Ecology Letters, (2004) **7**: 919–923 doi: 10.1111/j.1461-0248.2004.00651.x

LETTER

Algal contact as a trigger for coral disease

Maggy M. Nugues,[1]* Garriet W. Smith,[2] Ruben J. van Hooidonk,[1] Maria I. Seabra[1] and Rolf P. M. Bak[1]

[1]Royal Netherlands Institute for Sea Research (NIOZ), P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

[2]Department of Biology and Geology, University of South Carolina Aiken, Aiken, SC 29801, USA

**Correspondence and Present address: Maggy M. Nugues, Center for Marine Science, University of North Carolina at Wilmington, 5600 Marvin K. Moss Lane, Wilmington NC 28409-5928, USA.*

E-mail: nuguesm@uncw.edu

Abstract

Diseases are causing alarming declines in reef-building coral species, the foundation blocks of coral reefs. The emergence of these diseases has occurred simultaneously with large increases in the abundance of benthic macroalgae. Here, we show that physical contact with the macroalga *Halimeda opuntia* can trigger a virulent disease known as white plague type II that has caused widespread mortality in most Caribbean coral species. Colonies of the dominant coral *Montastraea faveolata* exposed to algal transplants developed the disease whereas unexposed colonies did not. The bacterium *Aurantimonas coralicida*, causative agent of the disease, was present on *H. opuntia* sampled close to, and away from diseased corals, indicating that the alga serves as a reservoir for this pathogen. Our results suggest that the spread of macroalgae on coral reefs could account for the elevated incidence of coral diseases over past decades and that reduction of macroalgal abundance could help control coral epizootics.

Keywords

Coral disease, coral reef, epizootic, *Halimeda*, macroalgae, overgrowth, white plague.

Ecology Letters (2004) 7: 919–923

INTRODUCTION

Infectious diseases are now acknowledged as a major force of change in marine ecosystems. They can lead to loss of keystone species and alter critical ecosystem processes (Aronson & Pretch 2001; Harvell *et al.* 2002). There is convincing evidence that they have recently increased in number, prevalence and virulence in most marine taxa (Harvell *et al.* 1999; Aronson & Pretch 2001; Porter *et al.* 2001). This trend has been particularly striking among scleractinian coral species. Within the last 30 years, the number of coral diseases has increased from two to 18 (Sutherland *et al.* 2004). Two of the most dominant reef-building corals, *Acropora palmata* and *A. cervicornis*, have largely vanished across the entire Caribbean-wide basin as a result of white band and white pox diseases (Aronson & Pretch 2001). More recently, new diseases have inflicted severe losses in a majority of coral species (Sutherland *et al.* 2004).

The growing impact of diseases in the ocean has raised great concern about the factors influencing marine epizootics. Elevated temperature, declining water quality and transport of aeolian dust from Saharan Africa have been proposed as potential causal agents of coral diseases (Hayes *et al.* 2001; Harvell *et al.* 2002; Bruno *et al.* 2003). However, unequivocal evidence has often been hampered by the multitude of confounding factors, lack of experimental studies and limited knowledge about reservoirs and modes of disease transmission (Harvell *et al.* 2002; Sutherland *et al.* 2004). Coinciding with the emergence of coral diseases, many coral reefs have experienced dramatic increases in abundance of benthic macroalgae (Hughes 1994; McCook 1999; Edmunds 2002). Greater macroalgal cover generally results in increased physical contacts between corals and macroalgae. While such encounters may not be lethal to corals *per se* (McCook 1999), it may increase their susceptibility to disease by compromising their resistance and increasing their exposure to pathogens. However, there is at present no experimental evidence for a causal connection between algal contact and coral disease.

