Global Change Biology

Global Change Biology (2014) **20**, 544–554, doi: 10.1111/gcb.12450

Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching

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Abstract

Nutrient loading is one of the strongest drivers of marine habitat degradation. Yet, the link between nutrients and disease epizootics in marine organisms is often tenuous and supported only by correlative data. Here, we present experimental evidence that chronic nutrient exposure leads to increases in both disease prevalence and severity and coral bleaching in scleractinian corals, the major habitat-forming organisms in tropical reefs. Over 3 years, from June 2009 to June 2012, we continuously exposed areas of a coral reef to elevated levels of nitrogen and phosphorus. At the termination of the enrichment, we surveyed over 1200 scleractinian corals for signs of disease or bleaching. *Siderastrea siderea* corals within enrichment plots had a twofold increase in both the prevalence and severity of disease compared with corals in unenriched control plots. In addition, elevated nutrient loading increased coral bleaching; *Agaricia* spp. of corals exposed to nutrients suffered a 3.5-fold increase in bleaching frequency relative to control corals, providing empirical support for a hypothesized link between nutrient loading and bleaching-induced coral declines. However, 1 year later, after nutrient enrichment had been terminated for 10 months, there were no differences in coral disease or coral bleaching prevalence between the previously enriched and control treatments. Given that our experimental enrichments were well within the ranges of ambient nutrient concentrations found on many degraded reefs worldwide, these data provide strong empirical support to the idea that coastal nutrient loading is one of the major factors contributing to the increasing levels of both coral disease and coral bleaching. Yet, these data also suggest that simple improvements to water quality may be an effective way to mitigate some coral disease epizootics and the corresponding loss of coral cover in the future.

Keywords: bleaching, Caribbean, coral disease, epizootic, eutrophication, outbreak

Received 1 August 2013; revised version received 24 September 2013 and accepted 27 September 2013

Introduction

Disease outbreaks are increasing worldwide and are a growing threat to biodiversity and ecosystem function (Daszak *et al*., 2000; Harvell *et al*., 2004). Several global change drivers may be potential mechanisms for this increase in diseases across disparate taxa (Harvell *et al*., 1999; Daszak *et al*., 2001; Rohr *et al*., 2010). In particular, nutrient loading may increase the prevalence or severity of diseases of a variety of organisms (McKenzie & Townsend, 2007), including plants (Mitchell *et al*., 2003), amphibians (Johnson *et al*., 2007), and fishes (Lafferty, 1997). However, the mechanisms and ubiquity of this effect are not well understood (Johnson *et al*., 2010). Given that rates of nutrient loading are increasing worldwide (Smith & Schindler, 2009), it is imperative to explore how increasing nutrient availability impacts the prevalence and severity of important diseases.

On tropical reefs, coral diseases appear to be fundamentally altering species diversity and ecosystem function. For example, in the Caribbean, often considered a hotspot for coral disease (Harvell *et al*., 2007), repeated outbreaks of several diseases, along with frequent episodes of coral bleaching, have dramatically reduced the abundance of coral across the region (Gardner *et al*., 2003; Schutte *et al*., 2010). Several factors may contribute to these coral disease outbreaks including: (i) temperature stress (Bruno *et al*., 2007; Brandt & McManus, 2009; Sokolow, 2009), (ii) alterations of reef fish abundance and functional diversity (Raymundo *et al*., 2009), (iii) high coastal human population (Aeby *et al*., 2011), (iv) proximity to algae (Nugues *et al*., 2004; Smith *et al*., 2006; Vega Thurber *et al*., 2012), and (v) nutrient availability (Bruno *et al*., 2003; Baker *et al*., 2007; Garren *et al*., 2009).

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Nutrient enrichment is predicted to be the fourth most impactful stressor to coral reefs after increased seawater temperature, high coastal population levels, and overfishing (Halpern *et al*., 2008). Increasing levels of nutrients may be increasing both the prevalence and severity of coral diseases as well as making corals more susceptible to coral bleaching. For example, field surveys suggest that the prevalence of coral disease is often correlated with nutrient concentrations (Kaczmarsky & Richardson, 2010; Haapkylä *et al*., 2011). Yet, it is often difficult to decouple nutrient loading from other co-occurring stressors such as temperature or sedimentation that also could impact disease. However, experiment suggests that increasing nutrient availability can negatively impact coral reproduction, growth, and mortality (Koop *et al*., 2001) and hasten the progression of coral disease (Bruno *et al*., 2003; Voss & Richardson, 2006). Evidence also suggests that nutrients may increase corals' susceptibility to bleaching as elevated nutrient availability may increase abundance of algal symbionts (Marubini & Davies, 1996), which may make corals more susceptible to bleaching when sea surface temperatures rise (Wooldridge, 2009b; Cunning & Baker, 2012; Wiedenmann *et al*., 2012). Despite these proposed links between nutrient availability and coral disease and bleaching, there has been no empirical evidence to date that nutrients can cause an increase in the prevalence of either disease or bleaching in the field.

Here, we describe a long-term nutrient enrichment experiment on a coral reef in the Florida Keys, USA, designed to examine the impacts of anthropogenic nutrient loading on benthic community structure. Over the course of 3 years, we continually added nitrogen and phosphorus to four 9 m^2 enrichment plots that were paired with control plots subject to ambient levels of nutrients. At the end of the experiment, we surveyed the coral community for signs of coral disease and bleaching. We showed that chronic nutrient enrichment significantly increased both the prevalence and severity of Dark Spot Syndrome in *Siderastrea siderea* and increased the prevalence of coral bleaching in *Agaricia* spp. Yet, we also show that 10 months after the enrichment treatment was removed (1 year after the initial survey), the increase in both disease and bleaching had disappeared.

