# Disease prevalence and bacterial diversity in stony corals

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# Abstract

Coral reefs are globally one of the most threatened ecosystems, both from natural as well as anthropogenic pressures. The Indian coastline harbours around 1% of the global reef area, which form an important part of our natural resource endowment, and are of high priority for conservation and management. Despite their usefulness, coral reefs are being degraded by destructive anthropogenic activities and natural causes such as competition, predation, diseases and bleaching. Coral disease is a rising problem within all reef areas in India which can cause significant changes in reproduction, growth, community structure, species diversity of corals and many reef associated organisms. Environmental stressors including elevated sea surface temperature, ocean acidification, variations in salinity, water quality depletion, increased pollution loads, sedimentation and eutrophication lead to incidence of diseases in corals and associated fauna. The coral bleaching and mortality event that occurred in 1998 was the most serious natural calamity ever recorded in the Indian Ocean. Currently, the coral reefs in India are showing signs of increasing prevalence of various coral diseases. As regular assessment of health of coral communities are fundamental to protection and conservation of marine biodiversity, the present study was undertaken aimed at identifying the health status and disease conditions in stony corals in the patchy reef areas from Enayam to Kollam along the south-west coast of India. Conditions such as white syndrome, pink line syndrome, sporadic/patchy bleaching, lesions due to trematode infestations, predation by corallivorous fishes, snails and sea urchins, infestation by sponges, zooanthids, mussels, filamentous as well as macroalgae were recorded in different locations along the study sites.

An attempt was also made to study the diversity of culturable bacteria associated with healthy as well as diseased corals. A total of 63 distinct bacterial strains belonging to 16 genera were identified. This section discusses the most prevalent disease conditions/syndromes affecting coral communities in the study area along with the bacterial diversity in healthy and diseased corals.

**Keywords:** Bacterial diversity, Bleaching, Coral disease, Stony corals, Southern India

# Introduction

Coral diseases are considered as one of the most destructive causes that impact corals and are responsible for decline of major reef ecosystems globally over the past couple of decades (Harvell et al., 2007; Miller et al., 2009). Coral disease is generally recognised as any abnormal condition affecting the coral holobiont, often described as a progressive loss of coral tissue caused by infectious/non-infectious agents and facilitated by environmental factors (Bourne et al., 2009). It usually manifests through tissue discoloration, necrosis and eventually tissue loss. Coral diseases were first studied intensively in the Caribbean where successive outbreak of disease seem to have contributed to the decline of major reef building species especially Acropora, resulting in major ecosystem changes. During the past two decades, considerable increase has been noticed in the frequency and distribution of coral diseases that have altered both total abundance and species diversity (Rosenberg et al., 2007). Diseases have now been described in all the major reef systems of the world. There has been recent intense interest in monitoring health of the world's reefs which partly explains the increased reporting of diseases. However there is a general consensus that the recent global increases in coral disease prevalence may be related to global warming, in particular elevated sea surface temperatures and a decline in reef environmental guality, which in turn facilitated proliferation and colonisation of disease causing microbes. Exact causes for most coral diseases remain elusive and the onset of most diseases likely is a response to multiple factors (Peters, 1997).

A wide range of disease-like syndromes have been reported to affect scleractinian corals (Willis *et al.*, 2004). Though more than 35 different coral disease conditions have been described, the increasingly confusing descriptions are hampering our understanding of the underlying pathology (Work and Aeby, 2006). Diagnosis of disease in corals is primarily based on characteristics such as loss of tissue, change in colour and exposure of coral exoskeleton. These limited macroscopic descriptions lead to imprecise names for the conditions which result in inaccurate subsequent diagnosis. It is

