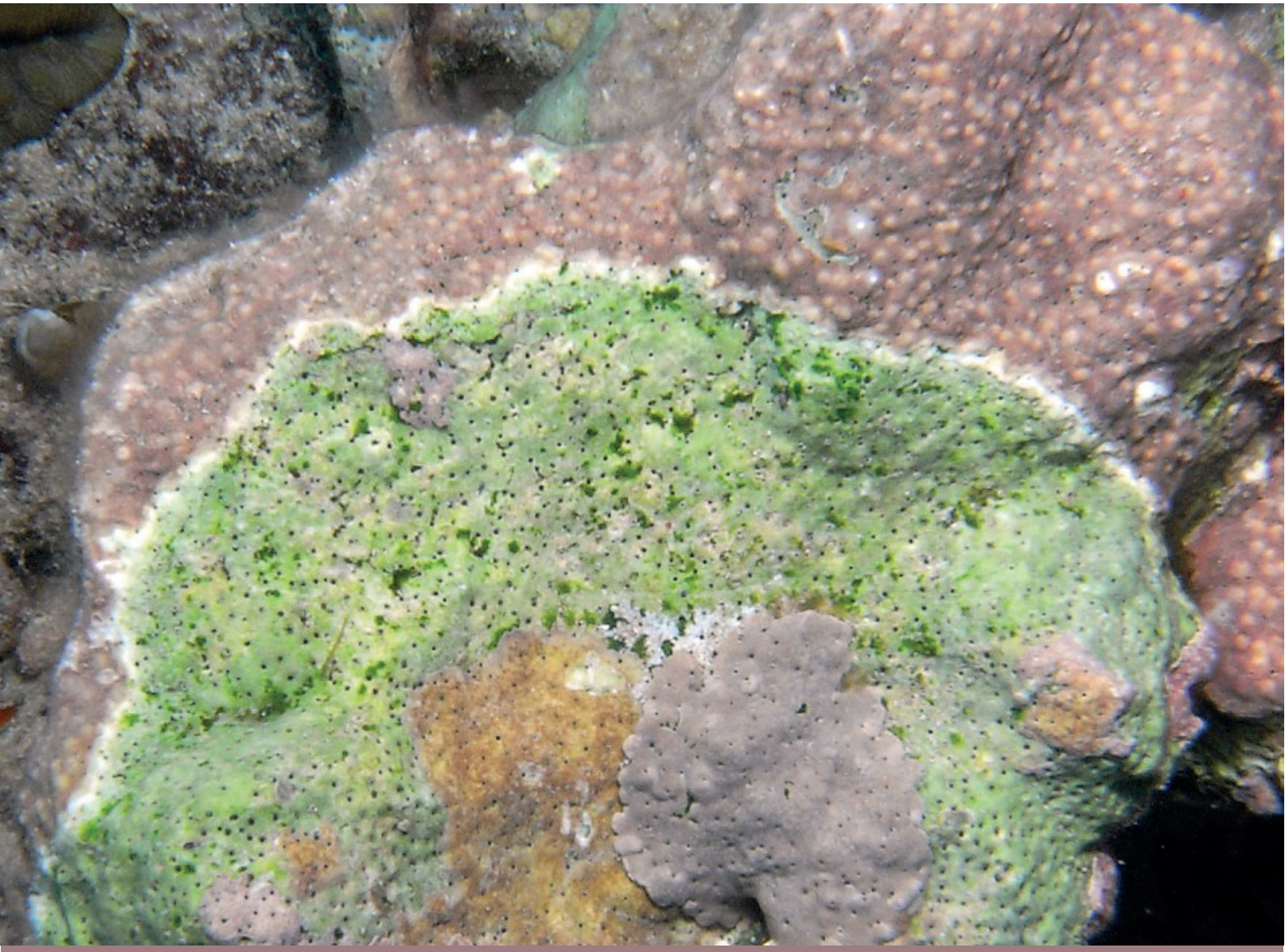

# Ecology of Diseases Affecting Crustose Coralline Algae

## characteristics, environmental drivers and effects on coral recruitment

Gaëlle Quéré

PhD thesis
University of Bremen
2015

# ECOLOGY OF DISEASES AFFECTING CRUSTOSE CORALLINE ALGAE

CHARACTERISTICS, ENVIRONMENTAL DRIVERS AND EFFECTS ON CORAL RECRUITMENT

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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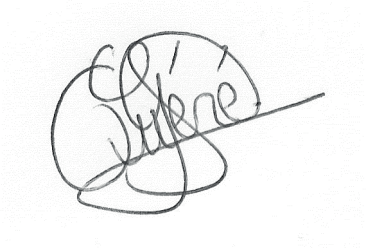
# Statement of Originality

I herewith certify that this thesis entitled Ecology of Diseases affecting Crustose Coralline Algae – Characteristics, environmental drivers and effects on coral recruitment does not incorporate without acknowledgement any material previously submitted for any degree or diploma in any University; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person, except where due reference is made in the text.

Gaëlle Quéré, Saint-Denis de la Réunion, February 22nd, 2015



# Erklärung

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel: Ecology of Diseases affecting Crustose Coralline Algae – Characteristics, environmental drivers and effects on coral recruitment selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe. Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

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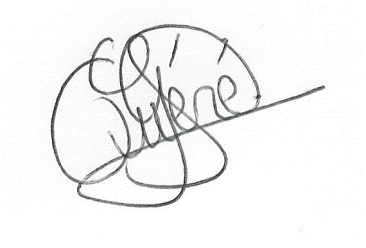

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# Abstract

Pristine coral reefs are characterized by high densities of large fishes, high cover of reef-building calcifiers and high densities of coral recruits. Such reefs have become rare and have been replaced in many places by macroalgal-dominated reefs. Marine diseases have been recognized as a key driver in coral reef decline worldwide, eradicating keystone species and altering fundamental ecological processes. In the past four decades, investigations aiming at a better understanding of diseases and their consequences on reef communities have multiplied. However, as most of these studies have focused on coral species, other key organisms of reef ecosystems have received little attention. Crustose coralline algae (CCA), along with scleractinian corals, are important primary producers and framework builders that play crucial roles in reef structure and function, including enhancing coral larval settlement. Like other reef organisms, CCA have been affected by striking disease outbreaks, yet, scientific knowledge on CCA disease ecology is lacking.

This thesis aims to investigate CCA diseases through different steps, from describing their characteristics and distribution to studying their causes and consequences, which are reflected in the three publications of the thesis. For that purpose, we combined various field and laboratory methods. First, **Publication 1** gives a detailed description of the type of diseases affecting CCA species on coral reefs of Curaçao, their macroscopic characteristics, their distribution and occurrences, and how they fluctuate under the influence of various environmental factors. Then, **Publication 2** investigates the microscopic characteristics and potential causal agents of the diseases. Finally, **Publication 3** deals with the subsequent effects of CCA diseases on important ecological processes such as the survivorship and settlement of coral larvae. The thesis includes a general introduction to the papers and concludes with a synoptic discussion.

In **Publication 1**, we conducted a survey along the coast and established a current baseline on the status of CCA diseases in Curaçao. Two types of diseases were recorded, Coralline White Band Syndrome (CWBS) and Coralline White Patch Disease (CWPD), the latter being reported here for the first time. Species-specific occurrences revealed that all CCA species encountered, i.e. 10 species among 9 genera, were vulnerable to diseases and that disease occurrences were not influenced by CCA community composition. We found that disease occurrences were

significantly higher during the warm/rainy season than during the cold/dry season and thus suggest that sea water temperature and rainfalls could be driving the increase in CCA diseases.

The investigation of disease manifestation at the cellular level in **Publication 2** revealed the complete depletion of protoplasmic content in diseased cells while cell walls remained intact. Diseased empty cells in CWPD were in the immediate vicinity of healthy cells, while a transition area appeared in CWBS in which diseased cells were only partially deprived of cellular content. We also found that various boring organisms (i.e. cyanobacteria, boring sponges, helminths and other macroborers) were more abundant in diseased thalli than in healthy thalli, which highlights a weakening of the skeleton in diseased CCA. We did not observe any signs of bacterial or fungal infection. Although the role of bioeroders in pathogenesis is not resolved, this study allowed to narrow down the range of potential pathogens for the diseases.

Finally, in **Publication 3**, we investigated the effects of diseases affecting three CCA species on the larval survivorship and settlement success of two common scleractinian corals. Our findings demonstrated a negative effect of disease affecting the well-known settlement inductive CCA species *Hydrolithon boergesenii* on larval survivorship of *Orbicella faveolata*. We also showed that the ability of *H. boergesenii* to activate coral settlement in *O. faveolata* and *Diploria labyrinthiformis* was reduced when diseased.

The outcomes of this thesis allow to better understand the ecology of CCA diseases by providing novel information on host, causative agents and environment, the three components of a complex disease triangle. This thesis indicates that a large range of CCA species is vulnerable to diseases, that disease occurrence may increase in relation to temperature and/or eutrophication, and that CCA diseases may have far reaching ecological impacts on coral reef ecosystems by weakening CCA skeleton and impairing coral recruitment. Our findings suggest that global action to limit ocean warming and acidification as well as local actions to reduce water pollution are essential to prevent disease outbreaks and their detrimental effects on coral reefs.

# Zusammenfassung

Intakte Korallenriffe zeichnen sich durch eine hohe Dichte großer Fische und Korallenrekruten, sowie einen hohen Bedeckungsgrad von riffbildenden Kalzifizierern aus. Solche Riffe sind rar geworden und wurden an vielen Orten durch Riffe ersetzt, die von Makroalgen dominiert werden. Marine Krankheiten, die Schlüsselarten ausrotten und entscheidende ökologische Prozesse ändern, wurden als einer der Hauptfaktoren für den weltweiten Korallenriffrückgang ausgemacht. Untersuchungen, die sich um ein besseres Verständnis von Krankheiten und ihren Konsequenzen für die Riffgemeinschaften bemühen, wurden in den letzten vier Jahrzehnten vervielfacht. Jedoch fokussierte sich die Mehrheit der Studien auf Korallen, während andere Schlüsselorganismen des Riff-Ökosystems wenig Aufmerksamkeit erhielten. Krustierende Kalkalgen (CCA, crustose coralline algae) sind zusammen mit Hartkorallen unter den wichtigsten Primärproduzenten und Riffgerüst-Konstrukteuren. Beide spielen eine entscheidende Rolle in der Struktur und Funktion des Riffs, wie beispielsweise der Erhöhung der Korallenlarvenbesiedlung. Wie andere Rifforganismen, wurden CCA von ausbrechenden Krankheiten beeinflusst, jedoch fehlen wissenschaftliche Erkenntnisse über die Ökologie von CCA Krankheiten.

Diese Doktorarbeit betrachtet CCA-Krankheiten durch unterschiedliche Blickwinkel: angefangen bei der Beschreibung ihrer Merkmale und ihrer Verteilung bis zu ihren Ursachen und Konsequenzen. Diese Ansätze sind in die drei Publikationen der Doktorarbeit aufgeteilt, die von einer allgemeinen Einleitung und zusammenfassenden Diskussion eingerahmt sind. Publikation 1 stellt eine detaillierte Beschreibung der Krankheitstypen von CCA in den Korallenriffen von Curaçao dar. Zudem werden makroskopische Charakteristika, Verteilung, Grad des Vorkommens und ihre Reaktion auf veränderte Umweltfaktoren beschrieben. Publikation 2 untersucht die mikroskopischen Charakteristika sowie potentielle Krankheitsursachen. Abschließend, beschäftigt sich Publikation 3 mit den Auswirkungen von CCA-Krankheiten auf die wichtigen ökologischen Prozesse: überleben und ansiedeln von Korallenlarven. Unterschiedliche Feld- und Labormethoden wurden verwendet, um sich den Themen zu nähren.

**Publikation 1:** mithilfe einer Bestandsaufnahme entlang der Küste wurde die aktuelle CCA-Krankheitslage in den Riffen Curaçaos erfasst. Zwei Typen von Krankheiten konnten hier zum ersten Mal beschrieben werden, Coralline White Band Syndrome (CWBS) und Coralline White Patch Disease (CWPD). Das artspezifische Auftreten zeigte, dass alle beobachteten CCA-Arten

(zehn Arten aus neun Gattungen) anfällig für Krankheiten waren und dass das Ausmaß der Krankheiten nicht durch die CCA Gemeinschaftszusammensetzung beeinflusst wurde. Wir fanden heraus, dass die Ausmaße der Krankheiten während der Warm-/Regenzeit signifikant höher lagen, als während der Kalt-/Trockenzeit. Dies deutet darauf hin, dass Wassertemperaturen und Niederschläge die Verbreitung der CCA-Krankheiten antreiben.

Die Untersuchung der Krankheitsmanifestation auf zellulärer Ebene in **Publikation 2** zeigte die komplette Entleerung des protoplasmischen Inhalts in kranken Zellen, während gesunde Zellen eine intakte Zellwand aufwiesen. Kranke leere Zellen in CWPD waren in direkter Nachbarschaft zu gesunden Zellen, wohingegen in CWBS eine Übergangszone erschien, in der kranke Zellen nur teilweise von zellulärem Inhalt geleert waren. Wir fanden außerdem heraus, dass diverse Bohrorganismen (Cyanobakterien, Bohrschwämme, Helminthen und andere Makrobohrer) häufiger in kranken als in gesunden Thalli anzutreffen waren. Wir konnten keinerlei bakterielle oder Pilzinfektionen feststellen. Wenngleich die Rolle von Bioerodierern in der Pathogenese nicht geklärt ist, erlaubt diese Studie eine Eingrenzung der Bandbreite an möglichen Pathogenen für diese Krankheiten.

Schließlich untersuchten wir in **Publikation 3** wie die Krankheiten drei CCA Arten in Bezug auf Larven-Überlebensrate und Besiedlungserfolg von zwei häufigen Hartkorallen beeinflussen. Unsere Ergebnisse zeigen, dass die Fähigkeit von *Hydrolithon boergesenii* Korallenbesiedlung zu aktivieren im Krankheitsfall reduziert wurde.

Die Resultate dieser Doktorarbeit erlauben ein besseres Verständnis der Ökologie der CCA-Krankheiten indem sie neue Informationen über Wirt, Pathogen (biotisch oder abiotisch) und Umwelt, den drei Komponenten eines komplexen Krankheitsdreiecks, bereitstellen. Diese Doktorarbeit weist darauf hin, dass 1) eine große Bandbreite an CCA anfällig für Krankheiten ist, 2) CCA-Krankheiten weitreichende ökologische Einflüsse auf Korallenriff-Ökosysteme haben, indem sie das CCA-Skelett schwächen und die Korallen-Besiedlung reduzieren. Aufgrund der Vorhersage, dass es im Zuge des Klimawandels zu einer Zunahme von Krankheitsausbrüchen kommt und der erlangten Resultate erscheint es dringend Gegenmaßnahmen einzuleiten. Diese Ergebnisse empfehlen, dass globales und lokales Handeln essentiell ist, um die Ozean-Erwärmung und -azidifizierung zu reduzieren, um dadurch Krankheitsausbrüche und ihreschädlichen Folgen auf Korallenriffe zu kontrollieren.

# Introduction

# Introduction

## 1. Coral reefs worldwide – from success to decline

### 1.1. Coral reefs – a highly productive ecosystem

According to geological records, the ancestors of modern coral reefs were formed at least 240 million years ago (Stanley and Fautin 2001) and flourished to the point of becoming today one of the most productive and diverse ecosystems on Earth (Hughes et al. 2003). Coral reefs cover only 0.1% of the ocean seafloor (Spalding and Grenfell 1997), but have been estimated to harbor one-quarter to one-third of the world ocean biodiversity (Knowlton et al. 2010). They are most commonly found in the photic zone where they thrive even though they are surrounded by oligotrophic waters (i.e. very low in nutrients necessary for primary production). In tropical seas, they are successful productive oases in the marine equivalent of a desert (Goeij et al. 2013). This paradox is only possible because pelagic and benthic organisms have developed strategies enabling nutrients to be stored and recycled efficiently within the system (Odum and Odum 1955; Hatcher 1990; Richter et al. 2001; Wild et al. 2004). Marine algae are the primary producers in the ocean, from the symbiotic algae in corals (zooxanthellae) to macroalgae through turf algae and encrusting coralline algae (Charpy-Roubaud and Sournia 1990; Haas et al. 2011).

At the origin of these large edifices are reef-building organisms, corals and coralline algae, which, thanks to their ability to deposit calcium carbonate, can build complex and massive 3D structures. Over time, these constructions attracted numerous and diverse organisms creating the complex ecosystem we know nowadays (Stanley Jr. 2003; Knowlton et al. 2010). Coral reefs grew into a key ecosystem that now delivers important ecological good and services (Cesar et al. 2003; de Groot et al. 2012). They provide food to millions of humans, protect and create land, supply natural compounds that can be developed in natural medicines and support commercial fisheries and tourism industry (Moberg and Folke 1999). It has been estimated that about 500 million people are dependent upon coral reefs at some level and about 30 million are virtually totally dependent on them (Wilkinson 2008). The total net benefit per year of the world coral reefs is estimated to multi-billion USD (Nunes et al. 2014).

Geological studies investigating fossilized corals have shown that species composition and zonation of coral assemblages have been stable over thousands to hundreds of thousands of years

(Jackson 1992; Aronson and Precht 1997). Extremely well adapted and adaptable, the reefs have survived dramatic environmental changes in the past (Stanley Jr. 2003). But, this image has now been shattered. Reefs have been under increasing human influence for millennia and their degradation has accelerated over the past ~50 years in a context of rapid climate change (Mumby and Steneck 2008; Pandolfi et al. 2011).

## 1.2. The decline of coral reefs

Several reports relate dramatic reversals in the health of coral reefs with considerable coral cover loss all around the world (Gardner et al. 2003; Pandolfi et al. 2003; Bruno and Selig 2007; De'ath et al. 2012). Coral reefs are one of the most threatened tropical ecosystems with over 60 % of the world's coral reef directly threatened by local human activities (Burke et al. 2011). Combined with global stressors such as warming seas and ocean acidification, the percentage of coral reefs classified as threatened reaches 75% (Burke et al. 2011).

Humans have altered the natural disturbance regime (e.g. hurricanes) by introducing new disturbances leading them from acute, short-term events to persistent or chronic, long-term events (Nyström et al. 2000). Eutrophication and increased sedimentation, over exploitation of marine species, mining and physical destruction of the reef are the main local drivers responsible for the decline of the reefs (Hughes and Connell 1999; Wilkinson 1999). In the context of climate change, coral reefs are also facing additional global stressors such as rising ocean temperature and ocean acidification. While the impact of these factors was first investigated individually, the scientific community recently focused its attention on the devastating effect of a combination of these factors that can interact putting the reefs under even higher threat (Blackwood et al. 2011; Melbourne-Thomas et al. 2011; Darling et al. 2013; Ban et al. 2014).

The most striking consequence of these threats is the shift from coral dominated to algal dominated reefs in many parts of the world (Knowlton 1992; Gardner et al. 2003; Bellwood et al. 2006). These shifts translate into extensive changes in populations, communities and ecosystem structure and function (Done 1992); with implications for the reef's architectural complexity (Alvarez-Filip et al. 2009) and the trophic relationships (Miller 2012). Reef-associated microbial communities also respond to disturbances. While resident microbes are essential for the healthy-functioning of the reefs (Rosenberg et al. 2007), studies have shown that pathogenic microbes became dominant within the coral mucus layer under stressful conditions (Mao-Jones et al. 2010). Furthermore, macroalgae exude high organic carbon that may in turn enhance microbial growth and activity of bacterioplankton in surrounding waters (Smith et al. 2006; Nelson et al. 2013).

They may also potentially serve as refuge to pathogens (Nugues et al. 2004). Degraded reefs thus appear as a favorable environment to microbes and could lead to an increase in coral diseases and coral mortality.

## 1.3. A particular case: the Caribbean basin

Among coral reefs worldwide, the Caribbean region shows the most severe degradation (Pandolfi et al. 2003). In this area, coral cover has declined from 50% in the 1970s to less than 20% today (Mumby et al. 2014). Caribbean reefs have been impacted by human activities prior to the 15$^{th}$ century mainly through the overexploitation of megafauna (e.g. dugongs, turtles, reef fishes) (Jackson 1997). Large declines in coral cover occurred across the region in the 1970s and corals have declined further since (Hughes and Tanner 2000). Recent studies have argued that reefs of the Caribbean basin may be more vulnerable to catastrophic phase shifts than other regions (i.e. Pacific ocean, Red Sea ) (Hughes 2009; Roff and Mumby 2012). Higher rates of algal blooms and missing or underrepresented key species (e.g. herbivores, three-dimensional coral species) are among the factors hampering the resilience of Caribbean reefs (Bellwood et al. 2004, 2006; Roff and Mumby 2012). Reefs are thus maintained in a degraded state where the dominance of macroalgae favors microbial development and disease outbreaks. For these reasons, the Caribbean basin has been dubbed a "diseased hot spot" (Harvell et al. 1999; Weil 2004).

### Key points

- **Coral reefs are one of the most diverse, productive and valuable ecosystem on Earth.**
- **Under rapid climate change and increasing human pressure, coral reefs are now facing an unprecedented decline leading to a coral-algal phase shift and favoring disease outbreaks, particularly in the Caribbean basin.**

# 2. Diseases in coral reef ecosystems

## 2.1. Definition of disease

Disease is any impairment of the normal structure or function of an organism manifested by characteristic clinical signs, the cause of which may or not be known (Work and Aeby 2006). According to this definition, diseases may be caused by biotic agents, pathogens such as viruses, fungi or bacteria or by abiotic factors such as unfavorable environmental conditions, nutrition or genetic mutations (Agrios 2005; Baudoin 2007). The disease doughnut (Fig. 1) can be used to illustrate in a simple manner this definition of disease (Baudoin 2007) and the importance of both biotic and abiotic agents. In order to diagnose diseases, all potential causes must be considered.

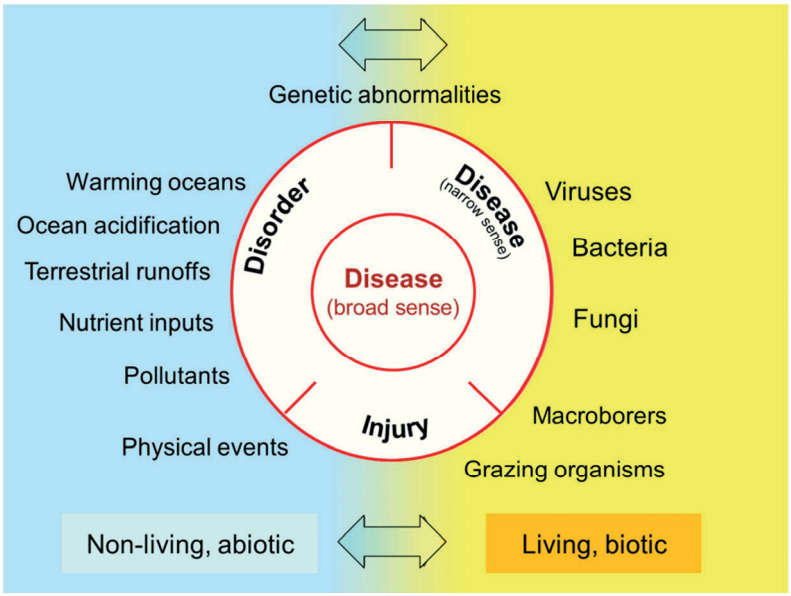


**Fig. 1** The disease doughnut modified from Baudoin 2007. The right side of the graphic illustrates the narrow definition of the term disease in which diseases are caused by biotic pathogens; the left side illustrates the broader definition including abiotic stressors as potential disease causation. The separations between both sides are deliberately left fuzzy to illustrate the interactions between both sides. All possible factors are not presented in order to keep the graphic simple.

## 2.2. The disease triangle

The disease triangle is a model initially proposed in plant pathology (McNew 1960; Stevens 1960) which describes the complex interactions between the environment, the host and an infectious agent (Scholthof 2007). While widespread in plant pathology, the use of this model is rare in studies investigating diseases in coral reefs and it is only recently that the disease triangle has been recognized as a good way to conceptualize interactions among environment-host-

causative agent when investigating disease causation in coral reefs (Cróquer and Weil 2009; Rosenberg et al. 2009; Fig. 2). Indeed, similarly to plants, benthic sessile reef organisms (e.g. corals, crustose coralline algae) are immobile and cannot escape an unfavorable environment. In the actual context of climate change, it is crucial to consider the three factors at the vertices of the triangle for a better interpretation of disease occurrence.

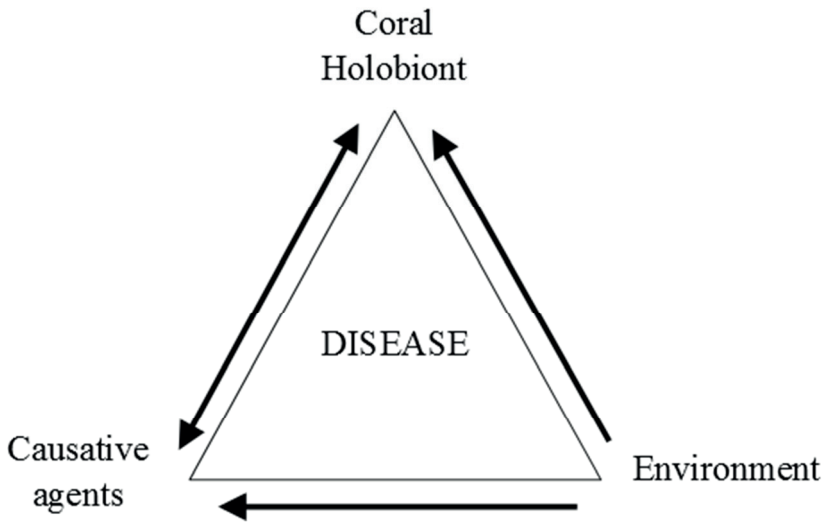


**Fig. 2** The disease triangle from Rosenberg (2009) represents the intricate relationships between host-pathogen and environment (black arrows). Factors may affect independently host and causative agent, and/or the interactions between them, leading to diseases.

## 2.3. Diseases in coral reefs

In the recent decades, an increasing number of scientific publications have raised awareness that marine diseases constitute a major threat to marine ecosystems, affecting a large spectrum of marine organisms from plants to invertebrates or vertebrates (Harvell et al. 1999; Ward and Lafferty 2004). In coral reef ecosystems, diseases have been reported for corals, crustose coralline algae (CCA), sponges and zoanthids (Weil et al. 2006; Harvell et al. 2007). Diseases have the potential to alter the structure and ecological function of the ecosystems, in particular coral reefs, by causing rapid population decline, species extinction or by disrupting key ecological processes (Harvell et al. 2007; Bourne et al. 2009). They can act directly by affecting major reef-building corals (e.g. Acroporid corals) (Gladfelter 1982) or indirectly by affecting important reef-associated organisms (e.g. *Diadema antillarum*). Mass disease-induced mortality of the herbivorous sea urchin *Diadema antillarum* in the late 1970s and of *Acropora palmata* and *Acropora cervicornis* in the early 1980s precipitated a coral to algal phase shift on many reefs in the Caribbean (Lessios et al. 1984; Hughes 1994; Bellwood et al. 2004).