Materials and methods

Study site and experimental nutrient treatments

The Florida Keys reef tract consists of a large bank reef system paralleling the island chain approximately 8 km offshore of the Florida Keys, USA. Coral cover on most reefs in the Florida Keys, including our site, is 5–10%, while macroalgal cover ranges from 5 to 30% depending on the season (Paddack *et al*., 2006; Schutte *et al*., 2010). Our study reef (25°00′05″ N, 80°24′55″W) was a 5–6 m deep spur and groove reef system located near Pickles Reef in the Upper Florida Keys off of Key Largo, FL. In June 2009, a nutrient enrichment experiment was established using eight 3 × 3 m (9 m^2) plots. Each 9 m^2 plot was delineated into 1 m^2 plots with metal nails driven into the reef at the corners and center of each plot. Plots were >5 m from each other. Of our eight, 9 m^2 plots, four plots were enriched with nitrogen and phosphorous and four were control plots with ambient nutrient levels. For the enrichment treatment, 175 g of Osmocote® (19-6-12, N-P-K) slow-release garden fertilizer was placed in 15 × 5 cm (length × inner diameter) PVC tubes or 'nutrient diffusers' with 6, 1.2 cm holes drilled into them. Diffusers were covered in mesh window screen to retain the Osmocote® within the tubes. This formulation of Osmocote® contains no trace metals that could confound the main effects of N and P enrichment and is an efficient method of enriching water column nutrients in benthic systems (Worm *et al*., 2000; Burkepile & Hay, 2009).

Nutrient diffusers were attached to each metal nail within the 9 m^2 enrichment plots for a total of 25 enrichment tubes per enrichment plot. This method of nutrient enrichment significantly increased the levels of dissolved inorganic nitrogen and soluble reactive phosphate approximately 3X above ambient water column values in 7 m^2 areas of seagrass beds (Heck *et al*., 2000) and can last for up to ca. 45 days (Sotka & Hay, 2009). Nutrient diffusers were replaced every ca. 30–35 days to ensure continued delivery of N and P. The enrichment experiment was run consistently for 3 years until August 2012 when Tropical Storm Isaac removed most of the experimental infrastructure.

Nitrogen and phosphorus levels were assessed in the water column above each enrichment and control plot. In July of 2009, 2010, and 2011, divers used 60 ml syringes to slowly draw water from ca. 3 cm above the benthos in either control or enriched plots. Samples were taken ca. 30 days after nutrient diffuser deployment to ensure that enrichment occurred across the duration of diffuser deployment. Immediately after collection, samples were filtered (GF/F) into acid-washed bottles, placed on ice, returned to the laboratory, and frozen until analyzed. Dissolved inorganic nitrogen [(DIN) = ammonium and nitrite + nitrate] and soluble reactive phosphorus (SRP) concentrations were determined via autoanalyzer. Tissue carbon:nitrogen (C : N) levels of the common alga *Dictyota menstrualis* were also assessed as nutrient content of macroalgae reflects ambient nutrient conditions over relatively long time scales (i.e., weeks to months) compared with ambient water column nutrients (Atkinson & Smith, 1983; Fourqurean & Zieman, 2002). *Dictoyta menstrualis* was collected from both enriched and control treatments during the same months as water samples, dried at 60 °C, ground to a powder, and analyzed for %C and %N content with a CHN Carlo-Erba elemental analyzer (NA1500). Nutrient data from both water and algal tissue were averaged across summers for each replicate for statistical analysis.

Disease surveys

In June 2012, we used a 5 m radial belt transect method modified from Santavy *et al.* (2001) to survey coral disease and bleaching in the control ($n = 4$) and nutrient enriched ($n = 4$) plots. (Santavy *et al.*, 2001). Transect tapes were attached to the central nail in each plot and measured out to 5 m along a given heading. A 1 m transect line was placed parallel to the center transect and used to identify every coral within nonoverlapping 1 m wide belts until all corals within the 10 m diameter circle of benthos were surveyed. Each survey covered ca. 79 m^2. Within each radial transect, all corals ≥5 cm in diameter were identified to species (or genus for some smaller corals) and surveyed for signs of disease and/or bleaching. If a coral showed a sign of bleaching or disease, its diameter (cm) was measured along its widest axis running through the center of the colony, and a photograph was taken to confirm the disease type or bleaching and to document disease severity. In addition to the enrichment and control sites, additional external sites ($n = 5$) were surveyed on randomly selected areas of adjacent reef using the same radial transect methodology. We surveyed these external sites to ensure that we had not inadvertently chosen sites with high disease prevalence for the control or enriched sites at the beginning of the experiment or influenced disease prevalence due to diver activity or experimental infrastructure (e.g., metal nails, etc.) around control or enriched sites. All survey sites were greater than 5 m from one another.

Disease presence was defined as any colony with known disease signs (e.g., dark brown spots, red coloration, black, white, or red band formation, white spots, yellow blotches, etc.), whereas the presence of bleaching was defined as the paling or absence of observable pigmentation in living coral tissue. Disease severity was measured as the percentage of a colony's living tissue area affected by the disease. Severity was calculated according to the following index: 0 = 0% of colony affected by disease, 1 = <10% of colony affected, 2 = 10–25% of colony affected, 3 = 25–50% of colony affected, 4 = 50–75% of colony affected, 5 = 75–90% of colony affected, and 6 = >90% of colony affected. Each photograph of diseased *S. siderea* in each plot was scored according to the disease severity index. Each *S. siderea* in each plot recorded as healthy (e.g. no signs of disease) in the field surveys was scored a 0 on the severity index. For each plot, the mean disease severity was calculated across all coral colonies in that plot for: (i) all *S. siderea* and (ii) only diseased *S. siderea*.

In August 2012, the experiment was terminated when Tropical Storm Isaac removed much of the experimental infrastructure thereby ending the enrichment treatment. In both February and June 2013, corals were resurveyed in the previously enriched and control plots to determine if removing the enrichment treatment had affected the prevalence of coral disease and coral bleaching. All *S. siderea* and *Agaricia* spp. were scored as described above. External surveys were not conducted in either sampling period in 2013 as initial surveys showed no differences between controls and external plots.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey post hoc tests was used to identify significant differences among survey types (control, nutrient enriched, and external in initial surveys in June 2012 and control vs. nutrient enriched in resurveys in February/June 2013) for the following: (i) disease prevalence for all corals (% of corals affected by at least one sign of disease), (ii) disease prevalence for individual coral species, (iii) disease severity (% of individual affected by disease), (iv) bleaching prevalence for all corals (% of corals with any sign of bleaching), (v) bleaching prevalence for individual coral species, (vi) diseased coral colony diameter size (cm), and (vii) abundance of individual coral species. The underlying assumptions of these tests were determined graphically (homogeneity of variance) and using a Shapiro–Wilk test (normality).