often difficult to meet Koch's postulates which are the usual criteria needed to prove etiology of the disease. This classic method is a tough challenge in the face of unculturable marine microorganisms and polymicrobial syndromes, requiring molecular approaches. Relatively little progress has been made in identifying and characterising the causative agents for these diseases and the etiological agents of most coral diseases as well as their modes of transmission are presently unknown (Loya et al., 2001). However, a small number of diseases have been well characterised with strong evidence of association with specific bacteria or fungi viz., black band disease caused by a microbial consortium (Carlton and Richardson, 1995), sea fan disease or aspergillosis caused by Aspergillus sydowii (Smith et al., 1996; Geiser et al., 1998), bacterial bleaching in Oculina patagonica by Vibrio shiloi and in Pocillopora damicornis by Vibrio coralliilyticus (Kushmaro et al., 2001; Ben Haim et al., 2003), white pox caused by Serratia marcescens (Patterson et al., 2002), coral white plague disease II (WPD-II) caused by Aurantimonas coralicida in Dichocoenia stokesi from the Caribbean (Denner et al., 2003) and by Thalassomonas loyana in Favia favus from the Red Sea (Thompson et al., 2006). For most of the diseases, knowledge of the mechanisms of pathogenensis and host responses is limited and many researchers remain sceptical about the role of microbes as primary causative agents of disease in uncompromised hosts.

The south-west coast of India is home to a diverse group of patchy corals and their associates. Unfortunately, a number of threats including fishing, pollution and sedimentation have caused serious declines in the distribution and health of these patchy reefs during recent years. Indiscriminate collection of ornamentally valuable species and destructive fishing practices, pollution, diseases, parasitic infestations as well as predation by fish, snails and echinoderms have led to the destruction of corals in the region. This section summarises the results of assessment and monitoring of health and disease conditions of the hard coral communities along the south-west coast of India from Enayam to Kollam.

# Materials and methods

Health and disease conditions of the hard coral communities in the patchy reef areas at 4 selected stations (Table 1) from Enayam to Kollam along the south-west coast of India was assessed during the period 2008-2012. Survey and sample collections were undertaken at the 4 sampling stations following the Line-Intercept Transect (LIT) method (English *et al.*, 1994) using 20 m long transects.

Station	Study area	Geographic coordinates
1	Enayam	08°12'818'' N; 077°10'860'' E
Ш	Vizhinjam	08°22'527'' N; 076°59'454'' E
Ш	Thirumullavaram	08°07'093'' N; 077°16'900'' E
IV	Thangassery	08°52'425'' N; 076°34'700'' E

Table 1.Geographic coordinates of the study areas

Discolouration/colour change or lack of pigmentation in tissues and tissue loss (absence of tissues with or without intact skeleton) were recorded. Representative samples of corals (normal, bleached and diseased) were collected for bacteriological analyses. Predominant bacterial colonies of culturable bacteria grown on general as well as selective media were purified and pure isolates were subjected to detailed morphological as well as biochemical tests and selected strains were also subjected to mitochondrial 16S rRNA gene sequence analysis. The bacterial strains were then identified to generic/species level as per Kreig and Holt (1984); Sneath *et al.* (1986); Staley *et al.* (1989); Brenner *et al.* (2005a, b); Vos *et al.* (2009) and Whitman *et al.* (2012).

# **Results and discussion**

Disease conditions were found more pronounced in massive corals as compared to branched corals which primarily impacted the genera *Porites* and *Montipora* in most of the study areas. Many other coral genera were found affected but to a lesser extent including *Pocillopora*, *Acropora* and *Goniastrea*. Tissue loss due to predation by coral reef fishes, sea urchins and sea snails were prevalent in most of the sampling areas.

# Station I (Enayam)

At Enayam, *Porites* affected by white syndrome and patchy bleaching along with lesions due to infestation with boring organisms, zooanthids and brown mussel were recorded (Fig. 1 - 11). Tissue loss due to predation by sea urchins (Fig. 12 and 13) as well as focal to multifocal, acute tissue loss due to predation by snails were prevalent.

The most striking observation was the prevalence of white syndrome, a collective term for describing disease like syndromes showing 'white symptoms' (Bythell *et al.*, 2004). White syndrome was observed in *Porites* spp., *Montipora* spp. and in *Acropora efflorescens*. White syndromes are distinguished by the presence of a diffuse, white lesion that develops into a linear/annular band or an irregular patch on the coral colony. Areas of exposed coral skeleton adjacent to apparently healthy tissue were found characteristic of the lesions and in selected cases a narrow band of bleached tissue was found to separate the denuded skeleton



Fig. 1. *Porites* ulcerative white spot syndrome with diffuse irregular tissue loss along with infestation by boring organisms in *Porites* sp. at Enayam

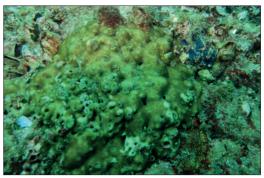


Fig. 2. Infestation by boring organisms and sponges along with ulcerative white spot syndrome in *Porites* sp. at Enayam



