

## LETTER

## Nutrient enrichment can increase the severity of coral diseases

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### Abstract

The prevalence and severity of marine diseases have increased over the last 20 years, significantly impacting a variety of foundation and keystone species. One explanation is that changes in the environment caused by human activities have impaired host resistance and/or have increased pathogen virulence. Here, we report evidence from field experiments that nutrient enrichment can significantly increase the severity of two important Caribbean coral epizootics: aspergillosis of the common gorgonian sea fan *Gorgonia ventalina* and yellow band disease of the reef-building corals *Montastraea annularis* and *M. franksii*. Experimentally increasing nutrient concentrations by 2–5× nearly doubled host tissue loss caused by yellow band disease. In a separate experiment, nutrient enrichment significantly increased two measures of sea fan aspergillosis severity. Our results may help explain the conspicuous patchiness of coral disease severity, besides suggesting that minimizing nutrient pollution could be an important management tool for controlling coral epizootics.

### Keywords

Aspergillosis, coral disease, coral reef, epizootic, nutrient enrichment, yellow band disease.

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### INTRODUCTION

Recent marine disease epizootics have dramatically reduced the abundance of a variety of endangered, commercially valuable and habitat-forming foundation species (Harvell *et al.* 1999). In many systems, this has caused a shift in community structure and the alteration of critical ecosystem processes (Harvell *et al.* 2002). The effects of diseases on Caribbean coral reefs are especially striking. In the 1980s, the most important herbivore, the urchin *Diadema antillarum* (Lessios 1988), and the two most abundant reef-building corals (*Acropora cervicornis* and *A. palmata*) (Aronson & Precht 2001a) were decimated by epizootics at a regional scale. The combination of increased coral mortality and decreased herbivory caused a Caribbean-wide shift from coral- to macroalgal-dominated communities (Aronson & Precht 2001b; Gardner *et al.* 2003). More recently, other important coral species have been affected by new epizootics, which is one reason that coral cover on most Caribbean reefs has continued to decline (Porter *et al.* 2001; Gardner *et al.* 2003).

Evidence from paleontological and ecological monitoring studies indicates that the prevalence and effects of marine diseases in general, and coral epizootics in particular, have

increased substantially over the last 20 years (Harvell *et al.* 1999; Aronson & Precht 2001a; Porter *et al.* 2001). One potential explanation is that environmental conditions have been altered via a variety of human activities, consequently impairing host resistance and/or increasing pathogen virulence (Hayes *et al.* 2001; Harvell *et al.* 2002). For example, nutrient enrichment (increases in the concentration of inorganic nitrogen and phosphorus) could affect coral disease dynamics by increasing pathogen fitness and virulence (Kim & Harvell 2002). Correlative evidence suggests that environmental factors such as nutrient availability, temperature and rainfall could affect the severity of animal and human diseases (Kim & Powell 1998; Harvell *et al.* 1999; Lenihan *et al.* 1999; Pascual *et al.* 2000; Kim & Harvell 2002; Kuta & Richardson 2002). However, field experimentation has rarely been employed to directly assess the influence of environmental variability on the dynamics of marine epizootics.

Here, we describe the first *in situ* test of the hypothesis that nutrient enrichment can increase the severity of coral diseases. We performed field experiments using two important Caribbean coral epizootics as model systems: aspergillosis of the common gorgonian sea fan *Gorgonia*

*ventalina* and yellow band disease in the reef-building coral *Montastraea* spp. Our results indicate that a modest increase in nutrient concentrations can significantly increase coral mortality and disease severity.

