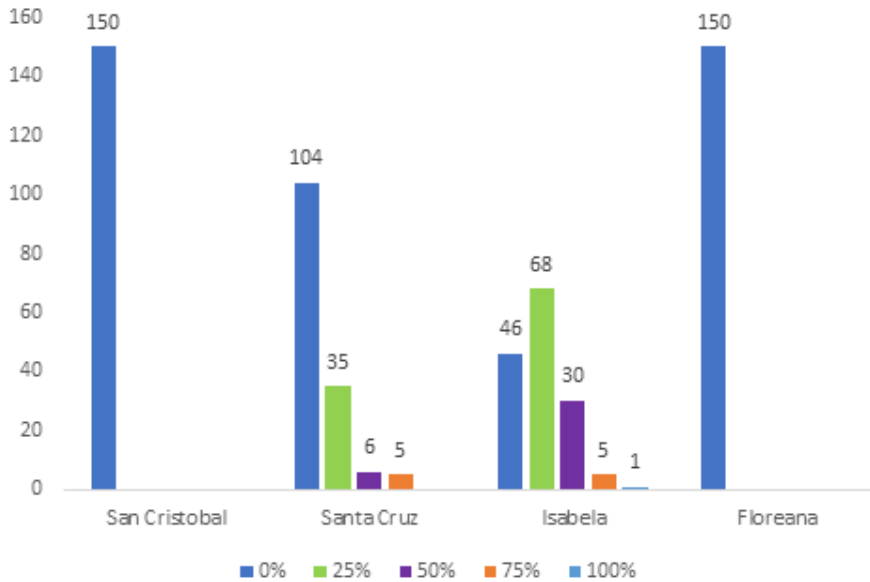


Figure 5. Propagule infestation percentage per site in the Galapagos Islands. Source: author's own elaboration.

A study conducted in 1998 about the presence of *C. rhizophorae* on the islands also reported its presence only on Isabela and Santa Cruz islands (Bright and Peck, 1998; Martínez-Zacarías et al., 2017). The Galapagos Islands, despite being a protected reserve and under particular conditions, is home to *C. rhizophorae* on the islands where it is presumed that there are both the highest levels of touristic exploitation and most hectares of mangroves. The difference between the infestation percentage and the absence of *C. rhizophorae* in some sites in the Galapagos Islands could be because of the quantity of nutrients in the soils, the tough environmental conditions of the islands, such as high salinity and strong wave action, and the height

and size of the trees. Past studies presented variations in infestation percentage in propagules of the genus *Rhizophora* by *C. rhizophorae* in two different sampling sites; the infestation percentage by beetles differed when the level of nutrients transported by guano (produced by birds) increased or decreased. During the season of more nutrients by way of guano, the infestation increased to 100 %, whereas was only 43.3 % when the nutrient levels decreased. Among other differences, it is speculated that the different infestation percentages at the various sampling sites are due to the size and height of the trees from which the samples were taken. For example, the trees at the periphery of the islands were between 4 and 5 m tall, whereas the continental forests were approximately 16.5 m (Onuf, Teal and Valiela, 1977; Sousa, Kennedy and Mitchell, 2003).

In Santa Elena, 231 infested propagules represented 25.6 % of the total sample (n = 900). With 168 infested propagules in the genus *Rhizophora*, which represented 18.66 % of the total sample (n = 900) and 56 % of the sample of the area (n = 300), El Palmar was the site with the highest infestation. Manglaralto, with 63 infested propagules, represented 7 % of the total sample (n = 900), and 21 % of the sample in the area (n = 300). Punta Carnero, with only one infested propagule, represented 0.11 % of the total sample (n = 900) and 0.33 % of the sample of the area (n = 300). As for the percentage of damage according to the scale from 0 % to 100 %, 25 % predominated with 128 propagules (14.22 of the total sample n = 900), followed by 50 % with 65 propagules (7.22 % of the total sample n = 900) (Figure 6).

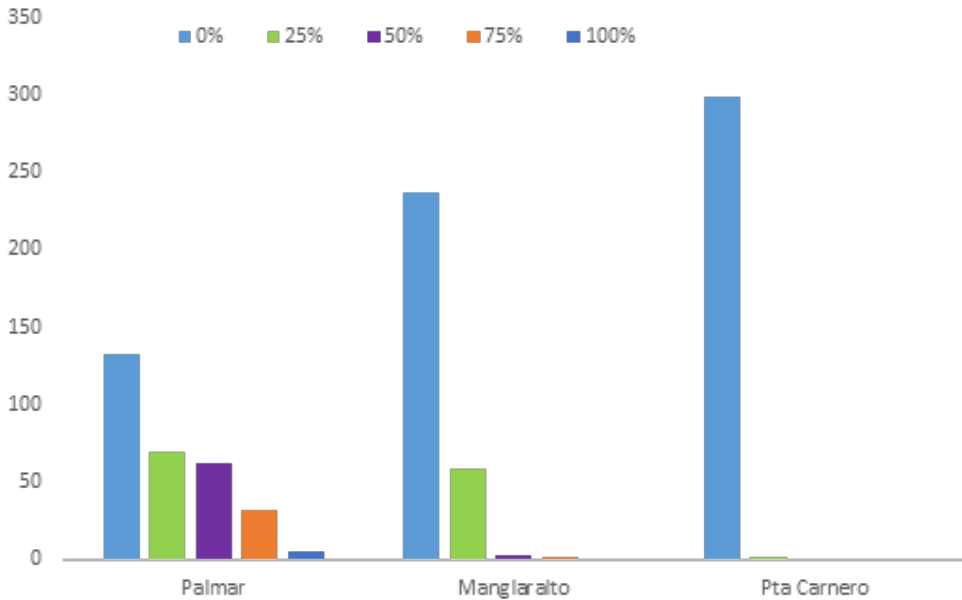


Figure 6. Propagule infestation percentage by site in Santa Elena. Source: author's own elaboration.

In the Santa Elena province, the percentage of infestation (25.6 % of the total sample $n = 900$) was very similar to that of the Galapagos. In comparison, previous studies in the Gulf of Guayaquil showed that the percentage surpassed 40, and in a study in Mexico it was higher than 70 % (Cassinelli, 2018; Martínez-Zacarías et al., 2017). Additionally, among the three areas established in Santa Elena, only Punta Carnero had an infested propagule. This can happen for multiple reasons, such as establishment next to the ocean where there is higher salinity than in estuarine areas. Such is the case in El Palmar, which has higher infestation. Another possible reason for the low infestation are the good hydrological conditions what allow the flow and reflow of water and the “young” mangrove structure, which is evidences by the thin shafts and low height (10 m on average).

Additionally, there are other causes, little studied, of other insect attacks in mangroves that can be related to their infestation. There

are different concentrations of nitrogen or other chemicals in the tissues of the propagules (Farnsworth and Ellison, 1997; Onuf, Teal and Valiela, 1977). Currently, the limited knowledge of insect species and other organisms, which feed on or otherwise need mangrove propagules in the study sites makes it difficult to evaluate the alternative explanations of the level of infestation of the different mangroves. It ought to be kept in mind that the insect attack levels in mangrove ecosystems depends on the properties of the propagules and the abundance of insects that vary over space and time (Sousa, Kennedy and Mitchell, 2003).

Another factor is that the development rate of the propagules can vary when the immature propagules are exposed to ovipositing insects. In the Galapagos Islands, it was observed that, in the propagules of *R. mangle*, the attacks by *C. rhizophorae* were carried out in the youngest and smallest propagules, where they were the most abundant in the whole sample (n = 600). It can be referred that these mangrove ecosystems were relatively young. A study that analyzed the impacts of propagule size and damage caused by insects on the growth and establishment of mangroves determined that the propagules of the genus *Avicennia* have the highest rates of insect attacks. They supposed that this was due to the thin pericarp and fleshy cotyledons of these propagules, which are easy to penetrate in comparison with the seeds of the genera *Laguncularia* and *Rhizophora* (Sousa, Kennedy and Mitchell, 2003). Some other studies suggest that the propagules size is related to the level of infestation; larger propagules have a lower propensity for infestation, and Punta Carnero has the highest average propagules length (Geritz, 1998; Onuf, Teal and Valiela, 1977; Sousa, Kennedy and Mitchell, 2003). With these data it can be inferred that the mangroves of this zone are found in a relatively healthy state for this pathogen, by considering *C. rhizophorae* as a bioindicator (Dangremond, 2013).

Identification of the most affected propagules

In the Galapagos, the radical area presented the highest level of infestation (Figure 7), with 72 affected propagules, representing 12 % of the total sample (n = 600). The lowest area of infestation was observed in the apical and apical-medial zones, with one propagule affected in each case. This represents 0.16 % of the total sample (n = 600). It was identified that the great majority of propagules (451, representing 75.16 % of the total sample) were healthy.

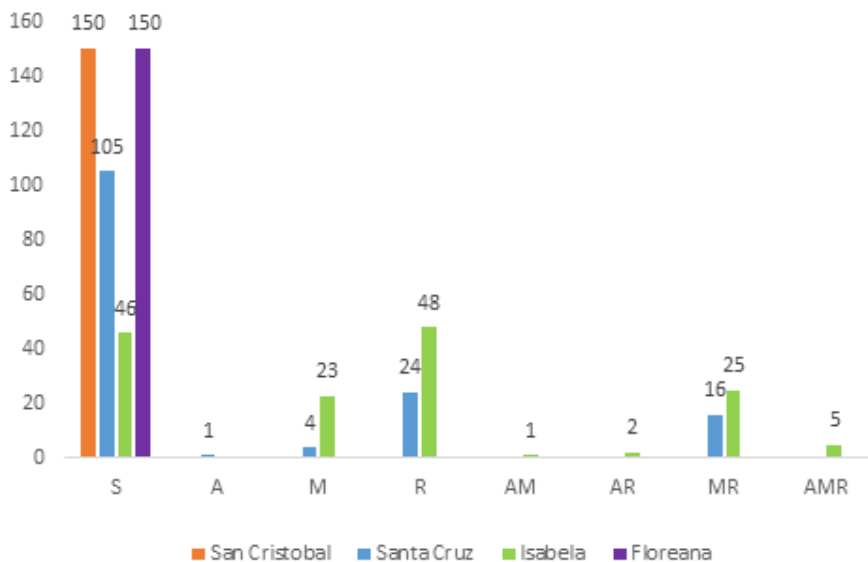


Figure 7. Infestation area of propagules in the Galapagos Islands. Source: author's own elaboration.

In Santa Elena, it was identified that the radical area is that which presents the highest level of infestation, with 73 % of propagules affected, representing 8.11 % of the total sample (n = 900), and 31.6 % of the total number of affected propagules (n = 231) (Figure 8). The apical-medial (AM) combination zone presented the lowest infestation, with only 15 propagules. This represented 1.6 % of the total sample (n = 900).

and 6.4 % of the total number of affected propagules (n = 231). It can be seen that the great majority (669) of the propagules, representing 74.3 % of the total sample (n = 900), were healthy.

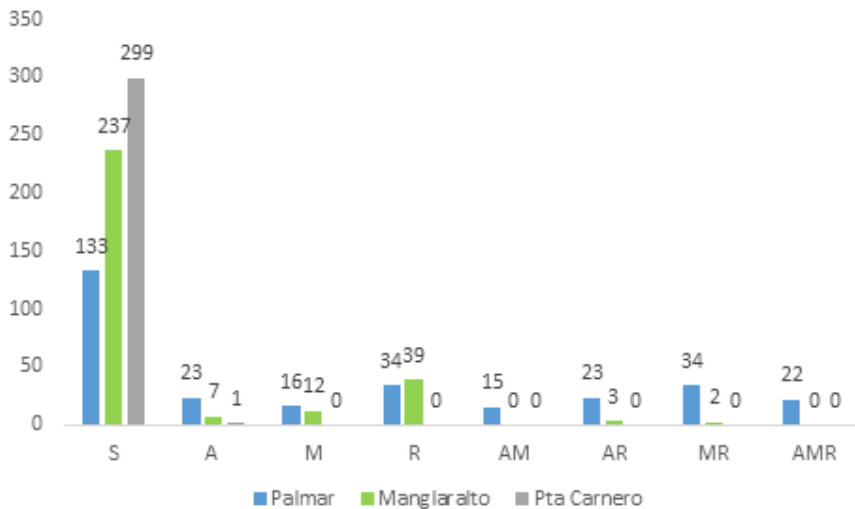


Figure 8. Propagule areas of infestation in the sites in Santa Elena. Source: author's own elaboration.

In the Galapagos Islands and Santa Elena, it was observed that the most affected zone of the propagule was the radical. This coincides with previous studies that also identified the radical area as the most affected (Arias de López & Molina-Moreira, 2019; Cassinelli, 2018; Martínez-Zacarías et al., 2017). It is known that the mangroves have lower probabilities of growing if *C. rhizophorae* drills into the apical zone of the propagule (Martínez-Zacarías et al., 2017).

Estimation of the number of individuals in each biological stage

A total of 1218 *C. rhizophorae* individuals were identified in the Galapagos Islands. Of these, 144 (11.82 %) were eggs, 444 (36.45 %)

were larvae, 177 (14.53 %) were pupae, and 453 (37.19 %) were adults (Figure 9). It can be seen that Isabela island presented the greatest number of total individuals, with a total of 874 (71.75 %) compared to Santa Cruz island's 334 (28.24 %).

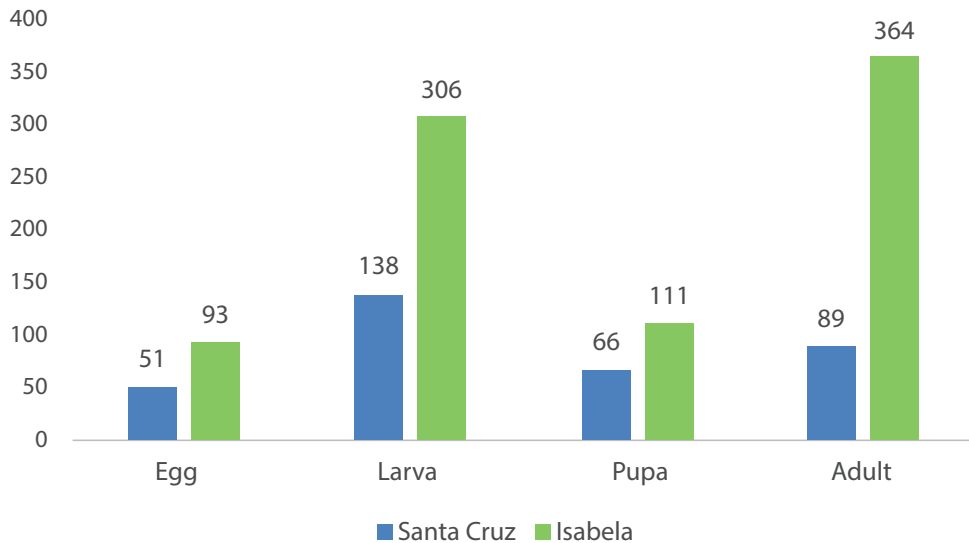


Figure 9. The number of individuals in each biological stage in the two infested islands in the Galapagos. Source: author's own elaboration.

A total of 2294 *C. rhizophorae* individuals were found in Santa Elena, of which 864 (37.6 %) were eggs, 781 (34 %) were adults, 389 (16.9 %) were larvae, and 260 (11.33 %) were pupae (Figure 10). The largest number of individuals was found in El Palmar (1649), compared to the lowest number in Punta Carnero (4).

