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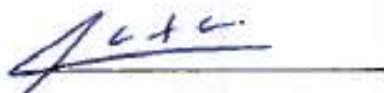
**Prevalencia de enfermedades en corales escleractinios del Caribe sur de
Costa Rica**

**Trabajo Final de Graduación para optar al grado de Licenciatura en
Biología Marina**

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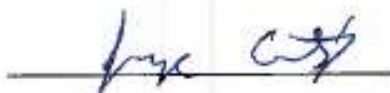


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Abreviaturas

Ssid	<i>Siderastrea siderea</i>
Pstr	<i>Pseudodiploria strigosa</i>
Apal	<i>Acropora palmata</i>
Pfur	<i>Porites furcata</i>
DS	Dark Spots
SCTLD	Stony Coral Tissue Loss Disease
WB	White Band
WPD	White Patch Disease
WP	White Plague
WS	White Syndrome
CNP	Cahuita National Park
PC	Punta Cocles
MA	Manzanillo
PU	Punta Uva
IUV	Uvita Island
PU	Punta Uva

Resumen

El mar Caribe ha sido una de las regiones marinas más afectadas por enfermedades en los corales escleractinios. Para Costa Rica, el conocimiento sobre las enfermedades coralinas es escaso. Comprender el estado de salud de estos ecosistemas es clave para tomar medidas de conservación efectivas. Por lo tanto, el objetivo de este estudio fue determinar la diversidad y prevalencia de enfermedades en corales escleractinios en el Caribe sur de Costa Rica y su posible relación con la cobertura coralina, las condiciones fisicoquímicas y el estado de protección marina. Las observaciones se realizaron durante septiembre y octubre de 2023, en los sitios de Isla Uvita, Cahuita, Punta Cocles, Puerto Viejo, Punta Uva y Manzanillo. Con base en la apariencia visual de las lesiones encontradas en las colonias, probablemente se identificó la presencia de cinco enfermedades infecciosas coralinas: la enfermedad de la banda blanca, la enfermedad de la mancha oscura, la enfermedad de la mancha blanca, la enfermedad de la peste blanca y, por primera vez en el Caribe de Costa Rica, la enfermedad de pérdida de tejido del coral pétreo (SCTLD, por sus siglas en inglés). Las especies de coral afectadas fueron *Acropora palmata*, *Siderastrea siderea*, *Porites furcata* y *Pseudodiploria strigosa*. *Porites furcata* fue afectada por el síndrome blanco, aunque no se pudo identificar el tipo específico de enfermedad. La enfermedad más común fue la peste blanca. No se encontró una relación significativa entre la prevalencia de enfermedades coralinas y la cobertura coralina, las áreas marinas protegidas (AMP) y los factores fisicoquímicos en las localidades de este estudio ($p > 0.05$). Se recomienda realizar análisis continuos de calidad del agua en cada localidad, como pruebas específicas de nutrientes, temperatura y sedimentación, ya que estos factores fisicoquímicos impactan directa e indirectamente la salud de los corales. Este es el primer reporte enfocado exclusivamente en enfermedades coralinas en el Caribe sur de Costa Rica. Debido a la posibilidad de presencia de SCTLD en el arrecife, es crucial mantener un monitoreo adecuado en la zona.

Palabras clave: Áreas Marinas Protegidas, cobertura coralina, Caribe suroccidental, peste blanca, *Pseudodiploria strigosa*.

Prevalence of Diseases in Scleractinian Corals of the Southern Caribbean of Costa Rica

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Abstract

The Caribbean Sea has been one of the marine regions most affected by diseases in Scleractinian corals. For Costa Rica, knowledge of coral diseases is scarce. Understanding the health status of these ecosystems is key to take effective conservation measures. Therefore, the objective of this study was to determine the diversity and prevalence of diseases in Scleractinian corals in the southern Caribbean of Costa Rica and their possible relationship with coral cover, physicochemical conditions, and marine protection status. Observations were made during September and October 2023, at the sites of Isla Uvita, Cahuita, Punta Cocles, Puerto Viejo, Punta Uva and Manzanillo. Based on the visual appearance of the lesions found in the colonies we probably found the presence of five infectious coral diseases: White Band Disease, Dark Spot Disease, White Spot Disease, White Plague Disease, and for the first time in the Caribbean of Costa Rica Stony Coral Tissue Loss Disease (SCTLD). The affected coral species were *Acropora palmata*, *Siderastrea siderea*, *Porites furcata*, and *Pseudodiploria strigosa*. *Porites furcata* was affected by the White Syndrome, although the specific disease type could not be identified. The most common disease was White Plague. No significant relationship was found between the prevalence of coral diseases against coral cover, marine protected areas (MPA), and physicochemical factors in the locations of this study ($p>0.05$). It is recommended to conduct continuous water quality analyses in each locality, such as targeted nutrient, temperature, and sedimentation tests, as these physicochemical factors directly and indirectly impact coral health. This is the first report focused solely on coral diseases in the southern Caribbean of Costa Rica. Due to the possibility of having SCTLD on the reef, it is crucial to maintain adequate monitoring in the area.

Keywords: Marine Protected Areas, Coral cover, Southwestern Caribbean, White Plague, *Pseudodiplora strigosa*.

Introduction

Scleractinian coral reefs cover less than 0.1% of the oceans; however, they are considered to be the equivalent of tropical rainforests as they harbor up to 35% of marine biodiversity (Knowlton et al., 2010; Fanning et al., 2021). Corals are characterized by providing protection to coastlines during storms and hurricanes, even acting as breakwaters to prevent beach erosion, and they capture significant amounts of CO₂ from the atmosphere (Ahmed et al., 2007; Burt, 2023). Additionally, they possess great economic relevance, with 850 million people worldwide directly dependent on coral reefs (Burke et al., 2011), and it is estimated that the economic contribution generated by corals is \$350,000 ha yr⁻¹ (Costanza et al., 2014).

Despite all the aforementioned, coral reefs are among the most threatened ecosystems (Rinkevich, 2014; Ferrigno et al., 2016). Nearly 40% have been lost due to natural and anthropogenic disturbances in the past decades (Bruno & Selig, 2007; Mbije et al., 2010; De'Ath et al., 2012; Rinkevich, 2015). Changes in temperature and nutrient availability have contributed to the increase in coral diseases (Ward & Lafferty, 2004; Aeby et al., 2021), resulting in significant mortality in the reefs (Randazzo et al., 2022).

The first coral disease was reported in 1965 in the Hawaiian Islands on *Madrepora kawaiiensis* (Squires, 1965; Sutherland et al., 2004). In the following three decades, four new diseases were identified, and since 1995, records of coral diseases have increased (Sutherland et al., 2004). In 2002, 13 additional diseases were identified, spreading at an accelerated rate (Sutherland et al., 2004). Between 2000 and 2020, 22 diseases and 165 affected coral species were recorded, the diseases that represented 76.8% of the reports were black band, white plague, white syndromes, skeletal erosion, dark spot and yellow band (Morais et al., 2022).