Fig. 3. White syndrome with lesions due to worm infestation, bordered by narrow distinct white lines in *Acropora efflorescens* at Enayam



Fig. 4. Lesions due to worm infestation and patchy bleaching in *Porites* sp. at Enayam



Fig. 5. Ulcerative white line / white spot syndrome, extensive loss of tissue and lesions due to to worm infestation in *Porites* sp. at Enayam

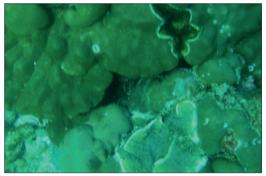


Fig. 6. Ulcerative white line / white spot syndrome and tissue loss in *Porites lichen* at Enayam



Fig. 7. White syndrome and tissue loss in  $\ensuremath{\textit{Pocillopora}}\xspace$  at Enayam

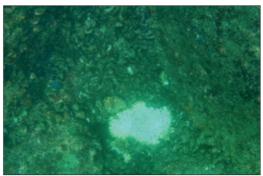


Fig. 8. White syndrome and brown mussel infestation in corals at Enayam



Fig. 9. Brown mussel infestation in Porites sp. at Enayam



Fig. 10. Encrustation of corals by zooanthids

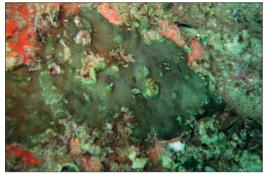


Fig. 11. Infestation by sponge and algae along with tissue loss in *Porites* sp. at Enayam

from the normal appearing tissue. In most cases, live tissue typically formed an abrupt margin at the lesion border and colonies usually lacked a zone of transition between live coral tissue and freshly exposed skeleton, though some amount of tissue sloughing was noticed. Lesion boundaries were observed to be free of pigmented bands or other colonising organisms. Algae and other microorganisms were found to

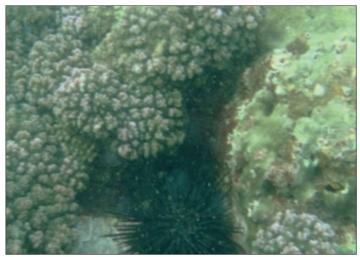


Fig. 12. Predation by sea urchins among Pocillopora verrucosa and Porites sp.

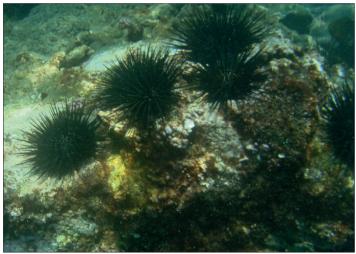


Fig. 13. Extensive damage and tissue loss due to predation by sea urchins at Enayam

colonise the initially exposed skeleton, distal to the actively progressing front of the lesion. Colonies often exhibited progression from sharply delineated white skeleton to greenish/brownish skeletal surfaces infested with filamentous algae, followed by later stages of colonisation by macroalgae and other organisms.

White syndromes characterised by diffuse patterns of tissue loss have been reported as 'White plague-like disease' in the Red Sea, 'Atramentous necrosis' in the Great Barrier Reef and as 'White band disease' in the Caribbean (Bythell *et al.*, 2004). The dynamics of white syndrome are currently unclear at local scales, but spatial analysis suggests that thresholds of thermal stress and coral cover are critical factors in determining the regional prevalence of the syndrome (Bruno *et al.*, 2007).

Sporadic coral bleaching and patchy bleaching was observed in *Pocillopora* spp. and *Porites* spp. However large-scale bleaching was not observed in any of the species. When corals are inordinately stressed, they often expel the symbiotic zooxanthellae which is manifested as bleaching (Glynn, 1996). Localised bleaching has been observed globally since at least the beginning of the twentieth century. However, since 1980s, regional and global bleaching events affecting numerous species have been reported from reefs worldwide. Reef-building corals are extremely susceptible to temperature stress as they have a narrow range of thermal tolerance between 18°C and 30°C and it is well known that corals "bleach" at high temperatures (Glynn, 1996). Bleaching is usually not uniform over single coral colonies within coral communities or across reef zones, and some species are comparatively more susceptible.