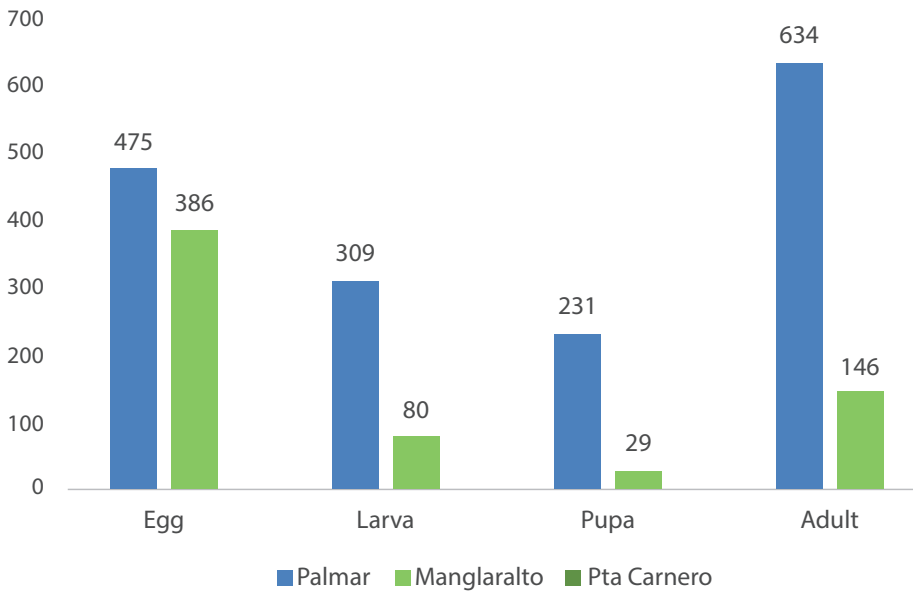


Figure 10. The number of *C. rhizophorae* individuals in each biological stage. Source: author's own elaboration.

Referring to the biological stages of *C. rhizophorae* individuals that affected the propagules, the sites present differences. In the Galapagos Islands, the adult stage that dominated in Isabela island was larval stage. This also presented the greatest number of individuals on Santa Cruz island. No relationship was found in the variation of the number of individuals in each biological stage. In Santa Elena, the quantity of individuals in each biological stage reflects that the eggs and adults represent the majority of individuals, with 71.6 % between the two (37.6 and 34 %, respectively). However, the variables that affect this distribution cannot be inferred because the biological cycle of *C. rhizophorae* lasts approximately one month (Arias de López & Molina-Moreira, 2019). The different species of the family Curculionidae have a variation from 12 days to 2 years, depending on the species. Additionally, the transformation process between the egg and adult stages lasts between 20 and 90 days (Wood, 1982). According to a recent study conducted in Ecuador, in the environmental conditions of

this specific study the minimum number of days from pre-oviposition to adult is 41 days, whereas the maximum is 54 days (Arias de López & Molina-Moreira, 2019).

Previous studies indicate the level of human intervention in the mangroves and the variation of environmental conditions can increase the percentage of *C. rhizophorae* infestation (Cassinelli, 2018; Menéndez, Guzmán & Núñez, 2006; Sousa, Kennedy and Mitchell, 2003). Therefore, comparing previous studies with this research, *C. rhizophorae* can be considered as a potential bioindicator of the state of mangroves in the genus *Rhizophora*, and that the infestation percentage is related to environmental conditions. However, these beetles are the only organisms identified in *Rhizophora* propagules. The presence of other arthropods such as *Acalles sablensis*, fungi such as *Penicillium sp*, and Lepidoptera in the family *Pyralidae* have been registered (Arias de López & Molina-Moreira, 2019; Cassinelli, 2018; Chamorro-Florescano et al., 2014; Feller and Mathis, 1997; Martínez-Zacarías et al., 2017; Posada, González & Romero, 2014; Wood, Stevens & Lezama, 1991). This study determined various solutions to mangrove attacks in eight species of Australian mangroves. It was demonstrated that some species had a lower propagule establishment and growth as a response to the insect attacks. On the other hand, some developed well whether or not the propagules were attacked. Also, the establishment and growth of other species was not seen to be affected by insect attacks. However, even still, attacked propagules grew more slowly than the propagules that were not attacked (Robertson, Giddins and Smith, 1990).

Conclusions

The evaluation of the damage caused by *C. rhizophorae* in mangrove propagules of the *Rhizophora* genus in the provinces of the Galapagos and Santa Elena concludes that the percentage of damage was lower than in the mangroves of the Gulf of Guayaquil in 2018. It is emphasized that a common factor in the incidence of damage is the human impacts that cause sedimentation in the mangroves. In

the sites studied in the Galapagos and Santa Elena, it is evidenced that the mangroves with greater human intervention had a higher percentage of damage. Furthermore, in the lower-populated islands such as Floreana and San Cristobal, where human intervention in the mangroves has not existed and natural tidal flow is maintained, the presence of *C. rhizophorae* is not observed. This reinforces the inference that greater human influence causes greater damage of the propagules by way of the insects. It is likewise evidenced that there was only one infested propagule in Punta Carnero in Santa Elena because of the constant tide which prevents sedimentation.

Since this insect was first reported in the Galapagos in 1998, it has only been present on the islands Isabela and Santa Cruz, where the mangroves have had greater pressure from human activities. However, this is the first evaluation of the harm caused by this insect in the propagules of *R. mangle*, which is the only species in the *Rhizophora* genus on the Galapagos.

Regarding the most affected area of the propagule, the radical area prevails in both the Galapagos and in Santa Elena. This brown area of the propagule is similar to the color of the insect and it is also thick. These factors could be the reason that the insect prefers the radical zone. The similar brown color of the insect and the radical area could lower visibility and the thickness could offer more space for creating the growth chambers and galleries for the larval stage, which is the stage that causes damage in the propagules because it is the stage in which the insect feeds most.

The number of individuals in each life stage inside the propagules was highly variable, which could occur because of the short lifecycle of the insect which lasts 21 days. As such, in the Galapagos the adult stage predominated on Isabela and the larval stage was greatest on Santa Cruz. In Santa Elena, the egg and adult stages prevailed.

Finally, the absence of *C. rhizophorae* on the Santa Cristobal and Floreana islands left open the question: to what extent is this insect an obligate parasite of the *Rhizophora* propagules? To obtain this

answer it will be necessary to further investigate the distribution of this insect. Similarly, it will be important to increase the number of studies of the natural and intervened mangrove conditions and their relationship with the presence and the damage caused by this insect which, owing to its relationship with *Rhizophora* propagules, ought to be considered as a bioindicator for the health of mangroves. Another relevant aspect to consider in future studies is the current structure of the mangroves in the Galapagos and Santa Elena. This kind of study would determine whether or not the arboreal structure and its relationship to *C. rhizophorae* change throughout time. This more integrated knowledge of mangroves will contribute to the achievement of better restoration and conservation strategies.

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VI

Mangrove forests of the National Wildlife Refuge Gandoca-Manzanillo, Limón, Costa Rica

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Lilliana María Piedra-Castro

Introduction

Mangroves are swampy forests located along tropical and subtropical coasts around the world. Their distribution is restricted to coastal areas where periodic flooding occurs. In addition, the species that dominate in these environments have different adaptations for occupying unstable substrates, tolerating salinity, exchanging gases in anoxic substrates, and reproducing through propagules (Kauffman et al., 2011; Díaz, Castro & Manjarrez, 2010).

These ecosystems are important because they offer a series of ecosystem services such as protection and stabilization of the coastline, which prevents coastal erosion and flooding. Likewise, they offer refuge to species of commercial and ecological importance in the critical stages of life cycles; they support fisheries by exchanging organic matter that is food for many species. Many species of fish, mollusks, crustaceans, use this area for their reproduction and the breeding (Yáñez-Arancibia & Lara-Domínguez, 1999).

In Costa Rica, mangrove ecosystems are included as estuarine systems of the intertidal subsystem and Woodland class. Costa Rica has important coastal areas with mangroves, especially in the Pacific where about 99 % of them are located. While on the Caribbean coast, only two mangrove forests have been reported: the one of Moín and

the one of Gandoca, which is found in the Refugio Nacional Mixto de Vida Silvestre Gandoca-Manzanillo. There are also come remains in the Cahuita National Park and the Tortuguero National Park.

In this text we are interested in the case of mangroves located in the town of Gandoca, which, unlike the majority of mangroves in the American Caribbean that suffer losses of 1 % of their area each year, has increased its area from 4.1 hectares in 1976 (Fonseca, Cortés & Zamora, 2007) to 24.7 hectares in 2017 (Álvarez-Sánchez, 2017).

The mangroves of Gandoca are one of the most preserved in Costa Rica and have a great diversity of both flora and fauna. Laguna Gandoca, associated with this ecosystem, is a breeding ground for the Atlantic tarpon (*Megalops atlanticus*) and its juveniles, and is inhabited by manatees (*Trichechus manatus*) and a large number of freshwater and brackish water fish. Mangroves are the only natural bank in which crabs, algae, sponges, and the mangrove scallop (*Crassostrea riphophorae*) can be observed (Coll, Fonseca & Cortés, 2001).

The study of the characteristics of these forests is essential to describe the history of a forest. Thus, the diameter of the trunks, height of the tree, and chemical composition of the wood are some of the variables that suffer temporary variations and are used to describe forest trends. Similarly, the distribution of the diametric classes allows us to understand the dynamics of forests, their relationship with environmental factors, and their response to management (Caritat, Molinas & Oliva, 1991; Bernadzki et al., 1998; Solomon and Gove, 1999).

The establishment of species lists alone provides limited information on the conservation status of an ecosystem. Therefore, it is necessary to use indicators based on structure and composition to differentiate conservation gradients (Lindemayer, Margules and Botkin, 2000). These indicators allow us to understand how species show different responses to environmental changes or disturbances. The functional group would be the species that use similar environmental resources and can overcome their ecological niche (Westoby and Leishman, 1997).

This text aims to analyze the plant biodiversity found in the mangroves of the Refugio Nacional Mixto de Vida Silvestre Gandoca-Manzanillo (REGAMA), Limón, Costa Rica. It is located between the mouth of the Cocles River and the Sixaola River and was created by Executive Decree No. 16614-MAG, in 1985. The refuge in total has 9449 hectares, where 5013 represent the continental part and the remaining 4436 correspond to the marine area.

Its geological composition is part of the Deformed Belt of northern Panama, which includes the sedimentary basins of Limón and Bocas del Toro in Panama, coral reefs, sandy beaches, fossil reef cliffs, estuaries, mangroves, soft bottoms, rock platforms, and seagrass beds. It is also a protection area for endangered species such as danta, various species of wild monkeys and felines, and the manatee. Furthermore, it is the only natural mangrove oyster bank (*Crassostrea rhizophorae*) in the country.

The refuge is divided into 15 zones that were established according to physical characteristics and land-use. Nine belong to terrestrial areas and six to coastal marine areas (Zamora, 2010). It was declared a RAMSAR site on December 11, 1995. Climate is influenced by a regime of heavy rains and constant temperatures for much of the year; according to the map of climatic regionalization, it presents temperatures from 24.9°C to 27°C and an average rainfall of 2500 to 3700 mm. The months of greatest rainfall range from May to August and from November to January and the relative humidity is between 80 % and 90 %.

The Gandoca area is where Gandoca lagoon is located and extends from Punta Mona to the mouth of the Sixaola River. Between the water's edge and the surrounding vegetation covers about 266 ha. The extension of the lagoon from the mouth to the entrance of the Gandoca River is 1.2 km and the maximum depth is 12.5 m.

This text describes the mangroves that are located in this refuge and whose secondary structure corresponds to a forest in constant renovation. There is a single dominant species and a series of environmental variations that favor the low diversity of tree species.

However, there is a great wealth of accompanying species that show a complex and resilient ecosystem.



Figure 1. Map of the mangrove sampling site in the Gandoca sector in REGAMA, Costa Rica, 2017. Green points are the sampled plots. Source: author's own elaboration.

Field Work

To determine the diversity and floristic composition of the site, six transects perpendicular to the water's edge were established, with six circular plots of 7 meters radius. The distance to the edge of the water and between each plot was 25 meters. A total of 36 plots were sampled, in an area of approximately 24.7 hectares (Kauffman et al., 2011).

The diameter at breast height (DBH) and height of individuals were measured. When the trunks of these species were not completely straight and presented bifurcations at 1.30 meters from the ground, each fork was measured separately.

The species were identified in situ. For those that could not be identified, a sample was collected, pressed, dried in an oven at 60 °C, and identified using the dendrological guides of Morales (2006) and Sánchez (2001) in the Anastasio Alfaro Herbarium of the Universidad Nacional, Costa Rica.

The basal area, dominance, and the total number of trees were calculated to obtain frequency and density. Dominance is the sum of all the basal areas of each species. As such, the absolute dominance is the sum of the asymmetric areas of a species expressed in m² and the relative one (Equation 1) as the quotient between the basal area of a species divided by the sum of the basal area of all species, expressed as a percentage (Lamprecht, 1990).

$$\text{Relative dominance} = \frac{\text{basal area (specie)}}{\text{basal area (all individuals)}} * 100 \text{ (Equation 1)}$$

Density corresponds to the number of trees per species. The absolute density is the number of individuals of a species; the relative is a percentage proportion of the number of individuals of a species divided by the sum of individuals of all species (Equation 2) (Lamprecht, 1990). Frequency is the existence or lack of a species within a given plot and is expressed as a percentage (Equation 3) (Lamprecht, 1990). Also, the class distribution was estimated using the Sturges formula (Equation 4). A total of 10 diametric classes were obtained. The general model of the distribution of the number of trees by diametric class is that of an inverted J (Valerio & Salas, 1997).

$$\text{Relative density} = \frac{\# \text{ ind of a species}}{\# \text{ total ind all species}} * 100 \text{ (Equation 2)}$$

$$\text{Relative frequency} = \frac{\# \text{ Plots containing species}}{\# \text{ Total plots}} * 100 \text{ (Equation 3)}$$

$$\text{Sturges class interval} = 1 + 3,322 * \text{LOG}(N) \text{ (Equation 4)}$$

The importance value index (IVI) corresponds to the sum of the relative dominance, abundance, and frequency (Equation 5), which indicates the ecological importance of the species in these wetlands and shows the indicator species of the forests, in their composition, structure, and relation to the site and its dynamics (Lamprecht, 1990).

$$IVI = \frac{\text{Relative dominance} + \text{Relative density} + \text{Relative frequency}}{3} \quad (\text{Equation 5})$$

For the frequency of occurrence of the accompanying species, all individuals in each plot were counted. Tables and graphs were made using the Microsoft Excel program. Wealth, frequency and relative dominance were calculated, as well as the IVI.

Floristic Composition

These mangroves are related to other plant associations. Based on the work of Coll, Fonseca and Cortés (2001), they are grouped into five associations: (i) swampy palm grove, (ii) palm grove with mangrove fern, (iii) mixed palm grove, (iv) very humid tropical forest, and (v) typical beach vegetation.

In this mangrove the following nuclear species have been described.

Rhizophora mangle: these trees can reach up to 45 m high. The outer cortex is light gray with dark spots and the inner face is pink. The wood is reddish and has no growth rings. The flowers are relatively small and are characterized by having four thick, fleshy lanceolate sepals. There are four narrow white or yellowish petals which come off easily. *R. mangle* generally has 2 to 4 flowers per inflorescence. Embryos are characterized as generally curved.

Laguncularia racemosa: this is a small tree and does not exceed 10 m high. The bark is grayish with long fissures. The leaves are opposite,

simple, whole, with a rounded apex and succulent aspect, reddish petioles, and cream-colored glands at the beginning of the leaf. The beam is characterized by an opaque green color, while the underside is characterized by a grass green color. The flowers are small and bell-shaped.

Rhabdadenia biflora: this is a creeper that can reach large sizes by occupying the forest canopy in low salinities. It is easily identified by its white latex and has simple, opposite, and leathery leaves. Floral peduncles may be longer than its leaves. It has white flowers with pink spots that are bell-shaped. Seeds are dispersed by the wind.