Multivariate analyses were used to identify differences in coral community composition among the different treatments. Bray-Curtis similarity was used to compare log-transformed relative coral abundance data. The similarity of these data was visualized using multidimensional scaling plots, and significant differences among these surveys were evaluated using an analysis of similarity (ANOSIM). These analyses were performed in PRIMER v. 6 (Clarke & Warwick, 2001).

Results

Chronic nutrient enrichment increased DIN and SRP

To evaluate the impact of nutrients on coral disease dynamics, we enriched four, 9 m^2 plots on a reef in the Florida Keys for 3 years. Measurements of water column nutrients across three different summers showed that DIN was approximately three times higher in the enrichment plots (3.91 ± 1.34 μM) than control plots (1.15 ± 0.05 μM) (ANOVA df = 1, $F = 13.5$, $P = 0.002$). Similarly, SRP was approximately nine times higher in the enrichment plots (0.27 ± 0.07 μM) as opposed to the controls (0.035 ± 0.006 μM) (ANOVA df = 1, $F = 10.7$, $P = 0.017$). Levels of both DIN and SRP in the control plots were within the range of concentrations for offshore reefs, as measured in a 15-year water monitoring program in the Florida Keys (Boyer & Briceño, 2010). For example, Molasses Reef had mean summer (June and July sampling dates) DIN levels of 1.12 ± 0.446 and SRP levels of 0.033 ± 0.004 from 2006 to 2011, almost identical levels to our control plots. Furthermore, levels of DIN and SRP in the enriched treatment were similar to those reported from other anthropogenically impacted reefs located around the world (Dinsdale *et al.*, 2008). In addition, nitrogen concentrations in the tissues of the common alga *Dictyota menstrualis* were 20% higher in the enriched plots compared with the control plots (ANOVA df = 1, $F = 14.7$, $P = 0.001$) suggesting that the nutrients from the

enrichment were consistently available to benthic organisms.

Chronic nutrient enrichment increased disease prevalence and severity

We used 5 m radial transects to survey the coral community in control plots (n = 4), nutrient enrichment plots (n = 4), and external control plots (n = 5) in June 2012. Across these three survey categories, a total of 1213 corals (control: 370; nutrient enriched: 387; external: 455) ≥5 cm were identified and assessed for visual signs of disease and bleaching. Twenty species of corals were identified (Fig. 1) with the most abundant species being: *Siderastrea siderea* (mean 34.14% ± 3.33 SEM), *Agaricia* spp., predominately *Agaricia agaricites*, (mean 24.71% ± 3.33 SEM), *Porites porites* (mean 13.94% ± 2.15 SEM), and *Porites astreoides* (mean 10.64% ± 1.96 SEM). When analyzed across the different treatments (control, enriched, and external), no differences were found in the relative abundances of each taxa (Fig. 1). Multivariate analysis also indicated that there were no differences in coral community structure among treatments (ANOSIM Global R 0.016, P = 0.437).

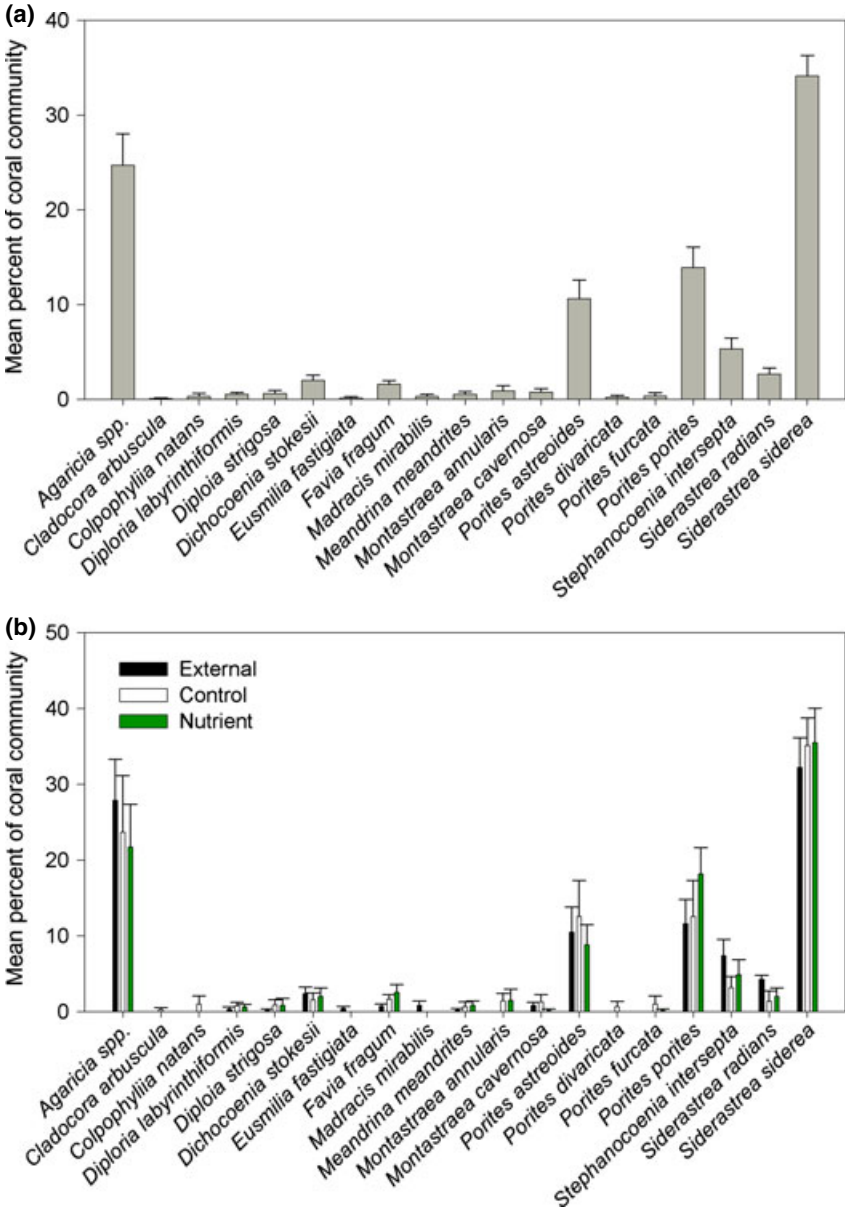

Fig. 1 Coral community composition is similar across experimental and external survey sites. Mean percentage (mean ± SE) of each coral species among all survey sites (a) and of each coral species within external (n = 5 black bars), control (n = 4 white bars), and nutrient-enriched (n = 4 green bars) sites (b).