In the face of climate change, reefs have been placed under additional stress and coral epizootics have multiplied (Weil and Rogers 2011; Pollock et al. 2014). Global stressors, e.g., rising ocean temperature and acidification, and local stressors, e.g., nutrient pollution, habitat deterioration, have all been identified as important drivers of disease dynamics (Harvell et al. 2007; Sokolow 2009). The potential interaction effect between them is still poorly understood, although increasingly studied (Buddemeier et al. 2004). These factors may act in synergy to increase the pathogen virulence and/or decrease the host fitness thereby increasing susceptibility to diseases (Harvell et al. 2002; Williams et al. 2010).

Even though studies investigating diseases in coral reef communities have for the most focused on coral species (Sutherland et al. 2004; Harvell et al. 2007), other key functional groups are not immune and crustose coralline algae are a prime example.

Key points

- **Disease is defined as any impairment of the normal structure or function of an organism. Disease can be infectious or non-infectious.**
- **The three factors of the disease triangle: environment, host and causal agent, must be considered to investigate diseases affecting coral reef organisms.**
- **Under rapid climate change and increasing human pressure, disease outbreaks have multiplied in the past decades, affecting a large spectrum of reef organisms.**
- **Most studies focused on coral species while other key organisms such as CCA have received little attention.**

# 3. Crustose Coralline Algae diseases

## 3.1. Crustose Coralline Algae

Crustose (Nongeniculate) coralline algae (CCA) are benthic algae that belong to the phylum Rhodophyta also referred to as red algae. Within this group, they form a distinct order, the Corallinales (Silva and Johansen 1986) characterized by the presence of high magnesium calcite within and between their vegetative cell walls (Bittner et al. 2011). This form of calcium carbonate is harder and denser than the aragonite produced by scleractinian corals. It is geologically resistant with a strong ability to become fossilized conferring the CCA a paleontological significance with an extensive representation in the geological record. Mg-calcite is also particularly hard which gives the CCA a crucial ecological role in coral reef construction and cementation (Steneck 1986). Coralline algae are exclusively marine plants and globally distributed from tropical over temperate to subpolar environments. They occupy a large depth range from the intertidal zone to great depths up to 268 m (Littler et al. 1986). They are among the most diverse and abundant organisms living on hard substratum within the photic zone (Steneck 1986). CCA are slow growers commonly growing prostrate on hard substrata and as epibionts on other plants and animals (Steneck 1986). They can build extensive carbonate structures called algal ridges (Adey and Vassar 1975) and their thick crusts provide microhabitats for various benthic organisms (Teichert and Freiwald 2014). Environmental factors such as substratum, temperature light, water motion and salinity influence greatly their abundance and zonation (Adey 1986; Steneck 1986). CCA show a great plasticity in their growth forms depending on environmental parameters (Steneck and Adey 1976). This phenotypic plasticity has hampered coralline identification (Steneck and Adey 1976) and made the taxonomy of this group extremely complex. Several classifications exist that use morphological and anatomical features as diagnosis characters and differ by the importance given to vegetative or reproductive characters (Adey 1970; Johansen 1976; Cabioch 1988; Woelkerling 1988; Bittner et al. 2011). However, the advances in molecular taxonomy have greatly improved the comprehension of the Corallinales lineage and of the species boundaries which are still evolving with new molecular phylogenetic analyses (Bittner et al. 2011; Kato et al. 2011).

CCA have developed strategies like photoacclimation allowing them to survive under a wide range of light regimes (Payri et al. 2001). They have the ability to adapt their photosynthetic apparatus leading to changes in light absorption efficiency, photosynthetic characteristics or pigment content depending on the light regime (Payri et al. 2001; Burdett et al. 2014). This could

explain their wide distribution in various habitats and their major contributions to the building of the reef.

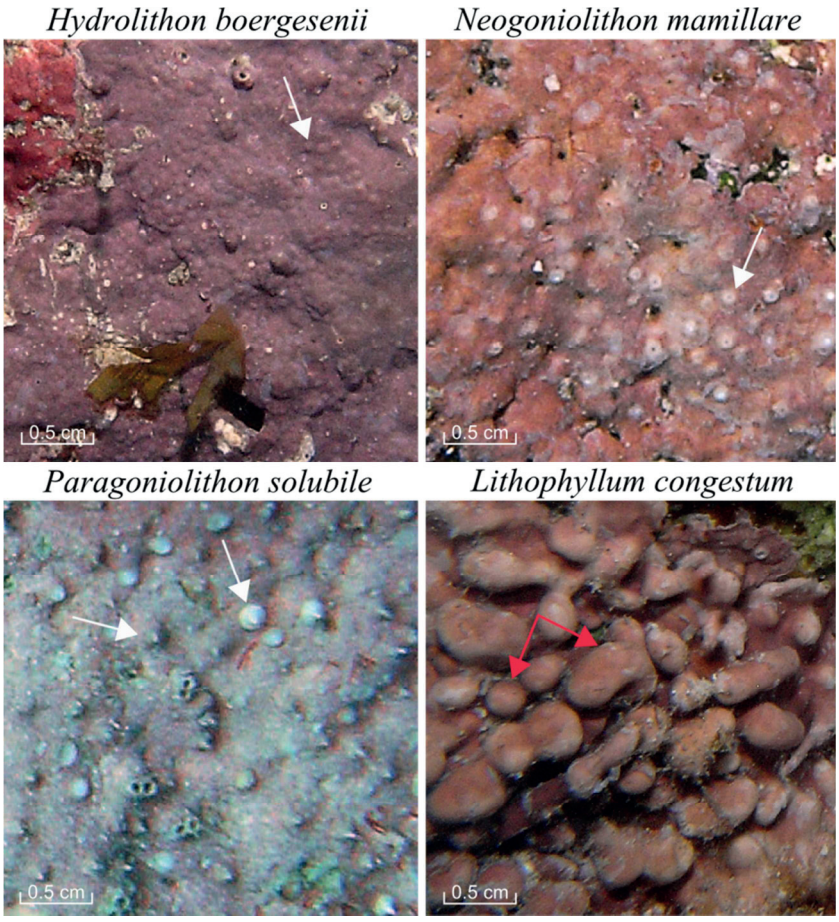


**Fig. 3** Morphology of different CCA species encountered in Curaçao. Conceptacles (reproductive structures) differ in size and shape (white arrows). Note the formation of "protuberances" also called "branches" in *L. congestum* (red arrows).

## 3.2. Crustose coralline algae and the reef ecosystem

Great abundances of crustose coralline algae, along with the presence of large fishes and reef-building corals, are an indicator of a healthy ecosystem not impacted by human presence (Bellwood et al. 2004; Knowlton and Jackson 2008; Sandin et al. 2008). Crustose coralline algae play a key role in the ecosystem functioning and perform numerous ecological services (McCoy and Kamenos 2015). As mentioned in the previous paragraph, CCA significantly contribute to calcium carbonate accretion during calcification and photosynthesis and are thus, together with scleractinian corals, major cementing and building agents of the reefs (Rasser and Riegl 2002). Crustose coralline algae play an active part in the marine food web. In addition to fish, several benthic organisms (i.e. urchins, limpets and chitons) feed on coralline algae which constitute an important food source in the energy web (Steneck 1983, 1986; McCoy and Kamenos 2015). Their

organic content per dry weight is higher than in many other algae (Hawkins 1981) and CCA are not destroyed by intense grazing pressure thanks to their stony thalli (Littler and Littler 2013). With filamentous algae and corals, crustose coralline algae therefore constitute one of the three major groups of primary producers of the shallow reef zone (Wanders 1976; Chisholm 2003). Conversely, coralline algae need grazing herbivores to prevent overgrowth by turf or macroalgae (Steneck 1997). However, they also have their own defensive mechanisms against macroalgae. They can shed their thalli or exude antifouling compounds preventing the growth and recruitment of macroalgae (Suzuki et al. 1998; Vermeij et al. 2011).

Even though their thallus is unfavorable to the growth of macroalgae, the opposite is true regarding coral larvae. CCA living surface constitutes a suitable settlement substrate and by excluding other space competitors it enhances the survival of coral settlers (Harrington et al. 2004). Among the different groups of algae occurring on the reef, crustose coralline algae are probably the only ones that have positive and not antagonistic interactions with corals (Barrott et al. 2011). CCA are also beneficial to coral recruitment as they produce cues activating coral larval settlement and metamorphosis (Morse et al. 1994; Heyward and Negri 1999).

## 3.3. Vulnerability of CCA to diseases

Despite their great resistance and flexibility that allowed them to radiate during geological periods, CCA have not been spared by the increase in marine disease outbreaks. The first reports on CCA diseases date back to the 1990s when coralline lethal orange disease (CLOD) and white band disease decimated populations of *Porolithon onkodes* in the Pacific and populations of *Porolithon pachydermum* in the Atlantic respectively (Littler and Littler 1995; Goreau et al. 1998). Diseases therefore have the potential to considerably reduce CCA abundance on many reefs. Five disease categories have been recorded so far (Vargas-Ángel 2010; Fig. 4), but the etiology of only two of them is known; the CLOD (Littler and Littler 1995) is caused by a consortium of bacteria and the coralline fungal disease is associated with a fungal infection (Williams et al. 2014). The etiology of the other categories remains largely unknown, in part due to a lack of scientific investigations.

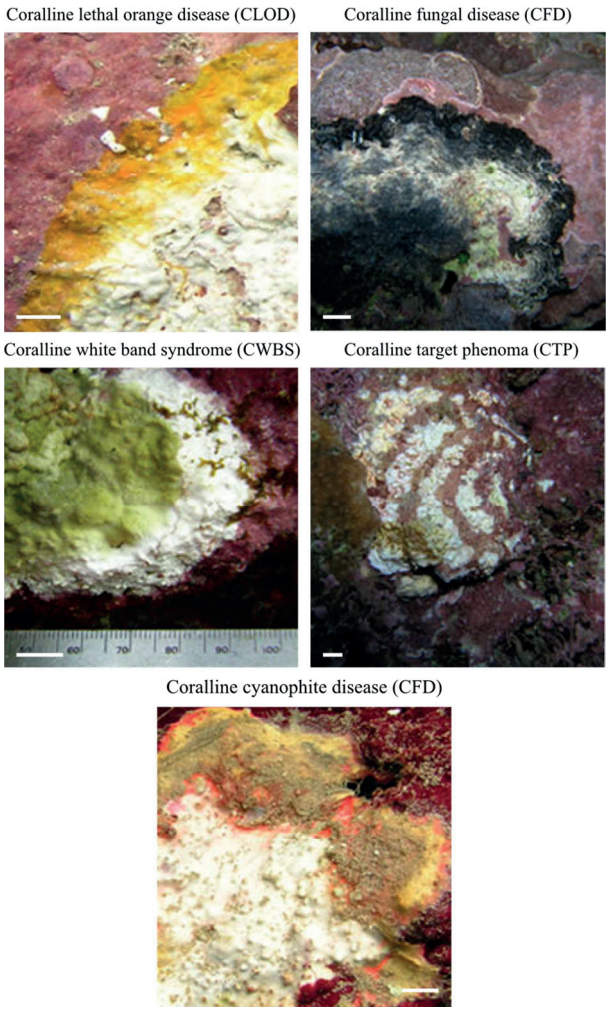


**Fig. 4** Five CCA disease categories taken from Vargas-Ángel (2010). Scale bar = 1 cm.

CCA diseases have now been observed worldwide (Vargas-Ángel 2010; Tribollet et al. 2011). Climate change has been proposed to be a key driver explaining CCA diseases outbreaks. However, it remains unknown whether global factors affect host directly or indirectly by increasing susceptibility to disease and/or pathogen virulence (Harvell et al. 1999, 2007). On one hand, CCA appear particularly sensitive to ocean acidification (Webster 2013; Kuffner 2007), rising temperature (Webster et al. 2011) or a combined effect of both factors (Diaz-Pulido et al. 2012; Williams et al. 2014). The direct consequences on the algae include a decrease in calcification rate, an increased skeletal dissolution and tissue mortality (Martin and Gattuso 2009; Diaz-Pulido et al. 2012), but also a shift in microbial community (Webster et al. 2011; Doropoulos et al. 2012). CCA are thus weakened, become more vulnerable to diseases and less resilient. On the other hand, laboratory experiments have demonstrated an increase in the virulence of coralline fungal disease under elevated temperature (Williams et al. 2014). Evidence of complex interactive effects between global stressors (e.g. warming temperature, ocean

acidification) also exist (Williams et al. 2014). Similarly, local drivers (e.g. grazing intensity by herbivores, sedimentation, exposure to sewage) are known to affect CCA growth and/or calcification, but their influence on CCA disease dynamics and the potential effects with global stressors are virtually unknown.

Despite their importance to coral reefs, CCA have so far received little attention. Their role in coral recruitment has been subject of several recent investigations (Ritson-Williams, 2010; 2014) but none has addressed the impacts of a decline in CCA health or abundance on this fundamental ecological process.

Given the ecological importance of CCA to coral reefs, greater understanding of the threat posed by diseases is critical. In the context of climate change, the issue is particularly urgent. Therefore, the knowledge gap in CCA algal disease etiology, the factors driving their dynamics as well as the impacts at the ecosystem scale must be addressed.

Key points

- **CCA participate in complex interactions with other algal functional groups, corals and herbivores. They provide important ecosystem services (food web, prevention of phase shift, coral larvae settlement) and partly regulate corals and macroalgae populations.**
- **In the past four decades, disease outbreaks have been particularly striking among CCA.**
- **Climate change is proposed to be one of the key driving factors explaining disease dynamics.**
- **Our understanding of the factors and mechanisms driving disease dynamics is only at its first stages and remains largely unknown at present.**

# Aims and outline of the thesis

The objective of this PhD thesis was to develop the knowledge and understanding of CCA diseases and diseases influence on coral recruitment. We investigated CCA diseases through different steps reflected in the three publications of the thesis. First, we identified the type of CCA diseases encountered, their macroscopic characteristics, their distribution and occurrences, and determined the potential environmental factors influencing their dynamics. Then, we used histopathology to investigate disease causation. Finally, we determined the subsequent effects of CCA diseases on two important ecological processes: the survivorship and settlement of coral larvae. To address these goals, we combined various field and laboratory methods. Our study area, Curaçao, Southern Caribbean, is located in the disease hot spot region and CCA diseases remain poorly studied in the area.

In **Publication I**, we aimed at establishing a current baseline on the status of diseases in Curaçao by conducting a quantitative survey during the wet and the dry seasons at six sites distributed along the leeward coast of the island. Number and type of lesions, benthic composition and CCA species encountered were recorded. Lesions were also monitored over a three weeks period to quantify their progression rate. The publication addresses the following questions:

- *What types of CCA diseases are present on the reefs of Curaçao?*
- *Are there sites more affected than others?*
- *Does disease primarily affect a particular genus or species of CCA?*
- *Do environmental factors influence disease occurrences?*
- *How fast do the diseases spread on the CCA crusts?*

Following the establishment of baseline levels of CCA disease occurrence, **Publication II** investigated the disease manifestation at the cellular level by conducting histological analyses of healthy and diseased CCA fragments collected in Curaçao. Although histopathology is a critical step in any effective disease survey, no study has investigated the manifestation of the two diseases encountered in Curaçao at the cellular level. The publication aimed at answering the following questions:

- *What are the cellular changes associated with gross lesions?*
- *What types of pathogenic organisms are present?*

- *Is there a link between potential causative agents and host response?*

Finally, **Publication III** tested the hypothesis that CCA diseases reduce coral larval survival and settlement success. CCA are well-known to benefit coral recruitment by activating coral settlement. However, the impact of CCA diseases on coral recruitment has not been investigated yet. Laboratory experiments were conducted in Curaçao in order to answer the following questions:

- *Do CCA diseases affect coral larval survival?*
- *Do CCA diseases influence coral larval settlement?*

## Aims of the thesis

1. **Establishing a baseline of CCA disease occurrence in Curaçao and investigating environmental factors explaining disease dynamics** (Publication I).
2. **Characterizing CCA diseases at the microscopic scale and determining the potential causative agents** (Publication II).
3. **Investigating the subsequent effects of CCA diseases on coral recruitment** (Publication III)**.**

# List of publications

## Publication I

Quéré G, Steneck R S, Nugues M M (2015) Spatio-temporal and species-specific patterns of diseases affecting crustose coralline algae in Curaçao. *Coral Reefs* 34: 259–273 [doi:406 10.1007/s00338-014-1225-3]

The collaboration with R. S. Steneck from the University of Maine was initiated by M. M. Nugues. G. Quéré and M. M. Nugues conceived and designed the study. G. Quéré collected and analysed the data. Identification of the collected CCA species was realized with the help of R. Steneck. Data interpretation and writing of the manuscript were conducted by G. Quéré in close collaboration with M. M. Nugues.

## Publication II

Quéré G, Meistertzheim A-L, Steneck R S and Nugues M M (Submitted) Histopathology of crustose coralline algae affected by white band and white patch diseases. *Diseases of Aquatic Organisms*

The idea of the study was developed by G. Quéré and M. M. Nugues. Data were collected by G. Quéré and analysed with the help of A-L. Meistertzheim in the laboratory. G. Quéré, R. Steneck, A-L. Meistertzheim and M. M Nugues interpreted the data. G. Quéré wrote the manuscript with improvements by M. M. Nugues.

## Publication III

Quéré G, Nugues M M (In revision) Coralline algae disease reduces survival and settlement success of coral planulae in laboratory experiments. Accepted in *Coral Reefs*

Experiments were designed by G. Quéré and M. M Nugues and conducted by G. Quéré. Data were analysed and interpreted by G. Quéré and discussed with M. M Nugues. G. Quéré wrote the manuscript with support of M. M. Nugues.

# PUBLICATION I

## Spatio-temporal and species-specific patterns of diseases affecting crustose coralline algae in Curaçao

Gaëlle Quéré, Robert S. Steneck and Maggy M. Nugues
2015

*Coral Reefs* 34: 259-273 [doi: 406 10.1007/s00338-014-1225-3]

# Spatio-temporal and species-specific patterns of diseases affecting crustose coralline algae in Curaçao

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## Abstract

**Distribution and abundance of coral diseases have been well documented but only a few studies considered diseases affecting crustose coralline algae (CCA), particularly at the species level. We investigated the spatio-temporal dynamics of diseases affecting CCA along the south coast of Curaçao, Southern Caribbean. Two syndromes were detected: the Coralline White Band Syndrome (CWBS) previously described and the Coralline White Patch Disease (CWPD) reported here for the first time. Diseases were present at all six study sites and our results did not reveal a relationship between disease occurrence and human influence. Both diseases were more prevalent on the shallower reef flat than on the deeper reef slope, and during the warm/rainy season than during the cold/dry season. The patterns observed were consistent with a positive link between temperature and disease occurrence. Reef flat communities were dominated by *Neogoniolithon mamillare* and *Paragoniolithon solubile* whereas deeper habitats were dominated by *Hydrolithon boergesenii*. Diseases affected all the species encountered and no preferable host was detected. There was a significant relationship between both disease occurrences and CCA cover. Monitoring of affected patches revealed that 90% of lesions in CWBS increased in**

**size whereas 88% of CWPD lesions regenerated over time. CWBS linear progression rate did not vary between seasons or species and ranged from 0.15-0.36 cm.month$^{-1}$ which is in the same order of magnitude as rates previously documented. We conclude that diseases have the potential to cause major loss in CCA cover, particularly in shallow waters. As CCA play a key role in reef ecosystems, our study suggests that the emergence of diseases affecting these algae may pose a real threat to coral reef ecosystems. The levels of disease reported here will provide a much-needed local baseline allowing future comparisons.**

**Keywords:** Crustose coralline algae species, disease, Caribbean, coral reefs, Curaçao, baseline transect survey

# Introduction

In the absence of human impacts, coral reef communities are commonly characterized by high densities of large fishes, abundant reef-building corals and crustose coralline algae (CCA; Rhodophyta, Corallinaceae), high densities of coral recruits, and low levels of disease (Hughes 1994; Jackson 1997; Pandolfi et al. 2003; Sandin et al. 2008). Such untouched reefs are rare and most coral reefs are now mildly to heavily degraded by local human activities as well as by global change (Sandin et al. 2008; Burke et al. 2011). Since the 1980s, an increasing number of studies have brought evidence that diseases are a key driver of the deterioration of coral reefs worldwide, eradicating keystone species and altering major ecological processes (Weil 2001; Harvell et al. 2007; Pollock et al. 2011; Tribollet et al. 2011). The long-spined sea urchin *Diadema antillarum* has largely vanished from the entire Caribbean-wide basin in the early eighties, due to an unknown waterborne pathogen, precipitating a coral to algal phase shift on many reefs (Lessios et al. 1984; Hughes 1994). Another striking example is the decimation of two primary framework builders, *Acropora palmata* and *Acropora cervicornis* by white-band disease from the late 1970s until the early 1990s that further contributed to the increase of algae on Caribbean reefs (Gladfelter 1982; Harvell et al. 1999; Aronson and Precht 2001). Since then, a wider range of corals as well as other groups of marine organisms have been affected by an increasing number of diseases (Green and Bruckner 2000; Aronson and Precht 2006). However, studies aiming to characterize and understand diseases affecting coral reef communities have for the most focused on coral species, and other benthic groups, such as crustose coralline algae, have received relatively little attention (Goreau et al. 1998; Sutherland et al. 2004; Harvell et al. 2007, but see Aeby et al. 2008; Vargas-Ángel 2010; Tribollet et al. 2011; Williams et al. 2014).

Crustose coralline algae are a key functional group in coral reef ecosystems for several reasons: (1) some species have a positive effect on coral recruitment by inducing settlement and metamorphosis of coral larvae (Ritson-Williams et al. 2010); (2) their living surface provides coral larvae with suitable settlement substrate and excludes other space competitors, thus enhancing the survival of coral settlers (Harrington et al. 2004); (3) they can shed their thalli to prevent overgrowth by macroalgae (Johnson and Mann 1986); (4) they produce antifouling compounds capable of reducing growth rate and recruitment success of macroalgae (Kim et al. 2004; Vermeij et al. 2011); and (5) together with scleractinian corals, they are considered as locally important framework builders and carbonate producers (Steneck and Adey 1976; Rasser and Riegl 2002; Gherardi and Bosence 2005).

The first reports of CCA disease date back to the 1990s when *Porolithon onkodes* in the Pacific suffered significant mortality from the coralline lethal orange disease (CLOD) (Littler and Littler 1995). During the same period in the Atlantic, *Porolithon pachydermum* populations were hit by white band disease which killed a quarter to three quarters of the *Porolithon* population at many Caribbean sites (Goreau et al. 1998). Despite the ecological significance of CCA and these striking examples of disease-induced mortality, few isolated reports on CCA disease exist at present and most concern the Indo-Pacific (Vargas-Ángel 2010; Tribollet et al. 2011; Miller et al. 2013; Williams et al. 2014). Surveys conducted in the US-Affiliated Pacific islands between 2006 and 2008 recorded five major disease categories affecting CCA (Vargas-Ángel 2010). Tribollet et al. (2011) reported two types of lesions in the lagoon of New Caledonia. More recently, two types of CCA disease were found to be widespread throughout much of the GBR, though their levels remained low and comparable to other Indo-Pacific reefs (Miller et al. 2013).

Our understanding of how global stressors, e.g. rising ocean temperature and acidification, and local stressors, e.g. nutrient pollution, affect CCA disease dynamics is still at its infancy. These stressors may increase host susceptibility to disease and/or pathogen virulence (Harvell et al. 1999, 2007). CCA appear to be more susceptible than corals to ocean acidification (Webster et al. 2013) and these effects are worsened by warm conditions (Diaz-Pulido et al. 2012). In the US-affiliated Pacific islands, island-wide overall CCA disease occurrence was positively correlated to mean annual sea surface temperatures (SSTs) (Vargas-Ángel 2010). At Palmyra Atoll in the Pacific, the occurrence of Coralline Fungal Disease (CFD) was associated with El Niño ocean-warming events (Williams et al. 2014). This relationship was supported by increased CFD lesion expansion rates under elevated temperatures in the laboratory, but lower pH mitigated these effects, suggesting complex interactive effects between global stressors on CCA disease dynamics (Williams et al. 2014). Local drivers can have a significant impact on the future state of

coral reefs and are intensified by global change (Gurney et al. 2013). Yet, their influence on CCA disease dynamics and potential interactive effects with global stressors are virtually unknown. Factors such as light availability, grazing intensity by herbivores, sedimentation and exposure to sewage can influence CCA growth rates and/or calcification (Steneck and Adey 1976; Steneck 1983; Björk et al. 1995; Fabricius and De'ath 2001; Wai and Gray 2005). Because they have the potential to affect CCA fitness, they could have cascading effects on CCA disease occurrence and dynamics.

Host density and species composition are also often important factors driving the spatio-temporal dynamic of diseases (Ostfeld et al. 2008). Recent research on CCA suggests that the relationship between host density and disease occurrence varies with scale. At the scale of archipelago in the US Pacific, Vargas-Ángel (2010) found a positive relationship between island mean CCA cover and overall CCA disease occurrence. However, spatial variation in CFD occurrence was independent of host abundance within Palmyra Atoll's forereef habitat (Williams et al. 2014). Variations in CCA species communities could control disease distribution patterns depending on the presence or not of a preferred host. However, this aspect has been so far completely left out due to the difficulty of identifying CCA species in the field (Williams et al. 2014).

In this context, CCA diseases appear as a serious cause for concern, yet major knowledge gaps exist in CCA disease ecology and spatio-temporal dynamics at local scales, but also especially at the species level. Fine-scale studies are needed to provide a baseline of actual reef conditions and to identify the most influential drivers. Here we present the first quantitative study of CCA diseases along the coast of Curaçao in the southern Caribbean, with the following objectives: (1) to characterize the different types of disease encountered, (2) to assess the species-specific occurrence of the different diseases, (3) to examine associations between disease occurrence and several potentially influencing factors, including depth, time period, human activity, CCA cover and community composition, and (4) to quantify the progression rates of disease lesion.

# Methods

### Study sites

To investigate the distribution and abundance of CCA as well as the occurrence and severity of diseases, quantitative surveys were conducted over two time periods, from September to December 2010 (warm/rainy season) and from March to May 2011 (cold/dry season), at six sites

along the leeward coast of Curaçao (12°N, 69°W) (Fig. 1). Leeward reefs were characterized by a shallow 50 to 100 m wide reef flat, a drop-off at 8 to 12 m depth, and a steep seaward slope extending to 50–60 m depth (Bak 1977). Survey sites included a gradient of human influence. Most of the population of Curaçao is concentrated in the capital city Willemstad, which also hosts an important urban center and a large commercial and military harbor. The city is a major point source of pollutants, including nutrients, organic compounds, metals and hydrocarbons (van Duyl et al. 2002; Klaus et al. 2007). Human impact is therefore greatest at the center of the island and decreases at reef sites located up current and down current from the main urbanized area (Barott et al. 2012; Fig. 1). The warm/rainy season is characterized by an increase in seawater temperature and rainfall in comparison to the cold/dry season (Martis et al. 2002; Bak et al. 2005).