The Caribbean has been one of the regions that has suffered the main consequences of coral diseases, 22 of the 40 reported diseases have been recorded in the Caribbean (Morais et al., 2022). In Puerto Rico, during a six-month period, coral cover loss was reported at nearly 60% due to a bleaching event followed by a white plague infection (Garcia-Sais et al., 2008). Florida reefs have experienced mortality since 2014 as a result of the stony coral tissue loss disease, at least 30% of its corals died (Walton et al., 2018; Muller et al., 2020). In the Caribbean at least 22 species of coral have been affected by this disease (Papke et al., 2024) Similarly, the yellow band disease nearly wiped-out all the colonies of the coral *Orbicella faveolata* in Tobago (Mallela & Crabbe, 2009). In the Virgin Islands, coral cover decreased by 60% after undergoing a massive bleaching event and an outbreak of white syndrome disease (Miller et al., 2009).

Similarly, in Costa Rica, coral degradation is also evident (Cortés et al., 1992; Alvarado et al., 2020); in the southern Caribbean, coral ecosystems have been primarily impacted by heavy sedimentation (Cortés et al., 1992; Fonseca & Cortés, 2002; Cortés & Jiménez, 2003; Fonseca, 2003; Roder et al., 2009). Moreover, the dynamic currents moving from northwest to southeast and the presence of counter-currents transport sediments originating from upstream coastlines and areas that have suffered deforestation and urbanization in watershed regions (Cortés, 1994). In the 1980s, coral cover in Cahuita National Park (CNP) decreased from 40% to 10% (Cortés, 1996); this degradation was associated with diversity factors such as the El Niño phenomenon that occurred between 1982 and 1983 (Cortés, 1984). Also, the sedimentation that arrived through the rivers as a result of deforestation and agricultural practices in the area (Cortés, 1996). Similarly, a mass mortality of the sea urchin *Diadema antillarum* occurred (Lessios et al., 1984), which is a key controlling algal populations that compete with corals (Alvarado et al., 2004). In 1995, another coral bleaching event was reported, related to the El Niño phenomenon (Jiménez, 2001). And recently, Quesada et al., (2023) mention that most of the sites visited in their research within the southern Caribbean of Costa Rica showed low coral cover and high cover of fleshy macroalgae, with an average (\pm SD) of $14 \pm 13\%$ and $31 \pm 28\%$ per site, based on censuses from 2019 to 2022.

Coral disease studies in Costa Rica's Caribbean are scarce, the existing information is concentrated at Cahuita National Park, where a proportion of colonies affected by diseases was reported as 4% in 2000 and 10% in 2004. During these years, White Plague (WP) and Dark Spots (DS) primarily affected the coral species *Siderastrea siderea* (Fonseca et al., 2006). Later, *S. siderea* was also affected by the Dark Spot Disease (DS) (Araya-Vargas & Nova-Bustos, 2017). Additionally, diseases have been reported in octocorals (Calderón-Hernández et al., 2021).

Understanding the health status of Scleractinian corals in Costa Rica helps to comprehend the current state of these ecosystems, the factors contributing to their degradation, and the measures that can be taken for their conservation. Given the limited information available on this subject in the country, the objective of this study was to determine the diversity of diseases in Scleractinian corals in the southern Caribbean of Costa Rica through visual and photographic surveys. Additionally, it aimed to quantify the prevalence percentages of these diseases in different sites and explore possible associations with factors such as coral cover, physicochemical conditions, and the marine protection category of the studied locations.

Materials and Methods

Study area

The Costa Rican Caribbean coast is located in the province of Limón, with a length of 212 km (Cortés, 2016) and is characterized by high sediment loads in the sea (Cortés et al.,

1998). The region is characterized by rainfall throughout the year, with a hot and humid climate (Cortés, 2016). It mainly has sandy beaches with remnants of fossil coral reefs (Cortés & Wehrtmann, 2009; Cortés, 2016). Coral reefs are primarily located in the southern section of this coast (Cortés & Jiménez, 2003; Cortés, 2016). The research was conducted in six locations in the southern Caribbean of Costa Rica (Fig. 1), visited once between September and October 2023. Monitoring was carried out at depths ranging from 2 to 5 m, with three to four sites visited at each location. A brief description of each location follows:

Uvita Island (Fig. 1A): This is an island of 6.4 ha (Fonseca, 2008), and is located less than 1 km from the port of Limón (Cortés & Jiménez, 2003). The island contains important coral reef and sponge formations (Pereira-Chaves & Sierra-Sierra, 2009). The terrestrial part of the island was declared a National Monument and National Heritage in 1985 and it is managed by the Limón municipality (Pereira-Chaves & Sierra-Sierra, 2009). There is no protection on its marine area. Three sites were studied on this island:

IUV1 (9°99'192''N, -83°00'897''W), IUV2 (9°99'103''N, -83°00'998''W), IUV3 (9°99'138''N, -83°01'193''W).

Cahuita National Park (Fig. 1B): The coral reef within the protected area of the CNP consists of three crests. The first is in front of Punta Cahuita, parallel to the shore, and extends 5 km long, reaching depths of up to 15 m. The other two crests are located on either side of the point (Fonseca et al., 2006). Punta Cahuita contains the best-developed coral reef in the Caribbean of Costa Rica (Cortés & Risk, 1984; Cortés & Jiménez, 2003; Fonseca et al., 2006). It was established as a National Park in 1978 due to its rich marine and terrestrial resources (Cortés & Risk, 1984). Four sites were studied at this location: Lechuga (PN1) (9°74'382''N, -82°82'044''W), Barrera Interna (PN2) (9°73'746''N, -82°80'774''W), Eduardo (PN3) (9°73'796''N, -82°80'584''W), and Perezoso (PN4) (9°74'771''N, -82°81'876''W).

Puerto Viejo (Fig. 1C): This reef is characterized by being shallow and having lagoons and scattered coral patches in some areas (Cortés & Guzman, 1985; Fansa-Fernández, 2021). Compared to the other sampling sites, the reefs at this location are under greater anthropogenic pressure (Cortés & Jiménez, 2003; Cortés et al., 2009). Additionally, this locality is not under any management category. Three sites were studied: PV1 (9°65'772''N, -82°75'678''W), PV2 (9°65'772''N, -82°75'678''W), PV3 (9°65'799''N, -82°75'668''W).

Punta Cocles (Fig. 1D): An area of 10.5 ha has been estimated for this reef (Fernández & Alvarado, 2004). It is a marginal reef with calcareous barriers close to the coast (Cortés et al., 1992). This reef is located within the Gandoca-Manzanillo National Wildlife Refuge (GMNWR), a marine protected area (Fernández & Alvarado, 2004). Three sites were studied here: PC1 (9°64'666''N, -82°71'928''W), PC2 (9°64'675''N, -82°71'862''W), PC3 (9°64'659''N, -82°71'783''W).

Punta Uva - El Arrecife Sector (Fig. 1E): The platform of the main reef has channels ranging from 1 to 4 m and is notably exposed to wave action and tourist influx (Cortés & Guzmán, 1985; Fansa-Fernández, 2021). It is also located within the GMNWR (Cortés & Jiménez, 2003). Four sites were explored: PU1 (9°64'368''N, -82°68'853''W), PU2

(9°64'368''N, -82°68'853''W), PU3 (9°64'421''N, -82°68'889''W), PU4 (9°64'332''N, -82°68'813''W).