While the external and control sites had similar mean percentages of corals with signs of disease (8.1% ± 1.64 vs. 7.78% ± 2.61 SEM prevalence), the nutrient enriched sites had >2 times (18.41% ± 2.17 SEM) the disease prevalence (ANOVA df = 2, $F = 7.819$, $P = 0.009$) of external or control sites. The dominant disease identified was 'Dark Spot Syndrome' (DSS) (89% of diseased individuals), which is known to infect more than a dozen species of corals (37). In these surveys, DSS was found on *S. siderea* (n = 88, prevalence = 22%), *Siderastrea radians* ($n = 7$, prevalence = 23%), *Stephanocoenia intersepta* ($n = 2$, prevalence = 7%), and *Agaricia* spp. ($n = 2$, prevalence = 1%) colonies. When individual species were analyzed independently, we showed that nutrient exposure significantly increased (ANOVA df = 2, $F = 7.33$; $P = 0.011$) the mean DSS prevalence in *S. siderea* colonies >2 fold, from ca. 17% in the external and 21% in control surveys to ca. 46% in the enriched sites (Fig. 2a). The only other major disease identified was black band disease (0.74% prevalence in all corals; 7.08% prevalence of diseased corals), which occurred on 8 *S. siderea* colonies (2.0%) and a single *Meandrina meandrites*. The mean colony size for all diseased corals was 9.17 cm, and no differences in mean size of diseased colonies were found among survey site types (df = 2, $F = 0.751$, $P = 0.497$). When only DSS-affected *S. siderea* (mean 10.25 cm ± 0.30 SEM) were analyzed, again no differences (ANOVA df = 2, $F = 1.655$, $P = 0.239$) were found among survey sites.

When *S. siderea* colonies were resurveyed in February 2013, DSS prevalence was marginally higher in the previously enriched treatment (25.1% ± 3.8 SEM of colonies affected) vs. controls, but the difference was no longer significant (14.1% ± 3.0 SEM of colonies affected; ANOVA df = 1, $F = 5.254$, $P = 0.062$; Fig. 2b). Four months later, in the June 2013 resurvey (exactly 1 year after the initial survey), DSS prevalence in *S. siderea* also was now no longer different between the previously enriched (32.9% ± 7.9 SEM of colonies affected) and control treatments (28.5% ± 4.0 SEM of colonies affected; ANOVA df = 1, $F = 0.249$, $P = 0.635$; Fig. 2c).

In addition to disease prevalence, the severity of DSS on individual *S. siderea* colonies showed a significant increase with nutrient enrichment. When diseased *S. siderea* colonies were considered, corals in the enriched treatment showed a higher score on the disease severity index (1.76 ± 0.09) than corals in the control (1.32 ± 0.13) and external plots (1.18 ± 0.08) (ANOVA df = 2, $F = 9.3$, $P = 0.005$). Similar to the DSS prevalence data, there also were no differences in disease severity between the two treatments in February (ANOVA df = 1, $F = 0.380$, $P = 0.560$, Fig 3b) or June of 2013 (ANOVA df = 1, $F = 0.380$, $P = 0.560$: Fig. 3c).

Nutrient enrichment increased bleaching in Agaricia spp

Along with signs of disease, signs of bleaching were recorded for every coral in the surveys. While no difference in the prevalence of bleaching (ANOVA df = 2, $F = 0.509$, $P = 0.616$) was found for all corals collectively (Fig. 4a), when analyzed on an individual species basis, nutrients increased the prevalence of bleaching in *Agarcia* spp. (ANOVA df = 2, $F = 7.424$, $P = 0.011$). The mean bleaching prevalence for *Agarica* spp. in the enrichment treatment (mean 16.6% ± 4.3 SEM) was ca. 4–7 times that of the control (4.26% ± 1.8 SEM) or external (2.44% ± 1.93 SEM) sites (Fig. 4b). No other species showed differences in bleaching across treatments. When corals were resurveyed in February 2013, *Agaricia* spp. showed no signs of bleaching as would be expected during cooler portions of the year when water

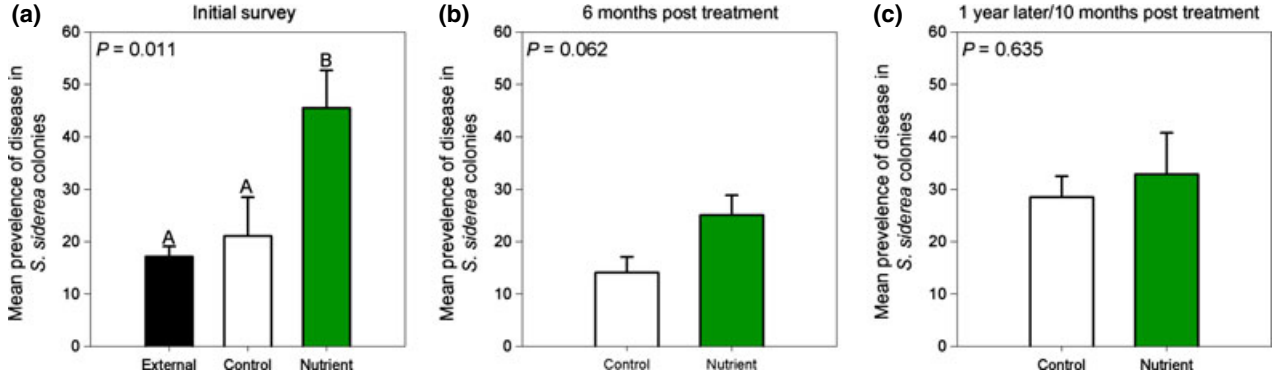

Fig. 2 Nutrient enrichment increases disease prevalence. Mean disease prevalence (mean ± SE) in the dominant coral *Siderastrea siderea* found within external ($n = 5$ black bars), control ($n = 4$ white bars), and nutrient-enriched ($n = 4$ green bars) survey sites in June 2012 (a), February 2013 (b), and June 2013 (c). External sites were not surveyed in 2013. *P*-values are from one-factor ANOVA. Letters above bars denote differences according to Tukey's post hoc tests.