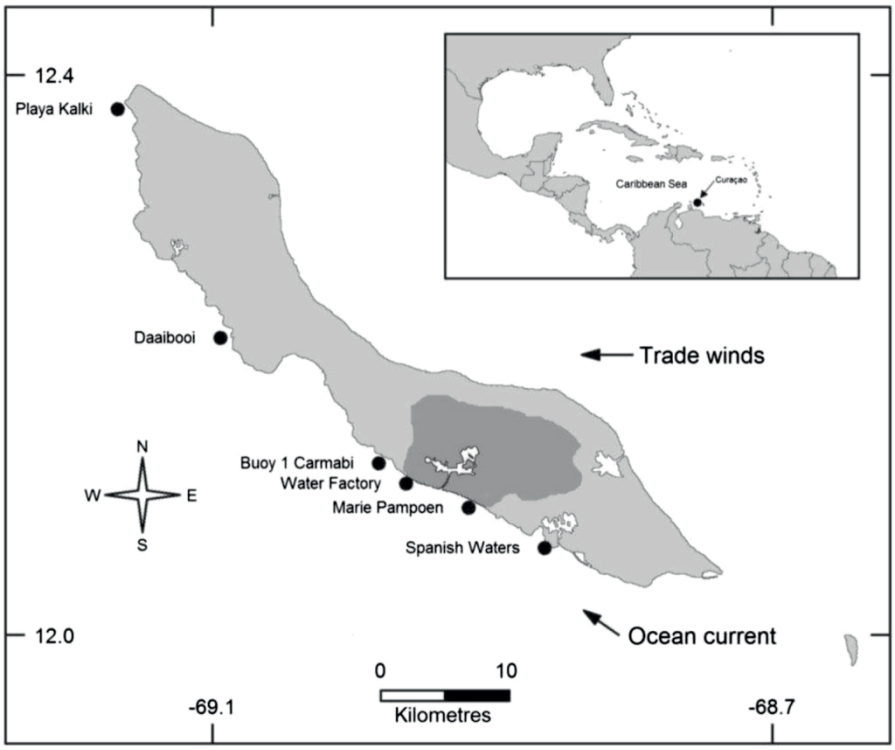


**Fig. 1** Map of Curaçao and location of the survey sites. Dark grey area indicates main urbanized area (capital city of Willemstad). Trade winds and current are indicated by arrows

**Disease and benthic surveys**

During each time period, spatial patterns of disease occurrence and benthic composition were examined across vertical and horizontal gradients: (a) by comparing different reef habitats/depths at one site (Water Factory; Fig. 1): i) the reef flat (3-10 m depth), ii) the shallow reef slope (10-15 m) and iii) the deep reef slope (15-25 m), and (b) by comparing six sites within the reef flat habitat, thus making a total of 8 locations pooling sites and habitats. Each location was subdivided into two zones in order to best represent intra-site variability. The abundance of CCA diseases was estimated in 3- or 6-m wide x 10-m long belt transects (i.e. 3 per zone, 6 per location) delimited using 10 m line transects laid haphazardly parallel to the shoreline. Transect width was 3 m at 'CCA-rich' sites (Spanish Water and Playa Kalki) and 6 m at all other sites so as to allow the completion of a minimum of one transect per 2-hr dive. We used the definition of diseases by Work and Aeby (2006): "Disease is any deviation or alteration from the normal structure or function of any body part manifested by a characteristic set of clinical signs of known or unknown cause". Work (2008) also defines a lesion as any injury to tissue or anatomic change associated with disease. Based on this definition, we recorded every lesion caused by disease encountered within each belt transect and assigned them to a disease type (Vargas-Ángel 2010). We did not take into account lesions due to predation or competitive overgrowth. Digital photographs of diseased CCA were taken and an objective description of the condition recorded (Work and Rameyer 2005). Using this methodology, two distinct crustose coralline algae conditions were found: Coralline White Band Syndrome (CWBS) and Coralline White Patch Disease (CWPD). CWBS has been the official name used to describe this condition since 2005 (Ballantine et al. 2005) and CWPD has never been documented before. Note that the terminology 'syndrome' and 'disease' are synonymous (Work et al. 2008). We used the terminology 'disease' for naming this new condition and left the original terminology for CWBS.

To estimate the percentage cover of CCA and major benthic groups, every cm of substrate falling directly underneath each transect line was assigned to one of the following benthic categories: hard coral, crustose coralline algae, macroalgae, turf algae, encrusting red algae, cyanobacterial mats, invertebrate, rubble and sand. Each CCA patch encountered during transects was sampled using hammer and chisel. In total, 704 CCA pieces (3-6 $cm^2$) were collected, rinsed in freshwater and dried in the oven at 60°C for minimum 6 hours. Morphological, anatomical and reproductive features were used as diagnosis characters under a dissecting microscope in order to identify the species (Steneck 1986). When identification down to species was not reached, samples were grouped to genera level whenever possible or left unidentified.

Surface area of CCA surveyed was extrapolated from cover measurements obtained from the line transects. CCA disease occurrence was then determined by calculating the number of CCA lesions per $m^2$ of CCA surveyed at each site or habitat. This measure was used in lieu of disease prevalence. Identification of CCA species is difficult in the field and enumeration of individual CCA is not possible, therefore this protocol is most suited for CCA and has already been used in similar studies (Aeby et al. 2008; Tribollet et al. 2011; Williams et al. 2014).

**Progression rate of lesions**

To determine the spatio-temporal dynamics of the diseases affecting CCA, CWBS and CWPD lesions on the species *Paragoniolithon solubile* were monitored during each time period at four sites (Buoy 1, Spanish Water, Daaibooi, Playa Kalki; Fig. 1). Inter-species differences were also investigated by monitoring two species *Neogoniolithon mamillare* and *Paragoniolithon solubile* (n = 4 per Species per Time period) at one site (Spanish Water). In total, 74 patches of CCA affected by diseases were tagged, photographed and analyzed at different time intervals up to 31 days. Images were imported into Adobe Photoshop CS3 and layered to form time series. A ruler placed next to the lesion during the monitoring was used to apply appropriate scale on each picture and to calibrate the measurements in Photoshop. For CWBS, the progression of active disease front was measured to the nearest mm using three points haphazardly located along the active disease band of each lesion and expressed in $cm.month^{-1}$. Since CWPD forms white patches without a well-defined and consistent area of disease progression, changes in the surface area of lesions which remained active (i.e. presence of white, non-pigmented zones) were measured and expressed in $cm^2.month^{-1}$. Of the 31 CPWD lesions, most regenerated and only 3 showed active disease progression. Those three were used to calculate a mean rate of disease progression.

**Statistical analyses**

The non-normal distribution of our response variables precluded a parametric approach. A permutation-based analysis of variance (PERMANOVA, Anderson 2001; McArdle and Anderson 2001) was used to test for differences between sites (6 levels, fixed factor), zones (2 levels, random factor) and time (2 levels, fixed factor) in both disease occurrence and CCA community composition. To analyze differences among habitats, the site factor was replaced by the habitat factor (3 levels, fixed) keeping the same design. When results were significant, pair-wise a posteriori comparisons were performed to resolve differences among levels. Differences in species composition among sites, habitats and between time periods were visualised with a

canonical analysis of principal coordinates (CAP) based upon Bray-Curtis similarity and 9999 permutations of the raw data. CAP provides a percentage of correct allocations by carrying out a ''leave-one-out'' procedure and indicating the robustness of the classification (Anderson and Willis 2003). Spearman rank correlations between the original variables and the canonical ordination axes were used to illustrate the CCA species that characterized the differences among groups. Correlations between CAP axes and disease occurrence were used to identify CCA community associated with each disease. To test the potential relationships between (a) disease occurrence and CCA coverage and between (b) community composition, we used Generalized Additive Mixed Models (GAMMs) with a Poisson distribution so we did not have to make any a priori assumptions on the shape of the relationships (Wood 2006). The number of lesions per transect was our response variable. CCA coverage and transect area were introduced as offsets in the model to account for their effects on the raw number of lesions and to obtain a dependent variable similar to a measure of "prevalence". The factors Site and Zone were used as fixed and random factors, respectively and (a) the overall coverage or (b) the relative coverages of each single species were alternatively used as covariables in two separate analyses. Transect (n = 36) was the unit of independent replication. Two species observed in only two transects throughout the survey were not included in this analysis. Covariables were smoothed using cubic regression smoothing splines. Model selection was performed by generating a set of models with all possible combinations of the terms in the global model using the *dredge* function (MuMIn package). The best-fit GAMM model was then selected among all possible models according to the corrected Akaike Information Criterion (AICc). Models were computed in R using the mgcv package (Ihaka and Gentleman 1996; Wood and Augustin 2002). Only data from the warm/rainy season were analyzed because occurrence was low for both diseases during the cold/dry season. Comparisons of disease progression rate between time periods (2 levels, fixed factor) and sites (4 levels, fixed factor) were performed using PERMANOVA with unbalanced design because the number of replicates per Site*Time combination was 3 to 4. Two sites did not present sufficient replicates and were excluded from the analyses. To analyze differences between species, a similar analysis was run with time periods and species (2 levels) as fixed factors. Data collected for CWPD were not sufficient to allow statistical analysis.

All PERMANOVA tests were run on untransformed data using 9999 permutations of the raw data from residuals under a reduced model (Anderson 2001; McArdle and Anderson 2001). Given the limited number of possible permutations especially in the individual pair-wise tests, we used the Monte-Carlo asymptotic p-values. The presence of zeros required using Euclidian distance in the analysis of disease occurrence. Bray-Curtis distance was used as the resemblance

measure in all analyses on CCA abundance. In cases when lesions were observed and no CCA cover was detected under a transect line (i.e. 3 out of the 96 transects for overall occurrence), disease occurrence was estimated by dividing the number of lesions by the mean cover of all transects from the same site and time period. All multivariate analyses were performed in R (v2.15.2) (R Development Core Team 2012), the FORTRAN computer program PERMANOVA (Anderson 2005), or PRIMER 6 and PERMANOVA + (Primer-E Ltd., Plymouth, United Kingdom).

# Results

### Overall disease occurrence

Table 1 provides a full description of the two diseases encountered and representative photographs are given in Figs. 2a & b.

**Table 1** Descritpion of the two crustose coralline algae diseases in Curaçao

| Name (abbreviation) | Description | Morphologic diagnosis | Etiologic diagnosis | References |
|---|---|---|---|---|
| Coralline White Band Syndrome (CWBS) | Diffuse, centrally but mostly peripherally situated, annular, smooth, circular, expanding white band leaving behind non-pigmented algal tissue becoming green (endolithic green algae) | Mild to severe, subacute to acute, tissue loss with white annular margin | Unknown | Goreau et al. (1998), Weil et al. (2006), Ballantine et al. (2005), Vargas-Ángel (2010) |
| Coralline White Patch Disease (CWPD) | Diffuse, both centrally and peripherally situated, distinct, smooth, irregular, non-pigmented tissue | Mild to severe, acute, white discoloration | Unknown | This study |

Morphological description of the lesions follows Work and Aeby (2006). See Fig. 2 for representative photographs of each disease

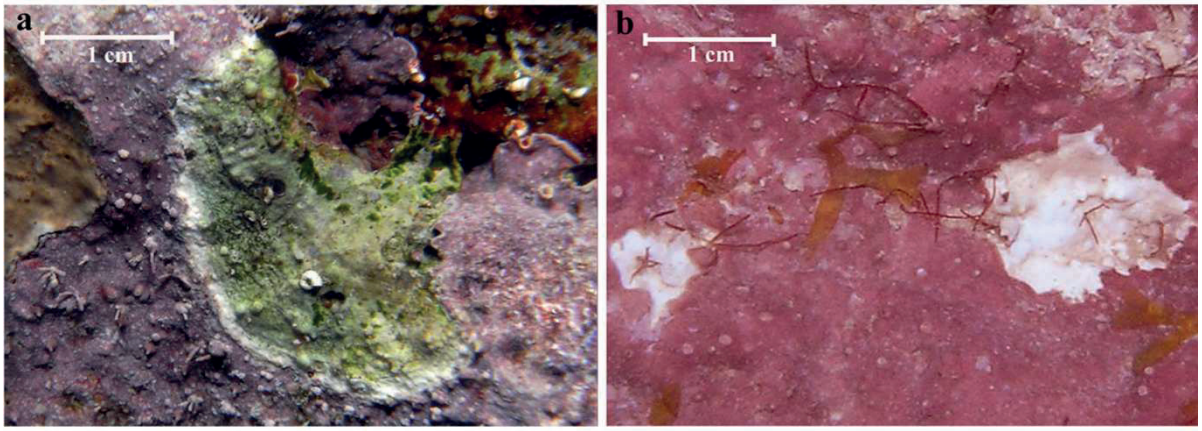


**Fig. 2 (a)** Coralline White Band Syndrome (CWBS) and **(b)** Coralline White Patch Disease (CWPD). All photographs taken on Curaçao

Overall we recorded 1067 lesions (676 for CWBS and 391 for CWPD) in 96 transects. Both diseases were spread along the leeward coast of Curaçao and were observed at all sites and habitats (Figs. 3a & b). Disease occurrence differed significantly among sites and between time periods for CWBS, and between time periods only for CWPD (Fig. 3a; Table 2a). There was no significant effect of zone nested within site and no significant interactions between time, site or zone within site. For both diseases, occurrence was higher in the warm/rainy time period than in the cold/dry time period. Pair-wise a posteriori comparisons of CWBS among sites indicated that Playa Kalki and Spanish Water generally had significantly higher disease compared to other sites (Table S1a). Since these sites are the furthest away from the capital city where human influence is the lowest, these trends did not support any positive association between CWBS and human influence. We found a significant relationship between both disease occurrences and overall CCA cover during the warm/rainy time period ($P < 0.05$; Fig. 4). In the 0 and ~7.5 % cover range which concentrated most data points, the relationship with CWBS occurrence was positive (Fig. 4a). It became negative at higher cover due to three transects. In contrast, the relationship with CWPD was negative within the 0-10 % cover range (Fig. 4b).

Significant differences in CWBS were observed among habitats and time periods (Fig. 3b; Table 2b). Interactions between time, habitat or zone within habitat were not significant. Disease was higher in the warm/rainy time period than in the cold/dry time period regardless of habitat and was higher on the reef flat compared to the 20 m reef slope (Table S1b), suggesting a decline in disease with increasing depth. These trends matched those of CCA cover during the warm/rainy time period, but not during the cold/dry time period when CCA cover increased across all habitats and particularly on the deep reef slope (Fig. 5a). Trends were similar for CWPD, but differences were not significant ($P < 0.1$).

CCA cover varied between 2 and 10 % in the warm/rainy time period (i.e. September-December 2010) and between 5 and 34 % in the cold/dry time period (i.e. March-May 2011) (Fig. 5a). This increase was most likely caused by a massive bleaching event affecting corals in October and November 2010 which opened up new substrates for colonization. Coral cover decreased from a 15-50 % range to a 16-30 % range over the two survey periods. Similar trends occurred across habitats (Fig. 5a).

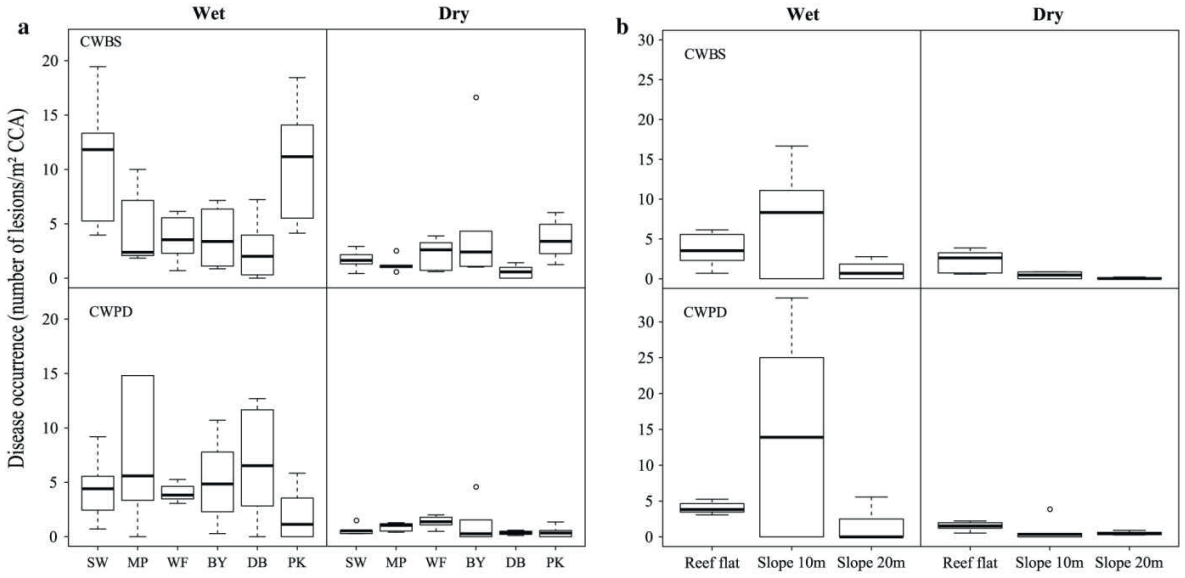


**Fig. 3** CWBS and CWPD occurrence (number of lesions per $m^2$ CCA) during the wet and dry time periods for all coralline algal species pooled **(a)** at the six study sites **(b)** at the three habitats. Study sites are Spanish Waters (SW), Marie Pampoen (MP), Water Factory (WF), Buoy 1 (BY), Daaibooi (DB), Playa Kalki (PK). Horizontal line indicates the median. The box represents the inter-quartile range (IQR) between the upper and lower quartile. Whiskers maximally extend 1.5 times beyond the IQR and outliers are indicated by circles

**Table 2** Permutational multivariate analysis of variance (PERMANOVA) testing for (a) the effects of site, zone, and time, and (b) the effects of habitat, zone, and time on CWBS occurrence, CWPS occurrence, and CCA community structure

| Source | *df* | CWBS | | | | CWPD | | | | CCA community | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | *F* | *P*(MC) | SS | MS | *F* | P(MC) | SS | MS | *F* | *P*(MC) |
| *(a)* | | | | | | | | | | | | | |
| Site | 5 | 0.0290 | 0.0058 | 32.0925 | **0.0006** | 0.0050 | 0.0010 | 1.0190 | 0.1995 | 98,891 | 19,778 | 17.5285 | **0.0001** |
| Zone(site) | 6 | 0.0011 | 0.0002 | 0.1410 | 0.9894 | 0.0030 | 0.0005 | 0.6411 | 0.6971 | 6,770 | 1,128 | 0.8450 | 0.7067 |
| Time | 1 | 0.0232 | 0.0232 | 20.5881 | **0.0049** | 0.0287 | 0.0287 | 63.7376 | **0.0004** | 8,631 | 8,631 | 6.5271 | **0.0004** |
| Site × time | 5 | 0.0222 | 0.0044 | 3.9457 | 0.0600 | 0.0049 | 0.0010 | 2.1560 | 0.1847 | 22,749 | 4,550 | 3.4410 | **0.0008** |
| Zo(site) × time | 6 | 0.0068 | 0.0011 | 0.8802 | 0.5213 | 0.0027 | 0.0005 | 0.5846 | 0.7451 | 7,934 | 1,322 | 0.9903 | 0.4802 |
| Residual | 48 | 0.0615 | 0.0013 | | | 0.0370 | 0.0008 | | | 64,092 | 1,335 | | |
| Total | 71 | 0.1437 | | | | 0.0812 | | | | 209,069 | | | |
| *(b)* | | | | | | | | | | | | | |
| Habitat | 2 | 0.0073 | 0.0036 | 10.9186 | **0.0387** | 0.0289 | 0.0145 | 6.3790 | 0.0763 | 47,687 | 23,844 | 27.0372 | **0.0002** |
| Zone(habitat) | 3 | 0.0010 | 0.0003 | 0.3562 | 0.7834 | 0.0068 | 0.0023 | 0.7048 | 0.5550 | 2,646 | 882 | 0.8672 | 0.5267 |
| Time | 1 | 0.0086 | 0.0086 | 18.2735 | **0.0229** | 0.0298 | 0.0298 | 8.8334 | 0.0559 | 1,158 | 1,158 | 0.8594 | 0.4901 |
| Habitat × time | 2 | 0.0068 | 0.0034 | 7.2259 | 0.0742 | 0.0280 | 0.0140 | 4.1544 | 0.1385 | 7,851 | 3,925 | 2.9140 | 0.0785 |
| Zo(habitat) × time | 3 | 0.0014 | 0.0005 | 0.5008 | 0.6941 | 0.0101 | 0.0034 | 1.0474 | 0.4025 | 4,041 | 1,347 | 1.3247 | 0.2431 |
| Residual | 24 | 0.0225 | 0.0009 | | | 0.0773 | 0.0032 | | | 24,406 | 1,017 | | |
| Total | 35 | 0.0476 | | | | 0.1809 | | | | 87,788 | | | |

Significant *p* values are shown in bold

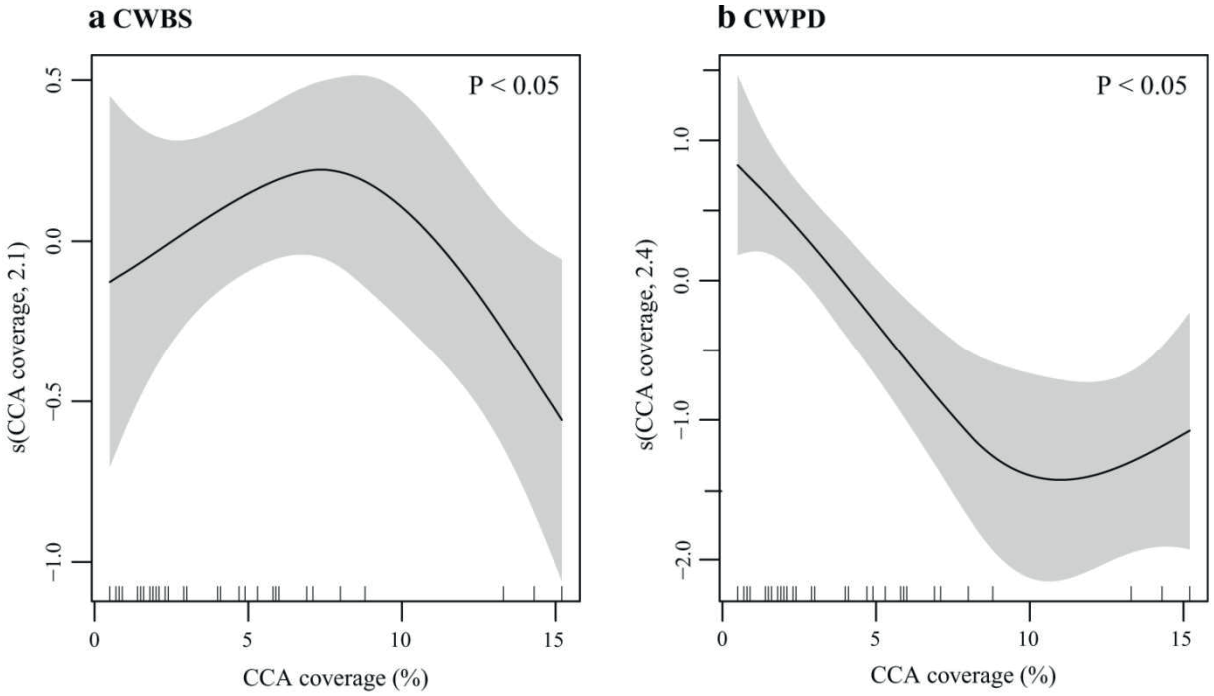


**Fig. 4** GAMMs smoothing curves showing the influence of overall CCA coverage on **(a)** CWBS occurrence and **(b)** CWPD occurrence during the wet time period (all sites pooled). Grey areas indicate 95% confidence intervals. The distribution of empirical data is shown by the inner ticks along the x-axis

**CCA community**

A total of 9 genera and 10 fully identified species were recorded during our surveys (Fig. 5). Pooling sites, habitats and time periods, 82% of the CCA cover was represented by three species: *Neogoniolithon mamillare*, *Paragoniolithon solubile* and *Hydrolithon boergesenii* (Fig. 5a). CCA community structure on the reef flat was influenced by the factors Site and Time (Table 2a). However, there was a significant interaction between Site and Time in the relative abundance of species. Between both time periods, Buoy 1 and Marie Pampoen showed a large increase in the relative cover of *P. solubile* and *H. boergesenii*, respectively (Fig. 5c). CAP achieved 69 % of correct allocations and revealed clear separations between sites (Fig. 5b). *P. solubile*, *N. mamillare, Paragoniolithon accretum* and *Lithophyllum congestum* were the most strongly discriminating species ($r_s > 0.4$). *P. solubile* characterized Buoy 1, Daaibooi and Playa Kalki, while *N. mamillare* was characteristic of Spanish Water. Marie Pampoen differed by the dominance of *Hydrolithon boergesenii*. Although *Paragoniolithon accretum* and *Lithophyllum congestum* were not the most abundant species, they were strong indicator species for Water Factory.

CCA communities differed significantly across habitats, but not between time periods (Table 2b). Communities from the reef flat clearly separated from those of the reef slopes at 10 and 20 m. CAP yielded 58 % correct allocations (Fig. 5b). *H. boergesenii* was the strongest

indicator species distinguishing habitats ($r_s > 0.8$). The reef flat was characterized by a higher species diversity and the dominance of *N. mamillare*, while *H. boergesenii* characterized the reef slopes, regardless of time periods (Fig. 5c).

**Species-specific disease occurrence and relationship with CCA community**

All the species encountered during the surveys were found to be affected by at least one lesion of either disease. We found cases of CWBS in 9 of the 11 identified CCA species and cases of CWPD in 8 species (Figs. 5b & c). The selected GAMM model did not reveal any significant relationship between overall disease occurrence and any species cover for both diseases ($P > 0.05$). This is also illustrated in the two CAP ordination plots with neither of the disease characterizing a specific CCA community ($r_s < 0.3$) (Fig. 5b). There was no significant relationship between the coverage and the disease occurrence of each species (Fig. 6; $P > 0.05$), supporting that CCA community did not influence disease occurrence. There was also no significant relationship between CWBS and CWPD occurrences of each species ($P > 0.05$).