In 1995, a virulent disease, called white plague type II, killed up to 38 % of *Dichocoenia stokesi* colonies in the Florida Keys (Richardson *et al.* 1998). Since then, this disease has spread throughout the Caribbean and affected most coral species (Nugues 2002; Weil *et al.* 2002; Miller *et al.* 2003). The causal agent of the disease was recently described as a marine ureolytic bacterium *Aurantimonas coralicida* (Denner *et al.* 2003). Infected corals are characterized by a sharp

©2004 Blackwell Publishing Ltd/CNRS

demarcation between apparently healthy coral tissue and freshly denuded skeleton that can migrate across the colony at several centimetres a day (Richardson *et al.* 1998). In 2002, we observed white plague on coral colonies on reefs along the south coast of Curaçao in the Netherlands Antilles. The green calcareous alga *Halimeda opuntia* was often present at the presumed center of origin of the infection, suggesting that the alga could be involved in transmission of the disease (Fig. 1). *Halimeda* is one of several genera of macroalgae that has greatly increased in abundance in the Caribbean over the last 2 decades (Hughes 1994; Edmunds 2002). It frequently interacts with living corals and utilizes dead coral skeleton as a primary point of attachment and growth.

To study a potential link between the presence of *Halimeda* and white plague incidence, we conducted a field experiment in which *H. opuntia* was transplanted next to healthy colonies of *Montastraea faveolata*, one of the primary reef-building corals in the Caribbean, and compared disease incidence with a control group unexposed to the alga. Our results show that contact with the alga can trigger the disease, providing a possible explanation for the emergence of coral diseases over last decades.

MATERIALS AND METHODS

Field experiment

Our experiment was carried out between December 2002 and February 2003 on the reef terrace (4–10 m depth) at Carmabi Buoy Zero (69°58′26″ N, 12°07′27″ W) in Curaçao, Netherlands Antilles. Eighty colonies of *M. faveolata* were tagged and randomly assigned to two treatments: algal transplant and control. Colonies were selected haphazardly by swimming along the depth contour and were used in the experiment if they showed no sign of disease, were not overgrown by *H. opuntia* and had dead substrate adjacent to colony margin to fix transplants and/or tags. A clump (*c.* 1000 cm^3) of apparently healthy *H. opuntia* was transplanted onto the colonies assigned to the algal treatment using cable ties and plastic nails inserted onto dead coral substrate. To simulate natural conditions, each plant was placed to overgrow a few centimetres of living coral tissue, allowing physical contact between the alga and the coral tissue. Control colonies had a tag and nail inserted on adjacent dead coral substrate. Colonies were monitored weekly or biweekly for 3 months. During each survey, all colonies were checked for the presence of white plague disease and, if applicable, the surface of coral tissue killed by the disease was measured using calipers. The living coral tissue of control colonies was kept cleared of all *H. opuntia*.

Sample collection for disease identification

Coral samples were taken using sterile 2.5 mL syringes on three experimental colonies showing signs of disease in February 2003. On each colony, one healthy tissue sample was obtained from an area of apparently healthy tissue at least 10 cm from the lesion boundary, and one diseased tissue sample was taken from the area of disease at the interface of healthy tissue and bare skeleton. Samples of *H. opuntia* were obtained from plants growing onto diseased *M. faveolata* and away from any diseased corals in February and July 2003. Each