Brassavola nodosa: this is one of the most common species in the lowlands, mangroves, and coastal cliffs, especially in dry and hot areas. Its leaves are similar to those of the onion and its flower is strikingly striking. It blooms almost throughout the year, but mainly from January to June.

In the interior of the Gandoca lagoon there is a strong mix with facultative freshwater species such as Yolillo (*Raphia taedigera*), Cativo (*Prioria copaifera*), and Sangrillo (*Pterocarpus officinalis*).

In the evaluation carried out on the floristic composition of this research, a total of 38 plant species, including herbaceous and woody, were observed in the mangrove ecosystem. Two of the species were nuclear, while 36 were accompanying species. Of the nuclear ones, the one with the most individuals was *R. mangle*, followed by *L. racemosa*.



Figure 2. Gandoca lagoon, at the RAMSAR Refugio Nacional Mixto de Vida Silvestre Gandoca-Manzanillo site, Costa Rica. Source: author's own elaboration.

The most highly represented species include four genera in the family Araceae: *Anthurium*, *Montrichardia*, *Philodendron*, and *Syngonium*. Meanwhile, Arecaceae presented three genera: *Chamaedorea*, *Asterogyne* and *Cocos*. Three genera (*Prioria*, *Pterocarpus* and *Zygia*) were identified from the family Fabaceae. Finally, Orchidaceae was represented by *Brassavola*, *Epidendrum* and *Vanilla*. The two species with the highest frequency of occurrence in the plots were *Hymenocallis littoralis* and *Acrostichum aureum*. The last species indicated presented the highest frequency of occurrence in all plots (Figure 3).

The floristic composition of tree species varies in response to climate and biogeography, so each species has different tolerance

and adaptations levels (Lieberman and Lieberman, 2007). In addition, the concentrations of Ca, Mg, C and N, pH, are important factors for the development of the species.

Nutrient removal occurs when a tree falls, which favors different species because changes in the composition of the soil causes the heliophytes to proliferate.

A. aureum to established itself in glade openings in this mangrove forest. According to Medina et al. (1990), this fern grows in greater density when exposed to sun and low salinity conditions. On the other hand, they can also grow at high salinity conditions, but in low densities.

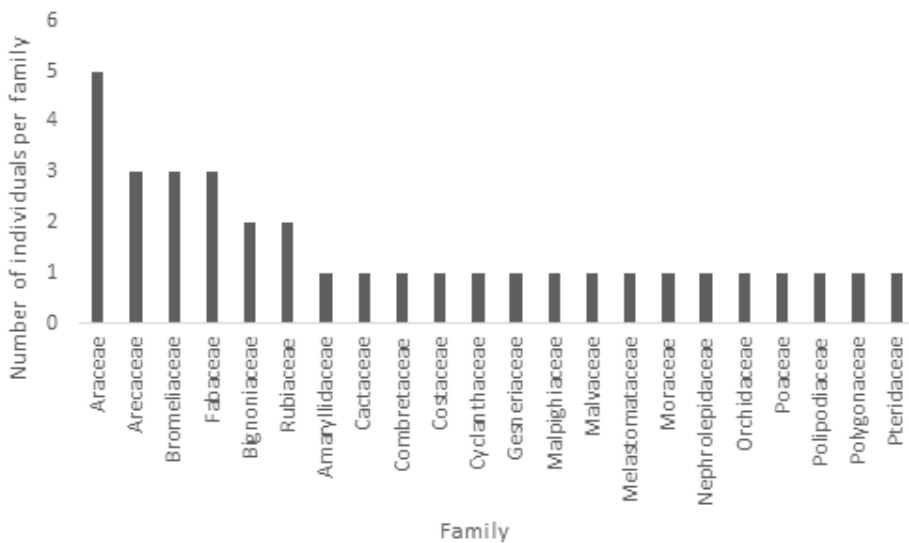


Figure 3. Number of species present in the different families found in the mangrove in the Gandoca sector in the REGAMA, 2017. Source: author’s own elaboration.

The mangroves presented a typical structure of secondary forests: an inverted J structure, where the largest number of individuals is in the first two diametric classes (Figure 4). This proportion of small

individuals seems to indicate a population in recovery phase after an event such as logging, strong winds, or a system that is in formation.

On the other hand, the distal structure is that which presents individuals that are distributed in various sizes and ages, with a distribution of inverted J. In addition, it occurs in humid forests, so the species are shade-tolerant (esciophytes). It is also a structure of young secondary forests, where most individuals have a small diameter and maintain stable conditions to compensate for changes in the environment. Consequently, there is a constant renewal due to the fall of mature trees that facilitates an ecological succession (Quirós & Quesada, 2003; Quispe, 2010).

In the mangrove of the Bahía de la Unión in the Pacific, in El Salvador, Chicas, González and Sayes (2016) reported an inverted J structure. There is potential for the forest to regenerate naturally because in this area they carry out the extraction of the wood from the shoots of the juvenile trees that are used as stakes to support the plastic membranes that are used in the salt mines.

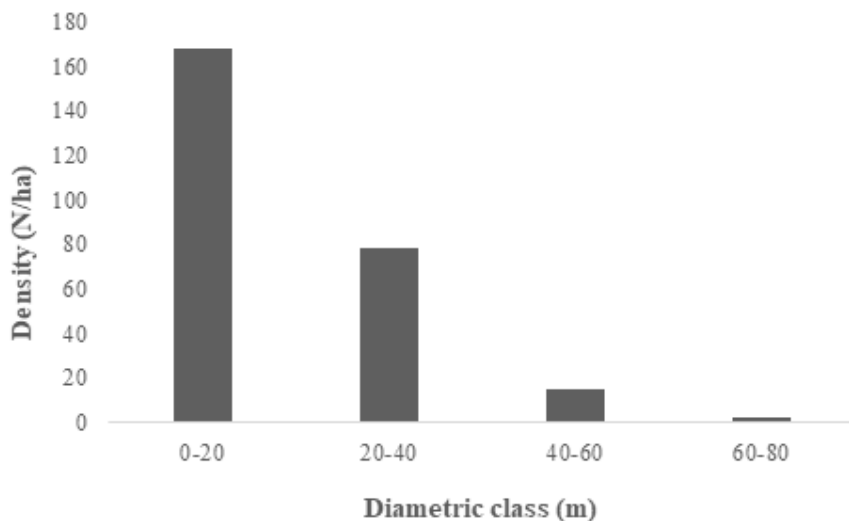


Figure 4. Diametric distribution of trees in the mangroves of the Gandoca sector in REGAMA, 2017. Source: author's own elaboration.

On the lower section, the individuals presented values of less than 10 meters. The greatest number of individuals was observed in the middle, presenting heights between 10 and 20 meters. The lowest number of individuals was in the upper section and had heights of greater than 20 meters (Figure 5).

Alterations in forest dynamics, soil, climate, and species strategies are determining factors of the horizontal structure and can be reflected in the diametric class of trees. Tree fall is a clear example of the transformations that occur in the forests; as a result, glades appear and allow the competition of individuals for light, space, and nutrients, among others, for the establishment of different species and their juveniles (Louman, Quirós & Nilsson, 2001). In these mangroves, sediment supply from the upper parts of the basin can form suitable territories where the juveniles of these plant species can establish.

Vertical distribution is determined by the distribution of organisms at the top of their profile. This responds to the characteristics of the species that compose them and to microenvironmental conditions of different heights. Therefore the tallest species are those that require more light intensity and are those that prevent the entry of light to understory plants (Louman, Quirós & Nilsson, 2001).

However, when glades are created by the fall of other trees, the dynamics of the forest change creating different microenvironments that allow ecological succession, hereby developing a new environment (Siteo, 1992, as cited in Zamora, 2010). However, there is a greater longevity of forest ecosystems in areas with higher concentration of tall trees; therefore, this phenomenon occurs more frequently in high areas than in those of lowland (Orozco, 1991).

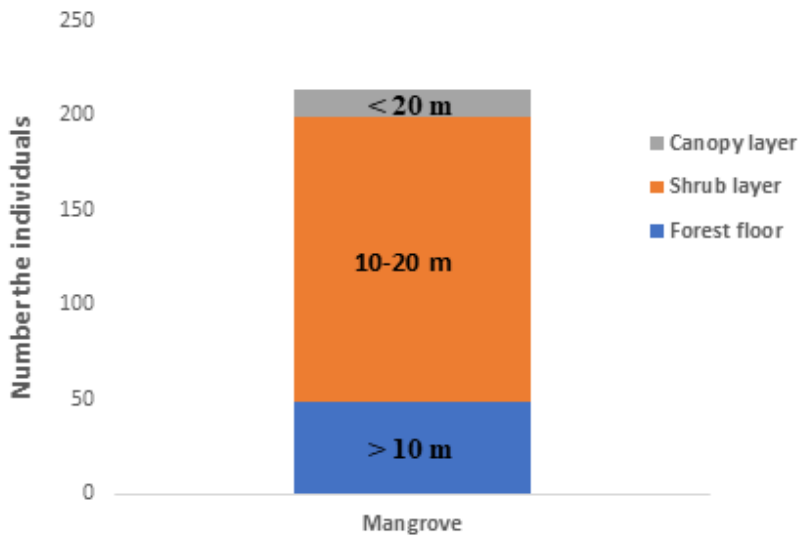


Figure 5. Distribution of heights (m) of the individuals present in the mangrove, in the Gandoca sector in the REGAMA, 2017. Source: author's own elaboration.

However, the characteristics of this mangrove are different from those described by some authors who report that there are seven different mangrove species in the Pacific mangroves of Costa Rica (Jiménez, 1999; Zamora-Trejos & Cortés, 2009). The opposite happens in the Caribbean of Costa Rica, where Coll, Fonseca and Cortés (2001), Manrow (2010) and Pizarro et al. (2004) report four different mangrove species, while only two of the four species were reported in this study. In general, mangroves of the genus *Rhizophora*, followed by the genus *Laguncularia*, are very exposed to the influence of the coastline. This could suggest that the increase in sea level, for the Caribbean Sea is affecting species composition due to the amount of salt that can accumulate in the soil.

The associated species in this study coincided with the reports by authors such as Coll, Fonseca and Cortés (2001) and Manrow (2011). The two most common species were the spider lily *H. littoralis* and the mangrove fern *A. aureum*; which it can displace other species by colonizing spaces with high light intensity.

In this study, 70 % of the individuals in the mangrove were found in the middle stratum; but Manrow (2011) reported 50 % of the individuals in this same stratum. This change may be due to the trend towards a mature environment in which individuals are old.

The species with high IVI in the Gandoca mangrove forest was *R. mangle*. In addition, it showed the relative frequency, density, and high basal area values that determined the IVI (Table 1). On the other hand, *R. racemosa* was below the value in all the variables.

Table 1. IVI for the nuclear species observed in the mangrove of Gandoca lagoon, 2017

Species	Density (%)	Frequency (%)	Basal area (m ²)	IVI (%)
<i>R. mangle</i>	78	66	8,5	74
<i>L. racemosa</i>	22	34	2,59	26

Source: author's own elaboration.

The mangrove fern *A. aureum* was the species that was observed in all mangrove plots and presented the highest percentage. The spider lily *H. littoralis* was observed with a 64 % occurrence frequency and the remaining 31 species had low occurrence frequency values (Figure 6).

The richness of species and their interactions in humid tropical forests are among the most important characteristics. These environments are dominated by trees, which interact with each other, and with other organisms whose presence and mixture are determined by climate and soil conditions (Louman, Quirós & Nilsson, 2001). Another variable that determines an environment is the altitude; ecosystems at higher altitudes have more species (Orozco, 1991). However, despite being located at low elevations, mangroves have low plant species richness. This particular condition is due to the stresses, such as high salinity and flooding, in which they develop (Domínguez-Cadena, León de la Luz & Riosmena-Rodríguez, 2018).

Although other authors have reported more species, we only managed to identify two individual species.

Mangroves have the best conservation status in the Costa Rican Caribbean, where they are constantly increasing in area (Coll, Fonseca & Cortés, 2001). There greater basal compared to *L. racemosa* evidences the dominance of *R. mangle* in most of the surface both near the lagoon and in the area that is farthest away. The dominance of two species and the fact that the traditional ecological zoning of mangroves is not fulfilled is concerning. However, some mangrove communities show particular patterns of distribution; some can occupy lowlands whereas others occupy higher areas but away from water.

Coll, Fonseca and Cortés (2001), and Manrow (2010) reported the presence of *A. germinans*, but in this study it was not observed. This could be related to chance or to the sampling effort. This could also be because their proliferation is constrained because certain nutrient limitations, such as phosphorus and nitrogen. Feller, Lovelock and Mckee (2007), showed that there was stimulation in growth and increase in biomass when phosphorus was added to *A. germinans*.

Another factor is the spread of propagules. According to Lema, Polanía and Urrego (2003), the propagules of *L. racemosa* and *A. germinans* are small and have an ovoid shape. They can die or move to other sites by water flow in less than two weeks, whereas those of *R. mangle* are larger, longer, and can remain in one place for a month or longer, increasing the establishment probability of this species.

Salinity may be another limiting factor in the growth of *A. germinans* because this species grows in soils where salinity reaches 90 PSU (Cintrón and Schaeffer, 1984). The salinity values in the lagoon reported by Benavides and Brenes (2010) were low and varied between 5 and 24 PSU, depending on the time of the year. The highest salinity occurs when the lagoon and the sea come together. When this study was carried out, the connection lasted a short time (about two months), so there was low salinity for most of the sampling (Fonseca, Cortés & Zamora, 2007).

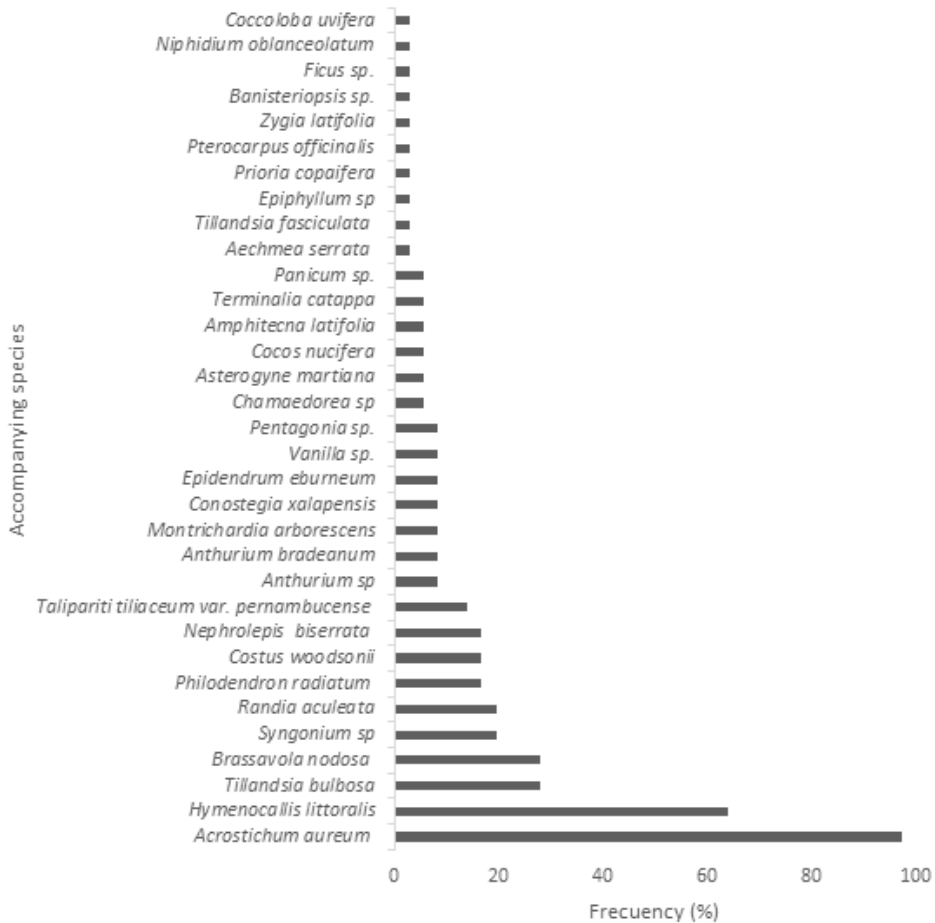


Figure 6. Frequency of occurrence of mangrove associate species in the Gandoca sector in REGAMA, 2017. Source: author's own elaboration.