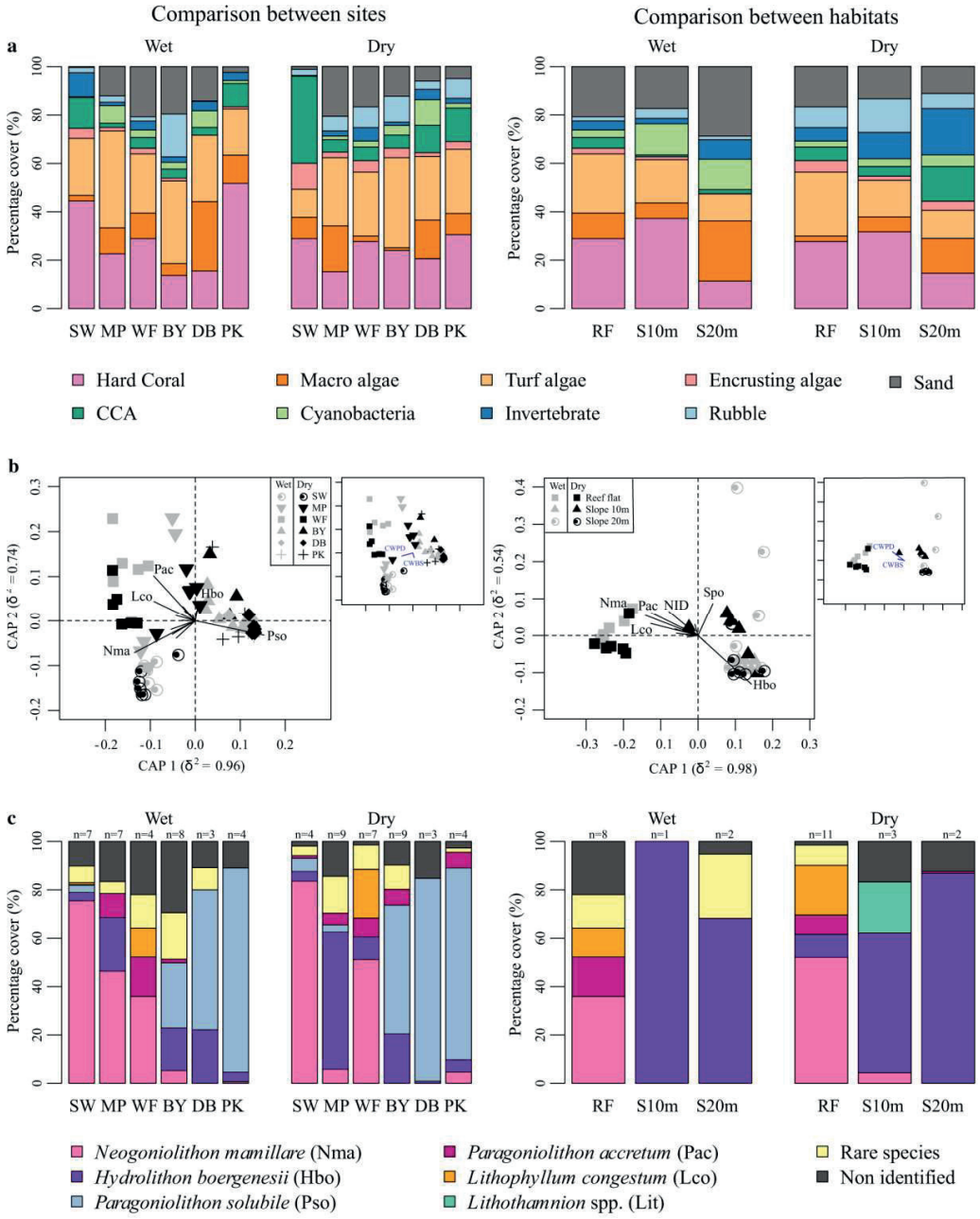


**Fig. 5** Benthos and CCA community composition at the six study sites (left) and at the three habitats (right) for the wet and dry time periods. **(a)** Percent cover of the main benthic category. **(b)** Canonical analysis of principal coordinates (CAP) for Sites and Habitats combining wet (grey symbols) and dry (black symbols) time periods. Vector overlays indicate Spearman rank correlations between the original variables and the canonical ordination axes. . The length of each vector is proportional to the strength of the correlation. The squared canonical correlation value for the first two ordination axes is shown in parenthesis. **(c)** Relative percent cover of the major CCA species. n = number of species present at each site/habitat. Rare species include: *Porolithon pachydermum, Neogoniolithon spp., Neogoniolithon munitum, Lithoporella atlantica, Sporolithon spp., Neogoniolithon affine, Titanoderma prototypum, Titanoderma spp*. See Fig. 3 for sites abbreviations. RF = Reef flat; S10/20m = Reef slope at 10 and 20m

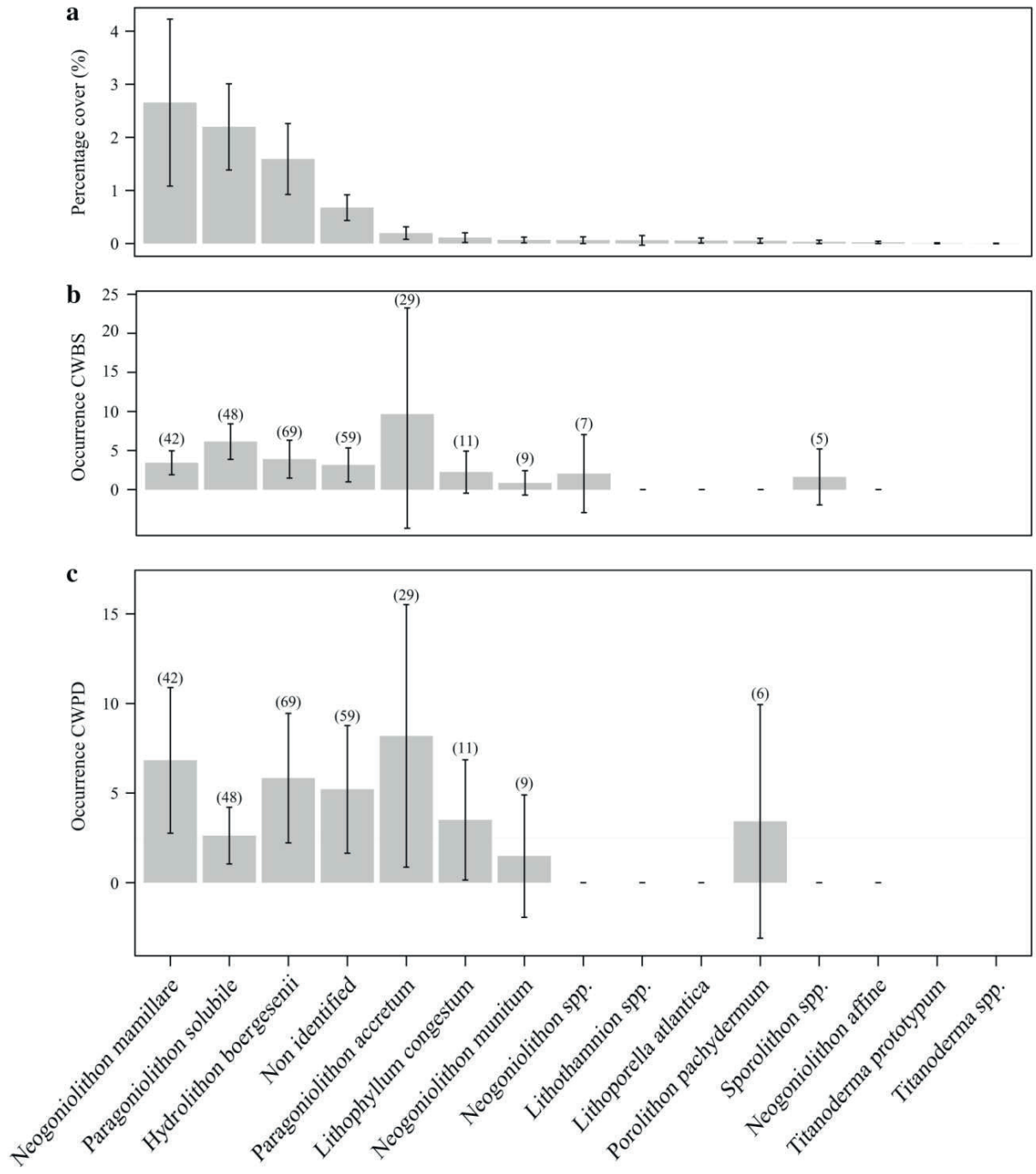


**Fig. 6 (a)** Absolute percent cover (n=96), **(b)** CWBS occurrence and **(c)** CWPD occurrence (number of lesions per $m^2$ CCA, mean ± 95% CI) of the different CCA species (or group of species from the same genera) pooling site, habitat and time period. Numbers in brackets above bars indicate number of observations (n) per species

## Progression rate of lesions

CWBS lesions were located either peripherally forming an arc of a circle spreading inwards, or centrally forming a full circle spreading outwards in a radiating pattern. However, the active progression was variable across the length of the lesion perimeter. Multiple rings may develop and merge resulting in irregular patterns. Despite simultaneous signs of regeneration and

degeneration, 90% of the patches affected by CWBS showed a decrease in healthy tissue area over time (Fig. 7a). We found no effect of time or site on CWBS lesion progression rate between the first and the last day of monitoring (Table S2a). Overall, CWBS linear rate of spread averaged 0.21 ± 0.06 cm.month$^{-1}$ (range: 0.15-0.36). Rate of lesion progression did not differ between *Neogoniolithon mamillare* and *Paragoniolithon solubile* (Table S2b).

CWPD lesions appeared as rather large, diffusive white patches also located both centrally and peripherally. Lesion area was highly variable ranging from 0.04 to 20.40 cm$^2$ on the first day of monitoring. Of the 25 lesions monitored for three weeks, only three resulted in higher tissue mortality at the end of the monitoring period. In those cases, lesion progression rate averaged 0.24 cm$^2$.month$^{-1}$ (range: 0.001-1.28). All the other lesions slowly recovered and two were completely sealed, replaced by healthy crust (Fig. 7b), suggesting that CWPD does not kill entire coralline crust.

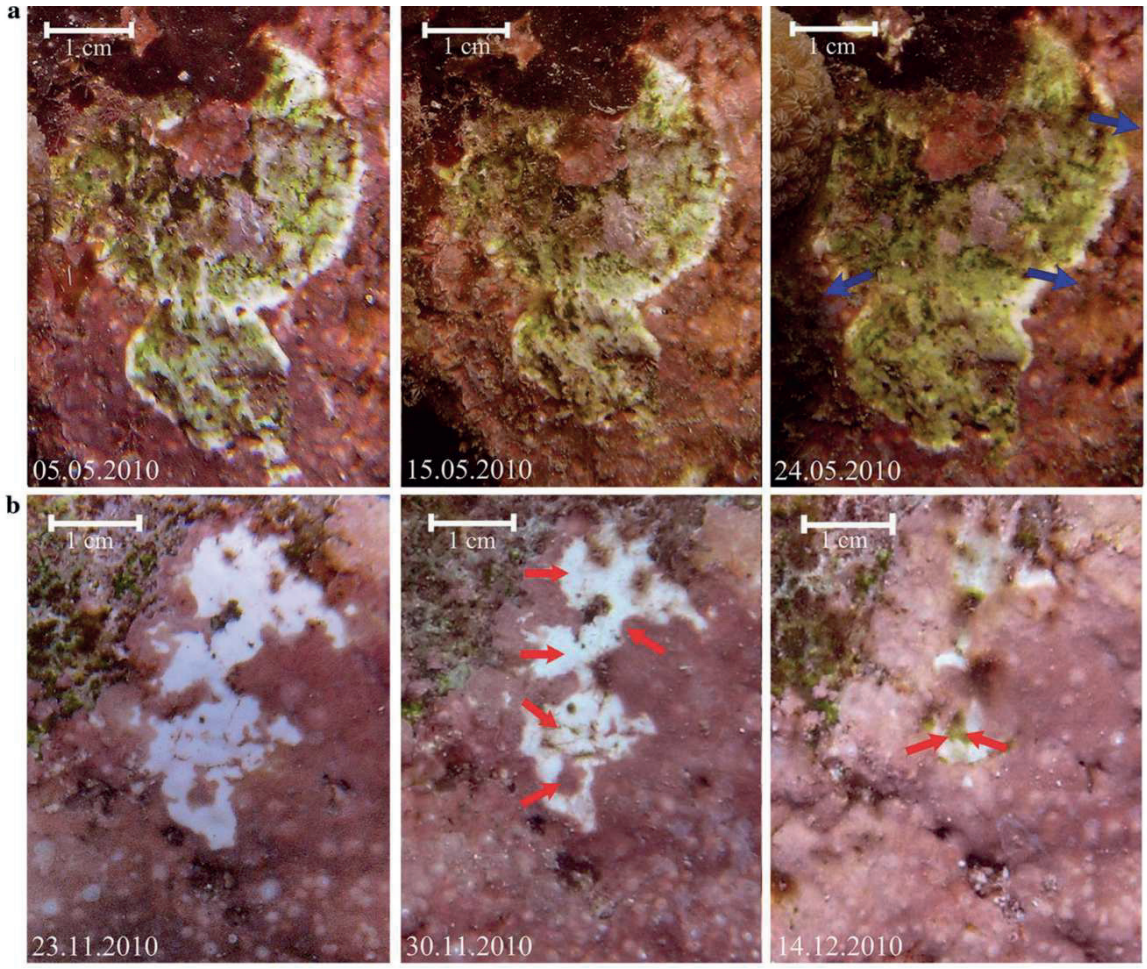


**Fig. 7** Time series images of **(a)** CWBS and **(b)** CWPD lesions on *Neogoniolithon mammilare* over three week intervals between November 2011 and May 2012. Blue arrows indicate active lesion spread. Red arrows indicate regeneration

# Discussion

A recent study looking at CCA diseases across coral reefs of the U.S Pacific enumerated five disease categories (Vargas-Ángel 2010). In contrast, we found only two diseases in Curaçao. This is few, especially that the Caribbean basin has been considered a disease "hot spot" since the late 1990s (Harvell et al. 1999; Haapkylä et al. 2010). However, the U.S.-affiliated Pacific Islands and Atolls surveyed in Vargas-Ángel (2010) represented a much wider area, and other studies conducted in the Pacific Ocean, but limited to smaller areas, also found a low diversity of CCA pathologies (Aeby et al. 2008; Tribollet et al. 2011). Populations of *Porolithon pachydermum* in the Atlantic were hit by white band disease and suffered major mortality in the Caribbean (Goreau et al. 1998). Surprisingly, we did not encounter any CWBS lesion on this particular species during our surveys, although this coralline was present at our study sites. White band symptoms are very similar to those of CLOD in the Pacific besides the fact that a white rim replaces the orange band, known to be a bacterial pathogen (Littler and Littler 1995). CLOD is abundant and widespread in the Pacific and has been reported in the Caribbean only recently on deep (20 m) CCA in Puerto Rico, the Cayman Islands and Mexico (Weil et al. 2009; Weil and Rogers 2011). In contrast, white band is now ubiquitous in the Caribbean (Goreau et al. 1998) and remains scarce in the Pacific Ocean. Why diseases spread in some regions of the world and not others remains unclear.

A major issue regarding the study of CCA disease is the lack of standardization in the calculation of CCA disease prevalence. This stems from the fact that CCA cannot be counted as individuals and CCA cover must be considered instead. Different studies have then used different terms in lieu of disease prevalence making comparisons between studies complicated. We used the same method and occurrence index formula as two previous studies in the Pacific (Aeby et al. 2008; Williams et al. 2014). Aeby et al. (2008) found CLOD as the most common disease in American Samoa, with a range of 0.01 to 0.37 lesions.$m^{-2}$ CCA. Williams et al. (2014) reported between 0.01 and 3.74 CFD lesions.$m^{-2}$ CCA at Palmyra Atoll, a remote toll in the central Pacific. These occurrences are lower than CWBS and CWPD occurrences in our study (CWBS: 0.6 to 10.9 lesions.$m^{-2}$ CCA; CWPD: 0.3 to 6.7 lesions.$m^{-2}$ CCA). Unlike these and our study, Vargas-Ángel (2010) studied CCA diseases on a much wider scale (i.e. between and within islands/atolls in the U.S Pacific Islands) and found a high spatial variability in disease occurrence. This suggests that Curaçao might in the end deserve the name of disease "hot spot" in terms of disease abundance rather than in terms of disease diversity, but studies conducted on a larger scale are needed to better characterize CCA disease levels in the Caribbean basin.

This study is the first to investigate CCA disease dynamics at the species level. There was no link between CCA community and disease occurrence. For instance, Spanish Water and Playa Kalki both had a high CWBS occurrence in the warm/rainy season, despite having distinct communities dominated by *Neogoniolithon mamillare* and *Paragoniolithon solubile,* respectively. A broad range of CCA species were affected by diseases and they all showed similar disease occurrence. This contrasts with the first observation of the disease in the Caribbean which indicated that 70 % of the *Paragoniolithon accretum* were affected by CWBS suggesting a higher vulnerability of this particular species (Ballantine et al. 2005). This species also found in our transects did not differ from other species in disease occurrence. Consistent with this finding, laboratory experiments have shown that, on 13 species of Corallinaceae tested, all could become infected with CLOD (Littler and Littler 1995). According to the disease-diversity hypothesis, the severity of host-specialist diseases can be higher in areas where species diversity is low (Aeby et al. 2011a). In our study, both diseases were clearly host-generalists. As a result, in the same reef flat habitat, sites with low diversity (i.e. Daaibooi and Playa Kalki) were not more affected than sites with high diversity (i.e. Spanish Water and Water Factory). Disease occurrence was also higher on the reef flat having high species diversity than on the deeper reef slope having low diversity. Environmental conditions such as light, UV and seawater temperature which are all higher at shallower depths might have compromised CCA fitness irrespective of the species present and favored CCA diseases in the reef flat habitat.

Disease distribution can be modulated by density or frequency dependent processes (Williams et al. 2010). We found a parabolic like relationship between CWBS occurrence and CCA cover. CWBS occurrence peaked at an intermediate level of cover which could represent favorable conditions for the disease to spread. In contrast, CWPD showed a declining trend with cover. However, considering the level of error indicated by the confidence intervals and the fact that the highest density of observations were between 0 to 7.5% CCA host cover, with only three data points above 10 %, these results should be taken with caution. The study of Williams et al. (2014) did not find any relationship between CCA cover and CFD occurrence within Palmyra Atoll's forereef habitat. This is inconsistent with the positive relationship between CCA cover and overall disease occurrence found by Vargas-Ángel (2010) at the scale of archipelago in the US Pacific. In the latter, the range of CCA cover (0-45.8%) was larger than the one reported by Williams et al. (2014) (19-39%). Together these results support that the relationship between host density and disease occurrence is complex and may vary with scale.

The results presented here did not reveal any clear spatial relationship between disease occurrence and human influence. Human-induced environmental factors can take the form of

overfishing, coastal development and coastal pollution leading to chronic pollution through terrestrial runoff or sewage pills and recreational activities, all having the potential to affect marine disease dynamics (Harvell et al. 1999; Aeby et al. 2011b). CCA diseases were present at all sites including Spanish Water, Daaibooi and Playa Kalki, which had a lower level of human influence than the three other sites (Barott et al. 2012). Sites located close from the main source of pollution in Curaçao (i.e. Willemstad) did not show a higher level of disease occurrence. These results corroborate those of the Pacific which found no relationship between CCA disease occurrence and human population density and reported high levels of disease in some remote uninhabited atolls (Vargas-Ángel 2010; Williams et al. 2014). However, Vargas-Ángel (2010) found a positive correlation between disease occurrence and human population density when considering only islands with diseases, suggesting that the impact of diseases may be worsened at sites more exposed to human disturbances. The absence of clear relationship between disease occurrence and human influence in our study could also come from the fact that all sites may have already reached a "threshold" level of human disturbances even those thought to be less impacted.

Disease occurrence was found to be higher during the warm/rainy season than during the cold/dry season. The same pattern occurred on the reef flat and on the reef slope down to 20 m depth. During the rainy season (October - January) on Curaçao, seawater temperatures down to 30 m commonly increase by 2 to 3 degrees (Bak et al. 2005) and precipitations are three to eight times higher than during the dry season (February - May) (Martis et al. 2002). Both factors could explain the increase in disease during the warm/rainy season. Rainfall and associated runoff may trigger coral disease outbreaks by increasing the availability of nutrients and organic matter, which in turn increase pathogen virulence and reduce host fitness (Bruno et al. 2003; Haapkylä et al. 2011). In 2010, rainfall during the warm/rainy season on Curaçao were particularly intense with an average of 207 mm of precipitation between September and December compared to 129 mm in 2008 or 70 mm in 2012 (mundomanz.com). Seawater temperature in 2010 also rose to record levels on the island, causing one of the worst coral bleaching event on Curaçao (Vermeij 2012). The positive relationship between SSTs and disease abundance, already known for corals (Harvell et al. 1999; Bruno et al. 2007; Haapkylä et al. 2011; Ruiz-Moreno et al. 2012), has been recently confirmed in CCA (Vargas-Ángel 2010; Williams et al. 2014) and is consistent with our study. Algal necroses similar to CWPD have been found under elevated temperature in aquaria (Martin and Gattuso 2009). Also in the laboratory, thermal stress has been shown to cause bleaching in both corals and CCA (Anthony et al. 2008). Since bleaching can make corals more susceptible to disease (Brandt and McManus 2009), the same may happen for CCA.

Due to the intricate nature of host-environment-pathogen interactions, CCA diseases, like most diseases, are likely to be the results of complex interactive effects among multiple environmental variables (Williams et al. 2010, 2014). For example, Williams et al. (2014) demonstrated a positive effect of temperature on CFD occurrence and a negative effect of acidification on lesion expansion rates. However, the authors suggested that lowered pH may still reduce survivorship by reducing calcification and increasing fungal bio-erosion. Several studies have demonstrated synergistic interactions between ocean warming and ocean acidification on CCA, including reduced calcification rates and increased frequency of necroses under both conditions (Anthony et al. 2008; Martin and Gattuso 2009; Johnson and Carpenter 2012). These observations make it clear that intricate relationships exist among global factors, but also probably with local stressors, that need to be incorporated when trying to understand spatio-temporal disease dynamics.

Unlike disease occurrence, lesion progression rates did not vary in time. This is inconsistent with the study of Williams et al. (2014) which found higher CFD lesion progression rates with elevated temperature under experimental conditions. The only available data on the CWBS rate of advance is provided by The Coral Reef Targeted Research & Capacity Building for Management (0.2-0.7 cm.month$^{-1}$) and is the same order of magnitude as our result (0.15-0.36 cm.month$^{-1}$). Linear progression rates reported for CLOD (1.5 ± 0.1 mm.day$^{-1}$, i.e. 45 cm.month$^{-1}$) (Littler and Littler 1995) and CFD (2.4 ± 0.5 mm.day$^{-1}$, i.e. 72 cm.month$^{-1}$) (Williams et al. 2014), were higher. However, no *in situ* data on the temporal variations of CLOD or CFD progression rate are available. This highlights the lack of study on CCA diseases. The macroscopic symptoms of CWBS observed in the field were consistent with those seen elsewhere in the Indo-Pacific region and in the Caribbean (Ballantine et al. 2005). Additionally, the similarity between the rate of advance for CWBS measured in this study and the previously reported rate, left little doubt on the characterization of CWBS. CWPD has never been reported before *in situ*. The same way CLOD has not been observed in the Caribbean until recently, CWPD may be a new syndrome yet still limited in space. Alternatively, CWPD might have been classified as CWBS in earlier studies. Several coral diseases can present almost similar gross symptoms, but in fact be distinct at the cellular level, highlighting the importance of histopathology. In the Institute of Marine Sciences (AIMS) long-term monitoring program, coral diseases producing white symptoms were grouped under the collective name "White Syndrome" even though they may be distinct pathologies (Willis et al. 2004). However, such grouping may lead to wrong conclusions when predicting disease prevalence as it may obscure interactions with environmental factors, especially if the grouped diseases have unknown etiologies and different environmental responses (Williams et al.

2010). In our study, both CWBS and CWPD presented different gross symptoms, spatio-temporal variations and spread of lesions. This suggests that they might be different diseases, but further investigations at the cellular level using histopathology and genetic analyses are needed to provide definite evidence.

The role played by CCA in reef ecosystems has been undervalued (Littler 1972; Adey and Macintyre 1973; Vermeij et al. 2011) and the decline or change in CCA communities could thus have important ecological consequences, especially for coral recruitment. The results from this study suggest that all CCA species are potentially vulnerable. Our study generates a first step in the establishment of a current baseline on the level of CCA diseases for Curaçao, which appears crucial to assess how diseases threaten coral reef integrity in a changing marine environment (Miller et al. 2013). Further research is needed to quantify the impact of these diseases on CCA population dynamics and to identify the complex, interactive effects between multiple factors driving their spatial and temporal variations.