## MATERIALS AND METHODS

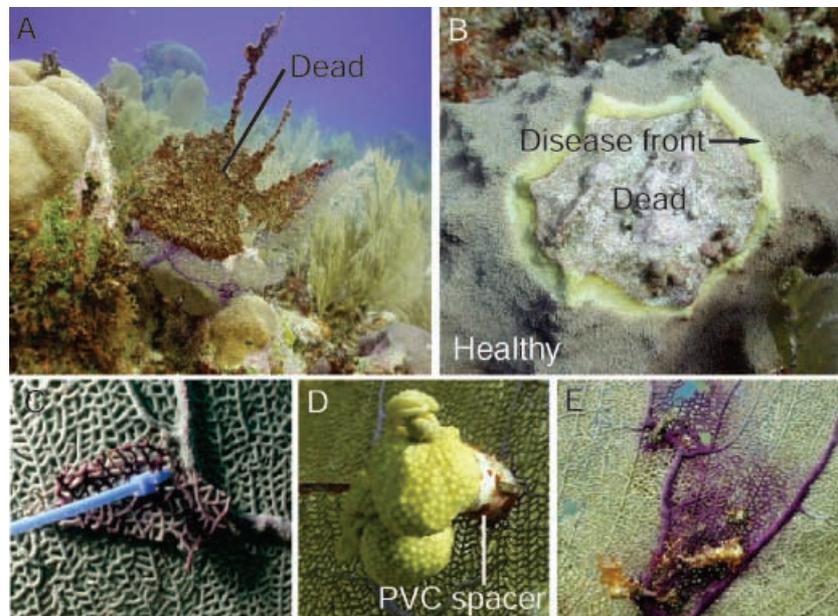
### Study location and systems

Both field experiments were performed on an exposed Caribbean fore-reef in Akumal, Mexico (on the Yucatán Peninsula) at 8–10 m depth and were run for 90 days beginning on 19 June 2002. Sea fan aspergillosis is caused by an infection by the fungus *Aspergillus sydowii*, a generalist pathogen of immune-compromised hosts (Smith *et al.* 1996; Kim & Harvell 2002). Signs of infection include darkly pigmented ('purpled') tissue adjacent to necrotic patches or lesions that can lead to partial or whole colony mortality (Smith *et al.* 1998; Kim & Harvell 2002) (Fig. 1A). Aspergillosis is currently distributed throughout the Caribbean, with the prevalence as high as 100% on some reefs at certain times (Nagelkerken *et al.* 1997; Kim & Harvell 2002). The prevalence and severity of aspergillosis is highly variable at spatial scales ranging from metres to hundreds of kilometres

(Nagelkerken *et al.* 1997; see 'Supplementary Material'). Yellow band disease is also found throughout the Caribbean. Prevalence in the *Montastraea* spp. complex ranges from 4 to 17% along the Yucatán Peninsula (J. Bruno, unpublished data) and has been reported to be as high as 91% on other Caribbean reefs (Cervino *et al.* 2001). The infection is putatively caused by the bacteria *Vibrio* spp. (G. Smith, unpublished data) and typically spreads across the colony surface as a yellow band of infected tissue, 1–3 cm in width (Fig. 1B), at a rate of 0.5–1.0 cm month<sup>-1</sup> (Santavy *et al.* 1999; Cervino *et al.* 2001).

### Aspergillosis experiment

Our approach in the sea fan aspergillosis experiment was to measure the effect of nutrient concentration on disease severity in experimentally infected sea fan colonies. The aspergillosis experiment included five treatments ( $n = 10$ ): (i) unmanipulated control, (ii) procedural control, (iii) experimental infection, (iv) nutrient enrichment, and (v) experimental infection with nutrient enrichment. Healthy sea fan colonies were inoculated by grafting on a piece of infected tissue from a neighbouring diseased colony (Smith *et al.* 1996). The procedural control treatment



**Figure 1** Photographs of the species and techniques used in the coral disease experiments. (A) The sea fan (*Gorgonia ventalina*) infected with aspergillosis caused by the fungus *Aspergillus sydowii*. A significant portion of the colony has died and is being overgrown by opportunistic algae, invertebrates and microorganisms. (B) The Caribbean reef-building coral *Montastraea franksii* infected with yellow band disease. The central portion of the colony is dead. (C) The tissue grafting technique used to experimentally inoculate healthy sea fans in the disease-addition treatments in the aspergillosis experiment. (D) The nutrient bags used to elevate nutrient concentrations in the nutrient-enriched treatments. (E) An infected sea fan exposed to elevated nutrient concentrations in the aspergillosis experiment. The darkly pigmented tissue is a characteristic reaction of sea fans to aspergillosis (Photos by J. Bruno).