Likewise, these differences can be attributed to the cycles and the size of the tides of the Caribbean. In addition, the salinity of both the mangrove soil and the water of the lagoon have variations because the river mouth is closed during the dry season whereas it opens in the rainy season, producing the exchange of fresh and salt water (Coll, Fonseca & Cortés, 2001). There is less development on the continental shelf of the Caribbean of Costa Rica, perhaps because it is very short. The growth of these ecosystems is associated with the

climate regime, salinity gradients, hydroperiod, tidal channel and the interior of the spatial distribution of the species. These last two factors can cause changes in biological functions and influence the growth of this type of forest (Jiménez, 1985; Monroy-Torres, Flores-Verdugo & Flores-de-Santiago, 2014).

For example, in the Pacific of Costa Rica, variation in the growth of mangrove trees has been observed when there are changes in salinity due to the abundant supply of fresh water. This occurs through runoff or a prolonged rainy season. There is consequently a decrease in salinity that allows greater development by presenting the highest heights, diameters, and basal areas (Jiménez, 1985). On the other hand, Ball (1998), in Northern Australian, observed greater growth of mangroves in places that were exposed to lower salinities, whereas the opposite occurred with individuals found in sites with greater salinities.

Regarding the tidal amplitudes, when mixed, an influx between sea and fresh water distributes the particles and forms sediment and beach banks where the mangrove is established (Mainardi, 1996). This is what happens in the Pacific Coast, but not in the Caribbean because the tidal amplitudes are smaller. In the particular case of Gandoca, the water of the lagoon is a greater influence than that of the sea, so there is no formation of beach banks.

These characteristics of the mangroves in the Gandoca lagoon could become vulnerable due to the changes in sea level because a greater volume of salt water will enter the estuary. This will allow greater water retention by increasing flood zones and salinity gradient. The mangrove will respond with a change in the vertical and horizontal structure of the vegetation and will recede as much as the terrain allows.

Conclusions

This mangrove forest presents a secondary structure with an inverted J distribution, which indicates that it is a forest in constant

renewal. The value of the importance value index demonstrates the dominance of *R. mangle*.

These mangroves constitute an ecosystem that presents environmental variations, which is why there is low diversity of tree species. Despite the dominance of a single species (*R. mangle*), the accompanying species significantly increase the diversity of the ecosystem, showing that it is a complex and resilient ecosystem. Future research needs should focus on the physical and chemical characteristics of the soil: the horizontal zoning of the species and how the physical-chemical factors determine their presence. At the same time, it is necessary to understand the regeneration processes and the role of exotic plants in the ecosystem.

Acknowledgements

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VII

Phylogeographical history of the Caribbean Mangrove Community based on Palynological and Molecular Data

Elsie Rivera-Ocasio

Introduction

The present-day spatial distribution of genetic diversity in plant species result from the interaction of multiple micro and macro evolutionary factors. These factors included mechanisms of pollen and seed dispersal, environmental heterogeneity, microhabitat selection, intraspecific interactions, life-history traits, breeding and mating systems, colonization history (bottleneck events, founder effects, and range expansions), geologic events and paleoclimate influences (Epperson, 2003; Vekemans and Hardy, 2004). The relative importance of these factors on the structure will depend on the spatial and temporal scale studied (Linhart and Grant, 1996; Rivera-Ocasio, Aide and McMillan, 2006). Factors such as mechanisms of pollen and seed dispersal and microhabitat selection are highly important in describing local and contemporary patterns of genetic structure (Proffitt and Travis, 2010; Cisneros de la Cruz et al., 2018). Whereas at larger spatial scales, historical factors (colonization events, founder effects, and genetic drift), are the ones that better explain patterns of genetic structure (i.e., regional) (Ouborg, Piquot and Van Groenendael, 1999; Rivera-Ocasio, Aide and McMillan, 2002).

The role of historical factors in shaping the spatial distribution of genetic diversity at the intraspecific level is fundamental in phylogeographic studies (Avice, 2000). Phylogeography is a sub

discipline of biogeography that studies the principles and processes that determine the geographical distributions of gene genealogies (Avice, 1998). In this discipline, individual genes (gene trees) are used to reconstructed genealogical histories that could reflect species population history (Knowles and Maddison, 2002). The phylogeographic approach complements the traditional population genetic approach by emphasizing a species biogeographic history (Knowles and Maddison, 2002). Phylogeographic studies have helped to identify population divergence in many species, which in some cases, have resulted in the discovery of cryptic species or the identification of evolutionary significant units or management units (Avice, 2004). The identification of units with unique geographic distributions and distinctive genetic patterns help to define and prioritized areas with a high conservation value. Furthermore, the phylogeographic approach has been used to make comparisons among multiple co-distributed species (i.e., comparative phylogeographic) and it has made possible to clarify the histories of biogeographical regions and to provide a historical perspective on the composition of present-day communities (Arbogast and Kenagy, 2001; Nettel and Dodd, 2007; Rivera-Ocasio, Aide and McMillan, 2002; Cerón-Souza et al., 2010, 2015).

The community of mangrove and associated species is an excellent model for comparative phylogeographic studies because it includes multiple co-distributed species which are not taxonomically related, but share many ecological characteristics (e.g., water dispersal, salinity tolerance). Previously, the lack of resolution of available molecular markers at low taxonomic levels (i.e., population) limited the extent of studies of plant comparative phylogeography. Recent studies, however, have proved that some molecular markers used for plant phylogenetics (e.g., nrITS) and population studies e.g., microsatellites) could be used, particularly for tropical species with broad geographical distributions (Nettel and Dodd, 2007; Dick, Abdul-Salim and Bermingham, 2003; Dick et al., 2007). Furthermore, the discovery of non-coding cpDNA regions with relatively high levels of variability at the intraspecific level has opened new possibilities to study the historical biogeography of plants (Hamilton, 1999; Shaw et al., 2005, 2007).

Moreover, the wetland community has one of the best pollen records for the entire Caribbean region (Graham, 1995, 2006; Gee, 2001; Ellison, 2008) (Table 1, Figure 1), which allows for comparisons of the concordance between the biogeographic histories revealed by paleo botanical and molecular techniques (Graham, 2006).

Palynological studies from the Caribbean had identified the historical presence of extinct (Gee, 2001) and contemporary (Graham, 1995, 2006; Duke, 1995; Gee, 2001) species of wetland plants in the region (Table 1, Figure 1) and a progressive increase in diversity from the Eocene (≈ 49 mya) to the present (Graham, 2006; Urrego et al., 2013). Some members of the community (e.g., *Rhizophora* sp. and *Avicennia* sp.) appeared in the fossil record in the Eocene (Figure 1). Since then, extinction (i.e., *Nypa* sp., *Pelliciera* sp., locally extinct in most of the Caribbean) (Gee, 2001) and colonization resulted in the present-day community composition; this community became established during the Quaternary, with the appearance of wetland-associated genera like *Conocarpus* sp. and *Pterocarpus* (Graham, 1995).

Table 1. Taxonomic identification of the wetland species, sampling design, and # of haplotypes from the psbB-psbF non-coding cpDNA region (in parenthesis)

Species	Family	*Colonization time (Millions of years)	No. of populations Caribbean Pacific		No. of individuals Caribbean Pacific		No. of haplotypes Caribbean Pacific	
Rhizophora mangle	Rhizophoraceae	Eocene (54.8-33.8)	10	(2)	43	(6)	4	(3)
Annona glabra	Annonaceae	M. Oligocene (28.5)	6	(-)	37	(-)	1	(-)
Avicennia germinans	Acanthaceae	L. Miocene (11.2-5.4)	9	(2)	101	(10)	3	(2)
Laguncularia racemosa	Combretaceae	E. Miocene (23.8-1.6)	9	(1)	38	(2)	3	(2)
Conocarpus erectus	Combretaceae	Quaternary (1.8-0.01)	9	(1)	46	(2)	1	(2)

Species	Family	*Colonization time (Millions of years)	No. of populations Caribbean Pacific		No. of individuals Caribbean Pacific		No. of haplotypes Caribbean Pacific	
Pterocarpus officinalis	Fabaceae	Quaternary (1.6-0.01)	10	(1)	50	(5)	7	(1)

*The estimated colonization time is based on the first appearance in the fossil record within the Caribbean Basin. No samples of *Annona* were collected from the Pacific coast. *Avicennia germinans* colonization time is not based on the first appearance in the fossil record (Eocene-Mississippi and Tennessee) but in the first appearance within the Caribbean fossil record.

Source: author own elaboration.

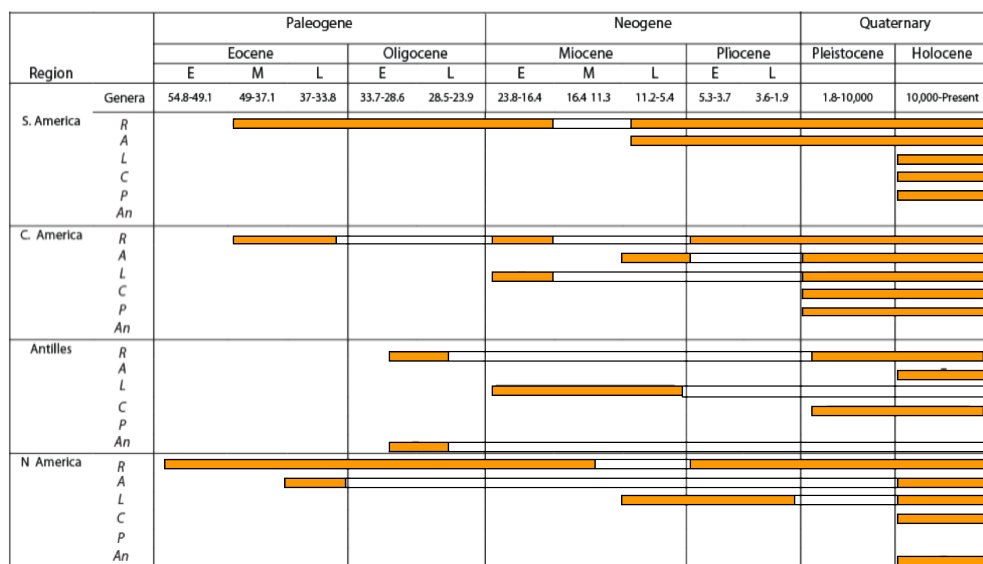


Figure 1. Palynological information for Caribbean wetland species at different geological time scales. Codes for genera are: R = *Rhizophora*, A = *Avicennia*, L = *Laguncularia*, C = *Conocarpus*, P = *Pterocarpus*, An = *Annona*. Dash-lines represent fossil records where the specific time (i.e., early, middle, or late) within the epoch was established; filled bars represent records that only report the epoch. Source: author own elaboration.

The main objective of the present study was to apply a comparative phylogeographical approach to reconstruct the colonization history and connectivity among six coastal wetland plants (e.g., *Rhizophora mangle*, *Avicennia germinans*, *Laguncularia racemosa*, *Conocarpus erectus*, *Pterocarpus officinalis* and *Annona glabra*) within the Caribbean region. Particularly, two major questions were considered: Are the phylogeographic patterns congruent among the different wetland plant species within the Caribbean Basin? Does the evidence from a cpDNA gene genealogy and the pollen record reveal a congruent colonization history?

The genetic evidence has revealed that the original colonization history of some coastal wetland plant species within the Caribbean Basin has been erased by local extinctions and recolonization due to climatic shifts. The present-day distribution of genetic diversity does not reflect the initial colonization history, but the result of historical and ecological factors acting at different temporal and spatial scales. The congruency found among the gene genealogies of the different species across the Caribbean region identify important historical events such as historical propagule and seed dispersal vectors (i.e., sea currents), geologic events (emergence of the Panamanian Isthmus), and paleoclimate influences as the generating forces of genetic structure. Moreover, three genetic units were identified for the region, result that could help to develop regional conservation efforts for the mangroves and associated species.

Phylogeographic patterns of coastal wetland plants based on chloroplast DNA

Gene networks representations from populations of six Caribbean wetland plant species using the non-coding intergenic region of the cpDNA (psbB-psbF) (Hamilton, 1999) revealed that four species had genetic differentiation within the Caribbean region (Table 1, Figures 2, 3). The variable species show the existence of three congruent genetic breaks within the Caribbean basin, with the exception of

C. erectus and *A. glabra* which showed no variation (Figures 2, 3). The phylogeographical units corresponded to a South American unit (Venezuela, Trinidad, and French Guiana), an Antillean Unit (Dominican Republic, Puerto Rico, and Guadeloupe), and a Central-North American Unit (Panama-Galeta, Panama-Bocas del Toro, Mexico, and the USA).

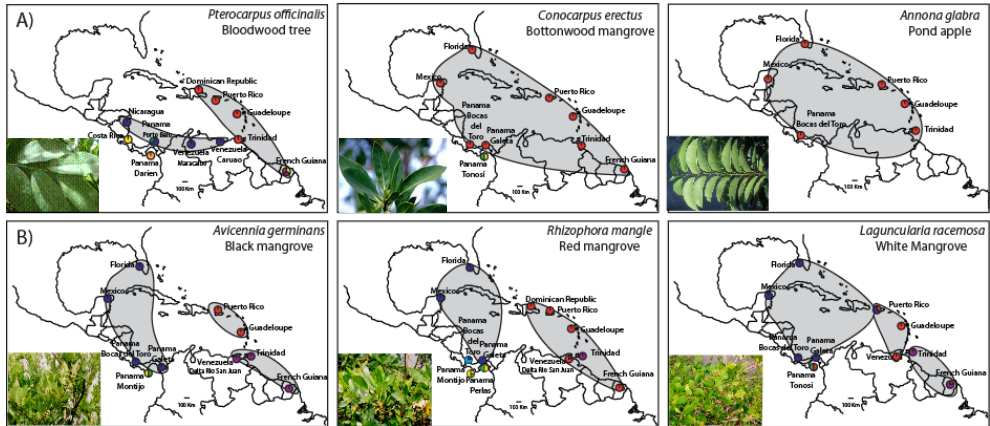


Figure 2. Distribution of cpDNA haplotypes within the Caribbean for six wetland plant species: **A.** Mangrove associated species, **B.** Mangrove species. The small colored pie chart represents haplotype proportions for all individuals in each population; each color identifies a different haplotype. Gray polygons identified major genetic breaks for each species within the region. Source: author own elaboration.