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# Supplementary material

**Table S1** Pairwise *a posteriori* comparisons of CWBS occurrence levels (**a)** Tests among levels of the factor Site and (**b)** Tests among levels of the factor Habitat. Significant P values are shown in bold

| Groups | Unique values | t | P(MC) |
|---|---|---|---|
| **a.** | | | |
| (WF, BY) | 3 | 1.9401 | 0.1790 |
| (WF, MP) | 3 | 0.8401 | 0.4940 |
| (WF, DB) | 3 | 2.1211 | 0.1860 |
| (WF, SW) | 3 | 10.8939 | **0.0060** |
| (WF, PK) | 3 | 12.2514 | **0.0060** |
| (BY, MP) | 3 | 2.4169 | 0.1410 |
| (BY, DB) | 3 | 3.1890 | 0.0850 |
| (BY, SW) | 3 | 3.5972 | 0.0620 |
| (BY, PK) | 3 | 4.8637 | **0.0390** |
| (MP, DB) | 3 | 1.5008 | 0.2900 |
| (MP, SW) | 3 | 10.5321 | **0.0060** |
| (MP, PK) | 3 | 11.8867 | **0.0090** |
| (DB, SW) | 3 | 8.0200 | **0.0160** |
| (DB, PK) | 3 | 9.1567 | **0.0100** |
| (SW, PK) | 3 | 3.4333 | 0.0630 |
| **b.** | | | |
| (Reef flat, Slope 10m) | 3 | 1.1079 | 0.359 |
| (Reef flat, Slope 20m) | 3 | 6.9391 | **0.029** |
| (Slope 10m, Slope 20m) | 3 | 3.8927 | 0.076 |

**Table S2** Permutational multivariate analysis of variance (PERMANOVA) testing for **(a)** the effects of site and time and **(b)** the effects of species and time on CWBS lesion progression rate

| Source | df | SS | MS | F | P(perm) |
|---|---|---|---|---|---|
| **a.** | | | | | |
| Site | 4 | 4.45E-02 | 1.11E-02 | 0.21814 | 0.935 |
| Time | 1 | 9.54E-03 | 9.54E-03 | 0.18696 | 0.647 |
| Site*Time | 4 | 6.70E-02 | 1.68E-02 | 0.32829 | 0.867 |
| Residual | 20 | 1.02E+00 | 5.10E-02 | | |
| Total | 29 | 1.14E+00 | | | |
| **b.** | | | | | |
| Species | 1 | 6.55E-03 | 6.55E-03 | 0.26955 | 0.604 |
| Time | 1 | 3.99E-03 | 3.99E-03 | 0.16409 | 0.693 |
| Species*Time | 1 | 5.72E-03 | 5.72E-03 | 0.23518 | 0.607 |
| Residual | 11 | 2.67E-01 | 2.43E-02 | | |
| Total | 14 | 2.85E-01 | | | |

# PUBLICATION II

## Histopathology of crustose coralline algae affected by white band and white patch diseases

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# Histopathology of crustose coralline algae affected by white band and white patch diseases

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## Abstract

**Crustose coralline algae (CCA) are major benthic calcifiers that play crucial roles in marine ecosystems, particularly coral reefs. Over the past two decades, epizootics have been reported for several CCA species on coral reefs worldwide. However, their causes remain often unknown in part because few studies have investigated CCA pathologies at a microscopic scale. We studied the cellular changes associated with two syndromes: Coralline White Band Syndrome (CWBS) and Coralline White Patch Disease (CWPD) from samples collected in Curaçao. Healthy-looking tissue of diseased CCA did not differ from healthy tissue of healthy CCA. In diseased tissues of both pathologies, the three characteristic cell layers of CCA revealed cells completely depleted of protoplasmic content, but presenting an intact cell wall. In addition, CWBS showed a transition area between healthy and diseased tissues consisting of cells partially deprived of protoplasmic material, most likely corresponding to the white band characterizing the disease at the macroscopic level. This transition area was absent in CWPD. Regrowth at the lesion boundary were sometimes observed in both syndromes. Tissues of both healthy and diseased CCA were colonised by diverse boring organisms. Bacterial and fungal infections associated with the diseased cells were not seen. However, other bioeroders were more abundant in diseased vs healthy CCA and in diseased vs healthy-looking tissues of diseased CCA. Although their role in the pathogenesis is unclear, this suggests that disease increases CCA susceptibility to**

**bioerosion. Further investigations using an integrated approach are needed to carry out the complete diagnosis of these diseases.**

**Keywords:** Disease, histopathology, crustose coralline algae, cell death, boring fauna, regeneration, lesion

# Introduction

Scientific awareness that marine disease represent a major threat to coral reefs has led to the multiplication of disease investigations over the past three decades (Weil 2001, Harvell et al. 2007, Pollock et al. 2011), principally through field monitoring surveys (Gladfelter 1982, Kuta & Richardson 1996, Hayes & Goreau 1998, Nugues 2002, Willis et al. 2004, Aeby et al. 2008, Weil et al. 2009, Haapkylä et al. 2010, Tribollet et al. 2011). Even though the documentation of disease signs *in situ* and the identification of drivers influencing disease dynamics are essential, they do not allow to elucidate the etiology of a disease. Histopathology is a vital step in any effective coral reef disease survey (Work & Meteyer 2014). It provides insight into cell pathology and host response to help resolving the question of disease causation (Work et al. 2014). It can detect etiological microorganisms and propose potential causative agents by their observation *in situ*. Furthermore, it provides a great amount of information on the cell and tissue damages associated with gross lesions (Peters et al. 1984, Ainsworth et al. 2007a, Burns & Takabayashi 2011, Williams et al. 2011, Sudek et al. 2012). Sometimes, even in the absence of pathogens, changes in the host tissue histology hint at the type of infection and lead to a diagnostic (Gupta et al. 2009). It is therefore the only current diagnostic tool that allows establishing a link between the potential causative agent and the specific changes in cell and tissue (Work & Meteyer 2014). For instance, histology has confirmed the presence of a fungal infection in the Coralline Fungal disease (CFD) (Williams et al. 2014). However, an integrated approach (i.e. combining microbiological, microsensor, molecular and physiological techniques) is necessary in order to incriminate infectious agents as disease causation and thus complete the diagnostic picture (Richardson et al. 2001, Work & Meteyer 2014).

Unfortunately, investigations at the cellular level are seriously lacking in diseases affecting crustose coralline algae (CCA) despite the importance of these calcifying algae in marine ecosystems, especially coral reefs. Along with scleractinian corals, CCA are important primary producers (Adey & Macintyre 1973, Chisholm 2003) and framework builders (Adey & Vassar 1975) delivering important functional services in coral reef ecosystems, including enhancing

coral larval settlement (Morse et al. 1988, Heyward & Negri 1999, Harrington et al. 2004, Ritson-Williams et al. 2010, 2014). CCA are not spared by the increasing intensity and severity of marine diseases (Littler & Littler 1995, Hayes & Goreau 1998) and field investigations on CCA diseases have multiplied in recent years (Aeby et al. 2008, Vargas-Ángel 2010, Tribollet et al. 2011, Miller et al. 2013, Quéré et al. 2014). At present, six disease categories have been reported (Vargas-Ángel 2010, Quéré et al. 2014, Williams et al. 2014), but only CFD and coralline lethal orange disease (CLOD) have known causation. Virtually nothing is known about the other CCA disease categories and they remain histologically uncharacterized. Further knowledge on the etiology of the remaining categories could be gained from studies at the tissue and cellular levels.

In Curaçao, CCA species are affected by the Coralline White Band Syndrome (CWBD) and the Coralline White Patch Disease (CWPD) (Quéré et al. 2014). CWBS lesions are defined by a white-band that appears centrally or peripherally and advances on the healthy tissue, while CWPD manifests by the presence of distinct white patches on the healthy crust (Fig. 1a & b). Both diseases result in tissue loss with subsequent colonization by endophytic algae often leading to the death of the diseased patch in the case of CWBS (Quéré et al. 2014). The visible symptoms may have a biotic or abiotic origin. On one hand, thermal stress has been shown to cause bleaching in both corals and CCA in the laboratory (Anthony et al. 2008) and algal necroses appear on CCA crust under elevated temperature in aquaria (Martin & Gattuso 2009). On the other hand, bacterial pathogens can also cause bleaching disease in the marine red algae *Delisea pulchra* (Fernandes et al. 2011). Gross symptoms in the shape of rings are known to be caused by a bacterial infection in the case of CLOD (Littler & Littler 1995) and by fungi in the case of CFD (Williams et al. 2014). The aim of this study was to characterize both CWBS and CWPD at the microscopic level to complete the diagnostic picture and increase our understanding of these two diseases and their effects on coralline tissues.

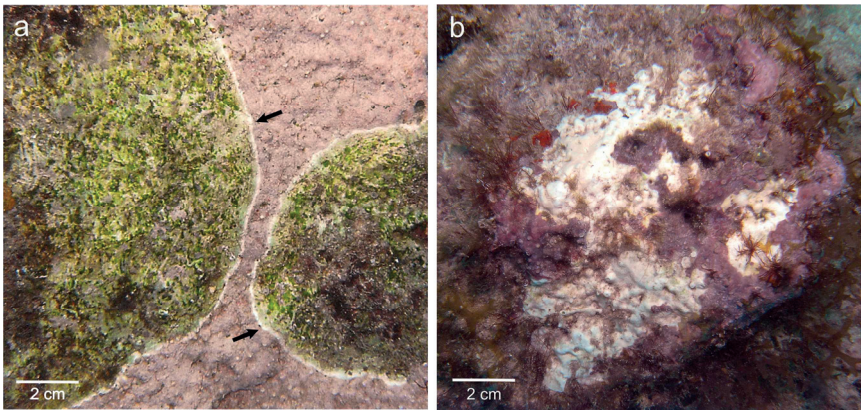


**Fig. 1** Gross lesions of **(a)** CWBS in *Paragoniolithon solubile* and **(b)** CWPD in *Hydrolithon boergesenii* from Curaçao in 2012. Black arrow shows the white band in CWBS.

# Materials and Methods

### Field collection

Crustose coralline algae were sampled in May 2012 at two sites along the leeward coast of Curaçao, Southern Caribbean (12°N, 69°W). Fragments (ca. 10-20 $cm^2$) from four CCA species were collected using hammer and chisel on the reef terrace at 5-10 m depth at two reef sites: *Hydrolithon boergesenii, Neogoniolithon mamillare* and *Paragoniolithon accretum* at Water Factory (12°06'32"N, 68°57'14"W) and *Paragoniolithon solubile* at Playa Kalki (12°22'30"N, 69°09'31"W). Sampling was not targeted towards particular species, but we sought to have an approximately equal number of healthy and diseased samples. A total of 23 fragments, including 7 healthy fragments, 8 fragments affected by CWBS and 8 fragments affected by CWPD, were sampled (Table 1). For each diseased fragment collected, we made sure to incorporate healthy-looking tissue. Each replicate was selected from a distinct patch. Healthy and diseased fragments of each disease were placed in separated collecting bags to avoid contamination and transported in the dark to the laboratory.

**Table 1**. Number of healthy and diseased fragments collected from each species

| | Healthy | CWBS | CWPD | Total |
|---|---|---|---|---|
| *Hydrolithon boergesenii* | 3 | 1 | 5 | 9 |
| *Neogoniolithon mamillare* | 2 | 4 | 3 | 9 |
| *Paragoniolithon solubile* | 1 | 3 | 0 | 4 |
| *Paragoniolithon accretum* | 1 | 0 | 0 | 1 |
| Total | 7 | 8 | 8 | 23 |

### Histology

Back in the laboratory, a sample (ca. 2-4 $cm^2$) of each fragment was kept for taxonomic identification. The pieces used for taxonomic determination were rinsed with freshwater and dried for six hours in the oven at 60°C before being checked under a dissecting scope for reproductive and morphological features (Steneck 1986). The rest was fixed in 4% Formalin-seawater solution and stored in the fridge until further use. Before decalcification, a small piece (ca. 1 $cm^2$) was cut from each fragment so that only the crust of the CCA and a thin (ca. 5 mm) layer of limestone underneath remained. All visible epibionts present on the surface of the coralline algae were removed. In the case of diseased fragments, each piece was chipped so that it included the boundary between healthy and disease tissues.

Each sample was then placed in an individual container with 5 % L-ascorbic acid solution to gently decalcify over a period up to one week. The solution within each container was refreshed every two days. Once the skeleton and limestone were dissolved, the tissue samples were placed in individual embedding cassettes and dehydrated at room temperature in ascending grades of ethanol (70°, 80°, 95°, 100°, 100°) for 40 minutes each, followed by an immersion in limonene (three baths of 40 minutes each). Samples that could not be processed immediately were stored in 70° ethanol. CCA tissue was then placed in three successive baths of paraffin (Paraplast® Plus™) each time 40 minutes before being embedded into paraffin blocks. Samples were orientated so that transverse sectioning was possible. The blocks were stored overnight at 4°C in the fridge to ease withdrawal from the cassette the following day. The blocks were sectioned (section thickness 5 and 7 μm) using low profile microtome blades (Leica DB80 LX) mounted on a calibrated rotary microtome (LEICA™ RM2245; Leica Mikrosystems GmbH, Wetzlar, Germany). Sections were floated onto water (20°C), mounted onto clean slides and dehydrated on a slide drying bench for minimum 40 minutes at 50°C.

Sections were then rehydrated and stained following the Sharman staining series (Sharman 1943) modified from Ruzin (1999). This method stains the cell walls of plant tissue in tannic acid and iron alum after the protoplasts have been stained in safranin and orange G (see Appendix 1 for detailed staining procedure). Sections were then dehydrated in successive baths of ethanol (45°, 90° and 100°) and cleared with limonene. Coverslips were finally mounted using adhesive resin. We examined and photographed 10 permanent histology sections of each CCA fragment using light microscopy (Leica DM750) with integrated camera (Leica ICC50 HD) using the Leica LAS EZ software.

**Analyses**

Host response was described at the microscopic level and interpreted by comparing normal healthy fragments paired with diseased ones. The presence of invading organisms, their type and localization within the tissue were recorded. In each fragment, organisms could be present in the CCA crust (*i.e.* epithallus, perithallus and/or hypothallus) or in the limestone underneath the crust. In addition, in diseased fragments, we noted whether they were located in the healthy-looking and/or diseased tissues of the fragments. The identification of the invading organisms was beyond the scope of this study and was restricted to two boring categories: macroborers (*i.e.* boring sponges, helminths and others) and microborers (*i.e.* cyanobacteria). All results are reported for pooled species of CCA as the number of replicates per species was too low to make robust comparisons between species, but species-specific data are listed in Table S1.

# Results

For both diseases, we observed no difference in cell structure and organization between healthy tissue of healthy CCA and healthy-looking tissue of diseased CCA. Cell walls and contents in healthy-looking tissue of diseased CCA were intact without any apparent damage (Figs. 2b & 3b). In contrast, the diseased part of the tissue showed distinct histological changes between diseases. Cells affected by both CWBS and CWPD presented no apparent damage of their cell walls, but showed a complete depletion of their protoplasmic content (Figs. 2d & 3d). However, in all cases of CWBS, we observed a transition area between healthy and dead cells consisting of cells that were partially deprived of protoplasmic content (Fig.2c).

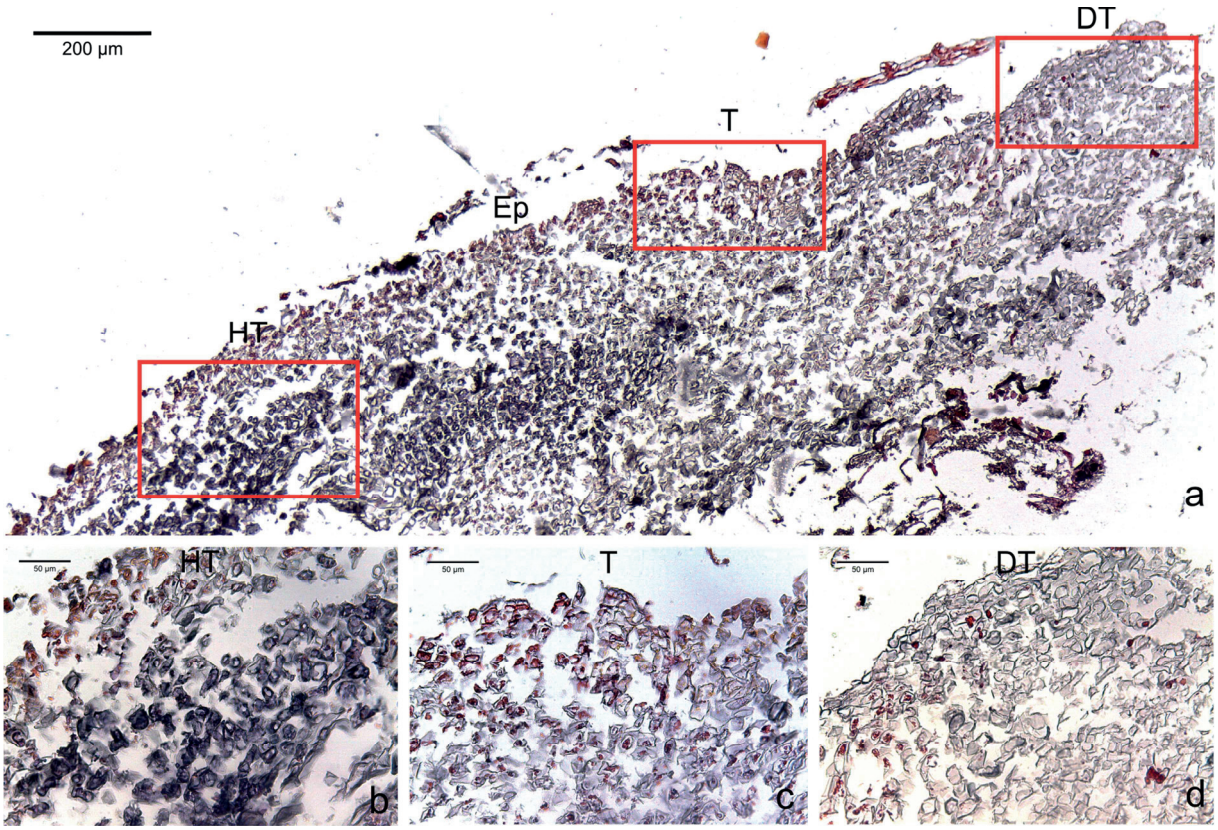


**Fig. 2** Cross-section of *Paragoniolithon solubile* affected by CWBS. **(a)** overview with locations of the healthy, transition and dead areas enlarged in **(b)**, **(c)** and **(d)**. Note the progressive loss of staining from left (healthy) to right (dead). HT, Healthy Tissue. T, Transition. DT, Diseased Tissue. Ep, epithallial cells.

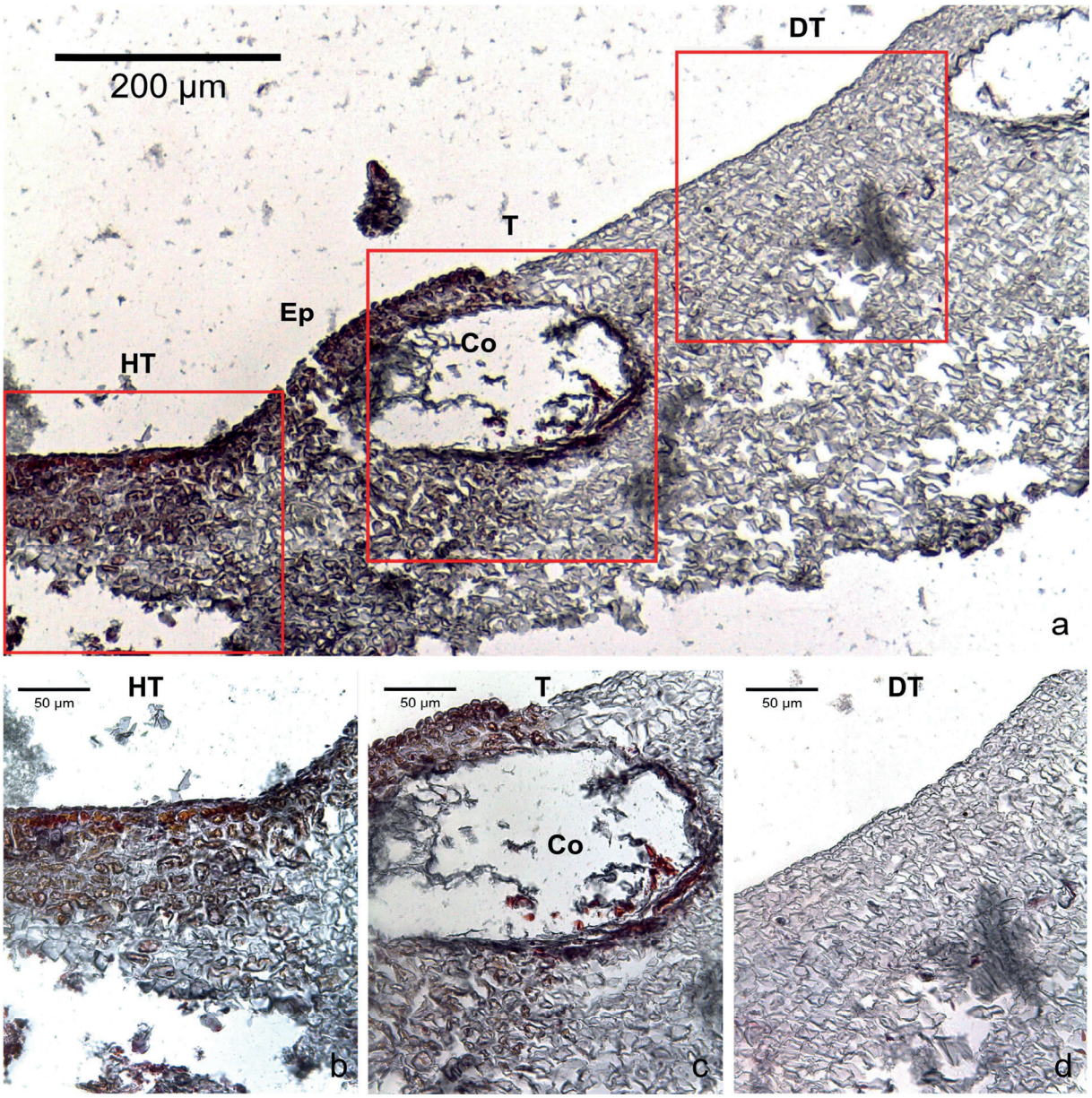


**Fig. 3** Cross-section of *Hydrolithon boergesenii* affected by CWPD. **(a)** overview with locations of the healthy and dead areas enlarged in **(b)** and **(d)**. **(c)** shows the boundary between healthy and dead areas. Note the absence of a transition area highlighted by sudden loss of staining. HT, Healthy Tissue. T, Transition. DT, Diseased Tissue. Co, conceptacle. Ep, epithallial cells.

Cells containing what appeared to be a condensed nucleus or balled up cytoplasmic materials were also frequently observed (Fig. 4a *insert*). This transition area most likely corresponds to the white band in the gross morphology (Fig. 1a). It did not exist in CWPD tissue where healthy-looking cells were in immediate vicinity of empty dead cells (Fig. 3c). In 2 cases of CWBS and 1 case of CWPD, we observed an overgrowth of the diseased/dead surface by the healthy crust, suggesting tissue recovery (Fig. 4a & b).

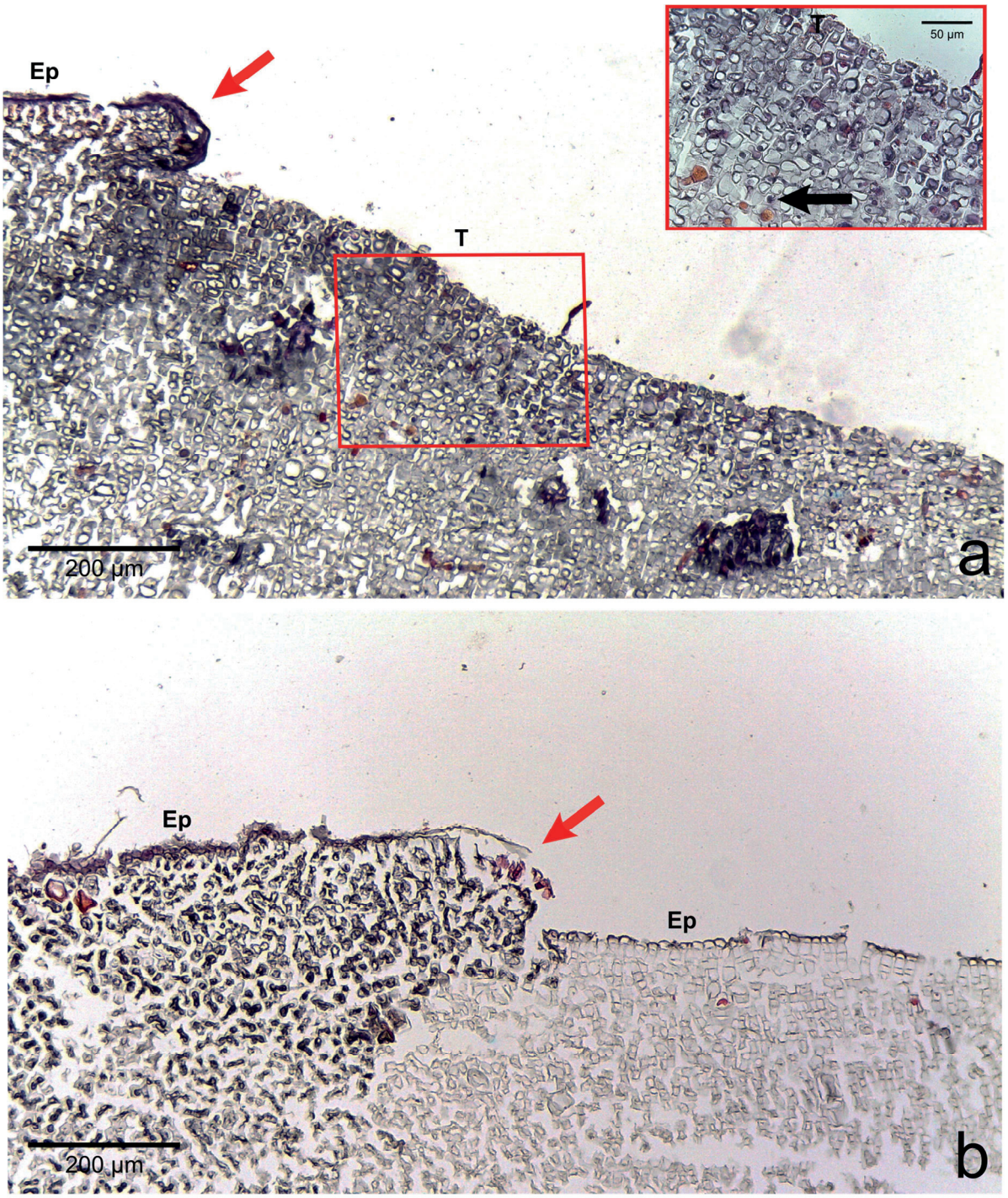


**Fig. 4** Regrowth of living crust in **(a)** CWBS and **(b)** CWPD. Remnant healthy crust (red arrows) regrew upward and laterally over dead/dying crust. *Insert* in **(a)** displays enlargement of transition area with cells showing a condensed nucleus or protoplasmic content (black arrows). T, Transition. Ep, epithallial cells.

Various macroborers and microborers were observed in the tissues (Fig. 5). They were more abundant in diseased fragments, particularly in CWBS. Of the 7 healthy fragments examined, 4 (57 %) had invading macro- and microorganisms versus all of 8 CWBS fragments and 5 (63 %) of the 8 CWPD fragments (Table 2). Of 13 diseased fragments with evidence of boring organisms, sponges were most common (62 %) followed by other macroborers (38 %) and cyanobacteria (31 %). Of the four healthy fragments with borers, 3 had sponges and two had other macroborers and cyanobacteria were not encountered. Within diseased fragments, borers were also more abundant in the diseased tissue of the fragments. Of 13 diseased fragments, 5 (38 %) presented borers in their healthy-looking tissue, whereas 12 (92 %) showed intrusion by borers in their diseased tissue (Table S1). However, boring organisms were rarely present within or in the immediate vicinity of diseased cells. Boring organisms were more abundant in the underlying limestone than in the CCA crust. In CWPD, borers were found exclusively in the limestone of all 5 diseased fragments containing borers. Cyanobacteria were never seen in the CCA crust. We did not visualize any bacterial and fungal infections associated with the diseased cells.