Manzanillo (Fig. 1F): This area is characterized by the presence of marginal reefs, fossil coral platforms, algal populations, and seagrasses (Soto & Ballantine, 1986). A total of 29 species of stony corals have been reported in this area (Cortés, 1992). It is located within the GMNWR (Cortés & Jiménez, 2003) three sites were studied: MA1 (9°63'527''N, -82°65'750''W), MA2 (9°63'488''N, -82°65'671''W), MA3 (9°63'591''N, -82°65'542''W).



Figure 1. Sampling points in the southern Caribbean of Costa Rica (highlighted in pink). Locations: A = Uvita Island, B = Cahuita National Park, C = Puerto Viejo, D = Punta Cocles, E = Punta Uva - El Arrecife sector, F = Manzanillo. MPAs = Marine Protected Areas (shown in dark blue). RVSGM = Gandoca-Manzanillo Wildlife Refuge.

Prevalence of Diseases Evaluation

The research was conducted following the protocol "Atlantic and Caribbean Reef Rapid Assessment" (AGRRA, 2021; <https://www.agrra.org/e>). This protocol includes a section dedicated to coral disease monitoring, which was carried out following the previously

established guidelines. At each location, at least three transects of 10 m in length and 1 m in width were performed. The coral species and their health condition were recorded: Healthy, pale, bleached, partial mortality, and disease identification. Additionally, photographs were taken with an Olympus TG-6 camera. The lesions found were characterized visually by comparing them with photos from disease guides and scientific papers, and consulting with experts.

Prevalence of Coral Diseases

Considering that a disease is any deterioration or disorder affecting the vital functions of an organism (Peters, 2015) and that coral bleaching compromises basic metabolic functions, such as reproduction and growth (Baker et al., 2008; Rosenberg et al., 2009; Hughes et al., 2018; Grottoli et al., 2020; Aguilera-Pérez & Gonzales-Díaz, 2024), coral bleaching was considered a coral disease in this study.

For each coral disease, the prevalence percentage was calculated per site and the average per location. Additionally, the total prevalence percentage of infectious diseases (excluding bleaching as the percentages are much higher, making it difficult to visualize the percentages of infectious diseases and compare the sites) was calculated for each site, as well as the corresponding average for the locality. Finally, to visualize the general situation of the Caribbean of Costa Rica, the overall average of diseases for the region was calculated. Disease prevalence is defined as the number of organisms that have a specific disease in relation to the total population at a given time (Moreno et al., 2000). Prevalence was calculated as follows (Randazzo et al., 2022):

$$\text{Prevalence} = (\text{Number of corals affected by a specific disease} / \text{Total number of corals}) \times 100$$

Coral Coverage

To determine the average percentage of coral cover at each site, a methodology using photo-quadrants was employed. Ten 0.25 m² quadrants were placed at each meter of the 10-meter linear transect, and a photograph was taken of each quadrant at a 90° angle using a PVC tripod. The software photoQuad (Trygonis & Sini, 2012) was then used to analyze each photograph using the random point method, for these 50 points were placed and the substrate was identified within each quadrant. The coral cover percentage was estimated by dividing the total number of points identified with coral substrate by the total number of points (Quezada-Pérez et al., 2023). Finally, the average coral cover for each locality was calculated.

Physico-Chemical Data

To describe the physico-chemical factors at the monitoring sites, monthly average concentrations of nutrients (nitrates, phosphates, and silicates) and temperature values were downloaded from the Copernicus platform (<https://www.copernicus.eu/es>), at a depth of 3.82 m, from January to December 2023. Since the nutrient data resolution ($0.25^\circ \times 0.25^\circ$) was too broad for detailed analysis by locality, the average monthly nutrient difference between the Uvita Island locality and the five other locations was compared. Similarly, the resolution of temperature data ($0.083^\circ \times 0.083^\circ$) It allowed the comparison of the sites of Uvita Island, Cahuita and, due to their proximity, Manzanillo-Punta Uva, Puerto Viejo-Punta Cocles shared the same temperature value. This study has been conducted using E.U. Copernicus Marine Service Information; <https://doi.org/10.48670/moi-00015> and <https://doi.org/10.48670/moi-00016>.

Statistical Analysis

To examine the relationship between the average coral cover percentages and total infectious disease per locality, Pearson's correlation was used, as the data were normally distributed according to the Shapiro-Wilk test. Similarly, to assess whether there was a relationship between total infectious disease per locality and marine protection category, a Welch ANOVA was performed, as the assumption of homoscedasticity was not met (Levene's test), but normality was confirmed (Shapiro-Wilk test).

The coral disease prevalence data by site were square-root transformed before performing an NMDS (Non-metric Multidimensional Scaling) using the Bray-Curtis dissimilarity matrix. Subsequently, an ANOSIM was applied with site as factor, followed by a SIMPER test to determine the factors that most contribute to data dissimilarity.

The normality of physico-chemical data was verified using the Shapiro-Wilk test. Nutrient data were normally distributed, and a t-Student test was conducted to compare nutrient levels in the southernmost Caribbean area against the concentrations in Uvita Island. To analyze temperature differences between localities, the Kruskal-Wallis test was used, as the data did not meet the normality assumption. All analyses were conducted in R version 4.3.0 (2023), using the following libraries: ggplot2, dplyr, car, and vegan.

Results

Throughout the study period, the probable presence of five infectious coral diseases was identified based on the visual appearance of the lesions found in the colonies 1) white band (WB), 2) dark spots (DS), 3) white patch disease (WPD), 4) white plague (WP), and 5) for the first time in the Southern Caribbean of Costa Rica what is probably Stony Coral Tissue

Loss Disease (SCTLD). The coral species affected by diseases included *Acropora palmata*, *Siderastrea siderea*, *Porites furcata*, and *Pseudodiploria strigosa* (Fig. 2, Table 1). A very similar pattern to SCTLD was found at Punta Uva, affecting colonies of *S. siderea*, and in colonies of *P. strigosa* on the internal reef at Cahuita. WP was present in five of the six study locations, being the most widespread disease. The coral species most affected by diseases was *S. siderea*, while *P. furcata* was only affected by White Syndrome, though the specific disease type could not be identified. Some colonies of *A. palmata* were found with diseases such as WB and WPD. *S. siderea* was found infected with DS at four different localities.