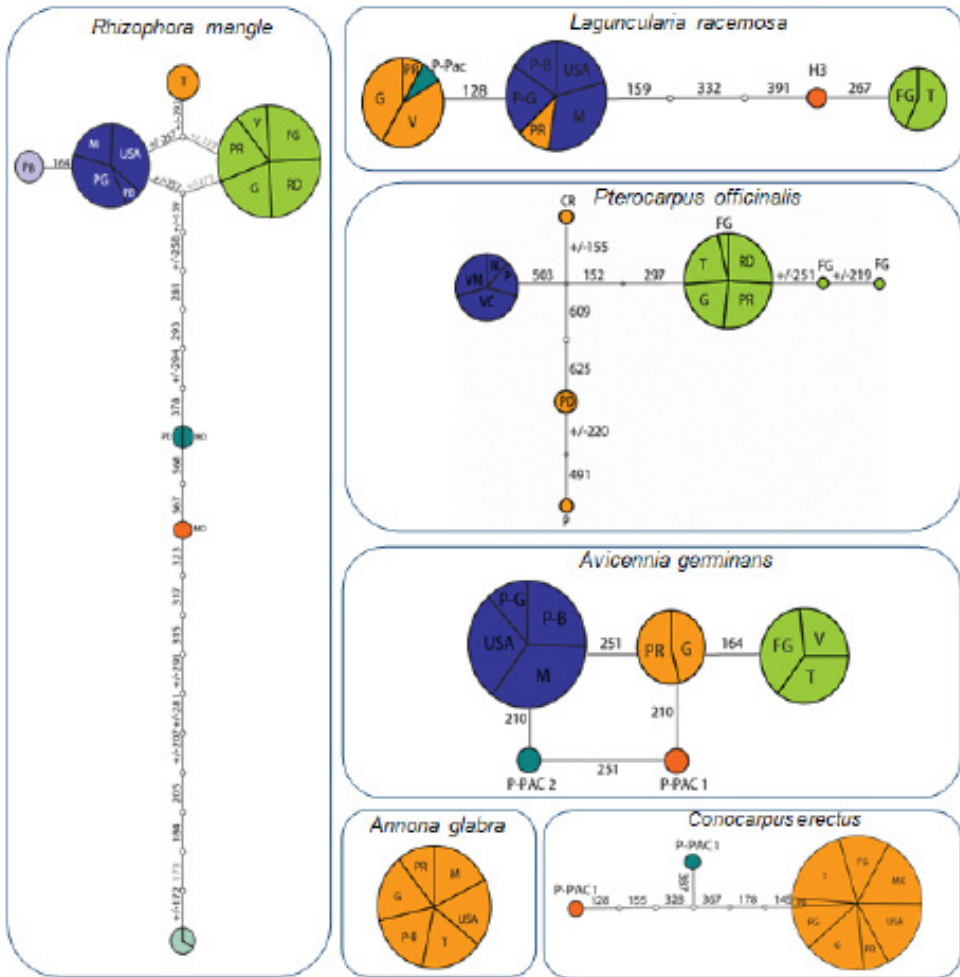


Figure 3. Haplotype minimum spanning network for all six wetland species based on cpDNA using TCS v1. 21 (Clement, Posada and Crandall, 2003). Circles represent haplotypes, and the sizes are proportional to the number of individuals in each haplotype. Small circles represent inferred haplotypes not recovered in the sampling. Each line between haplotypes represents a mutational difference, and the number next to the line is the position of the mutation. Indels are code as single character and are identified with a (+/-) symbol next to the mutation position. The limit of parsimony (Templeton, Crandall and Sing, 1992) was set at 95 %, and gaps were treated as a fifth character. Source: author own elaboration.

The congruency in cpDNA haplotype networks from the four variable species correlate with the dominant ocean currents within

the Caribbean in recent geological times (Kennedy et al., 2016). This correlation supports the hypothesis that despite the differences in life-history traits of mangrove and associated species, genetic patterns based on cpDNA show that seed dispersal through sea currents (i.e., hydrochory) is the most important factor influencing the structuring of species in these wetland plant communities (Rivera-Ocasio, Aide and McMillan, 2002, 2006). These results are consistent with studies that, although used different genetic markers (e.g., microsatellites), have also identified similar genetic separations in the Caribbean for certain mangrove species (Cerón-Souza et al., 2015; Kennedy et al., 2016). The microsatellites genetic markers are mainly used to determine connectivity among populations at an ecological time scale due to their fast mutation rate (Varshney, Graner and Sorrells, 2004). Accordingly, the similarity in the phylogeographic patterns revealed by cp-DNA, which operates at an historical (i.e., evolutionary) time scale, suggest that Caribbean wetlands plants have been connected through similar ocean currents since their historical colonization time.

For the species with no genetic variation (*C. erectus* and *A. glabra*), the lack of variation could be attributed to a low mutation rate of the used cpDNA region (Shaw et al., 2005). However, in the case of *C. erectus*, since there were seven mutational differences between the Caribbean and Pacific populations this explanation seems not suitable. Instead, the result supports the hypothesis that the original genetic pattern was erased from the Caribbean basin and that the lack of variation could be attributed to recent historical phenomena that obscured the older biogeographical pattern of population differentiation (Figures 2, 3). Based on the differences between the Caribbean and Pacific populations, we should expect a more complex genetic structure among the Caribbean populations because the presence of *C. erectus* along the Pacific coast of Central America suggests it has been in the region for more than 4 mya (i.e., age of closure of the Panamanian isthmus). This observation contradicts the pollen record, which only identified *Conocarpus* in the Quaternary or that the Caribbean populations started as a founder population containing an unsampled haplotype from the Pacific.

Nevertheless, when the spatial genetic patterns were considered at a smaller spatial scale there were some exceptions to the general pattern, particularly around northeastern Venezuela and Trinidad. For example, in *A. germinans* and *L. racemosa*, the populations of the South American and Antillean phylogeographic units had distinct haplotypes, but the populations of *P. officinalis* and *R. mangle* shared haplotypes between the two units. In *L. racemosa*, the differentiation between individuals from Trinidad-French Guiana and Caribbean populations was greater than their difference with individuals from the Pacific side of Panama, suggesting a stronger barrier to gene flow among the Northern South America and Antillean biogeographical regions or independent colonization events (Figures 2, 3). In *P. officinalis* the genetic break occurs between Venezuela and Trinidad despite their short distance (≈ 11 km). Individuals from Venezuela share a haplotype with those from Central America, while individuals from Trinidad share haplotypes with those from French Guiana and the Antilles. Similarly, in *R. mangle*, the most common haplotype among individuals from Trinidad is not shared with individuals from Venezuela. These exceptions to the general pattern show that in addition to ocean currents, there are other factors such as vicariance events and climatic fluctuations that also influenced genetic patterns; therefore, current patterns of genetic diversity result from the synergy of these historical and ecological factors.

Palynological records and the effect of paleoclimate shifts in Coastal wetlands plants in the Caribbean

The wetland community has one of the best pollen records for the Caribbean region since wetland environments provide favorable conditions for the preservation of pollen (Ellison, 2008). Many Caribbean sediment cores from coastal areas have identified the presence of pollen from contemporary coastal wetland plant species (Graham, 1995; Ellison, 2008; González et al., 2010). To contrast the colonization history based on genetic markers and palynological data, pollen records

from the selected wetland species were obtained from the literature and separated by age and geographic region (Table 1, Figure 1). The hypothesis tested was whether or not species that colonized the Caribbean earlier (e.g., *Rhizophora* and *Avicennia*) show higher genetic differentiation among populations than species that colonized more recently (e.g., *Pterocarpus officinalis* and *Conocarpus erectus*).

The summary of the pollen record of wetland taxa reveals that some species are not reported in the region on specific geological periods (e.g., *L. racemosa* disappeared from Central America from Middle Miocene until the Pleistocene) even when other mangrove species still occurred (Figure 1). Furthermore, even *R. mangle*, which has been present in most regions since the Eocene (Graham, 2006) showed population declines. The decline was assumed by the reduction in the percent of its pollen and a shift in the dominance of other mangrove species (Torrescano-Valle and Islebe, 2006), particularly in places where the rate of sedimentation was slower than the relative rise in sea level (Cohen et al., 2016; Khan et al., 2017). Pollen records show a very similar pattern in other regions of the Caribbean where pollen substitution of *L. racemosa*, *A. germinans*, and *C. erectus* is observed in the deeper layers of the *R. mangle* pollen-sediment, which is presented as indicative of a transgressional sequence associated with fluctuations in sea level (Ellison, 2019).

Accordingly, what the pollen record is showing is that wetland plant species fluctuated in density and distribution within the Caribbean region. Thus, the discontinuity showed by the pollen record concurs with the lack of support to the hypothesis that species with longer residence time in the Caribbean would show greater genetic diversity than late-colonizing species; since, no significant relationship was found between time of colonization based on pollen record and the genetic diversity of the studied species ($r^2 = 0.219$, $F = 1.13$, $df = 5$, $p = 0.35$) (Figure 4). For example, *R. mangle* has been present throughout most of the region since the Eocene, but it has low levels of genetic diversity within the Caribbean basin in comparison with *L. racemosa*, which appears in the fossil record approximately 25 million years later.

Annona glabra, which appeared in the fossil record 28 mya (Hollick, 1928; Graham and Palacios, 1996), showed no genetic variation (Figures 1, 3) and *P. officinalis* showed a relatively high genetic diversity, despite its relatively recent colonization time (1.8 mya).

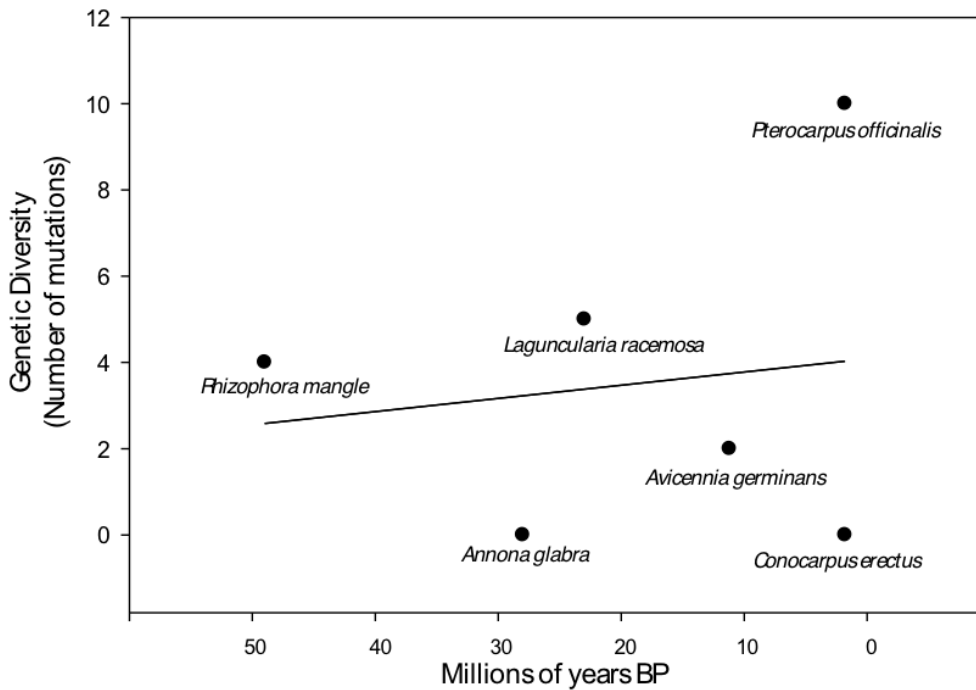


Figure 4. Genetic diversity represented as the number of mutations against the time of colonization. Source: author own elaboration.

The discontinuity found in the pollen record, and the lack of correlation between colonization time and genetic diversity supports the hypothesis that a historical reduction in population sizes and the potential local extinction of populations has erased the original colonization history. Seems that climate—rather than colonization time—is the primary driver of the pattern in genetic diversity in the Caribbean mangrove community.

Climate oscillations have been described as the most influential factor in the evolution of mangroves since the Eocene (Plaziat et al., 2001; Ellison, 2019). Paleoclimatic reconstructions (Eocene to Quaternary) have identified several periods of cooling/heating, which caused drastic changes in sea level (Plaziat et al., 2001; Torrescano-Valle and Islebe, 2012; Ellison, 2008; Aragon-Moreno et al., 2018; Ellison, 2019). The last period of severe climate fluctuations occurred during the Quaternary when several glacial-interglacial periods resulted in changes in temperature, rainfall patterns, and sea level (Plaziat et al., 2001; Woodroffe and Grindrod, 1991). These changes, in particular variations in sea level greatly affected coastal wetland species globally including the Caribbean (Woodroffe and Grindrod, 1991; Plaziat et al., 2001; Renner, 2004; Torrescano-Valle and Islebe, 2006; Vedel et al., 2006; Cohen et al., 2016; Ellison, 2019). Depending on the susceptibility of the different species, populations of coastal wetlands plants became locally extinct at specific locations, and probably re-colonized after more benign conditions were reestablished (Woodroffe and Grindrod, 1991; Schwarzback and McDade, 2002; Ellison, 2019).

Furthermore, a pattern of extinction and re-colonization during the Quaternary could explain the changes in the distribution of haplotypes and the relatively shallow phylogeographic structure of the present-day mangrove species (Figure 3). If re-colonization occurred at different rates among species, we could expect a distinct genetic structure in some areas of the species range. For example, the differentiation of *P. officinalis* and *R. mangle* from Trinidad and Venezuela populations could reflect different sources or time of colonization. Although the main direction of the major Caribbean currents have not changed since the breakup of West Gondwana (Vedel et al., 2006) there have been local changes associated with the closing of the Panamanian isthmus and the sea level fluctuations of the Quaternary, which could cause isolation of coastal plants and thus generate genetic differentiation as seen in Panama and Trinidad (Figures 2, 3). Although today Trinidad is an island, during the last glacial-maxima sea-level decline created a land connection between Trinidad and Venezuela that interrupted ocean currents.

These changes in the coastal morphology in this region could result in the isolation of these two localities or even a local extinction of coastal plants. Palynological evidence supports the presence of *P. officinalis* populations in Venezuela (Muller, 1959), but not in Trinidad, which could be associated with an incomplete fossil record from the area or could also mean that *P. officinalis* colonized or recolonized Trinidad later in the Quaternary in a second colonization event from a different source (Rivera-Ocasio, Aide and McMillan, 2002).

Furthermore, the levels of genetic variation seen in most species support the hypothesis that historical climatic shifts have influenced not only the genetic structure but the genetic diversity of wetland species (Figure 3). Climatic oscillations reduced genetic diversity levels of the studied species and the current day genetic patterns do not reflect founder effects as much as Quaternary re-colonization history (Figure 5).

Populations at the extreme of the distributions and in areas susceptible to fluctuations in sea level became locally extinct and recolonized when conditions became favorable for re-establishment (Figure 5). This colonization scenario is supported by genetic diversity data from multilocus markers (i.e., AFLP and microsatellite) of two coastal wetland species, *P. officinalis* (Rivera-Ocasio, Aide and McMillan, 2002, 2006) and *A. germinans* (Cerón-Souza et al., 2015). Both species showed a gradual decrease in genetic diversity from Southern to Northern populations within the Caribbean Basin, a pattern that support post-glacial colonization and seed dispersal following more contemporary sea currents.