Localised bleaching has been attributed to exposure to high light levels, increased ultraviolet radiation, temperature or salinity extremes, high turbidity and sedimentation resulting in reduced light levels, and other abiotic factors (Glynn, 1996). In addition, bleaching in some species has occurred in response to bacterial infection (Kushmaro et al., 1996). However, the seven major episodes of bleaching that have occurred globally since 1979 have been primarily attributed to increased seawater temperatures associated with global climate change and el Nino/la Nina events, with a possible synergistic effect of elevated ultraviolet and visible light (Hoegh-Guldberg, 1999). Debilitating effects of bleaching include reduced skeletal growth and reproductive activity, and a lowered capacity to shed sediments and resist invasion of competing species and diseases (Glynn, 1996). Prolonged bleaching can cause partial to total colony death. It has been reported that if the bleaching is not too severe, and the stressful conditions decrease after a short time, affected colonies can regain their symbiotic algae within several weeks to months (Glynn, 1996).

# Station II (Vizhinjam)

At Vizhinjam Bay, *Porites* ulcerative syndrome along with lesions due to fish bites and infestation with boring organisms were recorded (Fig. 14). Coral bleaching incidences were not recorded in the area during the study period.

Ulcerative syndrome includes lesions first described on *Porites* as *Porites* ulcerative white spot (PUWS) and more recently similar white spots (UWS) on other taxa. Raymundo *et al.* (2003) reported that in Philippines, PUWS affected more than 20% of *Porites* colonies in 8 reefs out of the 10 reefs surveyed. Kaczmarsky (2006) reported PUWS incidence as

high as 53.7% among *Porites* in selected Islands of Philippines and the higher prevalence was linked to anthropogenic factors. This disease is manifested as irregular depressed white patches of clean skeleton, devoid of tissue which seem to expand and coalesce eventually forming large areas of necrotic tissue covered by algae and sediments. Occasionally, the lesions coalesce and can cause whole colony mortality and in other cases full colony recovery may occur (Raymundo *et al.*, 2003).

Lesions in corals, similar to ulcerative white syndrome are also caused by fish bites. Lesions caused by fish bites were recorded especially among *Porites* spp. (Fig.14). Predominant corallivorous reef fishes like parrotfish, butterflyfish, filefish, pufferfish, triggerfish and damselfishes are common





Fig. 14. *Porites* ulcerative syndrome from Vizhinjam Bay along with lesions due to fish bites and worm infestation. Note large necrotic areas of tissue loss with subsequent algal colonization. in the study area. Most predators create distinctive scars marked by removal of tissue and underlying skeleton.

# Station III (Thirumullavaram)

At Thirumullavaram, only massive corals were present, predominantly *Porites lutea*. Corals in the area were relatively unaffected by bleaching. Most of the massive corals dominating in this area were covered with algae/seaweeds, sponges and sediments (Fig. 15 and 16), infested with parasites (Fig. 17) or affected by disease. Pigmentation response to parasitic infestation (by trematodes and polychaete tube worms) and pink spot/line syndrome was found widespread among *Porites* spp.

Pink line syndrome (PLS) was the most prevalent type of disease condition recorded in this area (Fig. 18). Pink lines, rings or spots were recorded in coral tissue bordering the margins of competing or boring organisms. The pink pigmentation appears to be symptom of a response mounted by the coral to invading or competing organisms such as cyanobacteria, polychaetes and parasites (Ravindran and Raghukumar, 2002). In some cases, the pigmented spots appeared as small raised pink areas surrounded by healthy tissue indicating parasitic infection and in other cases a variety of pink lines or rings were seen bordering dead patches



Fig. 15. Sedimentation, infestation by seaweeds and sponges in Porites sp. at Thirumullavaram



Fig. 16. Sedimentation, algal infestations and tissue loss in Porites sp. at Thirumullavaram



Fig. 17. Trematodiasis in *Porites lutea* from Thirumullavaram showing multifocal swollen lesions.

appearing as a response to competitive interactions. The pigmentation could be caused by many factors, including injuries and abrasions, fish bites and infestation by trematodes. Coral polyp infected by trematode larvae becomes swollen and therefore become more vulnerable to predation by fishes. Ravindran and Raghukumar (2002) reported pink line syndrome (PLS) in *Porites lutea* from Lakshadweep Islands and observed the presence of the cyanobacterium *Phormidium valderianum* exclusively in the PLS affected specimens.