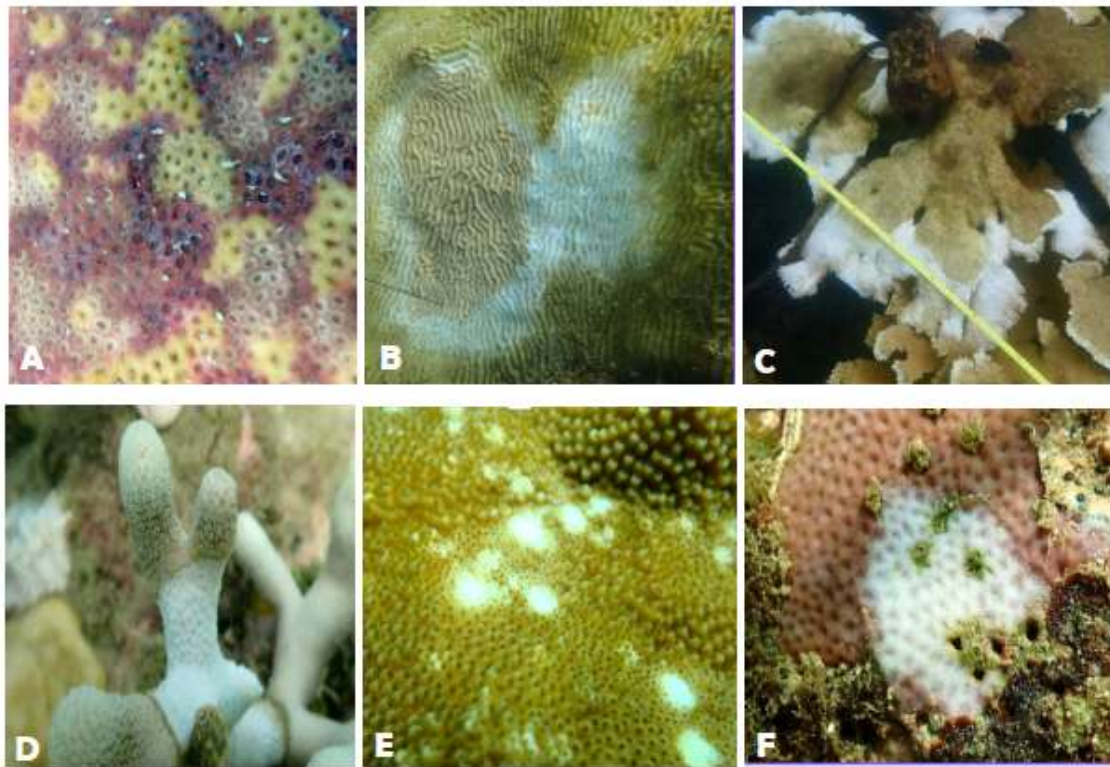


Figure 2. Coral diseases in the southern Caribbean of Costa Rica. *Siderastrea siderea*, *Pseudodiploria strigosa*, *Acropora palmata*, and *Porites furcata* showing signs of different coral diseases: (A) *Siderastrea siderea*, dark spots disease, (B) *Pseudodiploria strigosa*, Stony Coral Tissue Loss Disease, (C) *Acropora palmata*, white band disease, (D) *Porites furcata*, white syndrome, (E) *Acropora palmata*, white patch disease, and (F) *Siderastrea siderea*, white plague. Photographs taken in the field by Fabio Quesada Perez and Shanttal Rodríguez Esquivel.

Table 1. Summary of the infectious coral diseases found in the surveys conducted in the Southern Caribbean of Costa Rica between September and October 2023. Affected species: *Siderastrea siderea* (Ssid), *Pseudodiploria strigosa* (Pstr), *Acropora palmata* (Apal), *Porites furcata* (Pfur). Coral diseases: dark spots (DS), Stony Coral Tissue Loss Disease (SCTLD), white band (WB), white patch disease (WPD), white plague (WP), white syndrome (WS). Localities: Cahuita National Park (CNP), Punta Cocles (PC), Manzanillo (MA), Punta Uva (PU), Uvita Island (IUV), Punta Uva (PU).

Disease	Sites	Affected species	Characteristics	Photograph
DS	CNP2, PC1, MA1, MA2, PU1	Ssid	Purple patchy circles around the coral tissue	Fig. 2A
SCTLD	PU1, PU2, PU4, CNP2	Ssid, Pstr	A very characteristic attern, where live coral, dead coral, and coral affected by the disease can be seen, with the affected coral turning "Pale."	Fig. 2B
WB	IUV3	Apal	White band, visible from the edge of the branches towards the inside.	Fig. 2C
WS	CNP1	Pfur	White syndrome type disease, insufficient information to classify it.	Fig. 2D
WPD	IUV3, PU2	Apal	Patchy circles around the colony	Fig. 2E
WP	CNP2,PC2, PC3, MA1, MA2,MA3, PU3,PU4, PV2	Ssid Ptri	White area on an apparently healthy coral, with a very distinct "line" separating the healthy tissue from the diseased one.	Fig. 2F

Regarding the proportion of coral disease affected, it was observed that coral bleaching had the highest prevalence percentages (Table 2), followed by WP, WPD, DS, SCTLD, and with WB showing the lowest percentage. Punta Uva presented the greatest variety of diseases (5), followed by Cahuita (4), while Puerto Viejo was the location with the least variety of diseases (2). In Cahuita and Puerto Viejo, the highest average percentages of bleaching were recorded ($66.08 \pm 6.14\%$ and $66.05 \pm 4.62\%$, respectively). According to the overall average of infectious diseases by location, Puerto Viejo recorded the lowest percentage ($0.74 \pm 0.74\%$), while Manzanillo and Punta Uva had the highest levels ($10.57 \pm 5.46\%$ and $8.46 \pm 4.73\%$, respectively). The prevalence percentages of diseases for the entire Southern

Caribbean of Costa Rica, based on the average of the six locations, ranged from $0.001\pm 0.001\%$ to $2.14\pm 1.36\%$.

Table 2. Average percentage (\pm SE) of coral disease prevalence found in six locations of the Southern Caribbean of Costa Rica. Coral infections diseases: white patch (WPD), white plague (WP), dark spots (DS), Stony Coral Tissue Loss Disease (SCTLD), white syndrome (WS), white band (WB). Coral disease: bleaching (BL). Total = Overall average of infections diseases by location (without counting bleaching).

Locality	WPD %	WP%	DS%	SCTLD%	WS%	WB%	Total%	BL%
Cahuita	0	0.35 ± 0.35	0.69 ± 0.69	0.35 ± 0.34	0.007 ± 0.007	0	2.15 ± 1.34	66.08 ± 6.14
Cocles	0	1.73 ± 0.99	0.55 ± 0.55	0	0	0	2.28 ± 0.58	49.80 ± 6.51
Isla Uvita	2.08 ± 2.08	0	0	0	0	0.69 ± 0.69	2.78 ± 2.78	35.58 ± 11.86
Manzanillo	0	7.86 ± 3.22	3.24 ± 2.09	0	0	0	10.57 ± 5.46	37.71 ± 7.03
Puerto Viejo	0	0.74 ± 0.74	0	0	0	0	0.74 ± 0.74	66.05 ± 4.62
Punta Uva	5.19 ± 5.19	2.18 ± 1.28	0.51 ± 0.51	1.59 ± 0.54	0	0	8.46 ± 4.73	14.32 ± 2.29
Caribe	1.21 ± 1.51	2.14 ± 1.36	0.83 ± 0.67	0.32 ± 0.67	0.0012 ± 0.0012	0.12 ± 0.35	-	44.92 ± 8.15

On the other hand, the NMDS revealed variations in the spatial distribution of the sites in relation to coral diseases (Fig. 3). Diseases are spatially distributed across the locations, showing that the closest ones have more similar disease profiles. SCTLD is more closely related to the Punta Uva locality, while DS and WP are associated with Manzanillo, and coral bleaching with Cahuita. Significant differences in disease prevalence were detected between locations (ANOSIM: $R=0.26$, $P=0.003$).