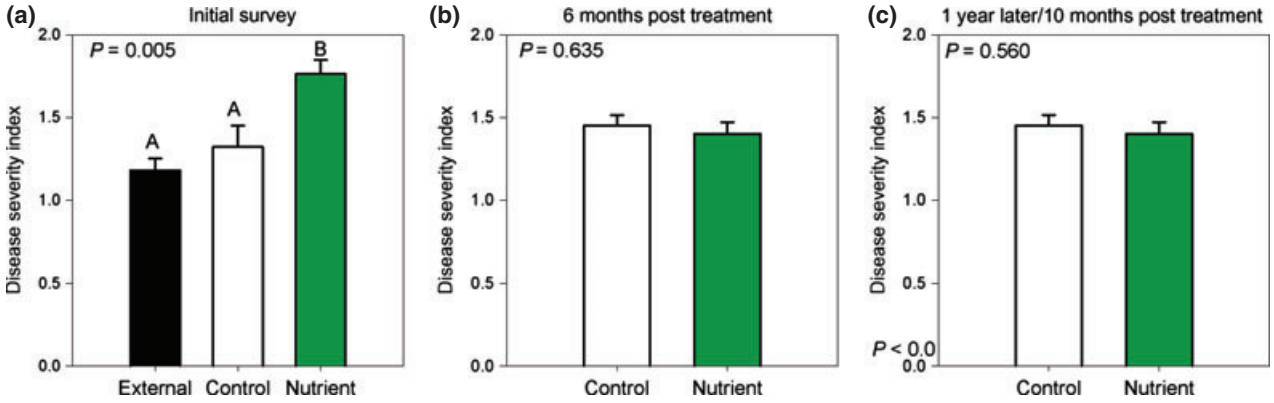

Fig. 3 Nutrient enrichment increases disease severity in *Siderastrea siderea*. Mean disease severity among external (n = 5 black bars), control (n = 4 white bars), and nutrient-enriched (n = 4 green bars) survey sites in June 2012 (a), February 2013 (b), and June 2013 (c). External sites were not surveyed in 2013. Severity index (mean ± SE) was a measure of the % of colony affected by disease: 0 < 10%, 1 = 10–25%, 2 = 25–50%, 3 = 50–75%, 4 = 75–90%, and 5 > 90%. Statistics as in Fig. 2.

temperatures are well below the bleaching threshold. In June 2013, however, minimal bleaching in corals was detected but with no differences between controls (2.7% ± 1.5 SEM of colonies bleached) and previously enriched treatments (2.8% ± 1.7 SEM of colonies bleached; ANOVA df = 1, F = 0.004, P = 0.949; Fig. 4c).

Discussion

Coastal nutrient loading and coral disease

The Florida Keys and wider Caribbean have been described as hot spots of coral disease (Harvell *et al.*, 2007). As disease epizootics in the region have continued, investigators have increasingly attempted to identify the ecological drivers responsible for the increased number and severity of the outbreaks. Although temperature anomalies often show clear connections to some disease outbreaks (Bruno *et al.*, 2007; Brandt & McManus, 2009), until now, no empirical data have definitively shown that chronic nutrient loading increases both the prevalence and severity of coral disease. More importantly, our data clearly show that increases in nutrient loading can cause a disease outbreak in the marine environment.

Overall mean disease prevalence in our control and external surveys (ca. 8%) was within the normal range (1–8%) for most reefs in the Caribbean (38). However, disease prevalence at our nutrient enrichment sites (17%) was on par with only the most disease-impacted reefs, such as those in the Mexican Yucatan (Ruiz-Moreno *et al.*, 2012) and Colombia (Gil-Agudelo & Garzón-Ferreira, 2001). We also found this high level of disease prevalence despite the near absence of

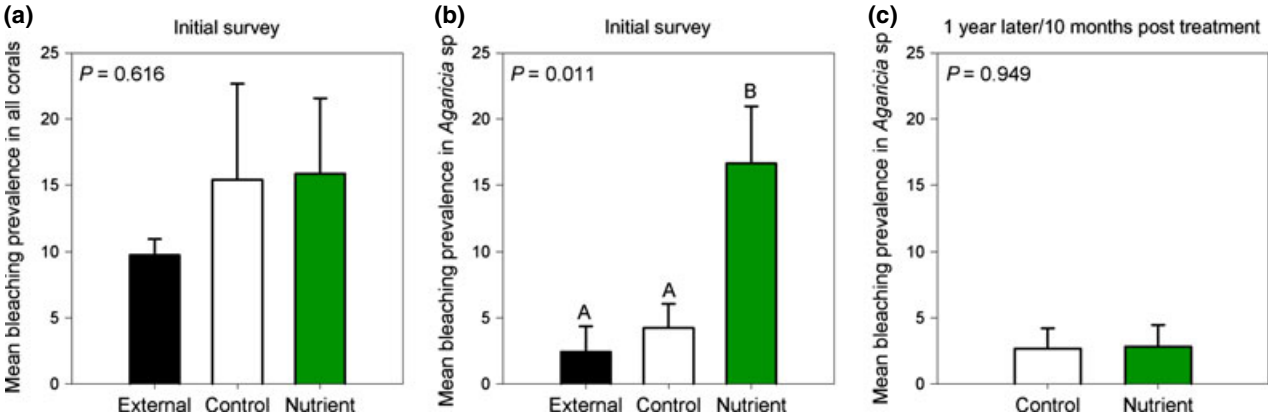

Fig. 4 Nutrient enrichment increases bleaching in *Agaricia* species of corals. Mean percentage (mean ± SE) of all corals species (a) and of *Agaricia* spp. in June 2012 (b) and June 2013 (c) with visual signs of bleaching within external (n = 5 black bars), control (n = 4 white bars), and nutrient enriched (n = 4 green bars) sites. External sites were not surveyed in 2013. Zero bleaching was recorded in February 2013 for both nutrient enriched and control sites. Statistics as in Fig. 2.