**Table 2**. Number of samples with boring organisms partitioned by health status of CCA fragments (i.e. healthy, CWBS vs. CWPD), health of tissue within fragment (i.e. healthy vs. diseased) and vertical layer within fragment (i.e. CCA crust vs. limestone). HT, healthy tissue. DT, diseased tissue. C, crust. L, limestone. T, total fragment. Note that the numbers can add up more than for the total fragments since the same fragment may have borers in different sections of the sample.

| Health of fragment | Healthy | | | CWBS | | | | | CWPD | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Health of tissue | HT | | | HT | | DT | | | HT | | DT | | |
| Vertical layer | C | L | T | C | L | C | L | T | C | L | C | L | T |
| Number of samples | | | 7 | | | | | 8 | | | | | 8 |
| Samples with borers | 1 | 3 | 4 | 2 | 4 | 2 | 6 | 8 | | 1 | | 5 | 5 |
| Samples with sponges | | 3 | 3 | 2 | 2 | 3 | 4 | 5 | | 1 | | 5 | 5 |
| Samples with helminths | | | | | | | 1 | 1 | | | | | |
| Samples with other macroborers | 1 | 1 | 2 | 2 | 3 | 1 | 3 | 4 | | | | | |
| Samples with cyanobacteria | | | | | 2 | | 2 | 3 | | | | 1 | 1 |

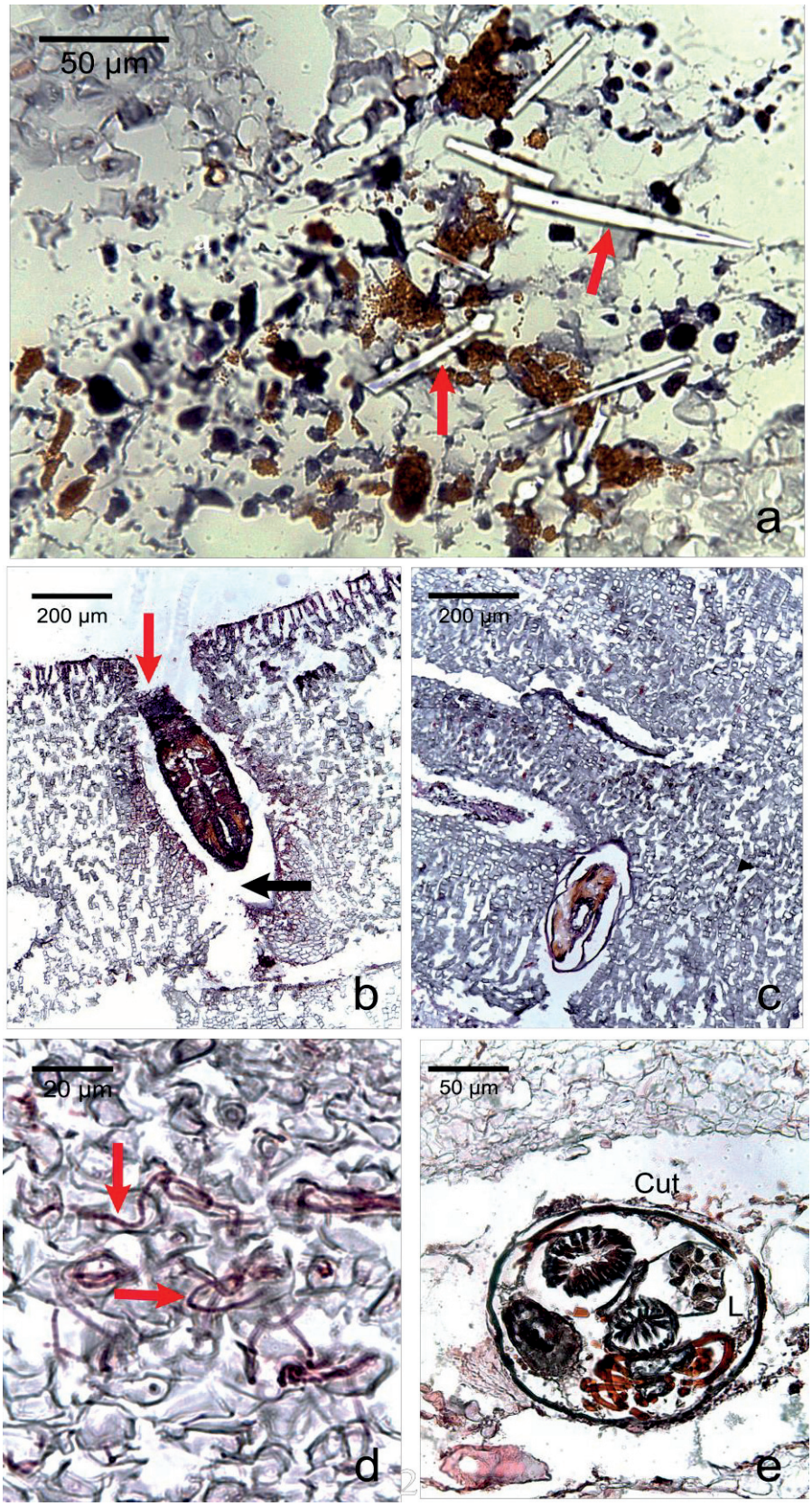


**Fig. 5** Photomicrographs of the most commonly encountered organisms in healthy and diseased CCA. **(a)** boring sponge characterized by siliceous spicules (red arrow) **(b)** Unidentified macroborer. Note the CCA cells lining up the burrow suggesting the growth of the algae around the invader (red arrow) and the acellular space around the organism (black arrow). **(c)** Unidentified macroborer, possibly a juvenile bivalve. **(d)** Cyanobacterial trichomes (red arrows). **(e)** Helminth. Cu, cuticule. L, lumen.

# Discussion

This is the first study providing histological information on CWBS and CWPD. We found no difference between healthy tissues of healthy and diseased crusts, which suggests that the action of the disease is localized, at least at the cellular scale. However, variations could occur at a smaller scale. For example, distinct differences in bacterial community between non-diseased corals and healthy-looking tissues of colonies affected by white band disease have been highlighted in *Orbicella annularis* using molecular techniques (Pantos et al. 2003). In tissue affected by both diseases, the three distinct cell layers characteristic of CCA (epithallus, perithallus and hypothallus) showed cells with an intact cell wall, but depleted from all cytoplasmic content as highlighted by a sudden change in the intensity of the staining. A plausible explanation to cell bleaching is the loss of pigments as already known in corals during bleaching events (Kleppel et al. 1989). CCA contain phycobilins (phycoerythrin and phycocyanin) pigments that are present in living tissue. Their loss could be followed by tissue necrosis and death (Fernandes et al. 2011).

In CWPD, healthy cells were in immediate vicinity of diseased empty cells whereas in CWBS, a transition area existed where cells had less protoplasmic content than healthy cells as highlighted by a weak silver stain within the cell walls. This transition area could reflect the slow but steady rates of CWBS progression on healthy tissue (*i.e.* $0.21 \pm 0.06$ cm month$^{-1}$ in Quéré et al. 2014). In contrast, CWPD generally manifests by a sudden and extensive loss of tissue (Quéré et al. 2014).

Different terms can be used to describe the causes of cell death (Franklin et al. 2006). Among them, cell necrosis and programmed cell death have been reported in various organisms (Berges & Falkowski 1998, Work & Aeby 2011, Dunn et al. 2012). The first phenomena is triggered by external factors, while the second also called apoptosis is triggered by intracellular signals activating specific gene expression (Greenberg 1997, Dunn et al. 2012). Both types have been highlighted during bleaching in the sea anemone *Aiptasia* sp. However, we did not observe the specific characteristics of apoptosis or necrosis such as cell shrinkage or compartmentalization of cell contents. In the transition area, the sudden high visibility of the nuclei or rounded cytoplasmic content could be related to the condensation of the nucleus during apoptosis. Similar cellular degradation has been observed in Acroporid corals affected by white syndromes (Ainsworth et al. 2007b). However, several other features that characterize apoptosis were not present in this study (e.g. DNA fragmentation, compartmentalization of cell contents into apoptotic bodies, cell shrinkage) (Franklin et al. 2006). Overall the aspect of the cells in our

study seems more likely to correspond to cell lysis, characterized by the rupture of the cell membrane and the dissolution of cell contents (Franklin et al. 2006, Ainsworth et al. 2007).

Interestingly, we observed regrowth of healthy-looking tissue over diseased tissue in both diseases. In reef-building corals, an immune response and repair mechanism consisting of a locally accelerated growth has been shown in wounded colonies (D'Angelo et al. 2012). We could interpret this regrowth as a response of the remaining healthy tissue to counteract the progression of the lesion like a wound healing response in CCA. Similarly, CCA are capable of healing wounds caused by herbivores grazing on their crust by regeneration of perithallial cells within the thallus (Steneck 1983). In several cases, we also observed CCA apparently growing around invading organisms (Fig. 5b).

We found various organisms associated with CCA tissue that are similar to the types of organisms reported in coral disease studies (Work & Aeby 2011, Séré et al. 2013) and in live and dead coralline thalli (Tribollet & Payri 2001). These organisms were more abundant in diseased than healthy CCA fragments, and, within diseased fragments, they were more abundant in diseased vs healthy tissue. It is not easy to determine whether invading organisms are the cause of the cell death or opportunistic secondary colonizers. Weakened or damaged coralline may have facilitated invasion by borers. The space around the different invaders was acellular. The presence of an empty cavity around them could be due to a digestive effect of the borer on the surrounding CCA cells creating a dead zone around them. The mechanical (chip production) and chemical (dissolution) bioerosion of calcium carbonate by boring sponges or bivalves has been reported (Lazar & Loya 1991, Zundelevich et al. 2007). Alternatively, although less likely, they could have taken advantage of an existing lesion to invade the CCA.

Among the organisms observed here, several have been identified as pathogenic in other species. This is the case for helminths known to cause tissue loss in *Montipora* (Jokiel & Townsley 1974) or cyanobacteria which appeared to cause tissue lysis and necrosis in black band diseased corals (Ainsworth et al. 2007a). Ciliates are also frequently associated with diseases and capable of invading animal and plant tissue by breaking cell membranes and walls using enzymes such as proteases (Work & Aeby 2011). In our observations, boring organisms did not seem to be associated with evident cell pathology. However, their presence reveals a weakening of the skeleton. Microborers are well-known agents of bioerosion in live and dead CCA thalli causing higher rates of erosion in dead versus live thalli (Tribollet & Payri 2001). The same way dead coral skeletons are colonised at the surface and bored inwards, diseased crusts could become rapidly vulnerable to invaders (Tribollet & Payri 2001). An increase in the presence of borers within coralline tissue could have a cascading effect by making carbonate substrata available for

new borers, thus increasing their eroding action. Ocean acidification also accelerates reef bioerosion without necessarily affecting the health of the boring organism (Wisshak et al. 2013). Furthermore, synergistic effects of ocean warming, ocean acidification and disease infection enhanced the reduction in calcification rate of CCA (Williams et al. 2014). In the face of climate change, disease outbreaks may thus, together with global stressors and boring organisms, aggravate reef degradation.

Histological observations of lesions from the two diseases did not reveal any evidence for the presence of bacteria and fungi. Yet, both are the only organisms that were identified as the pathogen agents responsible for the CLOD and the CFD diseases, respectively (Littler & Littler 1995, Williams et al. 2014). We could deduce that they are not implicated in CWBS and CWPD. Similarly, these pathogens were not observed in the white syndrome of Acroporid corals (Ainsworth et al. 2007b). Alternatively, they may not be easily detected using histology and their detection may require techniques such as Fluorescence in situ hybridisation (FISH) or transmission electron microscopy (TEM) (Work et al. 2012). The same applies for virus-like particules (VLPs) whose presence can be detected using TEM and flow cytometry (Davy et al. 2006). Viruses have been associated with the presence of syncitia inside cells (Work et al. 2012); however, they were not observed in this study. The potential implication of viruses in coral disease is still unknown but thermally stressed corals produce numerous VLPs (Davy et al. 2006, Rosenberg et al. 2009). Although this study did not identify the agents responsible of the diseases, it allows to narrow the pool of potential suspects. The use of an integrated approach is necessary for further progress on the complete diagnosis of CCA disease.

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# Supplementary material

**Table S1**. Type of boring organisms encountered and their localisation within the tissue for each CCA sample. HT, healthy tissue. DT, diseased tissue.

| | Microorganisms | Type | Tissue | Layer |
|---|---|---|---|---|
| *H. boergesenii* | | | | |
| Healthy 1 | Presence | Borer unidentified | HT | Crust |
| Healthy 2 | Presence | Sponge | DT | Limestone |
| Healthy 3 | Absence | - | - | - |
| CWBS 1 | Presence | Sponge | DT | Limestone |
| CWPD 1 | Presence | Sponge | DT | Limestone |
| CWPD 2 | Absence | - | - | - |
| CWPD 3 | Presence | Sponge | DT | Limestone |
| CWPD 4 | Absence | - | - | - |
| CWPD 5 | Presence | Sponge; Cyano | DT | Limestone |
| *N. mamillare* | | | | |
| Healthy 1 | Absence | - | - | - |
| Healthy 2 | Presence | Sponge; Borer unidentified | DT | Limestone |
| CWBS 1 | Presence | Sponge; juveniles macroborers | HT; HT | Crust and limestone; crust and limestone |
| CWBS 2 | Presence | Cyano | HT | Limestone |
| CWBS 3 | Presence | Sponge; juveniles macroborers | HT and DT; HT and DT | Crust and limestone; crust and limestone |
| CWBS 4 | Presence | Borer unidentified | DT | Limestone |
| CWPD 1 | Absence | - | - | - |
| CWPD 2 | Presence | Sponge | DT | Limestone |
| CWPD 3 | Presence | Sponge | HT and DT | Limestone |
| *P. solubile* | | | | |
| Healthy 1 | Presence | Sponge | DT | Limestone |
| CWBS 1 | Presence | Cyano | HT and DT | Limestone |
| CWBS 2 | Presence | Cyano; sponge | DT; DT | Limestone; Limestone |
| CWBS 3 | Presence | Sponge; Helminths; Borer | DT | Crust and limestone; limestone; Crust and limestone |
| *P. accretum* | | | | |
| Healthy 1 | Absence | - | - | - |

# Appendix

Sharman staining procedure (Sharman 1943) modified from Ruzin (1999)

**Procedure**
Tissues may be preserved with any fixative.

1. Deparaffinize in Limonene (3 baths of 3 minutes each), followed by hydration in a graded EtOH series (2 baths x 3 min in 100%, 2 baths x 3 min in 70%)
2. Transfer for 1 min to filtered 2% ZnCl2 (aq).
3. Wash in DI for 5 s.
4. Stain for 5 min in Safranin O staining solution.

*Staining*

5. Wash in DI for 5 s.
6. Transfer to Orange G staining solution and stain for 1 minute.
7. Wash in DI for 5 s.
8. Transfer to tannic acid solution for 5 min.
9. Wash in DI for 1–3 s.
10. Transfer to 1% aq iron alum [(NH4Fe(SO4)2, filtered] for 2 min.
11. Wash in DI for 5–15 s.
12. Dehydrate through 45, 90, 100% EtOH, about 10 s each step.
13. Transfer to Limonene (three baths of three minutes each)

Orange G staining solution:
Orange G 2 g Tannic acid 5 g HCl (conc) 4 drops DI to 100 ml
Add thymol, phenol, or azide (0.03% w/v) to inhibit microorganism contamination. Azide can be used at 0.03–0.1% w/
Filter before use

Tannic acid solution:
Tannic acid 5 g DI to 100 ml
Add thymol, phenol, or azide (0.03% w/v) to inhibit microorganism contamination
Filter before use

Safranin O stock solution:
Safranin O 1 g DI 50 ml

Safranin O staining solution:
Safranin stock (2%) 1–1.5 ml DI 500 ml

# PUBLICATION III

## Coralline algae disease reduces survival and settlement success of coral planulae in laboratory experiments

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# Coralline algae disease reduces survival and settlement success of coral planulae in laboratory experiments

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## Abstract

**Disease outbreaks have been involved in the deterioration of coral reefs worldwide and have been particularly striking among crustose coralline algae (CCA). Although CCA represent important cues for coral settlement, the impact of CCA diseases on the survival and settlement of coral planulae is unknown. Exposing coral larvae to healthy, diseased and recently dead crusts from three important CCA species, we show a negative effect of disease in the inductive CCA species *Hydrolithon boergesenii* on larval survivorship of *Orbicella faveolata* and settlement of *O. faveolata* and *Diploria labyrinthiformis* on the CCA surface. No effect was found with the less inductive CCA species *Neogoniolithon mamillare* and *Paragoniolithon accretum*. Additionally, a majority of planulae which settled on top of diseased *H. boergesenii* crusts were on healthy rather than diseased/dying tissue. Our experiments suggest that CCA diseases have the potential to reduce the survivorship and settlement of coral planulae on coral reefs.**

**Keywords:** coral recruitment, settlement cue, disease, crustose coralline algae

## Introduction

Marine diseases have increased in the past few decades and altered critical ecosystem processes (Harvell et al. 1999). Of particular concern is the decline of reef-building corals which are the foundation species of coral reefs (Bruno and Bertness 2001). While coral diseases have made a

direct contribution to this decline (Aronson and Precht 2006), diseases affecting other reef-associated organisms can indirectly affect coral populations by altering ecological processes such as coral recruitment, competition and predation. For example, in the early 1980s, an unknown pathogen eradicated most of the long-spined sea urchin *Diadema antillarum* in the Caribbean. The loss of this major herbivore facilitated a coral to algal shift on some reefs (Hughes 1994).

The emergence of diseases has been particularly striking among CCA. In the 1990s, coralline lethal orange disease (CLOD) caused massive mortality in *Porolithon onkodes* in the Pacific (Littler and Littler 1995). During the same period, *Porolithon pachydermum* largely vanished as a result of coralline white band disease (CWBS) in the Atlantic (Goreau et al. 1998). More recently, five CCA disease categories have been described in the Pacific (Vargas-Ángel 2010) and the Coralline White Patch Disease (CWPD) has been reported in Curaçao (Quéré et al. 2014). Although their prevalence remains low, disease hot spots have been found, and a link between coralline fungal disease (CFD) and ocean-warming events indicates that temperature anomalies will increase the susceptibility of CCA to diseases (Williams et al. 2014).

CCA produce cues enhancing coral larval settlement and metamorphosis (Morse et al. 1988, Heyward and Negri 1999, Negri et al. 2001). However, the impact of CCA diseases on coral recruitment is unknown. After survival through a pelagic phase, larval attachment and subsequent metamorphosis mark the start of the coral benthic life and constitute a critical ecological step to reef recovery and replenishment following disturbances (Vermeij and Sandin 2008; Ritson-Williams et al. 2009). Its success relies on the presence of effective sensory cues (e.g. chemical, spectral) associated with specific CCA and/or microbial films and the ability of coral larvae to respond to them (Morse et al. 1994; Heyward and Negri 1999; Webster et al. 2004; Mason et al. 2011). CCA diseases could reduce coral larval survivorship and settlement success by inducing shifts in the microbial community and/or the morphogens associated with CCA and disrupting the signal inducing metamorphosis or settlement in coral planulae. It is unclear whether the biofilms present on CCA or the CCA themselves are responsible for the settlement and metamorphosis of corals (Johnson et al. 1991; Webster et al. 2004). However, settlement in marine invertebrates can be hampered when CCA are treated with antibiotics, suggesting that benthic microbes may be necessary to induce settlement and metamorphosis (Johnson et al. 1991; Vermeij et al. 2009). Diseases can change the microbial or chemical profiles of marine organisms. For instance, the production of certain metabolites and the chemical and microbial profiles of the marine sponge *Aplysina aerophoba* were altered when affected by the *Aplysina* Black Patch Syndrome (Webster at al. 2008). Similarly, coral diseases typically induce shifts in the microbial community associated with the coral mucus or tissue (Pantos et al. 2003; Sunagawa

et al. 2009). Finally, coral larvae are also sensitive to conditions experienced in the water column prior to settlement (Vermeij et al. 2006). Diseased CCA could impact the survival of coral larvae by releasing noxious chemicals or by acting as reservoir and vector of pathogens.

The aim of this study was to test the hypothesis that CCA diseases reduce coral larval survival and settlement success. Survival and settlement of larvae from two major Caribbean reef-building coral species were quantified in no-choice laboratory experiments involving healthy, diseased and recently dead crusts from three CCA species which differed in their habitat preference, disease type and ability to induce coral settlement.

# Materials and methods

**Coral gametes collection**

The experiments were conducted on the island of Curaçao, Southern Caribbean at the Carmabi biological research station. Two common Caribbean reef building corals were used: *Orbicella* (formely *Montastraea*) *faveolata* and *Diploria labyrinthiformis*. Both species are broadcast-spawning corals. *O. faveolata* releases sperm-egg bundles during yearly mass spawning between August and November (Vermeij et al. 2006). *D. labyrinthiformis* spawns between April and July (Muller and Vermeij 2011). Planulae were obtained by collecting gametes from eight *O. faveolata* and eleven *D. labytinthiformis colonies* located in the shallow reef (3-8 m) at Seaquarium (12°04'59"N, 68°53'43"W). Gametes bundles from *O. faveolata* were collected at night on September 18, 2011. *D. labyrinthiformis* spawned just before nightfall on May 17, 2012. Gametes were pooled, fertilized and larvae were reared at Carmabi following Vermeij et al. (2009) until they reached competency on the fourth and second days after spawning for *O. faveolata* and *D. labyrinthiformis,* respectively.

**CCA collection**

Healthy and diseased fragments of three CCA species (*Hydrolithon boergesenii, Neogoniolithon mamillare, Paragoniolithon accretum*) were collected using hammer and chisel on the reef terrace (5-10 m depth) at Water Factory (12°06'32"N, 68°57'14"W). While *H. boergesenii* has been shown to be an effective coral settlement cue (Morse et al. 1991, 1994), *N. mamillare* appears less inductive (Ritson-Williams et al. 2014). The potential of *P. accretum* to act as a coral settlement cue has not been tested. *H. boergesenii* commonly grows in cryptic microhabitats between 8 and 20 m depth. *N. mamillare* is abundant at shallow depths (< 8 m) and colonizes exposed habitats, while *P. accretum* is less common and grows in cryptic microhabitats. All three

species showed frequent signs of disease (Quéré et al. 2014). All collected diseased fragments of *H. boergesenii and P. accretum* were affected by coralline white patch disease (CWPD) and all diseased fragments of *N. mamillare* were affected by CWBS. CWBS is characterized by a well-defined white band progressing over healthy algal tissue (Fig. 1a). The non-pigmented remains of tissue left behind initially appear white but turn greenish as it becomes colonised by endophytic green algae. CWPD is identified by the presence of distinct white patches on an otherwise healthy crust (Fig. 1b). The discolored area is irregularly shaped and can be located peripherally or centrally spreading in a random way which contrasts with CWBS (see Quéré et al. 2014 for further details on each disease).

Each replicate piece of CCA was selected from an individual patch, and fragments from each health category were placed in individual collecting bags in order to avoid contamination. After collection, healthy and diseased CCA fragments were maintained at Carmabi in separate aquaria with running seawater. A sample of each fragment was kept for taxonomic identification. The pieces used for taxonomic determination were rinsed with freshwater and dried for six hours in the oven at 60°C before being checked under a dissecting scope for reproductive and morphological features. The rest of each fragment was chopped into a 0.5 x 0.5 cm chip using angle pliers and cleaned so that only the crust of the CCA and a thin layer of limestone underneath remained. From the diseased fragment, three chips were chopped: one into the healthy-looking tissue (He-D), one into the diseased tissue leaving ca. 30-50 % healthy-looking tissue (Di-D), and one into the dead tissue (De-D). All chips were used in experiments within 48 h after collection.

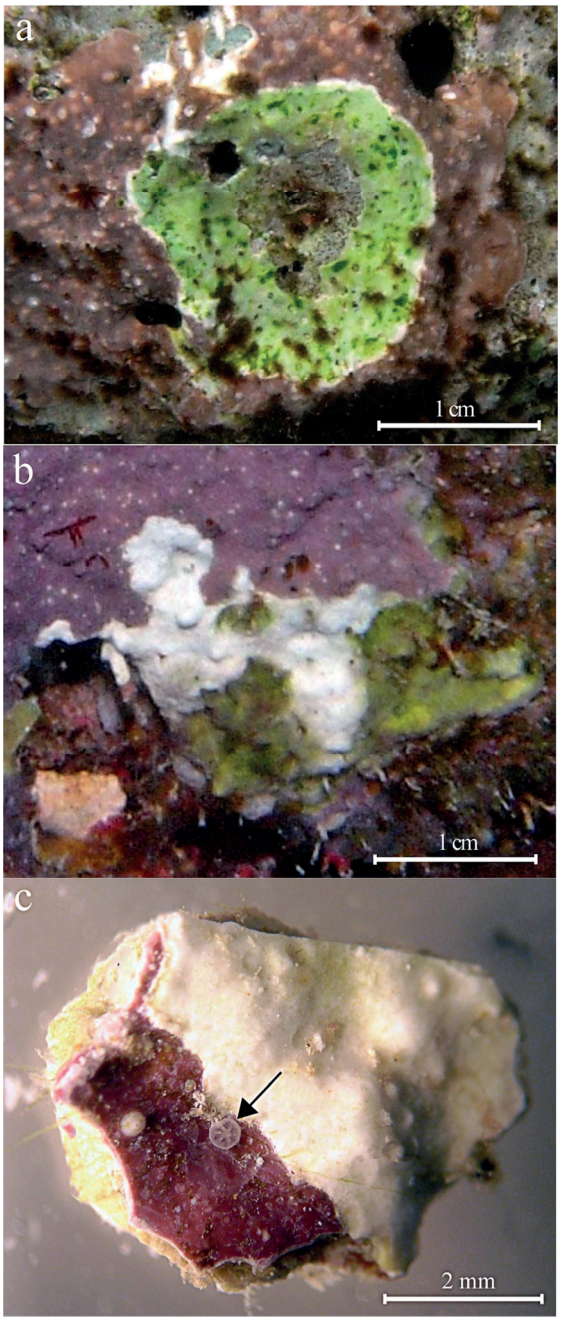


**Fig. 1 a** Coralline White Band Syndrome (CWBS). **b** Coralline White Patch Disease (CWPD). **c** Diseased crust (CWPD) showing *Diploria labyrinthiformis* settler (arrow) on healthy tissue

**Settlement experiments**

Larvae from each coral species were exposed in a no-choice experiment to six treatments: (1) a healthy fragment from a healthy CCA (He-H), (2) a healthy-looking fragment from a diseased CCA (He-D), (3) a fragment including an active disease lesion (i.e. presence of white, non-pigmented zones) from a diseased CCA (Di-D), (4) a recently dead fragment showing greenish tissue from a diseased CCA (De-D), (5) a fragment of dead coral skeleton (dead skeleton control or Sk-Ctrl) and (6) a treatment containing only filtered sea water (0.2 µm FSW) (seawater control or Sw-Ctrl). The dead coral skeletons were taken from long-dead and sun-bleached coral colonies lying on shore. The cut pieces were cleaned, rinsed in freshwater and left to dry under direct sunlight for 24 hours.

Larval bioassays were performed in sterile 6-well culture plates, with one treatment allocated to an individual well so each plate displayed all 6 treatments. Eleven coral larvae were added to each well with 10 ml of 0.2 µm FSW. After 48 h, the number of living larvae and settlers (firmly attached and beginning to calcify, Heyward and Negri 1999) were recorded using a dissecting microscope. We also recorded the substrate on which the larvae settled. In each well, except for the seawater control, larvae could settle on three different types of substratum: the top of the fragment (CCA crust or top of the dead skeleton), the bottom of the fragment (limestone rock underneath the CCA or bottom of the dead skeleton) and the plastic of the well. In addition, in the treatment consisting of a fragment with an active disease lesion (Di-D), larvae could settle on the healthy or diseased parts of the CCA crust (Fig. 1c). Ten replicate culture plates were used for each CCA species. The same experiment was repeated for each coral species separately. The experiments were carried out in an indoor laboratory under natural light cycles and with a constant ambient temperature of 29°C.