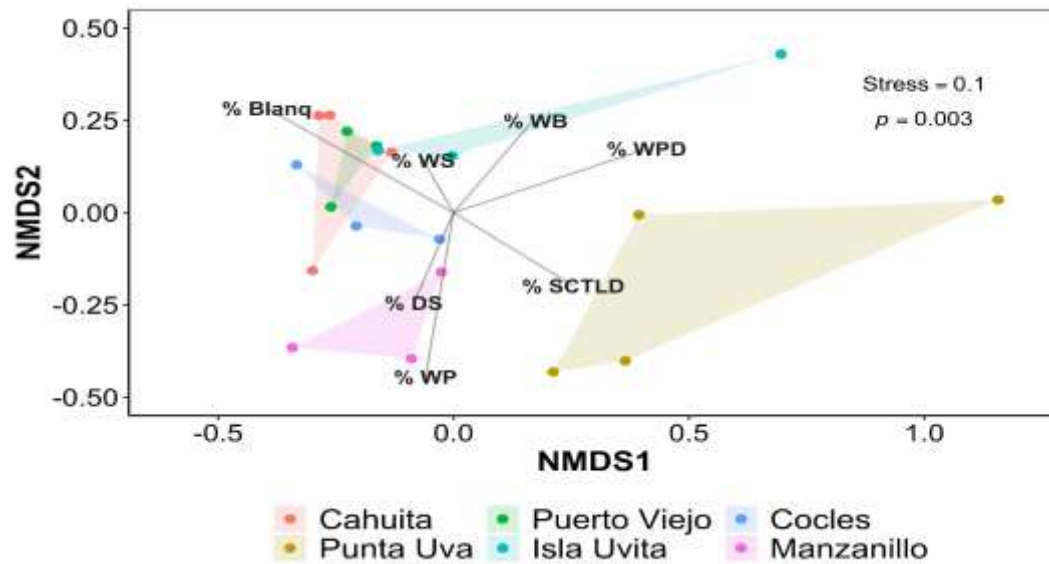


Figure 3. Non-metric multidimensional scaling (nMDS) analysis based on Bray-Curtis similarity matrix in six sampling locations in the Southern Caribbean of Costa Rica regarding the spatial distribution of coral diseases found in September and October 2023.

The SIMPER analysis revealed that bleaching is the primary factor contributing to the dissimilarity in distribution between locations. Additionally, WP and SCTL also contribute significantly to the dissimilarity primarily in Manzanillo and Punta Uva, respectively (Table 3).

Table 3. SIMPER analysis of percentage similarity showing the coral diseases that most contributed to dissimilarity between locations.

Localities	Disease	Average dissimilarity	SD	Contribution	Cumulative %
Cahuita-Punta Uva	BI	0.27	0.06	4.56	54.3%
	SCTLD	0.06	0.04	1.38	92%
Punta Uva-Puerto Viejo	BI	0.27	0.04	6.82	55%
	SCTLD	0.06	0.04	1.62	82.4%
PuntaUva-Isla Uvita	SCTLD	0.07	0.04	1.62	87.6%
Punta Uva-Cocles	BI	0.21	0.05	4.14	46.8%
	SCTLD	0.07	0.04	1.63	62.0%
Isla Uvita-Manzanillo	WP	0.15	0.02	6.33	39.6%
Puerto Viejo-Manzanillo	WP	0.12	0.05	2.24	38.7%
Cahuita – Manzanillo	WP	0.12	0.04	2.92	39.4%

Regarding coral cover, Cahuita had the highest percentage ($41.1 \pm 11.83\%$), followed by Isla Uvita with $32.3 \pm 6.13\%$, Punta Uva $27.2 \pm 5.63\%$, Manzanillo $27.0 \pm 3.77\%$, Punta Cocles $23.3 \pm 3.86\%$, and Puerto Viejo with the lowest coral cover at $12.16 \pm 2.72\%$. No correlation was determined between coral cover and the average percentage of total prevalence of infectious diseases by location ($t = 0.24$, $cor = 0.12$, $df = 4$, $P = 0.83$). Likewise, no significant differences were found in the average total prevalence of infectious diseases between locations based on their marine protection category ($F_{1, 3.91} = 2.9727$, $P = 0.1615$).

The physical-chemical factors for the five locations (Cahuita, Punta Uva, Punta Cocles, Puerto Viejo, and Manzanillo) concerning nutrient values, the monthly average concentrations of nitrate ranged from 0.09 to 0.98 mmol/m³, phosphate concentrations showed a range of 0.00016 to 0.00080 mmol/m³, while silicate levels fluctuated between 4.73 and 9.91 mmol/m³, with a slight increase in nitrates and silicates from June to October. Phosphates remained near zero (Fig. 4A). In Isla Uvita, nutrient concentrations were similar to the five other locations, with nitrates ranging from 0.06 to 1.10 mmol/m³, phosphates between 0.00019 and 0.00074 mmol/m³, and silicates between 4.84 and 10.70 mmol/m³. Similarly, phosphates remained nearly constant near zero, while nitrates and silicates showed a slight increase from June to October (Fig 4B). When comparing the nutrient levels between the five locations against Isla Uvita, no significant differences were found in silicic acid ($t = -0.53$, $df = 22$, $P = 0.60$), nitrates ($t = -0.14$, $df = 22$, $P = 0.88$), and phosphates ($t = -0.49$, $df = 22$, $P = 0.62$).

Furthermore, the monthly average sea surface temperature showed a similar pattern across all locations (Fig. 4C), ranging from 27°C to 31°C throughout 2023, with the hottest

months being from September to November. Cahuita recorded the highest temperatures, reaching 31°C in October and November. No significant differences were observed between sites (Kruskal-Wallis= 1.63, df=3, P = 0.65).