included a pseudonutrient bag (without nutrients; see below) and a grafted piece of healthy tissue from a neighbouring colony to control for artefacts resulting from the experimental inoculations. We measured the effect of the five treatments on disease severity by quantifying sclerite and tissue purpling at the end of the experiment (both are correlates of within-colony disease progression). Sclerites are small calcareous structures that form the internal skeleton of sea fans and other Octocorallians, providing structure and a generalized physical defence. Sclerites of *G. ventalina* are typically colourless but turn purple when the proximate tissue is infected with *A. sydowii* or other biotic agents (Nagelkerken *et al.* 1997; Smith *et al.* 1998; Alker *et al.* 2004). This purpling is thought to be associated with chemical and structural resistance (Alker *et al.* 2004). We quantified the proportion of purpled sclerites on each experimental colony by taking a 1.0 cm<sup>2</sup> tissue sample, removing the tissue with diluted bleach, and counting clear and purpled sclerites. The proportion of each experimental colony that was infected was estimated using digital photography and computer image analysis (with Image J software, US National Institute of Health) to measure the area that was darkly purpled, a correlate of the severity of *Aspergillus* infection.

### Yellow band experiment

We used two common species of *Montastraea* in the yellow band disease experiment: *M. annularis* and *M. franksii*. All colonies displayed signs of yellow band disease at the beginning of the experiment. We were not able to experimentally infect healthy colonies due to ethical and logistical constraints. The yellow band experiment included two fixed factors ( $n = 10$ ): treatment (control and nutrient enrichment) and species (*M. annularis* and *M. franksii*). The effects of both factors on rates of disease front advancement across the colony and of host tissue loss were quantified by making *in situ* measurements using callipers and reference nails placed in dead portions of the colonies (i.e. the change in the distance from a reference nail to the

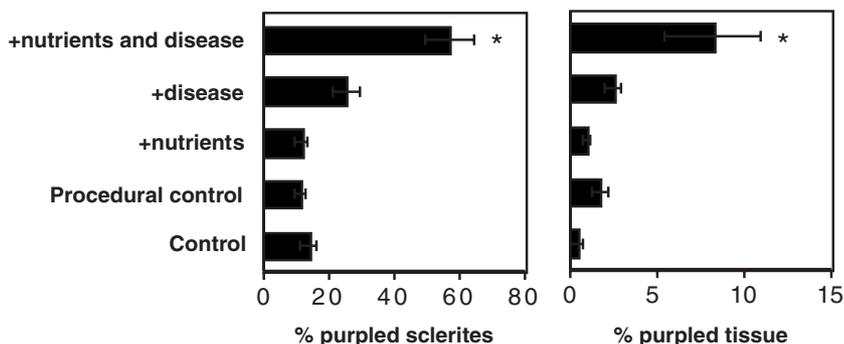
leading edge of the disease front during the experiment was the measure of disease advancement).

### Nutrient manipulations

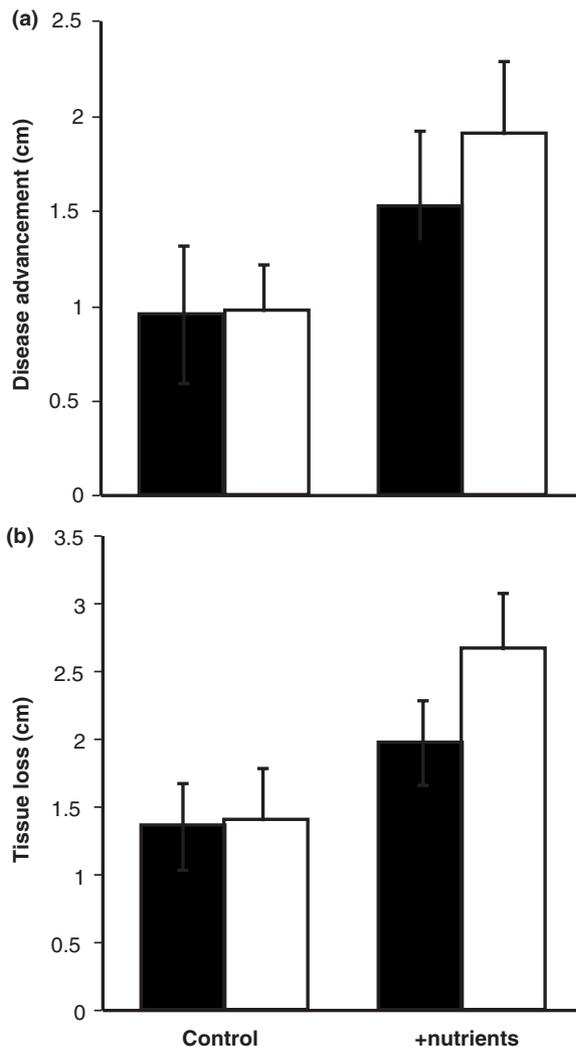
Water column nutrient concentrations were manipulated using nylon nutrient diffusion bags filled with 30 g of Osmocot<sup>TM</sup> (Scotts, Marysville, OH, USA) 9-6-12 time-release fertilizer pellets (Worm *et al.* 2000). Nutrient bags were replaced every 5–7 days. The sea fan nutrient bags were attached 3–5 cm from the inoculation graft. A 1-cm PVC spacer prevented the bag from directly contacting sea fan tissue (Fig. 1D). In the yellow band experiment, nutrient bags were attached 3–5 cm behind the disease front on dead portions of the *Montastraea* colonies (Fig. 1B). Neighbouring experimental colonies were spaced >3 m apart to prevent nutrient contamination of control colonies. Nutrient concentrations were increased by *c.* 10× immediately after nutrient bag replacement but generally levelled off to 2–5× ambient conditions within 12 h. Average concentrations in the nutrient addition treatments ranged from 1.0 to 6.4 µM for nitrate, 0.9 to 4.6 µM for phosphorus and 1.0 to 11.0 µM for ammonium (see ‘Supplementary Material’ for data on ambient nutrient concentrations at the study sites and on the treatment effects of experimental nutrient enrichment). These values are within the range of concentrations resulting from anthropogenic enrichment on coral reefs (Szmant 2002).