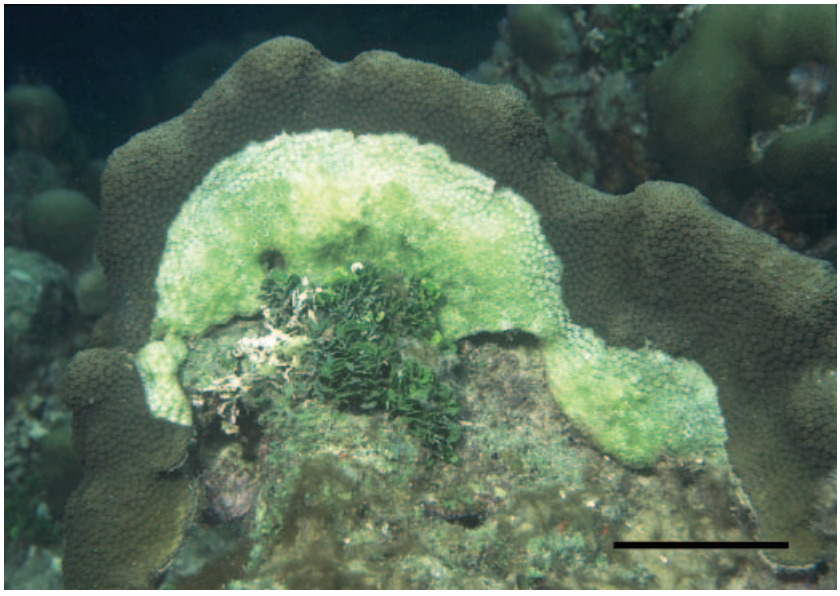

Figure 1 White plague on colony of the Caribbean reef-building coral *Montastraea faveolata* with the green alga *Halimeda opuntia* growing at the point of origin of the disease. The central portion of the colony has been recently killed by the disease. Photograph taken at 8 m depth at Carmabi Buoy Zero, Curaçao. Scale bar: 10 cm (Photo by M. Nugues).

©2004 Blackwell Publishing Ltd/CNRS

time, samples consisted of several branches taken from three plants and placed in separate zip-lock bags. During the February sampling, which was carried out at the same time as the above experiment, samples were selected among plants growing onto experimental colonies showing signs of disease. All samples were kept refrigerated and sent to a laboratory at Aiken, SC, USA within 24 h and processed immediately upon arrival.

Bacterial isolation and characterization

In the laboratory, all samples were streak plated on a permissive glycerol artificial seawater (GASW) medium (Smith & Hayasaka 1982) and incubated at 25 °C for 7 days. Each day, colonies arising on plates were restreaked to pure culture on GASW plates. Pure cultures were suspended in sterile artificial seawater and the density adjusted to an absorbance at 600 nm of 0.147 (±0.002) spectrophotometerically to assure consistent mass. Subsamples (0.15 mL) were distributed into microwells in Biolog™ GN plates. Each of the 96 microwells contained a different carbon source (except the first microwell which has no carbon source), mineral nutrients and a tetrazolium dye. Plates were incubated at 25 °C for 3 days after which they were read on an automated plate reader. Absorbance at 490 nm (reduced tetrazolium) and 580 nm (turbidity) was recorded for each microwell and an adjusted (for turbidity) increase of 40% over the control well was scored as positive. In this way, a carbon source utilization pattern (CSUP) was obtained for each isolate. A pure culture of *A. coralicida* was treated identically and all CSUPs were entered into a database from which matches with the *A. coralicida* reference culture were made.

RESULTS

The first signs of white plague appeared on colonies exposed to the alga within 2 weeks following transplantation. After 1 month, 22 (55%) of the 40 experimental colonies with algal transplants had the disease. The infection always originated from the area overgrown by the transplant, spreading rapidly over living tissues during the first 2 weeks of infection. No more colonies subsequently became infected during the next 2 months of observations after which the study was ended. In contrast, all control colonies remained healthy throughout the study period. There was a highly significant difference in disease incidence between the algal transplant and control treatments (chi-square test, $\chi^2 = 30.35$, d.f. $= 3$, $P < 0.001$).

At the end of the study, there was no sign of further coral tissue degradation. The total surface area of coral tissue killed by the disease for each infected colony averaged 54 (31/88) cm^2 (geometric mean and lower or upper 95% confidence limits). It varied from 12 to 570 cm^2 (Fig. 2), with the majority (64%) being < 54 cm^2, suggesting that colonies differed in their resistance to disease. None of the diseased colonies were totally killed.

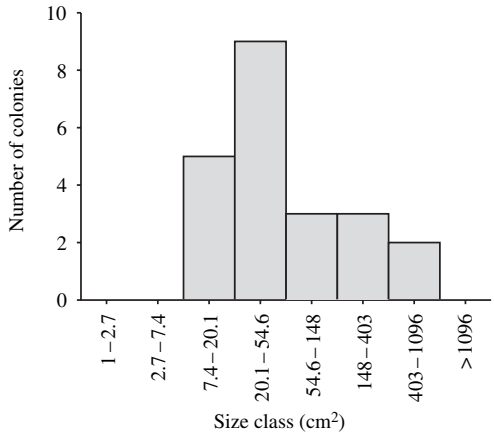

Figure 2 Size frequency distribution of the total surface area of coral tissue killed by white plague (logarithmic scale). $n = 22$ colonies.