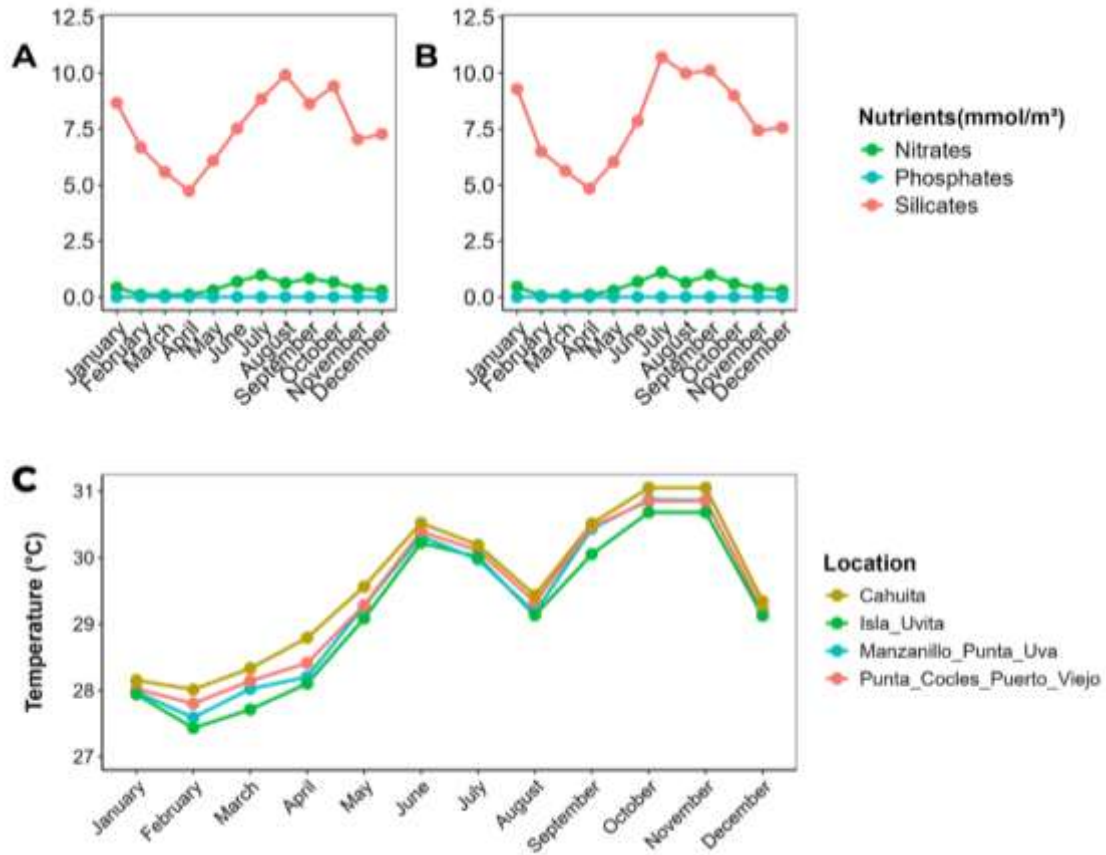


Figure 4. Surface physicochemical factors of the Southern Caribbean of Costa Rica downloaded from Copernicus (ref) for the year 2023. (A) Monthly average of nutrients, covering the localities of Cahuita, Punta Uva, Punta Cocles, Puerto Viejo, and Manzanillo. (B) Monthly average of nutrients for Isla Uvita. (C) Monthly average temperature (°C) for the localities of Cahuita, Isla Uvita, Manzanillo-Punta Uva, Punta Cocles-Puerto Viejo.

Discussion

Over the last 40 years, coral cover in the Caribbean has declined by 80%, while the reduction in the Indo-Pacific has been 50% (Bruno & Selig, 2007; Schutte et al., 2010; De'Ath et al., 2012). Coral diseases have contributed to the global degradation of these ecosystems (Loya, 2004), and the consequences can range from the loss of small tissue areas to the death of entire colonies (Wear & Thurber, 2015). Globally, 40 infectious coral diseases have been documented (Morais et al., 2022), 22 of which have been recorded in the Caribbean (Bourne et al., 2015), affecting at least 200 species worldwide (Riegl et al., 2009; Bourne et al., 2015).

Until 2022, white plague and dark spots were the only diseases reported for Cahuita in the Costa Rican Caribbean (Fonseca, 2003; Gateño et al., 2003; Fonseca et al., 2006; Cortés et al., 2010b; Araya-Vargas & Nova-Bustos, 2017). This study reports probably three additional diseases: white patch, white band, and Stony Coral Tissue Loss Disease. It also documents for the first time the presence of coral diseases in the localities of Manzanillo, Punta Uva, Punta Cocles, Isla Uvita, and Puerto Viejo. Given that previous reports were primarily focused on the Cahuita area (Fonseca et al., 2006; Araya-Vargas & Nova-Bustos, 2017; Calderón-Hernández et al., 2021), this study provides a broader view of the status of coral health with respect to coral diseases.

White plague is recognized as one of the most prevalent and virulent diseases in the Caribbean (Sánchez et al., 2010; Cróquer et al., 2021), affecting up to 42 species of coral (Cróquer et al., 2021). In this study, it was detected in the localities of Manzanillo, Cahuita, Punta Cocles, Puerto Viejo, and Punta Uva (Table 2) in two reef-building coral species, *S. siderea* and *P. strigosa*, which are known to be highly susceptible to this disease (Cróquer et al., 2021). These species are particularly abundant in the Costa Rican Caribbean, and a decline in their populations would result in a change in the structure and composition of the coral community in the region (Ward et al., 2006).

At the same time, *Siderastrea siderea* was found to be affected by the dark spot disease in Cahuita, Punta Cocles, Manzanillo, and Punta Uva. It is important to mention that this disease has been cataloged as one of the most common (Goreau et al., 1998; Gil-Agudelo & Garzón, 2001), with greater prevalence in reefs (Cervino et al., 2001; Borger, 2003; Porter et al., 2011), and it has also been associated with warmer sea temperatures, which are more common during summer months (Gil & Garzón, 2001; Borger, 2005; Gochfeld et al., 2006), as observed in this study (Fig 4C). The lesions caused by DS rarely lead to the total mortality of the colony. In turn, the net tissue loss is relatively low (Porter et al., 2011; Meyer et al., 2016) with injuries that can heal within a month (Borger & Steiner, 2005; Porter et al., 2011). However, coral diseases also have other negative effects, such as reduced resilience to future disturbances, low fertility, and changes in the microbiome (Lirman, 2000; Nugues et al., 2002; Borger & Colley, 2010; Pollock et al., 2017; Rippe et al., 2019; Page et al., 2023).

Moreover, at Isla Uvita, the diseases white band and white patch were found, both affecting *A. palmata*. White patch was one of the diseases with the highest prevalence percentage (Table 2). In fact, its rapid spread on the reef has been documented (Patterson et al., 2002; Sutherland et al., 2004), and it has also been observed to be more active during the summer months (Patterson et al., 2002). It is noteworthy that Isla Uvita is located near the submarine outfall of wastewater from Limón City, which was installed in 2005 for the treatment of solid waste. This system transports sewage 2 km offshore, between the island and the coast (Alfaro-Sandí et al., 2021). The fecal enterobacterium *Serratia marcescens* has been recognized as the etiological agent of white patch and has been associated with wastewater (Sutherland et al., 2010). However, Fonseca (2008) mentions that there is a

strong current that separates the island from the port and its pollution. Furthermore, Alfaro-Sandí et al. (2021) could not confirm that the discharges from the submarine outfall negatively impact the environment around Isla Uvita, and they note that marine currents may be homogenizing the pollutants from the outfall. Additionally, Mora-Alvarado et al. (2020) evaluated the sanitary risk of different beaches in Costa Rica, including the locations studied in the present research, during the period 2010-2017. In that study, they analyzed fecal coliforms/100 mL and determined the "Sanitary Risk Index" (IRS), with Isla Uvita classified as "very good," while Puerto Viejo was of great concern, classified as "very poor." It would be advisable to conduct a more up-to-date study of fecal coliforms in the waters surrounding the island and other locations, as well as other elements that may indicate the impact or entry of waters from the outfall onto the coral reefs.