**Statistical analyses**

The percentages of surviving and settled larvae were calculated for each well. Assumptions of parametric testing were not validated using diagnostic plots in R. Initial homogeneity of dispersion tests (PERMDISP, Anderson 2004) were conducted to ensure that there was no difference in spread among treatments (excluding the two controls). Since the same original diseased fragment was used to make one replicate of three of the six treatments (i.e. He-D, Di-D and De-D), we conducted a randomized block PERMANOVA to test for differences in larval survivorship and settlement rates for each coral and CCA species combination with treatment as a fixed factor (3 levels), 'sample origin' as a random effect (10 levels), and wells as replicates. However, sample origin did not have any significant effect (Tables S1 & S2); therefore, we did

not include this factor in further analyses. A one-way permutation-based analysis of variance (PERMANOVA, Anderson 2001) was used to test for differences in larval survivorship and settlement rates for each coral and CCA species combination with treatment as a fixed factor (6 levels) and wells as replicates. Settlement rates on top and bottom surfaces were also analysed using the same design, but excluding the control treatments since they were often zero. All PERMANOVAs were run on untransformed percentage data using 9999 permutations of raw data from residuals under a reduced model using Euclidian distance. When the effect of the treatment factor was significant ($P_{perm} < 0.05$), we performed individual pair-wise tests to detect which treatments were responsible for significant differences (Anderson 2005). When the number of possible permutations was low, we used the asymptotic Monte Carlo *p* values ($P_{mc}$). Analyses were performed in R (v2.15.2) (R Development Core Team 2013) and the FORTRAN computer program PERMANOVA (Anderson 2005).

# Results

Larval survivorship reached over 66 and 88 % across all treatments for *O. faveolata* and *D. labyrinthiformis*, respectively (Figs. 2a & 3a). Differences in survival rates were found among treatments for *O. faveolata* (all PERMAVOVAs, $P_{perm} < 0.05$), but not for *D. labyrinthiformis* ($P_{perm} > 0.05$) (see Table S3 for exact *F* and $P_{perm}$ values). *O. faveolata* survival in treatments containing *N. mamillare* or *P. accretum* ranged from 66 to 77 %. These values were significantly lower than in the FSW and dead skeleton controls, respectively (PERMANOVA pair-wise tests, $P_{mc} < 0.05$), which had above 81 % survivorship, suggesting a negative effect of these CCA on *O. faveolata* larvae regardless of disease status. In contrast, in treatments containing *H. boergesenii*, *O. faveolata* larvae survived as well in the presence of the healthy fragment of healthy CCA as in the controls, with over 87 % survivorship. However, survivorship declined below 74 % in the presence of diseased and recently dead fragments (Di-D and De-D) and was significantly lower than in the presence of the healthy fragment of healthy *H. boergesenii* (He-H) (PERMANOVA pair-wise tests, $P_{mc} < 0.05$), suggesting a negative impact of CCA disease on *O. faveolata* survivorship. The healthy fragment of diseased *H. boergesenii* (He-D) did not differ from the other treatments.

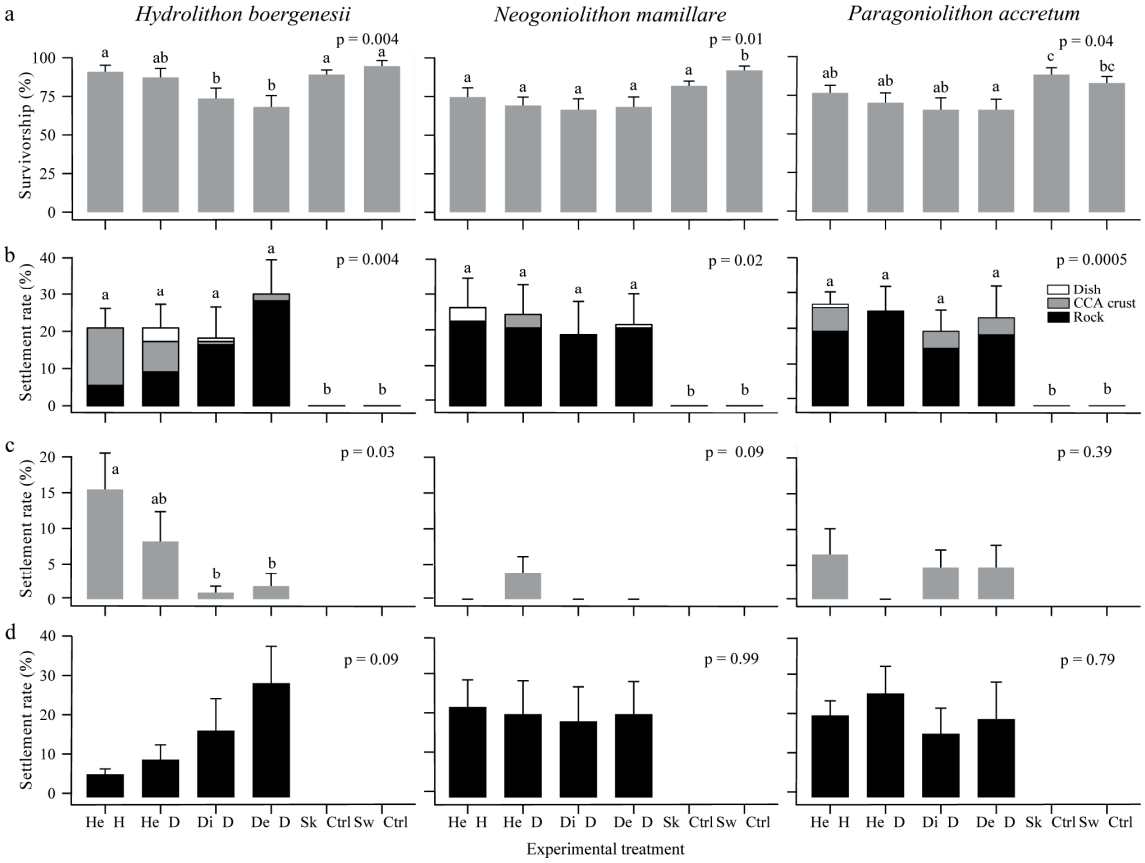


**Fig. 2** Survivorship and settlement rates (mean ± SE, n = 10) of *Orbicella faveolata* planulae. **a** Survivoship. **b** Total settlement. **c** Settlement on CCA surface. **d** Settlement on limestone rock underneath the CCA surface. Treatment abbreviations are described in the Methods. Analysed by PERMANOVA. Letters indicate homogeneous subgroups determined by pair-wise a posteriori comparisons. See Tables S3and S4 for full statistical results

Overall settlement rates in the controls was null for *O. faveolata* and less than 7 % for *D. labyrinthiformis* and significantly lower than all other treatments (Figs. 2b & 3b; see Table S4 for overall $F$ and $P_{perm}$ values; PERMANOVA pair-wise tests, $P_{mc} < 0.05$). No difference was detected among the remaining treatments except for the *D. labyrinthiformis* x *H. boergesenii* combination in which settlement in diseased treatment was significantly lower than in both healthy treatments (He-H and He-D) (PERMANOVA pair-wise tests, $P_{mc} < 0.05$). Settlement on dead fragment (De-D) was also lower than on healthy-looking fragment of diseased CCA (He-D).

In each well containing a CCA fragment, larvae had the possibility to settle on the top of the fragment (i.e. the CCA crust), which is the surface available to larvae in natural environment, or on the bottom of the fragment. The crusts of *N. mamillare* or *P. accretum* were clearly avoided by both coral species regardless of disease status (Figs. 2c & 3c). Settlement rates of both species ranged between 0-3.6 % on the crust of *N. mamillare* and between 0-11.8 % for *P. accretum* with no significant difference in settlement rates among treatments for these CCA species (PERMAVOVAs, $P_{perm} > 0.05$; Table S4). In contrast, with *H. boergesenii*, *D. labyrinthiformis*

larvae showed significantly higher settlement on top of both healthy fragments (He-H and He-D) (> 40 % settlement) than on top of diseased and dead fragments (Di-D and De-D) (< 20 % settlement) (PERMANOVA pair-wise tests, $P_{mc} < 0.05$). *O. faveolata* larvae showed a similar pattern, although only the healthy crust of healthy CCA (He-H) showed a higher settlement rate (15 %) than the crusts of the diseased and dead CCA (< 3 % settlement) (PERMANOVA pair-wise tests, $P_{mc} < 0.05$). The healthy crust of the diseased CCA (He-D) had intermediate settlement (8 %) and did not differ from the other treatments. Settlement on the bottom of *H. boergesenii* chips generally showed an inverse trend for both coral species (Figs. 2d & 3d). The high overall settlement in *D. labyrinthiformis* on healthy fragments of *H. boergesenii* clearly resulted from increased settlement induced by the healthy CCA crusts.

The importance of healthy *H. boergesenii* tissue was also visible on a smaller scale. When pooling all *D. labyrinthiformis* planulae that had settled on top of diseased fragments which displayed both healthy and diseased tissue, 15 out of the 20 settlers (75 %) were on healthy tissue (Fig. 1c). Too few *O. faveolata* larvae (n = 1) had settled on top of diseased fragments to make a similar comparison.

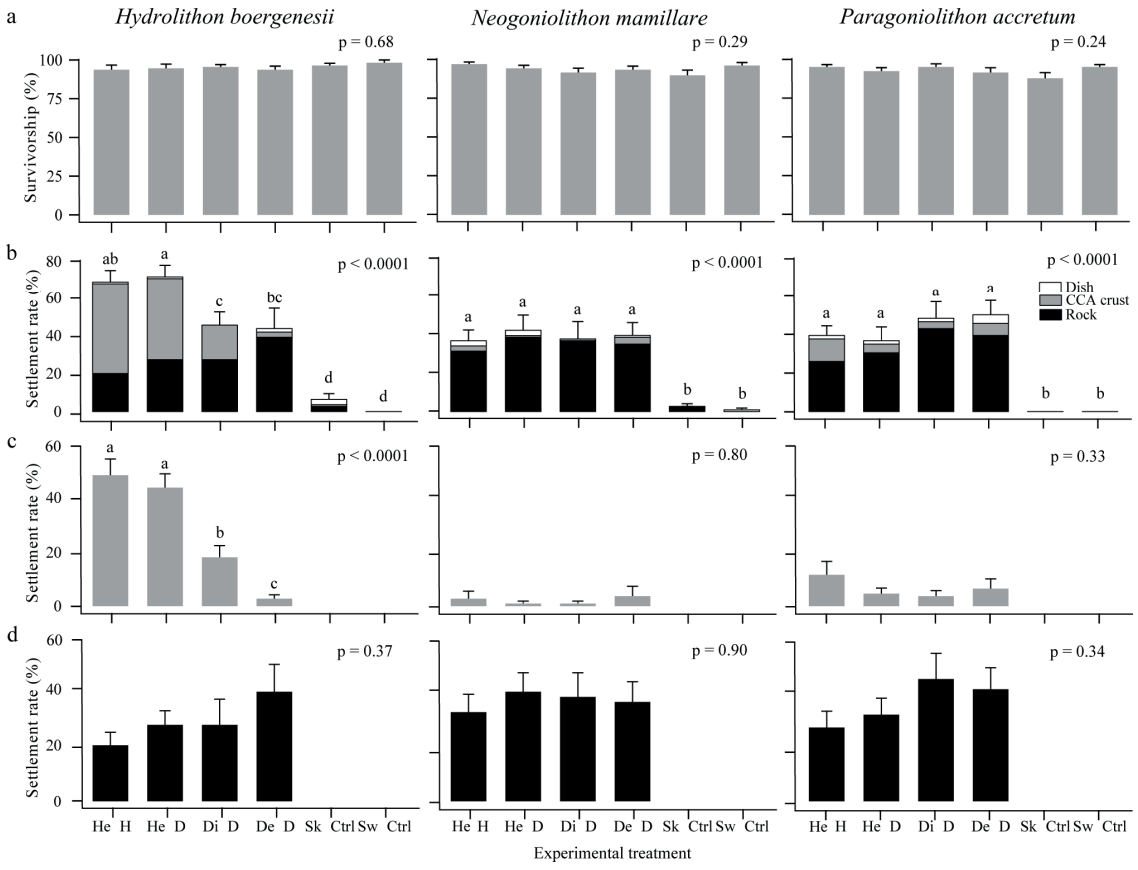


**Fig. 3** Survivorship and settlement rates (mean ± SE, n = 10) of *Diploria labyrinthiformis* planulae. Description and analyses as in Fig. 2

# Discussion

The survival of *O. faveolata* in the presence of two CCA species (*N. mamillare* and *P. accretum*) was lower than in one of the two controls regardless of their disease status. Although this suggests a negative effect of these CCA on *O. faveolata* larvae, we should consider that this effect was not consistent across both controls for both CCA species, and not observed in *D. labyrinthiformis*. To our knowledge, no negative effect of CCA on the survival of coral larvae has been reported to date. However, in the presence of healthy *N. mamillare* and *P. accretum*, settlement rates in both coral species were also low, especially on the top of the crust, indicating that these two species are ineffective cues for settlement. Negative effects of CCA on coral settlement are well-known and result mainly from chemical deterrents used as natural antifoulants (Harrington et al. 2004). An allelopathic substance from the crustose coralline algae, *Lithophyllum* spp., destroys zoospores from the brown alga *Laminaria religiosa* (Suzuki et al. 1998). It is thus plausible that these two CCA species release chemical deterrents which deter both larval survival and settlement.

In contrast, the survival of *O. faveolata* larvae was similar in the presence of healthy *H. boergesenii* than in both controls, suggesting no negative effect of this CCA species on coral larvae, but declined in the presence of diseased and dead fragments of diseased *H. boergesenii*, suggesting a negative impact of CCA disease on larval survival. *O. faveolata* larvae could be sensitive to microbes associated with the disease and/or metabolites produced by secondary invaders colonizing the dead CCA crust killed by the disease. To date, the causal agents responsible for CWBS and CWPD are unknown, but some CCA diseases are associated with bacteria and fungi (Littler and Littler 1995; Williams et al. 2014), which are well known coral pathogens (Sutherland et al. 2004). In addition, dead CCA are rapidly colonized by a wide variety of microorganisms (Tribollet and Payri 2001; Ghirardelli 2002). A comparison between live and dead thalli in coralline red algae revealed a more abundant and diverse community of microorganisms (cyanobacteria, chlorophyta and fungi) in dead thalli (Ghirardelli 2002) . Algal turfs, macroalgae and benthic cyanobacteria can negatively impact coral larval survival, most likely due to chemicals (e.g. allelochemicals or dissolved organic carbon) and/or pathogens associated with algae (Kuffner et al. 2006; Vermeij et al. 2009; Paul et al. 2011; Olsen et al. 2014).

The detrimental effect of CCA disease on larval survival was not found in *D. labyrinthiformis* larvae. *D. labyrinthiformis* reached competency after 2 days and exhibited high settlement rates (> 37 %), whereas *O. faveolata* reached competency after 4 days and exhibited

lower settlement rates (≤ 30 %). In a comparable study, *O. faveolata* settlement rates reached about 42 % in the presence of healthy *H. boergesenii* and 37 % in the presence of healthy *N. mamillare* (Ritson-Williams et al. 2014), while in our study the rates reached only 21 and 26 % for the same species, respectively. It is thus possible that larvae of *D. labyrinthiformis* were healthier than larvae of *O. faveolata*. Alternatively, coral species sensitivity towards CCA disease could vary. It has been demonstrated that coral species are not equal in the face of environmental stress (Miller et al. 2009, Hartmann et al. 2013, Miller 2014). This is the first report of *in vivo* larval survival and settlement rates in *D. labyrinthiformis*. The percentage of settlement reached over 60 % in the presence of healthy *H. boergesenii* which is in the same range as the rates obtained with 7-day old larvae from *A. palmata* in a comparable study (Ritson-Williams et al. 2010).

Our results confirm that *H. boergesenii* is a strong settlement inducer for corals, which is consistent with earlier studies (Morse et al. 1994; Ritson-Williams et al. 2010, 2014). However, they also show that the ability of this species to induce settlement is reduced when affected by disease. It is unclear whether this is the result of a loss in morphogens, bacteria or spectral signature associated with healthy CCA tissue (Morse et al. 1994; Heyward and Negri 1999; Webster et al. 2004; Mason et al. 2011). However, the lack of differences in settlement between healthy fragments of healthy and diseased crusts and the preference of larvae for healthy tissue when offered diseased fragments suggest that the mechanism operates on a millimeter scale.

Disease did not affect settlement rates in *N. mamillare* and *P. accretum*. Contrary to *H. boergesenii*, these two species may not possess the morphogen(s) responsible for the activation of coral larvae metamorphosis and settlement. Nevertheless, settlement still occurred on diseased and dead *H. boergesenii* as well as on the other less inductive species (though mostly on the bottom side of the fragments) regardless of their disease status. Thus living CCA are not essential to induce settlement. Microbes not associated with living CCA have been shown to induce coral metamorphosis (Negri et al. 2001, Webster et al. 2004). They may induce settlement without being influenced by the health of their underlying substrata.

The results of this study are experimental and need to be tested *in situ*. However, this study adds a further concern for the maintenance and recovery of coral reefs. Little is known about CCA communities *in situ* (Aeby et al. 2008; Vargas-Ángel 2010; Tribollet et al. 2011, Quéré et al. 2014). Disease outbreaks could lead to profound changes in these communities with cascading effects on coral recruitment (Doropoulos et al. 2012, Miller et al. 2013). Information on the status of CCA in the field, particularly the long-term effect of diseases on their populations, is also

needed to understand their potential for coral recruitment and the trajectory of reef communities in the face of climate change.

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# Supplementary material

**Table S1**. PERMANOVA block design results on the effects of treatment (i.e. He-D, Di-D, De-D) and sample origin on larval survival.

| Source | *df* | *H. boergesenii* | | | *N. mamillare* | | | *P. accretum* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | $P_{perm}$ | MS | *F* | $P_{perm}$ | MS | *F* | $P_{perm}$ |
| **a.** ***O. faveolata*** | | | | | | | | | | |
| Treatment | 2 | 439.55 | 0.99 | 0.471 | 374.66 | 0.90 | 0.5426 | 478.73 | 0.99 | 0.4758 |
| Sample Origin | 9 | 966.94 | 2.19 | 0.1423 | 19.28 | 0.05 | 0.9553 | 68.87 | 0.14 | 0.8671 |
| Residual | 18 | 440.47 | | | 414.14 | | | 485.15 | | |
| **b.** ***D. labyrinthiformis*** | | | | | | | | | | |
| Treatment | 2 | 8.26 | 0.13 | 0.8842 | 19.284 | 0.27 | 0.7681 | 35.81 | 0.57 | 0.566 |
| Sample Origin | 9 | 29.39 | 0.46 | 0.8924 | 35.507 | 0.50 | 0.8623 | 66.12 | 1.04 | 0.4395 |
| Residual | 18 | 63.36 | | | 71.319 | | | 63.36 | | |

**Table S2**. PERMANOVA block design results on the effects of treatment (i.e. He-D, Di-D, De-D) and sample origin on larval settlement.

| Source | *df* | *H. boergesenii* | | | *N. mamillare* | | | *P. accretum* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | $P_{perm}$ | MS | *F* | $P_{perm}$ | MS | *F* | $P_{perm}$ |
| ***a. O. Faveolata settlement*** | | | | | | | | | | |
| Treatment | 2 | 382.92 | 0.60 | 0.5668 | 74.38 | 0.09 | 0.9211 | 77.14 | 0.14 | 0.8724 |
| Sample Origin | 9 | 735.84 | 1.16 | 0.3613 | 421.18 | 0.48 | 0.8856 | 478.73 | 0.87 | 0.5648 |
| Residual | 18 | 633.91 | | | 870.22 | | | 548.52 | | |
| ***O. Faveolata Top*** | | | | | | | | | | |
| Treatment | 2 | 118.46 | 1.22 | 0.32 | 44.08 | 2.25 | 0.105 | 68.87 | 1.67 | 0.2117 |
| Sample Origin | 9 | 50.20 | 0.52 | 0.89 | 19.59 | 1.00 | 0.3958 | 73.46 | 1.78 | 0.1422 |
| Residual | 18 | 97.03 | | | 19.59 | | | 41.32 | | |
| ***O. Faveolata Bottom*** | | | | | | | | | | |
| Treatment | 2 | 966.94 | 2.21 | 0.1336 | 11.02 | 0.01 | 0.991 | 256.20 | 0.48 | 0.6368 |
| Sample Origin | 9 | 790.02 | 1.81 | 0.1359 | 490.05 | 0.55 | 0.84 | 692.99 | 1.29 | 0.2823 |
| Residual | 18 | 437.40 | | | 889.50 | | | 537.80 | | |
| ***b. D. labyrinthiformis settlement*** | | | | | | | | | | |
| Treatment | 9 | 2487.60 | 3.89 | **0.0392** | 52.34 | 0.09 | 0.9163 | 548.21 | 1.15 | 0.3307 |
| Sample Origin | 2 | 765.53 | 1.20 | 0.3501 | 802.27 | 1.40 | 0.2636 | 933.27 | 1.95 | 0.1104 |
| Residual | 18 | 638.81 | | | 572.70 | | | 477.81 | | |
| ***D. labyrinthiformis Top*** | | | | | | | | | | |
| Treatment | 9 | 4267.20 | 22.09 | **0.0001** | 24.793 | 0.47 | 0.6967 | 548.21 | 0.93 | 0.4131 |
| Sample Origin | 2 | 70.10 | 0.36 | 0.9383 | 44.077 | 0.84 | 0.6686 | 706.76 | 1.20 | 0.3512 |
| Residual | 18 | 193.14 | | | 52.342 | | | 591.06 | | |
| ***D. labyrinthiformis Bottom*** | | | | | | | | | | |
| Treatment | 9 | 465.56 | 0.78 | 0.4709 | 33.06 | 0.06 | 0.9408 | 429.75 | 1.02 | 0.3916 |
| Sample Origin | 2 | 863.48 | 1.45 | 0.2352 | 594.12 | 1.05 | 0.4318 | 943.99 | 2.24 | 0.0723 |
| Residual | 18 | 597.18 | | | 565.66 | | | 420.57 | | |

**Table S3**. PERMANOVA results on the effects of treatment (i.e. He-H,He-D, Di-D, De-D, Sk-Ctrl, Sw-Ctrl) on larval survival.

| Source | *df* | *H. boergesenii* | | | *N. mamillare* | | | *P. accretum* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | $P_{perm}$ | MS | *F* | $P_{perm}$ | MS | *F* | $P_{perm}$ |
| **a.** ***O. faveolata*** | | | | | | | | | | |
| Treatment | 5 | 1106.34 | 3.85 | **0.0044** | 969.97 | 3.34 | **0.0097** | 897.25 | 2.51 | **0.0037** |
| Residual | 54 | 287.42 | | | 290.02 | | | 357.97 | | |
| **b.** ***D. labyrinthiformis*** | | | | | | | | | | |
| Treatment | 5 | 31.13 | 0.61 | 0.7282 | 74.93 | 1.26 | 0.3071 | 85.12 | 1.39 | 0.2530 |
| Residual | 54 | 50.66 | | | 59.38 | | | 61.37 | | |

Significant p values are shown in bold

**Table S4**. PERMANOVA results on the effects of treatment (i.e. He-H,He-D, Di-D, De-D, Sk-Ctrl, Sw-Ctrl) larval settlement.

| Source | *df* | *H. boergesenii* | | | *N. mamillare* | | | *P. accretum* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | $P_{perm}$ | MS | *F* | $P_{perm}$ | MS | *F* | $P_{perm}$ |
| ***a. O. faveolata settlement*** | | | | | | | | | | |
| Treatment | 5 | 1509.92 | 3.97 | **0.0045** | 1465.84 | 3.11 | **0.0164** | 1605.51 | 5.71 | **0.0006** |
| Residual | 54 | 380.01 | | | 471.84 | | | 280.99 | | |
| ***O. faveolata Top*** | | | | | | | | | | |
| Treatment | 3 | 393.25 | 3.13 | **0.0333** | 33.06 | 2.25 | 0.0997[a] | 73.69 | 1.03 | 0.4275 |
| Residual | 36 | 125.57 | | | 14.69 | | | 71.40 | | |
| ***O. faveolata Bottom*** | | | | | | | | | | |
| Treatment | 3 | 993.80 | 2.36 | 0.0779 | 22.04 | 0.03 | 0.9937 | 170.80 | 0.36 | 0.7998 |
| Residual | 36 | 421.72 | | | 692.38 | | | 477.96 | | |
| ***b. D. labyrinthiformis settlement*** | | | | | | | | | | |
| Treatment | 5 | 9261.71 | 22.17 | **0.0001** | 3833.61 | 10.02 | **0.0001** | 5506.61 | 15.37 | **0.0001** |
| Residual | 54 | 417.81 | | | 382.61 | | | 358.28 | | |
| ***D. labyrinthiformis Top*** | | | | | | | | | | |
| Treatment | 3 | 4622.59 | 22.93 | **0.0001** | 18.595 | 0.3333 | 0.8035[a] | 134.30 | 1.19 | 0.3358 |
| Residual | 36 | 201.56 | | | 55.7851 | | | 112.72 | | |
| ***D. labyrinthiformis Bottom*** | | | | | | | | | | |
| Treatment | 3 | 624.66 | 1.10 | 0.3650 | 96.42 | 0.18 | 0.9099 | 586.7769 | 1.1736 | 0.3264 |
| Residual | 36 | 568.64 | | | 533.52 | | | 500 | | |

Significant p values are shown in bold. [a] indicate that Monte Carlo *P* values were used due to a low number of unique permutations

# General Discussion

# General Discussion

Diseases are a recognized threat to coral reef health and productivity worldwide (Harvell et al. 2007; Pollock et al. 2011). Scientists have investigated the characteristics, causes and consequences of disease outbreaks on coral reef ecosystems by focusing mostly on coral species. However, understanding the impact of diseases requires to study their effects on other components of the ecosystem and their subsequent cascading effects on communities. In this thesis, we explored the ecological significance of diseases affecting crustose coralline algae by combining different approaches, i.e. field and laboratory methods. Using CCA as a model organism, the three publications in this thesis follow the three steps, i.e. current state, causes and consequences, in order to improve our understanding of disease ecology. In the following paragraphs, the findings of this thesis will be discussed and the connections between them will be illustrated in Fig. 5.

## 1. Importance of establishing a baseline on CCA diseases

The first publication of this thesis establishes for the first time a current baseline on the status of CCA diseases in a Caribbean island. This is an important step forward in understanding CCA diseases. CCA diseases have been reported in the 1990s but only because obvious and devastating outbreaks led to the virtual disappearance of dominant CCA species in the Atlantic and Pacific oceans (Littler and Littler 1995; Goreau et al. 1998). This is one of the major problems with disease outbreaks. They are often characterized by a rapid onset, and are ephemeral (Hayes and Goreau 1998; Richardson et al. 2001). Thus, important changes in coral reefs populations and communities subsequent to disease outbreaks may be missed when no data prior to these events exist. In **Publication I**, we provided data on CCA disease occurrences in Curaçao during two time periods (wet and dry seasons). Diseases were found at all study sites, at all depths and during both monitoring periods. We compared our results to other CCA disease occurrences from reefs in the Pacific (Aeby et al. 2008; Williams et al. 2014) and found that Curaçao had among the highest occurrences reported to date, supporting the "coral disease hot spot" status of the Caribbean region (Harvell et al. 1999). However, similar baselines need to be conducted on a

larger scale and throughout the Caribbean to better characterize CCA disease occurrences in this area.