(a)





Fig. 18. (a): Pink line syndrome in *Porites lutea* from Thirumullavaram, (b): Multifocal lesions scattered across the colony are evident, (c): The lesions in the centre and bottom right have a wider area of affected tissue, with denuded skeleton colonised by filamentous algae

# Station IV (Thangassery)

At Thangassery Harbour area, the branched coral *Pocillopora damicornis* was predominant. Samplings were mainly done from the transects placed along the tripods in the breakwater area. Degradation of coral communities due to pollution, sedimentation and algal infestation was evident. Occasional incidence of bleached *P. damicornis* among algal settlement and sedimentation was recorded (Fig. 19 and 20).



Fig. 19. Bleached *P. damicornis* among sedimentation and algal infestation at Thangassery Harbour area, Kollam

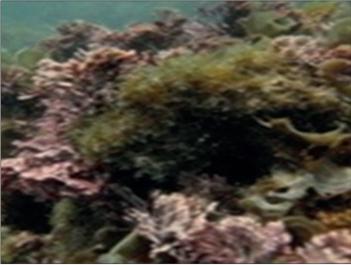


Fig. 20. Algal infestation among corals in Thangassery Harbour area, Kollam

#### **Bacterial diversity**

Corals live in association with endosymbiotic dinoflagellates of the genus *Symbiodinium* along with a rich microbial community, collectively referred to as the coral holobiont (Rohwer *et al.*, 2002). The associated microbial community contributes fundamentally to the functioning of the holobiont owing to its role in nutrition and host defence (Lesser *et al.*, 2004; Ritchie and Smith, 2004). Although bacteria have been isolated from corals since 1970's, rapid advances did not take off until 2002, since when culture independent methods have revealed a high diversity of bacteria associated with tropical corals with similar communities in corals of the same species from geographically very different regions. Recent studies have confirmed a very high diversity of metabolic functions associated with coral microbes including nitrogen cycling, sulfur cycling, photosynthesis, breakdown of complex proteins and polysaccharides (Munn, 2011).

In addition to the symbiotic microbial community, pathogenic microbes causing diseases in corals have also been reported (Geiser et al., 1998; Kushmaro et al., 2001; Patterson et al., 2002; Ben Haim et al., 2003; Denner et al., 2003; Thompson et al., 2006). Though the causative agents remain unknown for most coral diseases, compromised health in corals has been reported to be accompanied by shifts in the microbial community associated with the coral holobiont (Sunagawa et al., 2009; Kimes et al., 2010). However, it is uncertain whether infection of a single pathogen or opportunistic secondary infections following exposure to physiological stress, is responsible for triggering the restructuring of microbial communities in diseased coral colonies (Lesser et al., 2007). Though this can be attributed mainly to the complexity and dynamics of the host microbial assemblages, difficulty in conducting experiments underwater, an overall dearth of information on the structure and composition of the natural microbial community of corals, and differences in methodologies further complicate comparison of data.

Out of the total 216 bacterial isolates collected from healthy as well as diseased coral samples during the present study, 63 distinct strains belonging to 18 genera (17 known genera and one novel species) were identified. Forty two strains were identified up to species level and the rest 21 were characterised only up to generic level (Table 2). It was found that corals affected by diseases had increased bacterial loads and appeared to harbour higher abundance of microbes from a few select bacterial genera. Though several strains of bacteria were isolated from diseased corals, it was difficult to prove Koch's postulates in order to establish etiology of the disease conditions.

Station no.	Sampling site	Genus/ Species	Condition	Predominant bacteria isolated
I	Enayam	Pocillopora spp.	Healthy	Vibrio alginolyticus
				Vibrio neries
				Vibrio parahaemolyticus
				Vibrio EPOCH1
				Pseudomonas putida
				Pseudomonas fluorescens
				Alteromonas EPOCH2
				Bacillus megaterium
				Arthrobacter EPOCH3
				Micrococcus EPOCH4
l.	Enayam	Pocillopora sp.	Diseased (White	Vibrio EPOCD1
			syndrome)	Photobacterium EPOCD2
				Microbacterium EPOCD3
				Micrococcus luteus
П.	Vizhinjam Bay	Porites spp.	Healthy	Vibrio alginolyticus
				Vibrio vulnificus
				Vibrio VPORH1
				Aliivibrio fisheri
				Pseudomonas alcaligenes
				Pseudomonas VPORH2
				Alteromonas VPORH3
				Pseudoalteromonas VPORH4
				C29 (Novel strain)
				Alcaligenes faecalis
				Bacillus marinus
				Brevibacterium VPORH5
П.	Vizhinjam Bay	Pocillopora spp.	Healthy	Vibrio alginolyticus
				Vibrio campbelli
				Vibrio litoralis
				Serratia marcescens
				Pseudomonas alcaligenes
				Pseudomonas VPOCH1
				Alteromonas VPOCH2
				Alcaligenes faecalis
				Bacillus licheniformis
				Microbacterium arborescens
II	Vizhinjam Bay	Porites lutea	Diseased (Porites	Vibrio fluvialis
			ulcerative syndrome)	Alcaligenes faecalis
				Sphingomonas VPORD1