Laboratory analysis confirmed that the diseased corals had white plague type II. *A. coralicida* was not present in any samples collected from apparently healthy coral tissue, but was found in all samples collected from the surface of the disease line of experimental colonies (Table 1). Additionally, *A. coralicida* was present on all *H. opuntia* samples adjacent to diseased corals and on three (at least one for each sampling period) of the six samples of *H. opuntia* collected away from diseased corals (Table 1). This demonstrates that *H. opuntia* (or other organisms associated with the alga) serves as a natural reservoir for the pathogen.

Table 1 Carbon source utilization pattern matches with *Aurantimonas coralicida* from *Montastraea faveolata* and *Halimeda opuntia* samples from Carmabi Buoy Zero, Curaçao

		Sample number		
Sample	Sampling date	1	2	3
Diseased *M. faveolata* tissue	13 February 2003	+	+	+
Healthy *M. faveolata* tissue	13 February 2003	−	−	−
H. opuntia adjacent to diseased *M. faveolata*	13 February 2003	+	+	+
	1 July 2003	+	+	+
H. opuntia away from diseased coral	13 February 2003	−	+	−
	1 July 2003	−	+	+

A positive sign indicates the presence of *A. coralicida*.

©2004 Blackwell Publishing Ltd/CNRS

DISCUSSION

Our experiment demonstrates that physical contact with the macroalga *H. opuntia* can trigger white plague type II in the coral *M. faveolata*. How such contact promotes transmission of the disease is unclear. As the white plague pathogen was found on algae growing away from diseased corals, *H. opuntia* may be a vector for the disease. However, it is unknown whether the pathogen is transmitted by the plant itself. It cannot be excluded that organisms associated with the alga may be vectors for the disease. For instance, the fireworm *Hermodice carunculata* was recently shown to be the reservoir and vector for the coral-bleaching pathogen *Vibrio shiloi* in the Mediterranean Sea (Sussman *et al.* 2003). *Halimeda* provides associational refuge to a diverse community of small corallivorous invertebrates (e.g. worms, snails, crabs) (Naim 1988). This associated fauna may be transmitting the white plague pathogen while feeding on corals. There may be a causal relationship between outbreaks of corallivorous invertebrates and coral diseases (Antonius & Riegl 1998; Aronson & Pretch 2001).

The close proximity of *H. opuntia* may also cause an abnormal physiological stress or trauma to corals that facilitates invasion by the pathogen. The aforementioned predation from organisms associated with the alga may account for such stress, but other organisms present in *H. opuntia* may also damage adjacent coral tissue. For instance, filamentous algal turfs and cyanobacteria, common on the surface of *H. opuntia* segments, have been associated with coral tissue necrosis and are believed to exude chemicals toxic to corals (Littler & Littler 1997; Jompa & McCook 2003). The alga itself may damage coral tissue through physical abrasion or shading or by allelochemical effects (McCook *et al.* 2001). *Halimeda* holds potent secondary metabolites used to deter grazing (Paul & van Alstyne 1992). However, the possibility that these substances affect overgrown corals has never been investigated. Continued efforts should be made to identify which factors associated with *H. opuntia* are responsible for initiating and transmitting white plague.

At least three hypotheses can explain the large inter-colony variation found in coral tissue loss because of the disease. First, it may originate from innate immunity acquired during a previous infection. In the Florida Keys, corals infected by white plague type II, that survived the infection, were no longer susceptible to the disease (Richardson & Aronson 2002). This would strongly reduce the long-term impact of this disease. Second, it may reflect differences in the health status of corals. Colonies located in favourable environments are likely to have better health and presumably greater resistance to pathogens than colonies located in less favourable environments. Third, it may be caused by small-scale spatial variations in nutrient concentrations. A moderate increase (two to five times) in nutrient levels can significantly increase the amount of coral tissue loss from disease (Bruno *et al.* 2003). Sheltering fish schools are an important source of nutrients and organic matter on coral reefs. For instance, migrating haemulids can cause a two times increase in ammonium available to corals (Meyer & Schultz 1985). Therefore, differences in the abundance of sheltering fishes among colonies may cause localized variations in nutrient concentrations and subsequent disease spread.