### RESULTS

In the aspergillosis experiment, both measures of disease severity varied significantly among the five treatments (Fig. 2) (one-factor ANOVA results: % purpled sclerites  $F = 22.71$ ,  $P = 0.0001$ ; % purpled tissue  $F = 6.54$ ,  $P = 0.0003$ ). Additionally, percentages of purpled sclerites and tissue were significantly higher in the nutrient + disease addition treatment than in any of the other treatments, including the experimental inoculations performed at ambient nutrient concentrations (+disease treatment) and



**Figure 2** Effect of the five experimental treatments on the percentage of purpled sclerites and tissue (two measures of sea fan aspergillosis severity). Bars represent untransformed mean  $\pm$  SE ( $n = 10$ ). Asterisks denote significant differences ( $P < 0.05$ , Tukey–Kramer *post-hoc* tests) from the other four treatments.



**Figure 3** Effect of experimental nutrient enrichment on (a) yellow band disease front advancement, and (b) host tissue loss in the Caribbean reef building corals *Montastraea franksii* (black bars) and *Montastraea annularis* (white bars) during a 90-day *in situ* experiment. Bars represent untransformed mean  $\pm$  SE ( $n = 10$ ).

the procedural controls (Tukey–Kramer *post-hoc* tests  $P < 0.05$ ).

In the yellow band experiment, measured rates of disease advancement and host tissue loss were 0.3–0.5 cm month<sup>-1</sup>, which are concordant with previous results (Cervino *et al.* 2001). Nutrient enrichment had a significant effect on host tissue loss (2-factor ANOVA results:  $F = 6.38$ ,  $P = 0.02$ ) (an average increase of 1.8× relative to controls) and the advancement of the yellow band disease front ( $F = 6.04$ ,  $P = 0.02$ ) (Fig. 3) in both *Montastraea* spp. The species factor (tissue loss  $P = 0.33$ ; disease advancement  $P = 0.67$ ) and the species  $\times$  treatment interaction

(tissue loss  $P = 0.39$ ; disease advancement  $P = 0.71$ ) were not significant for either variable.

## DISCUSSION

Overall, both experiments suggest that a moderate increase in nutrient concentrations can substantially increase the severity of coral diseases. We hypothesize that the experimental pathogens, *A. sydowii* and *Vibrio* spp., utilized the additional nutrients in the enrichment treatments, thereby increasing pathogen fitness and virulence. Marine fungi and bacteria are generally nitrogen limited, especially in nutrient-poor oceans and are also known to take up and utilize dissolved inorganic nutrients, including ammonium and nitrate (Olutiola & Cole 1977; Smith 1988). The relatively minor enrichment was unlikely to harm the experimental coral hosts and possibly benefited them (as their symbiotic zooxanthellae are thought to be nutrient limited) (Szmant 2002). Healthy (uninfected) sea fans in the nutrient addition treatment did not exhibit any signs of infection, supporting the argument that the experimental enrichment alone did not cause the disease signs. Rather, this was evidently driven by an interactive effect of infection and nutrient addition.