Corallines are notoriously difficult to identify *in situ*, which has considerably hampered the characterization of CCA communities in tropical reefs. In **Publication I**, we identified CCA to the genus and species levels. Ten identified species and 9 genera were recorded among the approximately twenty species present in the Caribbean (Robert Steneck, personal communication). Species-specific vulnerability to diseases has been demonstrated among corals (Haapkylä et al. 2010). The white-band disease has been a major source of mortality in Acroporid corals throughout the Caribbean, substantially contributing to the algal phase shift (Aronson and Precht 2001). In CCA, Coralline Lethal Orange Disease (CLOD) has caused massive mortality of *Porolithon onkodes* in the Pacific (Littler and Littler 1995), while *Porolithon pachydermum* was depleted by Coralline White Band Syndrome (CWBS) in the Atlantic (Goreau et al. 1998). Interestingly, we documented diseases on all CCA species encountered and there was no link between CCA community composition and disease occurrence. Laboratory experiments showed that several species of Corallinaceae could be infested with CLOD (Littler and Littler 1995), while, in the field, it looked like *P. onkodes* was more vulnerable than other species (Littler and Littler 1994). Models simulating disease dynamics have demonstrated the increase of non-infectious (i.e. due to non-transmissible agents) as well as generalist infectious diseases in relation to environmental degradation (Lafferty and Holt 2003; Lafferty and Kuris 2004). We could argue that over time, reefs have degraded (e.g. eutrophication, overfishing) and environmental conditions have worsened (e.g. warming ocean, ocean acidification), potentially explaining the increase in disease outbreaks and the broader range of species affected.

The lack of background data prior to this survey prevent us from comparing the occurrences in this study to previous data in the same area and from determining whether the disease occurrences we observed can be defined as disease outbreaks (Miller et al. 2013). However, additionally to the high disease occurrences reported here, the implementation of a CCA disease baseline in Curaçao has allowed the description of a new condition, i.e. Coralline White Patch Disease (CWPD), and demonstrated the ubiquity of CWBS along the coast, as well as the large range of CCA species affected. Together, these results are clear signs of the still ongoing increase in disease intensity and the need to better understand the responsible drivers.

Key points

- **We found in Curaçao a new CCA syndrome, the Coralline White Patch Disease** (Publication I)**.**
- **CCA disease occurrence in Curaçao is among the highest reported to date** (Publication I)**.**
- **Diseases affect all CCA species and can be found in diverse habitats** (Publication I)**.**
- **Establishing baselines of CCA disease occurrence is critical to identify disease outbreaks.**

# 2. Environmental drivers and causes of CCA diseases

## 2.1. Abiotic factors

Human expansion in coastal areas and land use activities have considerably affected the health of coral reefs by bringing elevated concentrations of nutrients, suspended sediments and pesticides into the system (Mora 2008; Wilkinson 2008). In the Caribbean, field surveys have revealed that diseases were recorded in majority from coral reefs under medium to high human influence (Green and Bruckner 2000; Vargas-Ángel 2010). Poor water quality has often been suspected to exacerbate disease severity as a result of terrestrial inputs in nutrients and pollutants (Kim and Harvell 2002). Surprisingly, we did not find a clear relationship between human influence and disease occurrence (**Publication I**). CCA diseases were present at all sites along a gradient of human activity, with levels of human impact declining with increasing distance from the main urbanized area, Willemstad. All around the Caribbean, coral reefs are considered as being under either low, medium or high human pressure (Haapkylä et al. 2010; Ruiz-Moreno et al. 2012) and present-day reefs in this area can no longer be considered pristine (Pandolfi et al. 2003). Our study sites might have all already reached the threshold level of human disturbances allowing diseases to spread. Local factors are thus likely to play a role in CCA disease dynamics.

**Publication I** showed that CCA diseases were more frequent during the rainy/warm season. Heavy rainfalls characterize the wet season in Curaçao and enhance nutrients concentrations

along the coast (Den Haan et al. 2015). The link between nutrient enrichment and disease has been experimentally established for corals (Bruno et al. 2003), but it has never been studied in CCA. In contrast, it is known that sea surface temperature (SST) influences positively CCA disease abundance (Vargas-Ángel 2010; Williams et al. 2014). SST increases by 2-3°C during the wet season in Curaçao (Bak et al. 2005). So far, it is not known why warmer temperatures are beneficial to the development of diseases in CCA. In the case of CLOD, warmer SST appeared to be a critical factor in infection and rate of spread, while cooler temperature (< 23°C) led to the reduction of the lesion (Cervino et al. 2005). In our case, contrary to disease occurrence, the lesion progression rate of the CWBS did not vary in time (**Publication I**). It is unclear whether temperature increases the virulence of the potential pathogen involved in CWBS disease; or impair CCA fitness thus increasing its susceptibility to disease. Host-pathogen interaction can shift towards one or the other depending on the environmental conditions (Cróquer and Weil 2009; Fig. 1). Synergistic interactions between ocean warming and ocean acidification cause a reduction in calcification rates, an increase frequency of necroses and enhance the dissolution of the skeleton in CCA (Anthony et al. 2008; Martin and Gattuso 2009; Diaz-Pulido et al. 2012; Johnson and Carpenter 2012). Early reproduction stages of coralline algae can also be affected by marine acidification which increases mortality and rate of abnormalities (Bradassi et al. 2013). Exposure to sewage negatively affects CCA growth and calcification rates (Björk et al. 1995). In laboratory experiments, photosynthesis in CCA decreased in the presence of the marine herbicide diuron (Harrington et al. 2005; McCoy and Kamenos 2015). Here, the effects of nutrient inputs and warmer temperatures cannot be disentangled, but the interactions between different stressors probably pose a significant threat to coralline algal populations (Diaz-Pulido et al. 2012).

Key points

- **Temperature and terrestrial runoffs appear to be key drivers in CCA disease dynamics** (Publication I)**.**
- **Due to local human impacts, reefs in Curaçao may have already reached a level of deterioration that is favorable to high CCA disease occurrence** (Publication I)**.**

- **It remains unclear whether environmental factors influence the virulence of the potential pathogen, the fitness of the host, or both.**
- **CCA disease investigations need to integrate the complex relationships existing among global factors, as well as between global and local stressors.**

## 2.2. Potential biotic causative agents

The mechanisms behind the increase in CCA disease outbreaks in relation with environmental stressors such as temperature and rainfall (**Publication I**) remain unknown and whether environmental factors are direct or indirect causative agents still needs to be determined. On one hand, bleaching and necrosis in CCA crusts without obvious pathogens have been reported under stressful conditions in laboratory experiments (Anthony et al. 2008; Martin and Gattuso 2009). On the other hand, bacterial pathogens can also cause bleaching disease in the marine red algae *Delisea pulchra* (Fernandes et al. 2011). In **Publication II**, we shed light on the potential causes of CWBS and CWPD. Bleaching in red algae is associated with the loss of photosynthetic pigments, i.e. phycobilins, usually present in the superficial cell layers (Irving et al. 2004; Fernandes et al. 2011). Here, diseased cells in both pathologies showed a complete depletion of their protoplasmic content from the superficial epithallus to the basal hypothallus layer of the CCA crust. Without observations at the microscopic level, we could not have determined that the white tissue corresponding to the active disease lesion was not solely due to the loss of pigments but instead due to the loss of the entire cellular content and the potential presence of pathogens.

Interestingly, host response differed between CWBS and CWPD. In the former, we noticed the existence of a transition area between healthy and dead cells consisting of cells that were partially deprived of protoplasmic content. In contrast, healthy-looking cells were in immediate vicinity of diseased empty cells in CWPD, suggesting a more rapid course of action. These microscopic changes are concordant with the manifestation of the diseases *in situ* (**Publication I**). The transition area could be the sign of a chronic, slowly progressing disease reflected in a slow and stable progression rate of the white-band (i.e. 0.21 ± 0.06 cm month-1 in **Publication I**), while CWPD symptoms appeared suddenly, often with a rapid turn-over (personal observation), characteristic of acute diseases (Work et al. 2012; McCoy and Kamenos 2015).

Multiple organisms can play a role in disease outbreaks and recording their presence and type constitutes a key element in determining disease causation. Potential disease-pathogens span from microparasites (e.g. bacteria, cyanobacteria, fungi, virus) to macroparasites (e.g. helminths, arthropods) (Peters 1997). Most of them have been observed in corals associated with different types of tissue loss (Acropora White Syndromes) (Work and Aeby 2011). Other examples involve ciliates which can cause tissue loss in *Montipora* corals (Jokiel and Townsley 1974). In CCA, bacteria and fungi are known to be associated with CLOD (Littler & Littler 1995) and CFD (Williams et al. 2014), respectively. In **Publication II**, we showed that diseased fragments, in particular CWBS, were more frequently associated with boring organisms than healthy fragments. Previous studies looking at the association between host response and potential agents revealed that sponges, cyanobacteria and helminths were absent from acute lesions but often associated with chronic diseases such as the slowly progressing phases of White Syndromes in *Montipora capitata* (Work et al. 2012). Our observations confirm this pattern since sponges were often found in CWBS fragments, spreading through the crust and the limestone. They were also recorded in CWPD fragments, but exclusively in the limestone, suggesting that they may not have had time to invade CWPD tissues. The same rationale may apply for other macroborers. Their presence in both the crust and the limestone of CWBS and not CWPD might suggest that, similarly to sponges, they take longer to associate with gross lesions. There is evidence that macroborers such as bivalves or sponges could take a couple of years to colonize dead skeleton, as they are long-lived, slow-growing organisms (Tribollet and Golubic 2011). Microscopic observations seem to support the suggestions formulated on the basis of *in situ* observations (**Publication I**).

Surprisingly, contrary to CLOD and CFD, we did not observe any evidence of bacterial and fungal infections. One explanation could be that they were not detectable by light microscopy using the Sharman staining procedure. However, several histological studies support that bacteria and fungi can be detected using standard histological techniques, or that, at least, characteristic pathology should be evident (Work et al. 2012). Parallels with other studies can be made where bacteria were not associated with lesions (Ainsworth et al. 2007). If bacteria are not associated with the diseases, then, either the origin of the disease is abiotic or an organism smaller than bacteria, undetectable through light microscopy, is involved. This leads us to examine the potential role of viruses. Viruses can kill cells and tissues. They are suspected to be pathogenic agents in coral diseases (Marhaver et al. 2008; Vega Thurber et al. 2008). To verify this hypothesis, particular methods would need to be applied such as Transmission Electron Microscopy (TEM), metagenomics or flow cytometry (Davy et al. 2006). Presumably, if viruses

would have killed the tissues, subsequently, boring organisms would have colonized the thallus and would only be secondary opportunistic invaders and not the primary causes of the diseases. Alternatively, bioeroders could serve as reservoir and vectors for pathogens or transmission agents carrying the pathogen within the thallus. Boring polychaetes and sponges may be vectors of the shell disease in the abalone *Haliotis tuberculata* (Huchette et al. 2006). Finally, borers are capable of digesting calcium carbonate (Lazar and Loya 1991; Zundelevich et al. 2007). This process could weaken the CCA skeleton and provide an entry point for pathogens.

Key points

- **The bleaching of the crust in diseased CCA was due to the complete depletion of protoplasmic content and not solely to the loss of photosynthetic pigments** (Publication II)**.**
- **Our results suggest that CWBS is probably a chronic, slowly-progressing disease whereas bleaching in CWPD is characteristic of an acute disease** (Publication II)**.**
- **Boring organisms were more abundant in diseased CCA suggesting weakened diseased thallus and potential further erosion of the skeleton** (Publication II)**.**

## 2.3. Interplay between biotic and abiotic factors

We previously highlighted important information that can be collected from field surveys and laboratory investigations. Combining the results of **Publication I** and **Publication II** allowed to better understand the dynamics and etiology of CWBS and CWPD. Environmental parameters, gross lesions and microscopic signs of diseases are intimately linked. Fragments for the histological study were collected during the dry season (May-June 2012). It would be interesting to compare the microscopic lesions and boring organisms populations from samples collected during the warmer season, since environmental conditions during this time period seem to be favorable to disease agents and/or detrimental to disease host. Pathogens can enter the marine environment following rainfalls (Deslarzes and Lugo-Fernández 2007; Larsen and Webb 2009).

We followed gross lesions over time, but a temporal monitoring of changes at the cellular level could have also brought useful information (Work et al. 2012). The higher number of boring organisms associated with the thallus of diseased CCA, particularly in the case of CWBS, also raises awareness on the weakening of the thallus that would be enhanced during warmer months or if acute environmental disturbances became chronic.

Key point

- **Gross lesion, microscopic signs of disease and environmental conditions are intimately linked through complex interactions.**

## 3. Impact of CCA diseases on coral recruitment

Scientists all agree nowadays that diseases represent a serious threat for coral reef ecosystems. Diseases have increased in frequency and severity, spread worldwide and threaten to worsen with changing environmental conditions (Hoegh-Guldberg and Bruno 2010; Burge et al. 2014). To better understand the impacts of diseases on coral reefs, investigations focusing on diseases affecting coral species have multiplied (Aeby et al. 2008; Vargas-Ángel 2010; Tribollet et al. 2011; Miller et al. 2013). Scleractinian corals are the founders and engineers of the reef ecosystem (Jones et al. 1994). However, an ecosystem is an assemblage of diverse organisms all essential to its good functioning and persistence (Hatcher 1997). Within the reef ecosystem, CCA interact with other communities and thus part of our investigation also evaluated the potential consequences of CCA diseases on a broader scale. In **Publication III**, we focused on the interactions between CCA and coral communities, the two main reef-building organisms.

CCA represent important settlement cues for several coral species (Heyward and Negri 1999; Ritson-Williams et al. 2010). Whether the algae itself or its associated biofilms and microbial communities are responsible has not been determined yet (Morse et al. 1994; Webster et al. 2004). However, since evidence exists on the capacity of CCA to enhance coral larvae attachment on the reef and subsequent metamorphosis, it appears vital to understand the impact diseases may have on the interactions between these two communities. In **Publication III**, we showed that all CCA were not equal in their ability to enhance settlement. Our results confirm previous studies comparing settlement between several healthy coralline algae (Ritson-Williams et al. 2010,

2014). Our results clearly indicate a reduction in survival and settlement success in diseased *H. boergesenii*. This could come from the diverse organisms invading the thallus of diseased CCA (**Publication II**) via the release of chemical deterrents. Before settling at a specific spot, larvae spend some time exploring the reef bottom. During this phase, toxic compounds could be transferred to the larvae on contact, the same way toxic compounds from algae are transferred to corals (Nugues et al. 2004; Rasher and Hay 2010; Andras et al. 2012). Among the different CCA species tested in laboratory experiments, *H. boergesenii* clearly appears as one of the most inductive and beneficial to coral settlement (Morse and Morse 1991; Morse et al. 1994). As shown in **Publication I**, *H. boergesenii* can represent up to 50 % of the CCA cover at some sites in the shallow reef flats (<10 m) and is dominant in deeper habitats (>10 m). Some coral species in the shallows may depend on the provisioning of larvae from deeper habitats to maintain their populations (Van OPPEN et al. 2011), we could therefore imagine a negative impact of disease affecting CCA. However, the positive fact is that diseases were less frequent in deeper habitats. In contrast, disease may have a real detrimental effect in shallow waters (<10 m), where environmental conditions such as light, UV and seawater temperature are higher than in deeper habitats and might favor pathogens or compromise CCA fitness.

The decrease in survival and settlement could also come from a reduction in the activating capacity of the coralline algae due to changes at the microscopic scale affecting their associated biofilm. Damaged cells as observed in **Publication II** could be depleted from the inherent morphogen activating larval metamorphosis. If changes occur at the microscopic scale, we could also imagine that diseased CCA lose their anti-fouling ability and become less competitive towards macroalgae, which already benefit from changing environmental conditions (Hoegh-Guldberg 1999). Some larvae could still settle on the healthy-looking part of a diseased crust which supports the microscopic observations we made at the cellular level in which no apparent damages could be seen in healthy-looking cells (**Publication II**). Thus, microbial changes may not have yet occurred on this part of the crust. *H. boergesenii* was mostly affected by CWPD in our laboratory experiments. The rapid action rate of this disease as suggested earlier supports this hypothesis. Our study brings evidence that disease can alter a key ecological process, namely coral recruitment, and that impact on one population can have a broader impact on another interacting population that should be taken into account when investigating disease ecology.

Key points

- **CCA represent important settlement cues for several coral species** (Publication III)**.**
- **Disease in CCA reduces coral survival and settlement success, especially in *Hydrolithon boergesenii*, a well-known settlement inducer** (Publication III)**.**
- **We propose three hypotheses to explain the negative effect of diseases on coral recruitment:**
  - **a release of chemical deterrents by boring organisms invading diseased and dead CCA thalli.**
  - **a reduction in CCA-associated morphogens responsible for coral settlement activation.**
  - **a change in the microbial communities associated with CCA crust.**

## 4. Broader impacts of increasing CCA diseases – Future scenarios

In the actual context of climate change, if the future of coral reefs looks threatened, the future of disease outbreaks looks quite promising. We demonstrated in this thesis that diseases thrive in warmer and impacted reefs, that all CCA species are vulnerable (**Publication I**) and that once diseased they become less resistant to boring organisms (**Publication II**). Furthermore, the ecological role of CCA in coral recruitment can be altered (**Publication III**). Our results illustrate the intricate connections existing between host, disease agents and environment and allow us to complete the model introduced earlier with the findings of the thesis (Fig 1). Environmental conditions are predicted to worsen and coral reefs to degrade further (Knowlton and Jackson 2008, Jackson et al. 2014). Recently, studies investigating the combination of several stressors have multiplied and brought evidence that global and local stressors can act in synergy having larger consequences together than when acting alone (Diaz-Pulido et al. 2012; Johnson and Carpenter 2012). The effects of these global stressors on CCA are numerous: decrease in calcification, reduction in growth and increase in bioerosion. Simultaneously, positive effects on

pathogen virulence are observed (Cervino et al. 2004; Bally and Garrabou 2007). Microbes are better off in degraded environments and once benign microbes might become virulent when brought into an environment that has been degraded by human activities (Lesser et al. 2007; Ainsworth et al. 2010). Furthermore, negative effects of ocean acidification on calcifiers have been largely proven (Kleypas et al.; Orr et al. 2005; Hoegh-Guldberg et al. 2007). Forced to face pressure from all directions, CCA may not be capable to maintain both their metabolism and immune system leading to increase vulnerability towards more numerous syndromes.

Given the ecological importance of CCA to coral reefs, disease-induced damages or loss could potentially alter the state of the reef. CCA are calcifiers and reef-builders contributing to the maintenance of the structural integrity of the reefs (Bak 1976; Rasser and Riegl 2002). Diseases facilitate invasion by boring organisms (**Publication II**), which structurally weakens the algal thallus (Steneck and Paine 1986) making it more vulnerable to erosion. This may lead to increased susceptibility of coral reefs to physical disturbances (e.g. storms, cyclones) (Tribollet and Payri 2001; Ragazzola et al. 2012). In the photic zone, competition for hard substratum is high and competitive interactions exist between crustose coralline algae and other seaweeds (Steneck 1997; Figueiredo and Steneck 2002) that could be altered by diseases. When they are healthy, CCA can inhibit macroalgal growth and recruitment by shedding surface cells or by releasing toxic chemical compounds (Vermeij et al. 2011; McCoy and Kamenos 2015). Disease could decrease CCA ability to compete against other seaweeds for space and/or prevent their recruitment. The reduction of CCA coverage by diseased-induced mortality would also imply that new free substrata would be available for colonization by other seaweeds leading to their rapid spread. An increase in macroalgae would fuel the negative feedback precipitation towards algal phase shift. Furthermore, coral larvae survival and settlement is reduced when CCA are affected by disease (**Publication III**). All together and exacerbated under climate change conditions, the impacts of diseases on CCA could potentially hamper the resilience of coral reefs (Fig. 5).

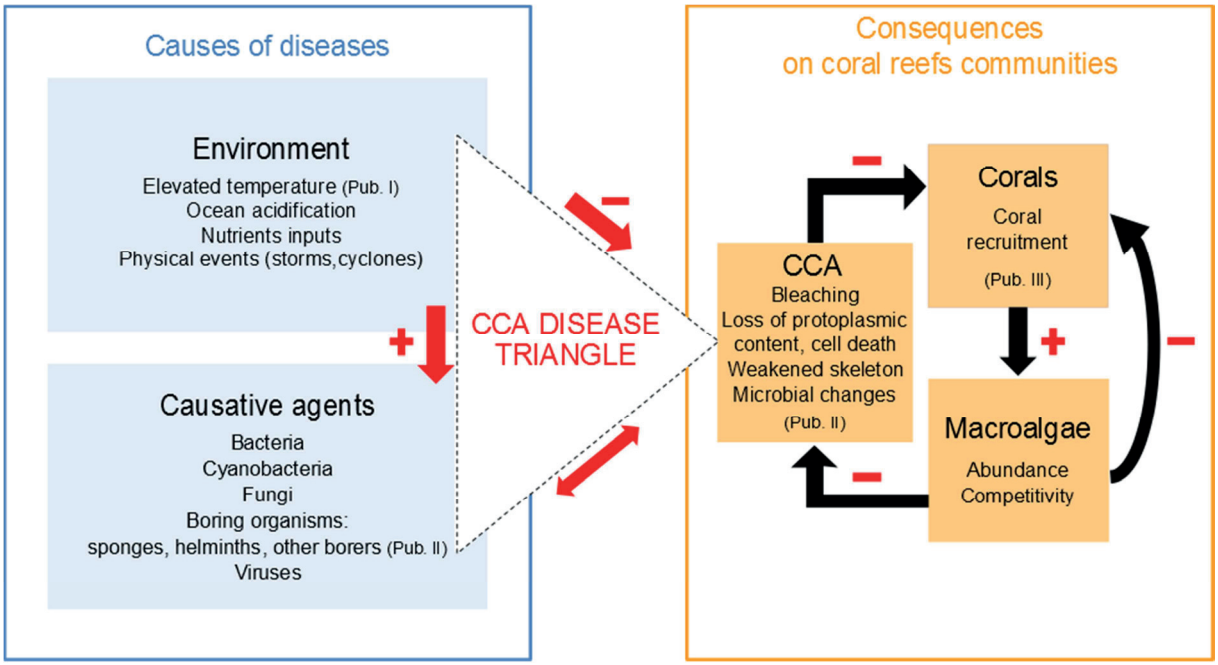


**Fig. 5** Schematic diagram illustrating the intricate relationships between environment, causative agents and hosts (red arrows); as well as the subsequent cascading effects of CCA diseases on coral reefs (black arrows). Pub.: Publication. Roman letters indicate the manuscript that provide elements supporting the illustrated connection. The symbol + stands for a beneficial effect while the symbol - stands for a detrimental effect.

## Key points

- **In the actual context of climate change and increasing local human impacts, CCA diseases are likely to become more frequent in coral reefs.**
- **Under such pressure, CCA might not be able to resist or recover from increasing disease outbreaks.**
- **CCA loss could weaken the structural integrity of coral reefs, increase abundance of macroalgae and reduce coral recruitment, precipitating a shift towards algal dominated reefs and hampering a potential reversal to coral dominated reefs.**

# 5. Management recommendations

Over the past four decades, marine diseases have been the subject of a worldwide effort which has generated a great amount of information. Marine diseases are not easy to stop through actions typically used on terrestrial systems once they are present. In open marine systems, medication, vaccination or quarantine are not possible (Burge et al. 2014). Microbes are numerous, ubiquitous and necessary components of the good functioning of coral reefs (Ainsworth et al. 2010; Rohwer et al. 2010). However, practical steps to manage coral reef diseases exist and management actions can be initiated whether the causative agent of a disease is known or not. The findings presented in this thesis support the fact that reducing local stress, in particular eutrophication, should be a priority, especially when a global action on climate change is so hard to implement. CCA are sessile benthic organisms which do not have the possibility to move to more favorable environments. Thus, acting at a local scale is crucial. The severity of some diseases on coral reefs have been linked to increased levels of nutrients in the seawater (Bruno et al. 2003; Voss and Richardson 2006). A reduction of coastal pollution and runoffs should prevent the intrusion of numerous pathogens potentially at the origin of diseases. Furthermore, bleaching of the CCA crust can be reversible (G. Quéré pers. obs.; Irving et al. 2004) and we showed in this thesis (**Publication II**) that healthy tissue can grow over diseased/dead tissue highlighting a potential for recovery. Therefore, management legislation should maintain or restore conditions favorable to resilience by reducing terrestrial runoffs, nutrients inputs and fishing.

## Key points

- **Management actions can be initiated whether the causative agent of a disease is known or not.**
- **Reducing human impacts at a local scale by limiting terrestrial runoffs and coastal pollution is essential to prevent disease outbreaks and maintain favorable conditions for recovery.**

# Perspectives for Future Research

The findings in the three publications provide new starting points for further research. An interesting direction for new research would be to investigate disease influence on CCA population dynamics. In **Publication I**, we monitored spatio-temporal variations of CCA diseases at the species level at two different time periods. Whilst this approach is a core component of most disease assessment programs (Aeby et al. 2008; Vargas-Ángel 2010; Tribollet et al. 2011; Miller et al. 2013), such studies do not address the dynamics of CCA at the scale of individual diseased patches (Mumby et al. 2005), which may either die or recover from disease. Monitoring the fate of bleached corals has provided important information on the nature and dynamics of the algal overgrowth that generally follows a bleaching disturbance (Diaz-Pulido and McCook 2002) Similar information on CCA would help understand the potential overgrowth of diseased or dead CCA crusts by macroalgae. Using a modelling approach, we could simulate the impacts of diseases on CCA populations and integrate environmental drivers (e.g. temperature) to identify which ones are the most influential. The population model would incorporate dynamic parameters (e.g. diseases initiation rate, recovery rate, recruitment rate, growth rate) combined with covariates (e.g. light and temperature measurements) and information on individual patch (e.g. habitat, orientation, grazing mark, competitors). Such models have been built to simulate the interplay between sponge, corals and macroalgae under different scenarios at different levels of disturbances (González-Rivero et al. 2011). The model could be used to determine the level of disease occurrences leading to extinction or on the contrary, to resistance and recovery, depending on changing environmental conditions. It would constitute a promising approach and a great tool for developing management recommendations.

According to previous studies, viruses are the best known pathogens of algae (Gachon et al. 2010). Yet, this path has not yet been explored in CCA diseases. Viruses can be extremely virulent and evolve at high pace with new taxa still being discovered (Davy et al. 2006; Lang et al. 2009). Viruses are ubiquitous in marine waters but their role in marine diseases remains to be determined. Since we did not visualize any obvious agents, the true responsible for the disease, if any, may not have been detected under light microscopy and could be a virus. To detect viruses and confirm their implication in CCA diseases, TEM, metagenomics or fluorescent cytometry could be used (Wilson et al. 2005; Davy et al. 2006).

Key points

- **Research on CCA diseases is emerging and disease dynamics remain largely unknown.**
- **A population model would a useful tool to simulate the impact of diseases on CCA populations while integrating environmental drivers.**
- **Viruses are ubiquitous in marine ecosystems and constitute a lead worth pursuing to determine disease etiology.**

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## for Introduction and General Discussion

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