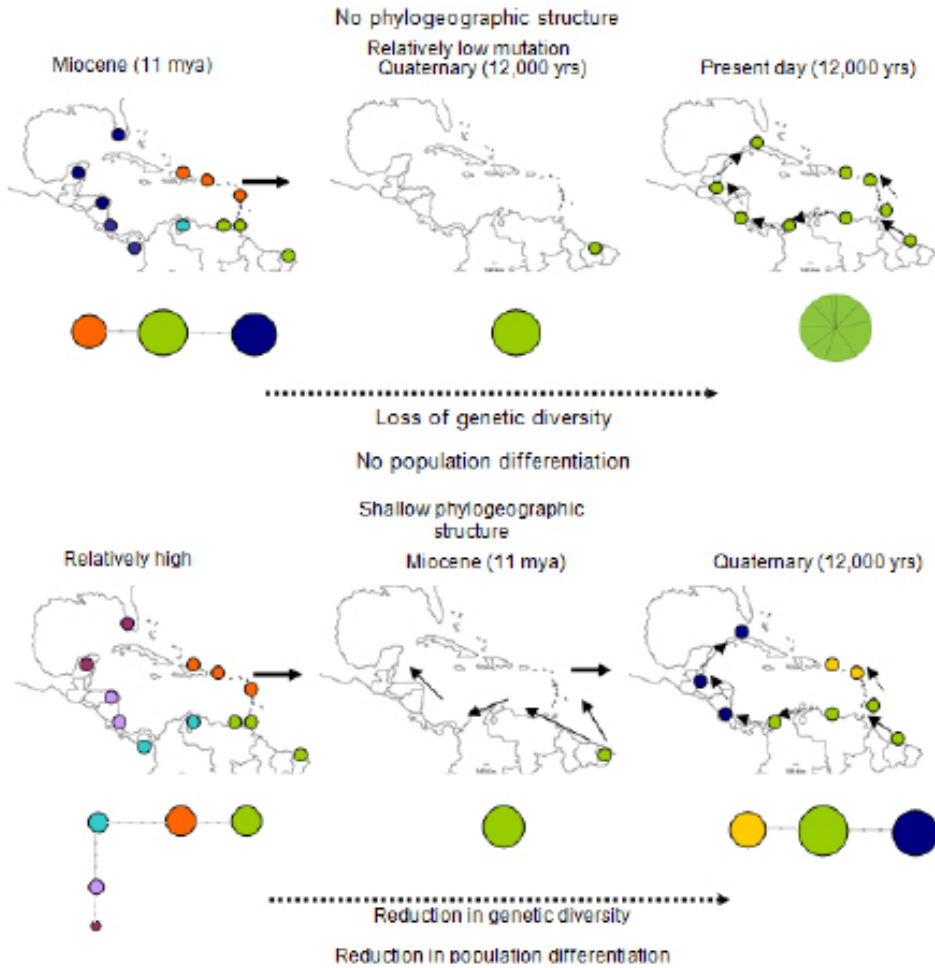


Figure 5. Hypothetic models of how climatic oscillations and changes in sea level during the Quaternary could have erased the original phylogeographic structure of coastal wetland plants: Model for species with relatively high mutation rates and high initial genetic diversity; Model for species with relatively low mutation rates and low initial genetic diversity. Source: author own elaboration.

Within the coastal wetland plant community, *P. officinalis* is the most inland species, and although it preferentially occurs in flooded areas behind mangroves, it can occupy non-flooded areas. Thus, the reduction in sea level and the disappearance of suitable areas for mangroves that presumably influenced responses of other

mangrove species in the Quaternary could have a mild or no effect on the distribution of *P. officinalis* populations and, consequently, in its genetic diversity pattern. Nevertheless, we understand that even though the mangrove fossil data is one of the best for the Caribbean region, there are gaps of information in the fossil record that could influence our interpretations of the colonization history, for example, there is limited data for the Antilles in general and particularly for the Neogene (Miocene and Pliocene) and early Quaternary. Thus, the lack of congruence between the pollen record and the cpDNA structure could result from the lack of resolution of the fossil record.

For example, *A. glabra* was identified early in the fossil record, but it was not observed for the next 28 million years from any geologic formation. Although the estimation of the colonization time—and, thus, the expected genetic diversity—of *A. glabra* could be incorrect (i.e., the identified fossil could be from other *Annona* species), the lack of genetic differentiation may also reflect climatic shifts. If a species range is restricted to some “refugial” areas that eventually act as sources for rapidly expanding populations, loss of diversity and homozygosity are among the expected genetic consequences (Hewitt, 2000).

The discontinuous presence of some taxa may also be explained by the lack of appropriate geological formations for pollen fossilization, non-detection of rare taxa due to low pollen counts (Chengyu, Hooghiemstra and Duivenvoorden, 2006), but once again the scenario of local extinctions and recolonization associated with climatic fluctuations could not be refused. However, when it comes to reconstructing the history of colonization using pollen records, we should be as conservative as possible. Although mangrove fossil data are one of the best for the Caribbean region, there are information gaps in the fossil record that could influence the interpretation of the history of colonization. For example, there are limited data for the Antilles, in general, and particularly for the Neogene (Miocene and Pliocene) and the early Quaternary (Flantua et al., 2015). It is for this reason that the combination of palynological data is strengthened

by the use of other tools such as molecular data that allow us to understand the evolutionary history of plant species.

Conclusions

In general, the geographic structure and patterns of genetic diversity of the Caribbean wetland community suggest that the main factor in the genetic structure of mangroves and associates are ocean currents. However, diversity patterns reflect more the extinction recolonization process associated with climatic fluctuations and changes in sea level. Although the diversity patterns of the species used were not consistent with the fossil record and no correlation was observed between colonization time and levels of genetic diversity, the fossil record shows changes in the community of mangrove species associated with climatic fluctuations. Local extinctions in historical times impacted the genetic diversity of the species, and we still see the signal in contemporary populations. If we use the historical scenario as a frame of reference to understand how present-day mangrove populations will respond to climate change, it is necessary to identify where the populations with the highest evolutionary potential are located. The survival of contemporary species will depend on the rate of sediment accumulation and the speed of sea-level rise. However, unlike the past, where the ability to survive climatic fluctuations depended mostly on geomorphological dynamics and climatic variables, we must now consider the anthropogenic factors on the survival models of mangrove lineages. The negative influences of anthropogenic factors (e.g., road proximity, fragmentation, isolation by changes in uses of land) and natural (e.g., increase in the frequency and intensity of storms) will constrained their capacity to migrate and adapt (Ellison and Farnsworth, 1996; Torrescano-Valle and Islebe, 2012). However, as in the past, there are areas where mangroves are more likely to persist (e.g., internal lagoons and higher elevation areas), and those are the areas that represent the best sources or gene-reserves (highly diverse) for future populations in the region. Therefore, the identification of regional genetically diverse units is of critical importance for the regional conservation of mangroves. Given

the existence of a significantly consistent phylogeographic structure among the different species of wetland plants in the Caribbean, it is relevant that the identified genetic units be considered in conservation plans for both, mangrove species and the conservation of other species of plants and coastal organisms associated with Caribbean mangroves.

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VIII

The Lords of the Mangrove. Ancestral knowledge of the Añú on the banks of Lake Maracaibo (Venezuela)*

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Dianora Richard de Mora
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Mangroves of the Maracaibo Lake Watershed

The Maracaibo Lake watershed was formed approximately 40 million years ago, as a consequence of the sinking of an immense forest in the Tertiary period. It is located in the Northwestern extreme of Venezuela, and occupies an area of 78,180 km², which contain the waters of the lake in its center. It constitutes a sedimentary watershed of tertiary and quaternary deposits, formed by all the plowed lands or dredges of the overflows that open into the lake, which includes all of the Zulia state, part of the Táchira, Mérida, Trujillo, Lara, and Falcón states, as well as an extension of the Northern Department of Santander of Colombia (Olier, 1997).

The abundance of freshwater tributaries in the watershed of Maracaibo Lake has made its shores an ideal space for the development of mangroves, an ideal habitat for the development of palafitic cultures, such as that of the añú (or paraujanos) who, as well as the mangroves, have benefitted from the fresh water that flows in this watershed. The availability of these generous

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mangrove properties and the diverse aquatic species, terrestrial and amphibian, that live in them has provided the paraujanos with sufficient resources for subsistence.



Figure 1. Map which, with a darker color, signals the distribution of mangroves in America.
Source: Wagner (1980).

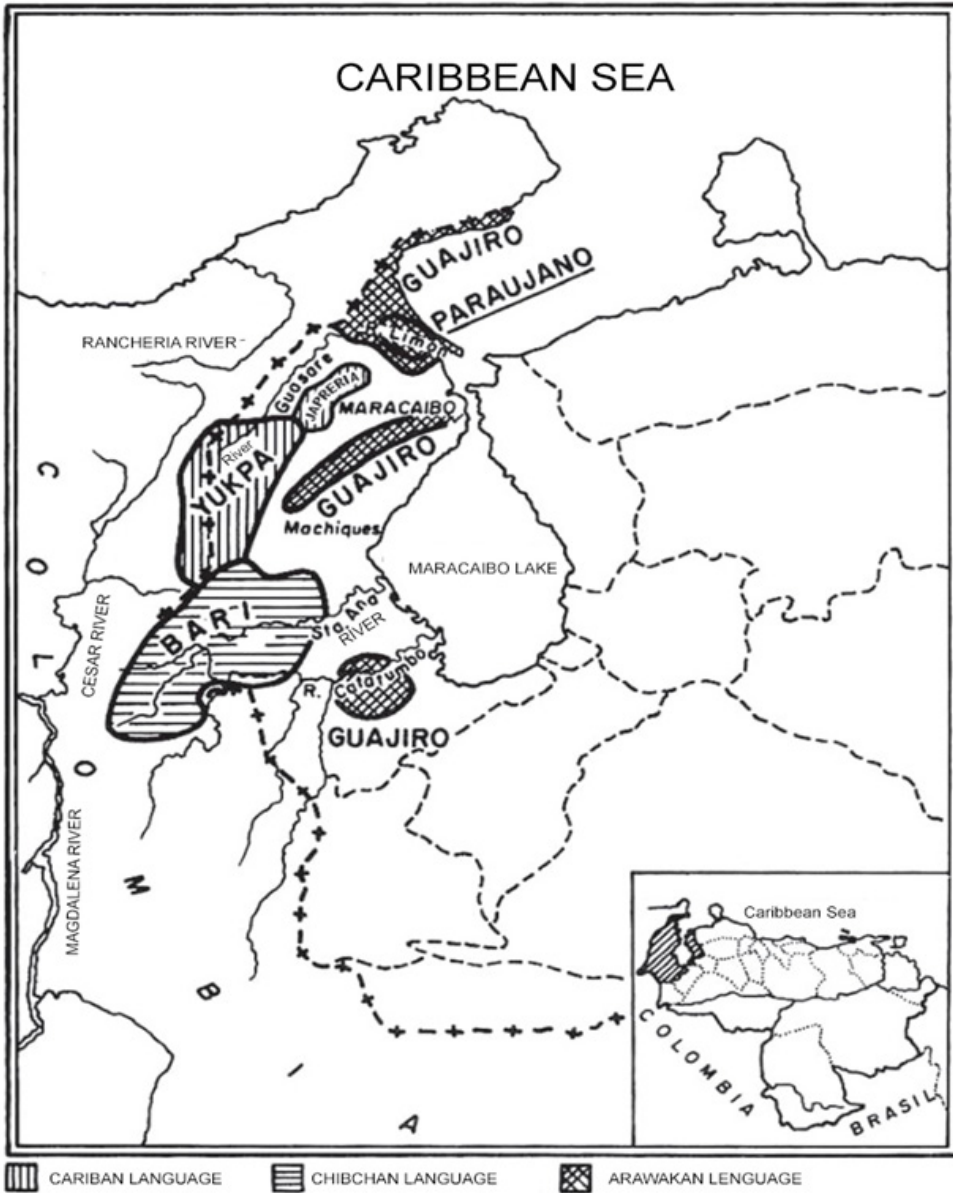


Figure 2. Map with the distribution of the indigenous ethnicities in Maracaibo Lake.
Source: Wagner (1980).



Figure 3. Map with the distribution of palafitic añ in the mangroves of Maracaibo Lake. Source: Olier (1997).

The añú (or *paraujanos*) made the mangroves of Lake Maracaibo their natural environment, where they constructed their homes: today the palafitics are the only surviving monument architects of their millennial cultures, which extended through the whole watershed of the lake. Historic sources indicate that, from the moment of contact with Europeans, the *paraujanos* have also lived in the maritime coast, the Western shore of Maracaibo Lake, and in the margins of the Southern rivers of Sinamaica, such as the Limón River (Wagner, 1980). Therefore, to study the importance and cultural significance of the mangroves in the Maracaibo Lake watershed, the añú constitute the principal source of ancestral knowledge.

In this study we propose to describe the diverse forms of the relationship between the añú and mangroves: a sacred space which covers a special importance for the añú, not only constituting the principal source of supplying their material needs, but also by representing their link with God, their ancestors, nature, and the “others” (the population that is not añú).

The añú language and its relationship to the arawak linguistic family

The traditional language of the añú is classified within the arawak linguistic family, just as with the language of their neighbors, the wayúu (Saler, 1988). But the difference between these is that the añú still do not use Amerindian language as a vehicle of everyday communication, they speak Spanish instead. Although the Amerindian language of the añú and wayúu have the same origin the arawak, statistical lexicon studies reveal that these languages diverged approximately 2000 years ago (Wilbert, 1959). Also, comparative studies of related groups show a close genetic relationship between both ethnicities (Layrisse, Layrisse and Wilbert, 1960), but despite linguistic and genetic similarities, they are clearly different ethnicities in their cultural point of view, which is evidenced in their relationship with geographic space, customs, social organization, and means of subsistence, among others.

The añú in the mangroves of the Lake Maracaibo watershed

The word añú has been translated into Spanish by members of this culture as “people of the coast” and “people of the lagoon,” so this word (añú) and its translations has been frequently substituted with the Spanish term *lagunero* (belonging to the lake), which has origins in the Sinamaica lagoon, the main population center of the añú.

Possibly to the wayúu we owe one more of the names attributed to the añú: the “paraujanos.” The wayúu and the añú differ in their territorial expansion. The wayúu migrated to the semi-deserted land of the Guajira peninsula, whereas the añú established themselves in riparian zones in the Maracaibo Lake. This difference in location clarifies why the wayúu, who live in the peninsula, have called the añú “Parróuja.” That is, poor people who depend on fishing (Jusayú & Olza, 1988). Moreover, in the language añú, the word “paraa” means “ocean” (Álvarez & Bravo, 2008), the word “paraujano” (it can also be written as paraujano, paraocan, parauhano, paraurano, parauxano, parawgwan, parhowka o prawkan) is the hispanized form of “Parróuja.” It is a pejorative term that the wayúu use to refer to the “towns of water” or “people of the sea,” characterizing them as “reeking of fish”, “fish-eater”, and “crocodile-eater,” among others (Fernández, 2002).

The inter-ethnic relationships of the añú

Since the 19th century, the añú spoke their arawak language and coexisted with a significant number of neighboring ethnicities in the riverbanks of Maracaibo Lake. Agustín Codazzi (1841) refers to this in the following manner:

The Zaparas, Aliles, Tamañares, Bobures, Toas, Quiriquires, Carates and Alcoholados, are nations and tribes living near the shore of Maracaibo Lake and Sucui river. Some others still exist in Sinamaica lagoon, Lagunilla, Moporo, and Ticaporo, in places which have a

variety of reunited families that live in huts that are elevated with pitchforks on the side in the middle of the water [...] the language in which these Indians still converse seems like a dialect of Goajira or of a common origin to that. (p. 256)

Currently, the paraujanos form one of the five indigenous ethnicities that live in the watershed of Maracaibo Lake. For each ethnicity the reference to their gods and ancestors is a fundamental element in cultural delimitation of their spaces, as from them they have received it. For the añú, Apañakai is the God Creator, which planted the mangroves under its summit, guaranteeing all that is necessary for the subsistence of his descendants: the añú.

An approximation of mangrove culture from archaeology

The Lagunillas site (population located in the mangroves on the East coast of the Lake), was archaeologically excavated between 1973 and 1975, which allowed the identification of a flourishing time period of this culture between 480 and 210 BC. The abundance of figures and adornments are traditional ceramics from Western Venezuela that are older and interesting. Regarding the results of the archaeological excavations in Lagunillas, Wagner (1980) said:

We had a total of 23,435 pots, of which 3,402 (approximately 15%) had plastic decorations [...] this ceramic, very elaborate and sophisticated, was very worn and fragmented [...] because it was under water for many centuries [...] what is called to attention is the great variety of forms, sizes, and techniques of the decorations and design elements. All of this notes, without doubt, that the ceramic was created by sophisticated artisans with an elevated sense of aesthetic. (p. 39)

The discovery of sitting figurines in hollowed, four-legged stools, are in accordance with the shamanic ceremonies in which offers to the gods are completed (Reichel-Dolmatoff, 1961). This practice, which responds to the gratitude and desire for retribution for the offering

received from the gods, was very present in the everyday life on the indigenous populations in the watershed of the lake.