On the other hand, white band (WB) had the lowest prevalence among all the diseases found in the southern Caribbean. This is encouraging, remembering that in the 1980, both *A. palmata* and *Acropora cervicornis* suffered mass mortalities across the Caribbean, with WB playing a significant role in this mortality, resulting in the loss of up to 95% of their colonies (Aronson & Precht, 2001; Miller et al., 2002). Later, Vollmer and Kline (2008) proposed that three out of every 49 colonies of *A. cervicornis* in Bocas del Toro, Panama, show immunity to WB infection. Considering that *A. palmata* is related to *A. cervicornis* (Vollmer & Palumbi, 2002), it is believed that this resistance to the disease could also be present in *A. palmata* (Vollmer & Kline, 2008). In this sense, it would be important to perform immunological tests on corals and analyze their specific resistance to pathogens. It is advisable to maintain monitoring on the reef and assess whether the prevalence percentage changes over time. WB progresses rapidly and it can cause the death of a colony in just a few months (Gladfelter, 1982).

This study documents the first report of what is probably Stony Coral Tissue Loss Disease (SCTLD) in the Caribbean of Costa Rica, specifically in *P. strigosa* and *S. siderea* in Cahuita and Punta Uva (Fig. 2B, Table 1). The discovery of this pathology raises concerns, as SCTLD has spread over hundreds of kilometers along the Caribbean coast, causing mass mortality in various species of reef-building corals (Precht et al., 2016; Walton et al., 2018; Muller et al., 2020). For the moment, it has been observed in 24 species of hard corals (Muller et al., 2020). Florida experienced massive coral mortality caused by SCTLD (Walton et al., 2018; Muller et al., 2020). Later, in Puerto Rico, in 2021, the prevalence of SCTLD ranged between 25% and 54%, depending on the species (Lucas et al., 2024). Initial observations of SCTLD transmission across reefs suggest that the pathogens involved are spread through water, with coastal currents possibly being the primary factor facilitating their diffusion (Precht et al., 2016; Muller et al., 2020; Sharp et al., 2020). While the prevalence observed in this study is relatively low compared to other reports (Costa et al., 2021), there is still uncertainty about whether the disease was already present in Costa Rica's Caribbean or if this marks the beginning of its emergence in the region. This involves

increased monitoring and follow-up of potentially infected colonies, as well as increased training in disease identification in the field for park rangers, volunteers and tour operators.

During monitoring in Cahuita National Park, at the "Lechuga" site, *P. furcata* was found infected by a white syndrome disease, although the exact type could not be identified. Tissue loss was severe (Fig. 2D, Table 1), but *P. furcata* has been postulated as a species less susceptible to SCTL (Estrada-Saldívar et al., 2021; United Nations Environment Programme, 2021; Coralmar, 2024). Additionally, the disease pattern closely resembles *White Band*, as the disease begins at the coral base and moves upwards towards the branches (Mayer & Donnelly, 2013). However, *White Band* disease has been postulated as being specific to *Acropora* spp. (Aronson & Precht, 2001). This phenomenon requires further study and monitoring of the colonies.

It is noteworthy that in Florida, the number of sites with coral diseases increased from 26 in 1996 to 131 in 1998, with a loss of 60% of corals and an increase in white syndrome diseases (Porter et al., 2001). In the Mexican Caribbean, in the Yucatán Peninsula, 19 species of corals were reported as infected in 2002, with a disease prevalence of $5.7 \pm 0.8\%$, which increased to $7.96 \pm 0.7\%$ by 2004 (Ward et al., 2006). Ruiz-Moreno et al. (2012) highlighted that 20% of Caribbean reefs have a disease prevalence above 10%. Page et al. (2023) emphasized that coral diseases have increased over the past 30 years. Although disease prevalence in Costa Rica remains below these percentages, it is important to consider that, in light of documented mortalities and impacts in other regions, constant monitoring of coral health should be maintained.

One of the main challenges in coral disease studies is the accurate diagnosis of diseases, associated pathogens, transmission dynamics, and strategies to control or mitigate disease impacts on reefs (Pollock et al., 2011; Shore & Caldwell, 2019; Beeden et al., 2012). The primary issue is the difficulty in identifying isolated pathogens. Various techniques have been documented, such as *Culture-Based Detection*, *Immunology-Based Detection*, *Nucleic Acid-Based Detection*, *Fluorescent in situ hybridization*, PCR-based methods, *Real-time quantitative PCR*, *DNA Target Selection*, *Ribosomal genes*, Microbiome and *Genomic phylogenetic marker genes* (Pollock et al., 2011). Each method has its limitations, but it is essential to implement one or more of these techniques in coral disease research in the country. A recommended strategy is to use model organisms like *Aiptasia* sp. to identify potential pathogens, determine virulence mechanisms, and validate findings in coral with fewer samples (Pollock et al., 2011; Zaragoza et al., 2014; Palmer & Traylor-Knowles, 2018).

Coral bleaching was observed in all the locations, with the highest prevalence levels recorded (Table 2). Some corals exhibited slight paling, while *Agaricia tenuifolia* and *Porites astreoides* showed severe bleaching. Fansa-Fernández (2021) notes that September and October are historically the hottest months in the Caribbean, which coincides with the increase in bleaching, additionally influenced by the El Niño phenomenon (Miller et al., 2024). During 2023, temperatures ranged from 27°C to 31°C (Fig 4C), peaking during these months and approaching the thermal tolerance limit for corals, which is estimated to be

between 30 °C and 32°C (Hoegh-Goldberg, 1999). Furthermore, the transects were conducted at depths of less than 5 m, where corals are more exposed to elevated temperatures and are more susceptible to bleaching (McClanahan et al., 2019). These high temperatures reduce coral resilience and promote pathogen growth, increasing their vulnerability to diseases (Aeby et al., 2020; Nalley et al., 2021; Randazzo et al., 2022).

Regarding coral coverage, Cahuita exhibited the highest percentage of live coral. This location has been distinguished by its coral formations, despite experiencing seasons where a notable decline has been observed (Cortés, 1996; Cortés et al., 2010b; Quezada-Pérez et al., 2023). Isla Uvita also presents a high percentage of coral coverage, which is consistent with the data presented by Quezada-Pérez et al. (2023). The location with the lowest live coral coverage was Puerto Viejo, followed by Punta Cocles; however, for both, the coverage is higher than what was reported in previous years (Cortés & Guzmán, 1985; Cortés & Jiménez, 2003; Fernández & Alvarado, 2004; Fansa-Fernández 2021). In Cuba and the Greater Caribbean, coral coverage between 20% and 40% is considered indicative of a healthy reef (Alcolado & Duran, 2011). However, Puerto Viejo, shows a record of 12.16% coverage. As previously mentioned, the corals in this locality are subjected to greater pressure from waste, urban development, unrestricted collection, and sedimentation (Cortés et al., 2010a). No correlation was found between coral coverage and disease prevalence.