Montastrea spp. and complete absence of *Acropora* spp., which are some of the most disease-prone corals in the Caribbean, and the abundance of *Porites* spp. and *Agaricia* spp., which are some of the most disease-resistant species (Ward *et al*., 2006).

'Dark spot syndrome' (DSS) was the most common disease, and nutrient enrichment increased DSS prevalence >100% in the most common coral *S. siderea*. Compared with another recently published survey, we found that within our control surveys, the prevalence of DSS for *S. siderea* was comparable to that recorded in this region of the Florida Keys from 2002 to 2004 (ca. 17 in ours vs. 13%) (Porter *et al*., 2011). In contrast, we found that *S. siderea* in the nutrient-enriched plots had DSS prevalence of 46%, which was >3.5x the mean prevalence rates typically recorded for this species in the Florida Keys, strongly suggesting that nutrients play a major role in the etiology of this disease and perhaps other diseases. The elevated prevalence rates of DSS for *S. siderea* in our study were, however, similar to some rates of this disease previously recorded on reefs in Grenada (42%), Puerto Rico (50%), Bonaire (53%), and Turks and Caicos (56%) (Cervino *et al*., 2001). It is tempting to suggest that such high rates of this disease at these locations may be due to locally high nutrient loading, but there were no assessments of nutrient concentrations at these sites.

The link between nutrient availability and increased disease prevalence is often suggested for coral reefs, but direct evidence without confounding factors has been elusive (Harvell *et al*., 2007). Large-scale surveys of reefs have shown that the prevalence for some diseases is often positively correlated with nutrient availability (Dinsdale *et al*., 2008; Kaczmarsky & Richardson, 2010; Haapkylä *et al*., 2011). However, studies such as these often cannot disentangle the effects of nutrients from the potential impacts of other stressors. For example, the prevalence of the disease atramentous necrosis on *Montipora aequituberculata* on the Great Barrier Reef, Australia, is positively correlated with organic nutrients (Haapkylä *et al*., 2011). Yet, nutrient dynamics are driven by seasonal rainfall and also are negatively correlated with changes in salinity. Thus, it is impossible to disentangle the effect of increased nutrients from the stress of decreased salinity in affecting disease dynamics. In contrast, small-scale nutrient enrichment experiments have shown that increasing nutrient availability to corals, while controlling for other potential confounding factors, increases the severity of existing disease and rates of coral tissue loss (Bruno *et al*., 2003; Voss & Richardson, 2006). For example, Bruno *et al*. (2003) showed that experimental additions of N and P in the field almost doubled the rate of advancement of yellow band disease on *Montastrea annularis*.

Using experimental nutrient enrichment, we, here, were able to remove any potential factors (e.g., depth, salinity, temperature) that often confound interpretation of the relationships between nutrients and disease in large field surveys. Thus, our data suggest that increasing nutrient loading can cause some corals to become diseased without the presence of any other obvious chronic stressor. This finding is significant in that it strengthens the link between nutrient loading and some diseases seen from many correlative field surveys of reefs around the globe. Furthermore, our work expands on previous small-scale studies on the impact of nutrients on disease severity. Our data suggest that disease severity increases with increased nutrient availability. Unfortunately, Tropical Storm Isaac ended the experiment before we could determine if this increase in disease prevalence and severity would result in higher rates of tissue loss or morality for corals under nutrient enrichment. However, our resurvey data suggest that efforts at reducing or remediating nutrient loading could significantly impact the abundance and severity of coral diseases and that such local efforts could have significant positive effects on the health and integrity of reef ecosystems.

One potential driver of the differences in disease prevalence among our different treatments could have been if DSS preferentially affected specific size classes of corals and these size classes were more abundant in our nutrient-enriched treatment. However, our analyses of the size distribution of corals infected with DSS suggest that the disease is evident on corals across the size spectrum. Furthermore, the distribution of disease across the size frequency of corals was similar across the enriched, control, and external sites suggesting that enrichment did not induce disease on size classes of corals that normally do not get the disease, but rather that enrichment merely increased the frequency with which these corals acquire the disease.

Unfortunately, we did not initially score disease or bleaching prevalence when the experiment was established. It is therefore a possibility that the control and nutrient-enriched plots differed in disease/bleaching prevalence when the experiment started and that our June 2012 survey data merely reflected initial differences unrelated to nutrient enrichment. Three lines of evidence suggest that this is not the case. First, in June 2012, we randomly chose five other 5 m radius portions of reef and surveyed as external controls. These external controls were indistinguishable in coral community composition from our experimental controls and nutrient-enriched sites. Second, disease and bleaching prevalence were also not different in the external and

control plots, but were significantly lower than the nutrient enrichment treatment. These data suggest that it would have been highly unlikely, although not impossible, that at the beginning of the experiment, we chose four plots to be our nutrient-enrichment treatments that consistently had more disease and bleaching than our controls. The third, and most compelling piece of evidence to refute this possibility, is that when the nutrient-enrichment treatment was taken away, the differences in disease prevalence, disease severity, and bleaching prevalence between enriched and control plots disappeared. If the differences between control and enriched plots that we showed in June 2012 had been due to initial differences at the outset of the experiment and not the enrichment treatment, then removing the enrichment treatment should have had no effect on the differences between the treatments. Thus, we are confident that the patterns of increased disease and bleaching in the enrichment treatment were due to the effect of nutrient addition to the corals.