The evidence of these sitting figurines that included a variety of four-legged, hollow stools in the place of the adhesion of the figure [...]. Also we have modern evidence of the importance of ceremonial banks that have many indigenous tribes from the Amazon region. They are made of wood, and the shamans sit over them in the moment of a ritual. The witch or shaman invokes the spirits, sitting in a bank which has extremes that are shaped like the head of a caiman, turtle, or jaguar, or also they use them to ritually smoke [...]. (Wagner, 1980, p. 51)

The diversity of characters who participated in these ritual exchanges and their link with the spirits of nature and with divinities, have left material evidence in the figures of a variety of animals present in the archaeological discoveries.

The excavations of Lagunillas produced a great variety of animal and human-shaped adornments, some clearly realistic. We have been able to identify snakes, birds, monkeys, a rabbit, a toad, a spider, a possible cat head, a turtle, and heads and tails of caimans. Some adornments seem to be combinations of animals, for example a bird with a serpent, an animal head with a bird claw, what is similar to the famous alter-ego sculptures of San Agustín, Colombia. Some of the adornments that seem anthropomorphic tend to have asymmetric features that are sometimes twisted in a grotesque form. (Wagner, 1980, p. 51)

The artifacts found in the excavations of Lagunillas are from a complex culture that had an intense spiritual life. Anthropomorphic, zoomorphic, hybrid, feminine, androgynous or with exaggerated sex, and ambivalent figures highlight the ceremonial accompaniment of the critical moments of bodily, mental, and spiritual transformation of each individual, such as birth, childhood, puberty, and death (Amodio, 2008). The personal rites of passage of these moments, the various prohibitions and prescriptions vis-a-vis illness, and the

chaos of nature, just as the different experiences of the shaman and his allied spirits during his activities and roles: they speak to a culture whose spiritual life was intense and filled with rituals with important meaning, for existence and for the relationship between the group with its natural surroundings and its spiritual beings (Boza & Pineda, 1990; Fernández, 2002).

The collection of Lagunillas includes an entire closed figure, which unfortunately, lacks a head (Figure 4). The feminine sex is indicated by mammary appendages and incisions. Another type of hollow figure, of indeterminate sex, consists of a big head and a flat part joined with a round body with a low plane (Figure 5). The apparent lack of legs and arms reveal that the hands are united near the center of the body. The height of the shoulder presents perforations that possibly stop its suspension. Between the atypical clay pieces are rattling hollows in the shape of a drum (Figure 6), with a concave superior surface, perforations, and a perforated and flat bottom, which probably has to do with ceremonial markings (Wagner, 1980).



Figure 4. Entire closed figure. It measures 10.5 cm long and 12 cm wide.
Source: Wagner (1980).



Figure 5. Type of hollow figure. It measures 14 cm in length and 13 cm at its maximum width. Source: Wagner (1980).



Figure 6. Atypical clay piece. Source: Wagner (1980).

An approximation of the añú culture from a historical perspective

The European conquistadors were captivated by the watershed of Maracaibo Lake with the beauty of its mangroves, dwellings, and reed beds. The perception that the conquistadors had of these things was influenced by the medieval image that the Garden of Eden was found among this land of such beauty. “The edenic models of medieval literature are totally reproduced in Colón, imposing its characteristics in the description of the beauty of new lands” (Amodio, 1993, p. 92). Thus, a new age in the dream of Apañakai and his descendants was initiated: confrontation with the worldview of the conquistadors.

It is generally accepted that the lake villages encountered by European conquistadors in the Western coast of Maracaibo Lake inspired the name of Venezuela in Ojeda and Vespucci: Little Venice. These dwellings—constructed above the water—were and continue to be today the traditional houses of the añú, indigenous descendants of populations that took in the conquistadors on Terra Firme in 1449.

The work of the chroniclers of this encounter offers limited data: fragments and, in many cases, contradictions. Each chronicler shows diverse denominations of the indigenous people in this place. Some of the denominations used were Onotos (especially in the first documents), Aliles, Toas, Cinamaicas, and later Zaparas. Nevertheless, in the first chronicles of the *Conquista* there were interesting descriptions of what these populations were. In 1528, Ambrosio Alfinger left in an expedition from Coro to the Western coast of Maracaibo Lake and encountered three populated lake villages by Ontos in the Limón River (called Macomiti and earlier Sucuy or Socuy) along the riverbank. The chronicler’s description reaffirms the development of this previously described myth: the descendants of Apañakai are the lords of the mangrove, they do not sow, and the mangrove provides all of their necessities.

The people that lived in the lagoon were of the Ontos nation [...] these indians do not sow, they are lords of the lake, with nets and hooks they fish many genera of fish that are in the lagoon [...] these Indians have their houses inside the same lagoon, armed with their stages; they work with canoes in the lagoon, they are valiant men, they fight with bows and arrows and macanas. (de Oviedo y Baños, 1885, p. 229)

The chronicles of the conquistadors inaugurate a large series of descriptions and interpretations of the añú culture, produced from the Western viewpoint. From this viewpoint, the añú have passed through an extensive and diverse itinerary of collective representations, that goes from the recognition as “lords of the lake” (15th century) to their denomination and classification as “the revolting Indians that populate the watershed of Maracaibo” (17th century). This change in the collective representation, attributed to the mangrove inhabitants, took place from the *Conquista* to the 20th century. This has been used to justify all types of atrocities, including the hunting and the enslavement of the añú, not only by the Europeans and their descendants, but also by their indigenous neighbors, the wayúu. Despite this cultural debacle and the additional environmental problems of sedimentation and contamination in the water of Maracaibo Lake, the añú have survived through 20th century and into the 21st. This surpasses the most optimistic prognoses, including that of the academics and researchers who forecasted the inevitable extinction of the añú culture.

Predictions about the extinction of the Añú in the 20th and 21st centuries

One of the first and most complete scientific works about the añú in the 20th century is the text written by the engineer, ethnographer, and pioneer of anthropological studies in Venezuela, Alfredo Jahn (1867-1940), who revised historic-graphic sources, executed fieldwork, and edited the results of his investigation in his book *The Aborigines of Western Venezuela*, in 1927. In his work, Jahn estimates the añú

population as about 2,500, located principally in three populations in the Sinamaica Lagoon (Boca del Caño, El Barro, and Sinamaica), in Santa Rosa (in the Northeast zone of Maracaibo city), and a town North of San Rafael del Moján, called Nazareth. Since then until the present day, the study and predictions about the cultural extinction of the añú (*or paraujanos*) have been many and varied.

In 1959, after his fieldwork with the añú, Helmuth Fuchs indicated “their original culture can hardly be considered in action” (p. 47).

In 1965, Angelina Pollak-Eltz writes: “the study of the Paraujano confronts us with an urgent task, now that they appear to have reached their final phase before their complete disappearance” (p. 97). One year later, Antonio Pérez-Esclarín (1984) spoke of “the last Paraujanos.” And, five years later, Marie-France Patte also expressed the same concern.

The Añú have resisted the consequences, first of the Conquista, later of colonization, and finally of the different ages of modernization and industrial development. If it is certain that they were able to survive until this time period, it is unclear whether they will be able to escape the cultural conflict in which they actually live. (Patte, 1989, p. 16)

In 1991, Isabel Aretz published an interesting view of indigenous music in Venezuela, in which a similar form to that of previous investigators is expressed:

The Añú [...] inhabited the Sinamaica Lagoon [...]. At present their descendants are creolized (adapted to the customs of Latin America) [...] they no longer speak their indo-american language. Today they live in their palafitic houses [...] their music which was able to be registered while they were conserving their traditions does not exist. (Aretz, 1991, p. 227)

However, further from the predictions and affirmations about their cultural extinction, the añú have continued to exist. Certainly, as was indicated, the Amerindian traditional language is no longer the vehicle

of communication between them. The pre-hispanic socio-political organization does not constitute an operating unit. Their cultural and traditional beliefs and practices are strongly minimized or influenced by cultural elements of the Creole society, or have been suppressed and confined to private use after centuries of clandestine existence. Nevertheless, in these small, clandestine spaces, important rites of passage were carried out until about twenty years ago. These rites of passage marked the beginning of the girl's adult life. Aerophone musical instruments were played, and important knowledge that defined being añú was transmitted through various songs in their Amerindian language.

Away from public view, traditional identity rites and the staging of their corresponding power structures have been suppressed. However, the spaces of private life have remained as daily settings for the horizontal transmission of knowledge and representations of the world, especially through various stories, tales and legends, often conveyed and learned through songs in Spanish musical style and language, as such is the case of the tens of spinels (Villalobos, 2008; Mora, González & Richard de Mora, 2010).

Such traditional stories as the general teachings are transmitted in castellan, which is the language that the añú use in their daily lives. From [...] they speakers of the añú language [...] there are only a few older speakers and one younger [...] the children spend a great part of their time playing in groups, which allows them to share information and histories. This horizontal transmission of knowledge [...] is carried out mainly between siblings, for example, at night when they play in their house or are already in their hammocks [...] do not seem to be special ritual moments, that mark the times of childhood growth or are utilized to transmit particular knowledge. The elders relate that, formerly, the arrival of menstruation among young women marked the end of infancy. These young women were lock up for a certain time, during which they were made aware of some feminine knowledge. (Amodio, 2005, p. 308-309)

The mangrove continues to be the paradise that Apañakai created for the añú

Despite all the cultural processes that we have described from a Western, materialist perspective, the Añú ethnic group not only exists, but it is actually in the process of strengthening, and the rest of the national society recognizes them as carriers of a specific and differentiated from creole and other indigenous cultures. The añú recognize each other as part of the same ethnic group, bearers of an ancient culture, enriched with a significant number of non-traditional characteristics. Faced with this scenario, the interpretative lectures of the cultural processes and the actual reality of the añú are diverse and contradictory. It could be said that those who predicted the cultural extinction of the añú were very pessimistic. Also could be said that the añú are the most relevant example of the resistance and stoicism of a society in its struggle to survive and maintain its validity.

Nevertheless, our investigation highlights that certain mechanisms by which the añú have redefined themselves throughout time, maintaining their everyday cultural practices with symbolic elements that allow them to preserve their cultural borders and achieve recognition of their ethnic identity, have not been considered. It deals with cultural practices, regarding ancient spirituality of their ancestors and their definitions, to which this society consciously responds: mangroves are sacred places, their own and sovereign; created by the god Apañakai for the lives of his descendants: the men, the people, the lords of the lagoon, and the lords of the mangrove.

Future investigations ought to deepen the study of the environmental, economic, and social implications that the añú worldview has with respect to the mangroves. The sentiments of gratitude and concrete retribution of the population towards the mangroves, their gods, and their ancestors are fundamental. This is translated into the care and respect for each being that lives in the dream of Apañakai.

It is this collective representation of the mangroves that today continues to be a vehicle for the añú through their songs. They do not still speak of Apañakai, rather they use the term “Sovereign God.” Despite the myths that are not sung anymore with the traditional melody of “airriei” or “adiechi,” rather in the Spanish tenths, learned from the Spanish conquistadors and their descendants.

This can be appreciated in the tenth entitled “Santa Rosa is a Star,” composed by the añú Aurelio Ortega Sánchez (Santa Rosa, 10/27/1941-01/28/2006), and considered a hymn of the Lords of the Mangrove.

SANTA ROSA ES UNA ESTRELLA

(Décima Paraujána)
Himno de Santa Rosa

A: Aurelio Ortega Sánchez

Sa - na - ta Ro - sags u - naes - tre - lla Ta n ru - ti - lan - tey ga -

6 8 Dm Dm Dm Dm E7 E7 Am
la - na Quea ___ las co - s - tas ve - ne - cia - nas La s com - pa - ra - o n con e - lla

13 8 Am E7 Am Gm A7
Pe - ro cre - o quee s más be lla La ___ cos - ta de su ri - be -

18 8 Dm Dm E7 E7 E7
ra A - dor - na - da de pal - me - ra s Yun am -

23 8 E7 Am Dm Am E7 Am
bien - te tro - pi - ca | Con el do - te na - tu - ra | De la in ___ día sa n - ta - ro - se - ra

II
Santa Rosa es el Edén
Donde el Poderoso quiso
Construir un paraíso
Para los indios también
Y quiso para su bien
Que su lago marabino
Majestuoso y cristalino
Fuera su limpio velo
Donde el zigzagante cielo
Se posara diamantino

II
Cual legendaria Manoa*
Del majestuoso Orinoco
Es mi tierra que yo evoco
Reina del Coquivacoa
Y la india en su canoa
Adorna su panorama
El lampo de la mañana
La cobija cariñosa
Es mi bella Santa Rosa
Venecia venezolana

IV
Como fúlgida carroza
Que baja en su manso vuelo
De los albores del cielo
Fue bajada Santa Rosa
De ese jardín fue la hermosa
Rosa que santificada
Quiso Dios fuera poblada
Con inocencia y cariño
Yo le canto como el niño
A su madre venerada

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IX

Regeneration and structure of a disturbed mangrove forest in Ramsar Site No. 1602 “Manglares y Humedales de Tuxpan”, Mexico

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Introduction

In Mexico the extent of mangrove forests is estimated to be about 775555 ha; of those that are in the state Veracruz, there are 38311 ha. Approximately 1740 ha are considered disturbed, ranking the state as 4th at the national level in this category. It is important to note that, since 2005 there has been a trend towards regeneration (Valderrama-Landeros et al., 2017). From the cases reported for Veracruz, Mexico, Valiela et al. (2009) identifies new activities causing disruption of mangroves at the global level. Moreno-Casasola et al. (2002) indicates the irrational exploitation of forest, the modification of hydrology by riverbed diversion, and the substitution of mangrove areas for that of aquaculture and tourism infrastructure.

The consequences of hydrological modification are changes in salinity (Blanco, Vilorio and Narváez, 2006; Foti et al., 2013; Röderstein et al., 2014), which causes stress to mangrove populations (Perdomo et al., 1998). In fact, exposure to high levels of salinity can affect seedling establishment, survival, and growth (Lugo and Snedaker, 1974; Cintrón et al., 1978; Smith and Snedaker, 1995; Bider, 2006; Krauss et al., 2008; Bosire et al., 2008; Chan-Keb et al., 2018; Kodikara et al., 2018).

This study presents a case to understand the behavior of species in a mangrove forest that, since 1991, has been hydrologically modified by the construction of embankments that divide the mangroves into three sections. Consequentially, the forest exhibits a pattern of degradation because of stress caused by high salinity, from the interior of the mangrove to the main body of water, with four observed conditions (isolated, completely degraded, partially degraded, and not degraded). The objective of this study is to characterize the classes of regeneration and the mangrove species structure, with the goal of identifying the population behavior and defining its viability given the present condition, as well as confirming the hypothesis that the three fragments form parts of one continuous mangrove forest.

Characterization of the southern, central and northern fragments with isolated mangroves, totally and partially degraded and not degraded

This study was conducted in the northern state of Veracruz, Mexico, in the Ramsar Site 1602 “Mangroves and Wetlands of Tuxpan”, which consists of 3600 ha of mangrove. Inside this Ramsar Site there is Tampamachoso Lagoon, and to the east of this lagoon there is a mangrove community with *Rhizophora mangle*, *Avicennia germinans*, *Laguncularia racemosa* and *Conocarpus erectus*.