Although the southern Caribbean of Costa Rica has a large portion of marine protected areas (Fig 1), no significant difference was found between the protected areas (Cahuita, Manzanillo, Cocles, and Punta Uva) and those lacking protection (Puerto Viejo and Isla Uvita) in relation to the prevalence of recorded diseases. However, this region of the Caribbean is generally exposed to high sediment loads and coastal erosion (Cortés et al., 1998; Barrantes et al., 2021a; Barrantes et al., 2021b). In fact, sediment can affect corals in various ways: finer sediments can cause coral suffocation, block light, and hinder larval settlement (Hodgson, 1990; Risk, 2014). These factors impair coral immunity, making them more susceptible to pathogens (Sharma & Ravindran, 2020). Similarly, the southern Caribbean is affected by the Panama-Colombia cyclone currents, which distribute contaminants and nutrients throughout the coast in a northwest-southeast direction (Mooers & Maul, 1998), and coastal development has contributed to widespread water pollution in this region (Quezada-Pérez et al., 2023).

On the other hand, in addition to temperature, high concentrations of nutrients promote the growth of pathogens (Aeby et al., 2020; Randazzo et al., 2022) and affect corals in various ways. For example, they reduce growth, skeletal and reproductive capacity, alter pH levels, increase the growth of macroalgae that compete with corals (Schaffelke & Klumpp, 1998; Holmes, 2000; Fabricius, 2005; Dunn et al., 2012; Silbiger et al., 2018), reduce resilience to thermal stress, and increase susceptibility to bleaching (Wiedenmann et al., 2013; Angelo & Wiedenmann, 2014).

For 2017 and 2018, nitrate levels in Cahuita were reported to average 1.7 μM , silicates 11.6 μM , and phosphate levels 0.3 μM (Samper et al., 2021). These values were higher than those reported in 1997 and 2004-2005 (Muller-Parker & Cortés, 2001; Roder et al., 2009;

Samper et al., 2021), suggesting nutrient enrichment (Samper et al., 2021). However, in 2023, lower nutrient concentrations were reported (Fig 4) compared to those indicated by Samper et al. (2021). It should be noted that Samper's data were more specific, as samples were taken in the field and analyzed by locality, offering greater accuracy than the satellite data reported in this study (Fig 4A, 4B).

Furthermore, Kleypas et al. (1999) mention that over 90% of areas containing reefs have nitrate levels lower than 0.60 μM and phosphate levels lower than 0.20 μM , and that the maximum tolerance levels for nutrients would be 3.0 μM for nitrates and 2.0 μM for phosphates, although they note that further studies are needed to define the correct maximum tolerance threshold. But it is mentioned that phosphate levels greater than 0.1 μM are already detrimental to coral larvae (Fabricius, 2005; Nalley et al., 2023). Since nutrient excess significantly contributes to the spread of coral diseases (Harvell et al., 2007) by making corals more susceptible to stress events and pathogens more virulent (Bruno et al., 2003), understanding how the dynamics of these factors change over time is essential as they directly influence the presence of coral diseases (Bruno et al., 2003; Aeby et al., 2021).

Although the NMDS analysis showed significant differences in the percentage of disease prevalence using locality as a factor, none of the variables evaluated in this study including management category, nutrient concentration, temperature, or coral cover showed significant differences. These results suggest that other factors not considered in this study may be determining the observed patterns.

For example, sedimentation levels could play an important role. Cahuita has been reported as a locality that has experienced a high sediment load (Cortés et al., 2010b; Samper-Villarreal, 2021). Sediments not only introduce pathogens responsible for coral diseases but also alter the reef benthos and displace herbivorous fish, which are key to maintaining ecosystem balance (Gordon et al., 2016; Wenger et al., 2017; Sura et al., 2021). These changes can promote the growth of fleshy macroalgae, as documented in other reef areas, altering ecosystem dynamics (Quezada et al., 2021).

Similarly, in the Costa Rican Caribbean, banana plantations have historically been associated with excessive deforestation and high sediment input (Cortés et al., 1985). It would be relevant to include water quality analyses of nearby rivers to detect contaminants derived from the use of agrochemicals and plastics in these plantations (Aragón et al., 2012), as well as to assess the presence of heavy metals (Guzmán & Guevara, 1998). In fact, high concentrations of heavy metals have been found in coral reefs in Bocas del Toro, carried by rivers due to banana farming activities (Guzmán & Jiménez, 1992).

In the case of Puerto Viejo, the Suárez River flows into its waters and comes into contact with coral reefs; in the upper parts of the river, indicators of pollution have already been identified (Aragón et al., 2012). On the other hand, the Estrella River carries sediments and contaminants from upstream to Cahuita National Park (CNP) (Aragón et al., 2012).

Finally, analyzing wastewater and solid waste management in nearby communities could provide a more comprehensive understanding of the factors affecting coral health in these areas. In the future, an integrated approach will allow us to identify external factors contributing to the observed differences and develop more effective strategies for coral reef conservation.

Conclusions

The southern Caribbean of Costa Rica has gone from reporting two infectious diseases in a specific locality to documenting five different types of diseases spread across six localities. These findings highlight an increase in both the spread and diversity of coral diseases. It is recommended to conduct continuous water quality analyses in each locality, such as targeted nutrient, temperature, and sedimentation tests, as these physicochemical factors directly and indirectly impact coral health. Additionally, complement the study of coral diseases with biotechnological techniques that enable proper diagnosis of the diseases and their associated pathogens. This is the first report focused solely on coral diseases in the southern Caribbean of Costa Rica, and it is advisable to continue this research in the coming years. Only through systematic monitoring will it be possible to understand whether the dynamics and prevalence rates of these diseases experience significant changes over time and if more stringent management measures are needed in the area.

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Conclusiones

Este estudio documenta, por primera vez, la presencia de cinco tipos distintos de enfermedades coralinas en seis localidades del Caribe sur de Costa Rica, lo que evidencia un aumento tanto en la diversidad como en la distribución espacial de estas afecciones en comparación con reportes anteriores. Los resultados obtenidos permiten establecer una base de referencia para el monitoreo futuro de la salud coralina en la región, y resaltan la necesidad de seguir evaluando la dinámica y prevalencia de estas enfermedades a lo largo del tiempo. Esta investigación representa un aporte importante al conocimiento local sobre enfermedades coralinas y marca un punto de partida para estudios más detallados en el área.

Recomendaciones

Se recomienda complementar las evaluaciones de campo con técnicas de laboratorio que permitan una identificación más precisa y confiable de las enfermedades coralinas. El uso de herramientas moleculares y microbiológicas contribuiría a superar los retos actuales en la caracterización de estas enfermedades y en la comprensión de sus mecanismos de transmisión. Asimismo, incluir el estudio de posibles fuentes de contaminación cercanas, como aguas residuales y residuos sólidos, ayudaría a identificar factores externos que influyen en la salud de los arrecifes. Finalmente, es fundamental mantener un monitoreo sistemático y sostenido en el tiempo, lo que permitirá detectar cambios en la prevalencia, distribución o severidad de las enfermedades, y facilitar la toma de decisiones orientadas a la conservación y manejo efectivo de los ecosistemas coralinos.

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Conflicto de Interés

Esta investigación no representó ningún conflicto de intereses