Yellow band disease and aspergillosis were first reported in the mid-1990s (Nagelkerken *et al.* 1997; Santavy *et al.* 1999). It is unlikely that nutrient enrichment caused the overall emergence of either epizootic, as there is no evidence for a Caribbean-wide increase in nutrient concentration prior to these outbreaks. However, local nutrient enrichment could potentially affect the dynamics of these and other epizootics on smaller spatial scales and could partially explain the observed variability of coral disease severity. Two recent studies indicate that nitrogen concentrations are positively related to the prevalence of sea fan aspergillosis and black band disease in brain corals in the Florida Keys (Kim & Harvell 2002; Kuta & Richardson 2002). Nutrient enrichment can occur on reefs on a scale of metres to kilometres as a result of human activities and a variety of natural processes, including input from rivers, the seepage of injection-well effluent via groundwater, local upwelling and internal tidal bores (Shinn *et al.* 1994; Nixon 1995; Szmant 2002; Leichter *et al.* 2003). For example, breaking tidal bores on coral reefs in the Florida Keys can increase nitrate concentrations by 10–40× to 4.0  $\mu\text{M}$  (Leichter *et al.* 2003).

Regional-scale marine epizootics with highly uniform patterns of prevalence and severity, such as white band disease in *Acropora* spp. (Aronson & Precht 2001b), are unlikely to be substantially influenced by local-to-landscape-scale environmental variability. Furthermore, the dynamics and effects of most coral diseases are likely controlled by a variety of factors, the relative importance of which probably varies among scales and species. Nevertheless, our results

indicate that moderate nutrient enrichment can significantly increase the mortality of three important Caribbean species by facilitating disease. *Gorgonia ventalina* is one of the most abundant species on shallow Caribbean reefs (Kim & Harvell 2002), and the *Montastraea* spp. complex that includes *M. annularis* and *M. franksii* is critical to reef growth, especially since the collapse of Acroporid populations in the Caribbean (Aronson & Precht 2001b).

The global nitrogen cycle has been dramatically altered over the last 100 years (Vitousek 1994; Nixon 1995). Deforestation and inputs from agriculture and sewage have significantly increased nearshore nutrient flux (Nixon 1995). The degree of nutrient pollution on many Caribbean reefs is currently thought to be minor (Szmant 2002), but will likely increase with human population density and coastal development over the next century (Nixon 1995). Thus, reefs that are still 'pristine' could eventually experience nutrient enrichment and increased coral mortality. Epizootics in the ocean will be more difficult to manage than human, agricultural, or wildlife diseases, because standard management tools of culling, quarantine and vaccination are all unavailable. Managing water quality to minimize nutrient pollution could be an important remediation option; thus, understanding the link between nutrient enrichment and disease severity is a top priority. Identifying other aspects of global change that could influence marine disease dynamics (e.g. increasing ocean temperature) and devising policies to mitigate their impacts are major challenges for ecologists and resource managers.

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#### SUPPLEMENTARY MATERIAL

The following material is available at <http://www.blackwellpublishing.com/products/journals/suppmat/ELE/ELE544/ELE544sm.htm>

**Figure S1** The prevalence (a) and severity (b) of sea fan aspergillosis at three reefs in the upper Florida Keys, USA in October 2000.

**Figure S2** The effect of the nutrient addition treatment (i.e. the addition of nylon bags filled with 30 g of Osmocot<sup>TM</sup> 9-6-12 time-release fertilizer) on nitrate, ammonium and phosphorus concentrations in each of three trials performed *in situ* in Akumal, Mexico.

**Table S1** Nutrient concentrations ( $\mu\text{M}$ ) at eight sites in Akumal, Mexico on the central Yucatán Peninsula where the field experiments were performed.

**Table S2** The effect of the nutrient addition treatment on nutrient concentrations ( $\mu\text{M}$ , trial 2, 20–21 September 2002,  $n = 3$ , Akumal Back Reef, 2 m depth).

**Table S3** The effect of the nutrient addition treatment on nutrient concentrations ( $\mu\text{M}$ , trial 3, 21–25 January 2003,  $n = 3$ , Las Redes Reef, 10 m depth).

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