Vector transmission of disease is well known among human and other terrestrial hosts (Dye 1992). Insects have been known to be disease vectors in agricultural and forest ecosystems for some time (Anderson & May 1980). Vector-borne pathogens can be very virulent as vectors tend to increase the efficiency of transmission (Ewald 1983). Although much less is known of vectored diseases in marine environments, this is probably because of the lack of knowledge of the disease process in the marine environment. Leeches and isopods have been identified as vectors of some marine fish diseases (Davies & Smit 2001), but this is the first report of an alga acting as a vector or reservoir of a pathogen.

Our study highlights macroalgae as a new factor influencing coral epizootics and suggests that the increase of macroalgal abundance on coral reefs may have contributed to the recent emergence of coral diseases. Declining levels of herbivory, increasing supply of nutrients and coral mortality have been suggested as the main factors promoting macroalgal abundance (McCook 1999; Aronson & Pretch 2001; Szmant 2002). Unless remediation measures are adopted, these factors are likely to worsen as human populations grow. We anticipate that coral epizootics will become even more common and widespread as macroalgal abundance increases on reefs. Measures to reduce macroalgal abundance may be essential if significant coral populations are to survive on coral reefs.

ACKNOWLEDGEMENTS

We thank the staff of CARMABI and NIOZ institutes – W. Bakhuis, A. Debrot, L. Pors, B. Leysner, C. Winterdaal and G. Nieuwland – for logistical support. Comments from J. Hawkins, C. Roberts, J. van der Meer, A. Dyer and four anonymous reviewers improved this manuscript. This study was supported by a Marie Curie Postdoctoral Fellowship (EVK3-CT-2001-50007) to M. Nugues, and a NOAA CRES (0648-0384) grant and a NSF-COP Emerging Diseases (OCE-0326269) grant to G. Smith.

REFERENCES

Anderson, R.M. & May, R.M. (1980). Infectious diseases and population cycles of forest insects. *Science*, 210, 658–661.

©2004 Blackwell Publishing Ltd/CNRS

Antonius, A. & Riegl, B. (1998). Coral diseases and *Drupella cornus* invasion in the Red Sea. *Coral Reefs*, 17, 48.

Aronson, R.B. & Pretch, W.F. (2001). Evolutionary paleoecology of Caribbean coral reefs. In: *Evolutionary Paleoecology: The Ecological Context of Macroevolutionary Change* (eds Allmon, D. & Bottjer, D.J.). Columbia University Press, New York, NY, pp. 171–233.

Bruno, J.F., Petes, L.E., Harvell, C.D. & Hettinger, A. (2003). Nutrient enrichment can increase the severity of coral diseases. *Ecol. Lett.*, 6, 1056–1061.

Davies, A.J. & Smit, N.J. (2001). The life cycle of *Haemogregarina bigemina* (Adeleina: Haemogregarinidae) in South African hosts. *Folia Parasitol.*, 48, 169–177.

Denner, E.B.M., Smith, G.W., Busse, H., Schumann, P., Narzt, T., Polson, S.W. *et al.* (2003). *Aurantimonas coralicida* gen. nov., sp. nov., the causative agent of white plague type II on Caribbean scleractinian corals. *Int. J. Syst. Evol. Microbiol.*, 53, 1115–1122.

Dye, C. (1992). The analysis of parasite transmission by blood-sucking insects. *Annu. Rev. Entomol.*, 37, 1–19.

Edmunds, P.J. (2002). Long-term dynamics of coral reefs in St. John, US Virgin Islands. *Coral Reefs*, 21, 357–367.

Ewald, P.W. (1983). Host–parasite relations, vectors, and the evolution of disease severity. *Annu. Rev. Ecol. Syst.*, 14, 465–485.

Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J. *et al.* (1999). Emerging marine diseases – climate links and anthropogenic factors. *Science*, 285, 1505–1510.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S. *et al.* (2002). Climate warming and disease risks for terrestrial and marine biota. *Science*, 296, 2158–2162.

Hayes, M.L., Bonaventura, J., Mitchell, T.P., Prospero, J.M., Shinn, E.A., Van Dolah, F. *et al.* (2001). How are climate and marine biological outbreaks functionally linked? *Hydrobiologia*, 460, 213–220.

Hughes, T.P. (1994). Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science*, 265, 1547–1551.

Jompa, J. & McCook, L.J. (2003). Coral-algal competition: macroalgae with different properties have different effects on corals. *Mar. Ecol. Prog. Ser.*, 258, 87–95.

Littler, M.M. & Littler, D.S. (1997). Epizoic red alga allelopathic (?) to a Caribbean coral. *Coral Reefs*, 16, 168.

McCook, L.J. (1999). Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. *Coral Reefs*, 18, 357–367.

McCook, L.J., Jompa, J. & Diaz-Pulido, G. (2001). Competition between corals and algae on coral reefs: a review of available evidence and mechanisms. *Coral Reefs*, 19, 400–417.

Meyer, J.L. & Schultz, E.T. (1985). Migrating haemulid fishes as a source of nutrients and organic matter on coral reefs. *Limnol. Oceanogr.*, 30, 146–156.

Miller, J., Rogers, C.& Waara, R. (2003). Monitoring the coral disease, plague type II, on coral reefs in St. John, U.S. Virgin Islands. *Rev. Biol. Trop.*, 51, 47–55.

Naim, O. (1988). Distributional patterns of mobile fauna associated with *Halimeda* on the Tiahura coral-reef complex (Moorea, French Polynesia). *Coral Reefs*, 6, 237–250.

Nugues, M.M. (2002). Impact of a coral disease outbreak on coral communities in St. Lucia: what and how much has been lost? *Mar. Ecol. Prog. Ser.*, 229, 61–71.

Paul, V.J. & van Alstyne, K.L. (1992). Activation of chemical defenses in the tropical green algae *Halimeda* spp. *J. Exp. Mar. Biol. Ecol.*, 160, 191–203.

Porter, J.W., Dustan, P., Jaap, W.C., Patterson, K.L., Kosmynin, V., Meier, O.W. *et al.* (2001). Patterns of spread of coral disease in the Florida Keys. *Hydrobiologia*, 460, 1–24.

Richardson, L.L. & Aronson, R.B. (2002). Infectious diseases of reef corals. *Proc. 9th Int. Coral Reef Symp.*, 2, 1225–1230.

Richardson, L.L., Goldberg, W.M., Kuta, K.G., Aronson, R.B., Smith, G.W., Ritchie, K.B. *et al.* (1998). Florida's mystery coral-killer identified. *Nature*, 392, 557–558.

Smith, G.W. & Hayasaka, S.S. (1982). Nitrogenase activity associated with *Halodule wrightii* roots. *Appl. Environ. Microbiol.*, 43, 1244–1248.

Sussman, M., Loya, Y., Fine, M. & Rosenberg, E. (2003). The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*. *Environ. Microbiol.*, 5, 250–255.

Sutherland, K.P., Porter, J.W. & Torres, C. (2004). Disease and immunity in Caribbean and Indo-Pacific zooxanthellae corals. *Mar. Ecol. Prog. Ser.*, 266, 273–302.

Szmant, A.M. (2002). Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries*, 25, 743–766.

Weil, E., Urreiztieta, I. & Garzón-Ferreira, J. (2002). Geographic variability in the incidence of coral and octocoral diseases in the wider Caribbean. *Proc. 9th Int. Coral Reef Symp.*, 2, 1231–1237.

Editor, David Liddell
Manuscript received 20 April 2004
First decision made 20 May 2004
Manuscript accepted 22 June 2004

©2004 Blackwell Publishing Ltd/CNRS