#### Table 2. Diversity of culturable bacteria in coral samples collected from the study sites

Station no.	Sampling site	Genus/ Species	Condition	Predominant bacteria isolated
III.	Thirumullavaram	Porites lutea	Healthy	Vibrio parahaemolyticus
				Vibrio alginolyticus
				Pseudomonas alcaligenes
				Acinetobacter TPORH1
				Roseobacter TPORH2
				Bacillus licheniformis
				Microbacterium arborescens
111.	Thirumullavaram	Porites lutea	Diseased (Pink line syndrome)	Vibrio splendidus
				Sphingomonas TPORD1
				Bacillus megaterium
				Microbacterium TPORD2
				Micrococcus roseus
IV.	Thangassery Harbour	Pocillopora damicornis	Healthy	Vibrio alginolyticus
				Vibrio parahaemolyticus
				Shewanella indica
				Pseudomonas putida
				Alteromonas THPOCH1
				Alcaligenes faecalis
				Bacillus subtilis
				Bacillus cereus
				Brevibacterium THPOCH2
IV.	Thangassery Harbour	Pocillopora damicornis	Bleached	Vibrio THPOCB1
				Alcaligenes faecalis
				Micrococcus THPOCB2

# Conclusion

Corals in selected sites in the study area suffered extensive degradation and the differences observed in the types and prevalence of coral diseases/ syndromes between different study sites could be a result of differences in the levels of direct/indirect human stressors and natural changes in the environment. Extensive fishing and collection of fish for the aguarium trade continue to degrade coral communities in selected locales in the study area. Besides the contribution of direct damage by fishing, pollution and climate change related factors in degradation of tropical coral reefs, the role of microbes in bleaching and diseases of corals is now evident (Rosenberg et al., 2007). Coral disease field is truly in its infancy and many important basic questions remain to be answered. Despite increasing efforts, the identification of etiological agents of coral diseases has proved problematic. Study of coral disease has suffered from a lack of standardisation in nomenclature (Work and Aeby, 2006), leading to confusion in the number of true diseases (Richardson, 1998). One of the major challenges faced by coral disease researchers is to establish a framework to systematically study coral pathologies. As majority of the coral diseases are described predominantly by gross morphology, a systematic approach to describing lesions in corals is required (Work and Aeby, 2006). Research efforts to identify pathogens, complying with Koch's postulates and to study mechanism(s) causing tissue damage are needed. Not much is known on how coral disease prevalence relates to multiple interacting variations in host densities, abiotic stressors, and levels of anthropogenic impact. In particular, there is dearth of information on coral disease dynamics under changing abiotic conditions in the absence of direct anthropogenic stressors.

Since temperatures are expected to rise considerably during the 21<sup>st</sup> century, it is likely that coral diseases will become even more prevalent (Ben-Haim *et al.*, 2003). Thus, there is an increasing need to identify and characterise coral pathogens and it is vital that we develop a better understanding of the role of microbes in the health of corals. With a multitude of factors affecting corals, the etiology of diseases needs to be investigated in detail to evolve suitable management plans for preservation of coral reefs. The intrinsic value and high level of dependency of coastal population on reef associated fisheries call for evolving adequate conservation strategies for the protection and management of coral reef ecosystems. A more holistic approach with concerted effort of all stakeholders is needed to control habitat deterioration and also to better understand the dynamics of disease conditions. Understanding how disease dynamics vary in relation to shifts in environmental conditions is crucial for the successful management and preservation of coral communities.

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