Another potential drawback of the study is that we quantified coral disease in 5 m radial transects, which extended beyond the edges of our 9 m^2 enrichment plots. The larger disease surveys suggest that corals at the outer edges of the survey may not have experienced the same nutrient-enriched environment as those corals in the center of our enrichment plots, although the enrichment treatment spread beyond the absolute boundaries of the 9 m^2 plots due to transport by wave action and currents. Thus, our test of the role of enrichment on disease and bleaching is actually likely to be more conservative than if we had limited the corals surveyed to those just within the boundaries of the enrichment treatment. However, the fact that we still showed statistically significant differences in disease prevalence and bleaching with nutrient enrichment despite including corals that would have received less nutrients over time would suggest that the impact of nutrients on DSS and bleaching may be larger than actually observed.

Mechanisms behind nutrient-induced disease

Currently, controversy exists as to whether DSS in *S. siderea* is a physiological disease or one caused from a pathogen. Although DSS was first identified ca. 20 years ago and is one of the most common coral diseases in the wider Caribbean (Solano *et al.*, 1993; Weil, 2004; Gochfeld *et al.*, 2006; Porter *et al.*, 2011), the etiology of DSS is currently unknown. This lack of understanding of the origins of this syndrome makes it difficult to establish the potential mechanism whereby nutrient enrichment increases disease prevalence and severity. Hypotheses about the origin of DSS range from a physiological response to stress to a specific pathogen. For example, while endolithic organisms have been suggested as potential pathogens for DSS in the Caribbean (Renegar *et al.*, 2008) and the Indo-Pacific (Work *et al.*, 2008), others have claimed that bacterial or fungal disease, and even to a disease afflicting the *Symbiodinium* (Cervino *et al.*, 2001; Borger, 2004; Gochfeld & Aeby, 2008) are responsible. Regardless, one of five scenarios is the likely pathway whereby nutrient loading induces this disease: (i) an effect on the host physiology, (ii) an effect on its algal or other microbial symbionts via changes in abundance, physiology, or taxonomic identity, (iii) infection by a putative pathogen, (iv) competition with other benthic community members such as algae, or (v) the combined effect of more than one of these mechanisms.

Even though we do not know the origins of this disease, we can still hypothesize how nutrients may induce and exacerbate DSS. For example, if signs of DSS were the result of pathogen infection, then nutrient exposure could be directly responsible for altering pathogen invasion, growth, and/or virulence pathways. Such metabolic alterations of the coral microbiota have been shown in coral specimens exposed to elevated N and P (Vega Thurber *et al.*, 2009). The fact that DSS was clustered around our nutrient enrichment treatment may suggest a potential pathogen infection that can be transferred from coral to coral; however, spatial analyses of DSS in the Florida Keys suggest that there is rarely clustering of diseased corals either at small (m) or large scales (km) suggesting that these diseases do not follow a standard contagious disease models (Muller & van Woesik, 2012). Thus, our nutrient enrichment treatment may have simply lowered the threshold of corals to resist pathogens that were already present and/or increased the pathogenicity of these microbes. Nutrient exposure may provide the mechanism of pathogenesis by altering the physiology of the host and/or its symbionts. If this disease is not caused by a pathogen, then nutrients may be altering the holobiont's homeostasis, ultimately inducing and/or exacerbating the signs of a physiological disease. Finally, nutrient exposure could alter both holobiont and pathogen physiology simultaneously, ultimately leading to the production of the signs of the disease.

Nutrient-induced bleaching

In addition to disease prevalence and severity, we demonstrated that nutrients also increase the prevalence of coral bleaching. Coral bleaching is largely correlated with anomalous sea surface temperatures. Yet, we showed that *Agaricia* spp. corals, which tend to be fairly susceptible to bleaching (Manzello *et al.*, 2007), were >3X more likely to show signs of bleaching in

our nutrient-enrichment treatments as compared with controls. This pattern was surprising, given that sea surface temperatures were within the normal range for June during our surveys (June 2012 mean water temperature 28.13 ± 0.44 °C) and that the temperature threshold for bleaching in the Florida Keys is ca. 30.5 °C (Manzello *et al*., 2007). Compared with high temperature years, where bleaching prevalence can exceed 40% in the Upper Keys, our bleaching rates were significantly lower in 2012, ca. 15% for all corals combined. However, this bleaching was recorded in June, whereas most bleaching in the Florida Keys occurs in July–September (Manzello *et al*., 2007). Yet, in June 2013, when the nutrient enrichment treatment was not ongoing, bleaching prevalence was similar in the control and previously enriched treatments. Water temperatures in June 2013 (mean water temperature 27.79 ± 0.16 °C) were similar to those for June 2012 suggesting that water temperature was not the driver of these interannual differences. Thus, it appears that our enrichment treatment may have lowered the temperature threshold for bleaching for *Agaricia* spp. Had Tropical Storm Isaac not prematurely ended our experiment, we may have shown that this pattern of nutrient-induced bleaching would get stronger and ultimately result in coral mortality during the warmer late summer months when bleaching is typically most intense (Manzello *et al*., 2007).

The population dynamics of the coral's algal endosymbionts, or *Symbiodinium*, under enrichment conditions may be driving the increased susceptibility to bleaching. Nutrient enrichment often results in an increase in *Symbiodinium* densities within corals (Hoegh-Guldberg & Smith, 1989; Muscatine *et al*., 1989), which may then produce more reactive oxygen species under stressful conditions (Lesser, 1996), one of the triggers for coral bleaching. Thus, the threshold for bleaching threshold can vary in dependence on nutrient levels and symbiont densities and/or growth rates (Wooldridge, 2009a; Wiedenmann *et al*., 2012; Cunning and Baker). For example, Cunning & Baker (2012) used lab experiments to show that increased *Symbiodinium* density in corals results in higher susceptibility to bleaching. Furthermore, recent work from both the Great Barrier Reef (Wooldridge & Done, 2009) and Florida Keys (Wagner *et al*., 2010) suggests that DIN levels are positively correlated with bleaching prevalence, particularly during normal temperature years rather than extreme years.