The mangrove forest of this study is situated inside the conservation area of the thermoelectric complex “President Adolfo López Mateos.” In 1991, due to the construction of three embankments with electrical towers, south-north-south laminar fluid has been modified in the 699 ha mangrove forest, generating substrate to the north of the embankments, and an hyper-saline (120 parts per million) shadow, which generated chronically damaged zones or massive mortality (López-Portillo et al., 2014). In these three areas, there is a pattern of degradation among individuals from the shore of the body of water to the interior of the forest, as well as to their topographical limit. The width of the forest is ± 500 m; from the shoreline of the

lagoon to 200 m the mangroves do not exhibit degradation in their population (non-degraded mangrove), behind this zone there is approximately 70 m of perturbed mangrove with living and dead mangroves (partially degraded), next is 260 m with total mortality (completely degraded), and finally, at their topographical extent, a mangrove forest with a 10 m width that does not feature evidence of perturbation (isolated mangrove).

In 2011, the embankments were opened, forming a series of canals that permitted the passage of laminar fluid that provided the southern part of the river with the initiation of a desalination process of the soil. However, after seven years, natural regeneration and a recovery process in these areas has not been observed, neither in the mangroves with partial mortality nor in those with total mortality.

To understand how the population behavior relates to regeneration and forest structure, eight perpendicular transects were established along Tampamachoco lagoon, distributed in three transects in the southern fragment, two in the center fragments, and three in the northern fragment. There was a total of four points for each transect. At each point a plot of 10 x 10 m was established; inside this, was also a 5 x 5 m and a 1 x 1 m transect. The species, height (Brunton clinometer), and the diameter (graduated, flexible girth in centimeters) were recorded. The regeneration measurement categories were established by height (CT I < 40 cm; 40 < CT II < 150 cm; 150 < CT III < 300 cm, < 2.5 cm diameter) according to Kairo et al. (2002), and the forest structure was based on the diameter (2.5 < CT IIIb < 5 cm; 5 < CT IV < 10; CT V > 10 cm), based on Cintrón-Molero and Schaeffer-Novelli (1992).

For the regeneration (height) and structure (height and diameter) data, a non-parametric test by Kruskal-Wallis was utilized; the structural and regeneration density and the basal density were analyzed with a variance test (ANOVA). Comparison tests of Mann-Whitney and Tukey averages were applied, according to the case.

Multivariate analyses were used to identify the differences and/or similarities in the sampling sites (not degraded, partially perturbed,

completely perturbed, and isolated) as a function of the fragments, mangrove species, and measurement classes. A hierarchical grouping was applied to conglomerates or clusters, with a Bray-Curtis similarity matrix, grouping the data with the average bonding strategy. The groups obtained were found with a graphic produced using a non-metric multidimensional scaling (NMDS) analysis, establishing its stress value, taking into consideration the criteria that signaled stress values less than 0.05 represent an excellently-ordered data. The percentages of dissimilarity reported by the condition type (not degraded, partially perturbed, completely perturbed, and isolated) and contributed differences to each measurement and species class, were obtained from a Similarity Percentage (SIMPER) test (Clarke and Warwick, 1994). To determine the significant differences among the sites with the species measurement classes being the basis of comparison, a Similarity Analysis (ANOSIM) was used, with a level of significance at 0.05. All of the multivariate analyses were executed with the software Past, version 3.22 (Hammer, Harper and Ryan, 2001).

696 individuals were measured, pertaining to all of the species and classes of measurement. Of these, *Avicennia germinans* presented 231 organisms in its regeneration class and 217 in the forest structure; *Rhizophora mangle* counted 98 individuals in regeneration and 103 in forest structure; *Laguncularia racemosa* only had seven representatives of the regeneration class and 40 of structure. By fragment, all classes of these three species were present, but by the fragment type, only *A. germinans* was sampled as having many in the conserved, partially degraded, and isolated conservation types. *R. mangle* was not found in the partially degraded condition, and *L. racemosa* was exclusively in the isolated type. The highest class represented was mature trees (223), then seedlings (208), then juveniles (100), of which *A. germinans* contributed the majority of this representation.

The mean maximum height present in the isolated type in its northern fragment was 7.05 m; the minimum was 4.05 m and was located in the partially degraded condition in their central fragment.

The individual with the largest diameter was found in the degraded type of the southern fragment (14.73 cm measured at DBH). 8.16 cm (DBH) was the smallest and was found in the center of the isolated fragment. The height in the structural ($p < 0.05$) and regenerated ($p < 0.05$) components demonstrate significant differences between the condition types and fragments (Kruskal-Wallis test). Regarding the diameter, there were significant differences ($p < 0.05$) in forest structure. Although the density component in the forest structure did not exhibit significant differences ($p = 0.607$) by the type and condition of the fragment (by means of ANOVA), there were significant differences in regeneration ($p = 0.0285$), with the partially degraded type in the southern fragment having the lowest density. In the application of a ANOVA of the total basal areas and density by the type and fragment, there were no significant differences ($p = 0.355$).

The contribution of species for the regeneration density emphasized that the southern fragment with the isolated condition contained 100 % of the *R. mangle* individuals. 100 % of *A. germinans* were in the southern and central perturbed fragments and in the northern isolated fragment. *Laguncularia racemosa* only had a 10 % density in the central isolated fragment. With regards to the structure, only *A. germinans* had an 100 % representation; it was present in the perturbed forests in the three fragments (southern, central, and northern). *L. racemosa* was found at 61, 40, and 20 % in the three fragments in the its isolated condition. *R. mangle* only presented a large contribution to density in the isolated southern fragment, with 67 %.

Table 1. Data of the forest structure and regeneration (32 sampling points) in three condition types

Location	Forest structure							Regeneration				
	Height (m)	DBH (cm)	AB (m ²)	Density (ind/ha)	Contribution to density			Height (cm)	Density (ind/ha)	Contribution to density		
					Rm (%)	Ag (%)	Lr (%)			Rm (%)	Ag (%)	Lr (%)
C_FS	6.67	14.73	14.8	867	38	62	-	44.91	10,667	56	44	-
C_FC	5.40	10.27	11.0	1,325	33	67	-	79.39	6,000	27	73	-
C_FN	6.11	11.15	13.5	1,383	39	61	-	50.55	43,667	83	17	-
P_FS	4.59	10.63	6.8	767	-	100	-	197.5	800	-	100	-
P_FC	4.05	10.73	10.9	1,200	-	100	-	54.68	4,400	-	100	-
P_FN	6.96	11.19	5.9	600	-	100	-	50.86	3,467	62	38	-
A_FS	5.79	9.15	8.8	1,333	67	13	20	30.05	2,800	100	-	-
A_FC	5.62	8.16	10.5	2,000	-	60	40	50.33	7,000	-	90	10
A_FN	7.05	10.61	24.8	2,800	30	9	61	44.78	14,533	-	100	-

Note: not degraded (C), partially degraded (P), and isolated (A), southern fragment (SF), central fragment (CF), northern fragment (FN), basal area (AB), *Rhizophora mangle* (Rm), *Avicennia germinans* (Ag), *Laguncularia racemosa* (LR).

Source: author's own elaboration.

The multivariate analysis resulted in a data matrix with an abundance of measurement categories by species and the location of these sampling sites (fragments and types), also allowed a conceptual perspective of the behavior of the regeneration and structure of the forest.

The cluster analysis indicated the locations of the transects by the type of condition (not degraded, partially degraded, and isolated), showing distinct groups with their associates and assemblages: they highlight two well-defined associates, one integrated with the isolated mangrove in the northern fragments and its three transects, and those which preferentially integrate with species *L. racemosa*. A second association subdivides the data in three: a central composed exclusively of partially degraded mangrove in their fragments at the north and south, association of the non-degraded mangroves, and associations with the isolated mangrove (Figure 1).

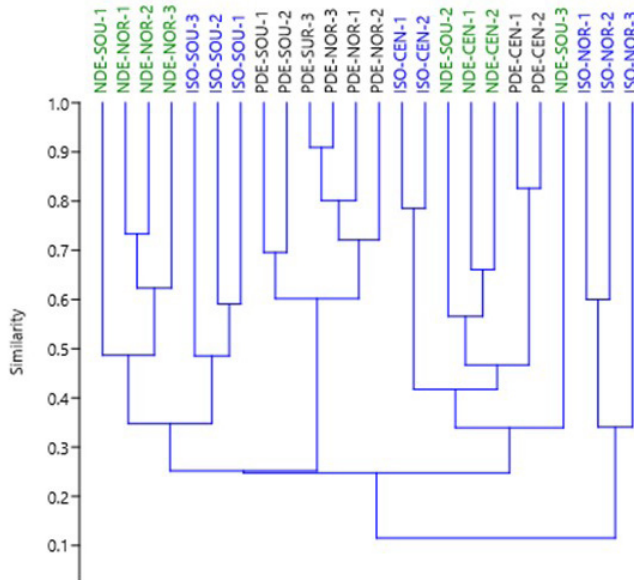


Figure 1. Grouped dendrogram (cluster), sampling by abundances of size classes. Showing the condition types: NDE (non-degraded), PDE (partially degraded), and ISO (isolated); and fragments: SOU (southern), CEN (central), and NOR (northern). This is based on a Bray-Curtis similarity and the UPGM rule to form groups. Source: author's own elaboration.

By processing the abundance information in an nMDS, three orders were established: (i) one from the left that corresponds to the samples obtained in the isolated mangrove (southern and northern fragments, with their respective transects, except the third southern fragment, because it did not contain *L. racemosa* individuals); (ii) all transects of the non-degraded mangrove are located in the center of the graphic, with an emphasis on the southern fragment in the third transect that has the highest number of seedlings in the system, and the central fragment of the isolated mangrove in its two transects which has the second-greatest quantity of seedlings; (iii) to the right, there are locations with partially degraded mangrove with all the fragments and all the transects. The nMDS result orders the three types of forest conditions in the field site, independent of the fragments and their transects. It can be considered that the three fragments are found in an unified location that integrates the fragments of the entire forest. Only some transects are integrated in orders of the types which do not contribute to present regeneration classes. Regarding the reported stress of the test (0.2036), the interpretation is considered rigorous.

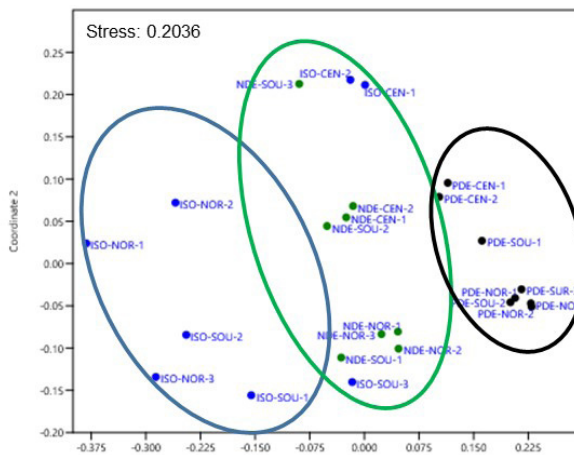


Figure 2. An ordering graphic (nMDS) of sampled collected by abundances of size classes. Showing the types: NDE (non-degraded), PDE (partially degraded), and ISO (isolated); and fragments: SOU (southern), CEN (central), and NOR (northern). The three elliptical figures demonstrate the grouping by type of condition in the forest: left (blue) is isolated mangrove, central (green) is non-degraded mangrove, and right (black) is the partially-degraded mangrove. Source: author's own elaboration.

The similarity analysis (ANOSIM) demonstrates that there were significant differences ($R = 0.613$, $p = 0.0001$; Bray-Curtis index of similarity) with grouping by type of condition. When a Bonferroni correction is applied, the three types were distinct.

To determine the contribution of the classes by species and type of condition, a percentage similarity (SIMPER) analysis was executed, with the size category CT V Ag (mature) of the *A. germinans* species that has 19.77 %, then with 16.27 % of the same species, but the size category of the seedling (CT I Ag). Next the contribution of the *R. mangle* seedlings (CT I Rm), which are located in the third contribution, with 8.66 %. To arrive at 50 % accumulation, *L. racemose* is in its size category CT V Lr (mature) and the adult *R. mangle* (CT IC Rm). In the first five contributions, the participation of the three present species was present in the analysis of the classes of > 5 cm diameter for the forest structure and the seedlings < 40 cm for regeneration in *A. germinans* and *R. mangle*, which means that there is little representation of these intermediate classes of juveniles and young trees.

Structural responses to environmental conditions

Significant changes in the environmental conditions in mangroves are generally followed by alterations in the vigor and zonation of their species; if the alterations continue for a prolonged time there can be generalized mortality of individuals (Jiménez and Lugo, 1985). The response of mangroves to factors that influence their growth and survival are organized in areas where the geomorphological aspects and hydrological gradients are maintained (Lugo, 1980). Moreover, the structural characterization of mangroves is a valuable tool for understanding responses and conditions of the changes in processes (Soares, 1999; Estrada et al., 2013). It is known by various authors (Wolanski et al., 2009; Feller et al., 2010) that areas with abiotic factors with little variation of optimal ranges, with geomorphological stability and little direct influence of adverse effects, produce better mangrove development. In this study, different types of forest condition (non-degraded, partially degraded, completely degraded, and isolated)

are presented, with obvious variations in their structure, such as regeneration, which exemplified how hydrological modification is causing stress in diverse sections of the forest. The individuals located close to the shoreline of the lagoon, in those where their stability depends on the tide, presented better structural and regeneration development. On the other hand, individuals that are 250 m inland, where the tide is not a clear hydrological factor, rather laminar fluid in the interior of the forest is needed to bring stability, experienced deterioration due to the hydrological modification driven by obstruction. Farthest inland, by 300 m, there was a general mortality.

Height, DBH, basal area, and density data for the forest structure, and that of height and density for regeneration, also identify different intensities of alteration of the area, which for their part provided results of individual processes of seedling mortality and establishment, as demonstrated in Lugo (1980), Jiménez (1990), and Carvalho et al. (2016). The average height and diameter in the partially degraded condition, in comparison with the non-degraded and isolated conditions, experienced stress (Blanco et al., 2001). Regarding seedling establishment, the reported density for regeneration in the partially degraded condition is 2.889 ind/ha and 20.111 ind/ha. The scarce seedling establishment in the partially-degraded forest, but above all the null regeneration in the completely perturbed condition, is principally due to the hydrological barrier established by the constructed embankments, which impedes propagule expansion and dispersal (Day et al., 2018). Additionally, the inland mangroves experience less water exchange, consequently driving mortality in inland areas (Lewis et al., 2016) and their inland setback provides a more disaggregated passage, increasing edge area (Day et al., 2018).

The differentiation of the three classes of mangrove species in the diverse forest types and their fragments indicates the presence of stress. As a result, there are perturbations in the partially degraded conditions, above all else because of the lack of natural regeneration (Lugo, 1980; Jiménez, 1990; Carvalho et al., 2016). This tendency presents a reduction in the structure and densities of mangrove species of the

partially-degraded type, which could be due to the modification of the hydrological regimen from 1991 to the present, a situation reported by Estrada et al. (2013), Yang et al. (2013) and Carvalho et al. (2016).

According to Cunha-Lignon et al. (2011), the stability at each site is the principal factor responsible for the differences in the types of forests. The multivariate analyses do not exhibit differences between the northern, southern, and central fragments. Rather, there are differences between the types of conditions of where the fragments are found, and this can be attributed to hydrological modification, as reported in Carvalho et al. (2016), associated with mangroves that have anthropogenic influences.

Conclusions

The analyses carried out for the classes of species provided knowledge about the population status presented in the study area. The completely degraded type did not present living individuals of any species. There were seedlings in the partially degraded type, but there was a lack of individuals in other developmental stages, and the abundance of adult and mature trees with signs of stress predicts the null recuperation of this mangrove forest. The non-degraded and isolated mangroves, with the presence of regeneration and tree categories, give certainty to the viability of this type of forest condition.

Regarding the northern, central, and southern fragments, the analysis shows that this forest can be considered as a continuous unit because it does not form groups or orders that separate the fragments. Differences in size categories better separates the conserved, perturbed, and isolated mangrove types.

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