Our enrichment treatment not only increased the availability of N and P but also altered the stoichiometric ratios of these nutrients from a 33 : 1 N : P ratio in the controls to 14 : 1 N : P in the enrichment treatment. Such alterations of stoichiometric ratios of nutrients can also potentially impact coral physiology and increase susceptibility to bleaching and increase their mortality under heat stress (Wiedenmann *et al*., 2012). However, the pattern we show is opposite to that of Wiedenmann *et al.* (2012) who used laboratory experiments to show that a higher N : P ratio makes corals more susceptible to bleaching. Yet, in our long-term field experiments, we showed that increasing overall loading of N and P but lowering the N : P ratio vs. ambient levels resulted in more coral bleaching. Regardless of the direction of change, alterations of ambient N : P appear to alter coral physiology in ways that make them more susceptible to bleaching and/or disease.

Like many field surveys of coral disease, studies linking nutrient loading to higher susceptibility of coral bleaching often cannot disentangle the impact of nutrients with other factors that co-occur, such as variation in salinity or sedimentation that may also influence bleaching. Our field experiment provides a critical and unconfounded link between lab experiments and field surveys confirming that nutrient loading lowers bleaching thresholds in some coral species. Ultimately, these data suggest that local-scale nutrient loading may exacerbate regional or global-scale thermal stressors that influence the dynamics of some coral diseases and bleaching, but importantly that reducing nutrient inputs may be a viable management tool for mitigating these threats to corals.

Future directions

Several future experiments should be conducted to better evaluate how chronic nutrient exposure can lead to increased disease and bleaching prevalence. First, it would be ideal to determine whether this disease is truly the result of a pathogen, physiological alterations, or both. Comparative metagenomic amplicon sequencing (e.g., bacterial/archaeal and fungal specific markers) and analysis of samples taken directly from healthy and diseased corals may confirm the presence of the pathogen. Physiological tests could also be conducted. However, unless the pathogen or physiological change and the signs of disease are coincident, lack of an observable taxonomic or physiological signature could be inconclusive. A better strategy would be to continuously sample and evaluate the microbiomes and physiology of healthy individuals under nutrient stress until signs of the disease are manifested. Analysis of those temporal samples may then indicate the appearance/elevation of a new microbe or a signature alteration in host or symbiont physiology. Secondly, evaluation of whether the resulting increases in disease and bleaching were the result of nitrogen, phosphorus, or both should be conducted. As nitrogen and

phosphorus have variable effects on different metrics of coral health and microbial physiology (Koop *et al.*, 2001; Fabricius, 2005; Wiedenmann *et al.*, 2012), experiments that expose corals to nitrogen and phosphorus in different ratios or different forms (e.g., ammonium vs. nitrate) would identify a more specific disease–nutrient link. Finally, chronic vs. episodic nutrient stress may have fundamentally different effects on corals and coral disease, and more detailed studies of how the timing and intensity of nutrient enrichment affects disease dynamics are clearly needed.

Corals are essential habitat-forming species on tropical reefs, but are rapidly declining, particularly across the Caribbean, due to local and global stressors. In the Florida Keys where this study was conducted, coral cover is at historical lows from highs of ca. 60% in the 1970s down to 5–10% recently (Gardner *et al.*, 2003; Schutte *et al.*, 2010). As reefs have degraded, managers have sought out the causes and solutions to these declines. For example, Sutherland *et al.* (2011) found that one disease of corals, white pox or acroporid serratiosis, was directly linked to the human pathogen *Serratia marcescens* invading near shore reefs from human wastewater and infecting and killing the once dominant, but now rare, elkhorn coral *Acropora palmata.* One of the new dominant corals in this region, *S. siderea*, was the most affected by disease in our study, with nutrient enrichment increasing disease prevalence by 170% and almost half the individuals in the nutrient-exposed areas getting disease. Of the remaining *S. siderea* coral cover in the Florida Keys, ca. 1% is lost annually from DSS under normal conditions (Porter *et al.*, 2011); our data suggest that, at a minimum, chronic nutrient exposure could triple this loss rate to 3% per year given the increase in both disease prevalence and severity.

Given that coral disease and bleaching are some of the primary killers of corals worldwide (Bruno *et al.*, 2007; Harvell *et al.*, 2007), conservation efforts that reduce nutrient loading and lower the prevalence and severity of disease and bleaching may be effective strategies for helping preserve reef ecosystems. However, a key step will be to understand which coral diseases respond to nutrient availability. In the Caribbean, yellow band disease (Bruno *et al.*, 2003), black band disease (Voss & Richardson, 2006), and DSS (this study) have all been shown to increase in prevalence or severity with increasing nutrient availability. However, broad-scale outbreaks of diseases like white-band disease on *Acropora* spp. would seem unlikely to be influenced by nutrient availability, given that disease has killed corals across both heavily impacted and relatively pristine reefs throughout the Caribbean (Aronson & Precht, 2001). Thus, a more in-depth understanding of how water quality affects the dynamics of different coral diseases and bleaching will be required, so that proactive measures to improve water quality can be targeted to help minimize the effects of specific diseases.

Acknowledgements

We thank the numerous members of Team HERBVR for long-term maintenance of the experiment, assistance with disease surveys, and preparation of the samples. We are grateful to the Florida Keys National Marine Sanctuary for supplying the permits to conduct this study (permit #'s FKNMS-2009-47 and FKNMS-2011-90). Molasses Reef DIN and SRP data were provided by the SERC-FIU Water Quality Monitoring Network, which is supported by EPA Agreement #X994621-94-0 and NOAA Agreement #NA09NOS4260253. Special thanks also are given to Marilyn Brandt for stimulating discussion on these data. This research was supported by National Science Foundation grant OCE-1130786 to D.E.B and R.V.T., a National Science Foundation, Research Experience for Undergraduates (REU) supplement to support the training of C.F. and the College of Arts & Sciences at Florida International University. The authors confirm that they have no potential conflicts of interest regarding the data and experiments described in this manuscript.

Author contributions

R.V.T and D.E.B. designed the research, analyzed the data, and wrote the manuscript; all authors performed the research and contributed to editing the manuscript.

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