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Nutrient manipulation methods for coral reef studies: A critical review and experimental field data

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Abstract

The results reported in this paper demonstrate suboptimal experimental designs in some of the previously published manipulative methods and provide insights for the improvement of in-situ nutrient studies on coral reefs. Overgrown 0.5-liter porous clay-pot diffusers ("mini-reefs"—following a decade of recruitment, colonization and competition) were utilized to evaluate protocols for studies of controlled nutrient enrichment on coral reefs. A commonly used fertilizer, Tree Food Stakes resulted in detrimental 11-fold and 20-fold decreases of fleshy algae and calcareous coralline algae, respectively, relative to the Control treatments; while blue-green algae (Cyanobacteria) became significantly (6 times) more abundant. Osmocote-filled mini-reefs showed no such negative differences in mortality from the Controls for any functional group. By avoiding the pitfalls of inappropriate sources of enrichment, insufficient durations of colonization/competition studies, suboptimal study areas and inadequate nutrient detection limits in future research, the potential to provide new insights into the nutrient status of coral reefs is greatly increased.
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1. Introduction

A fundamental goal in ecology is to understand the mechanisms by which natural and anthropogenic factors may alter or maintain interactions and structure in biotic communities. The concepts of "top–down" and "bottom–up" controls have been used (e.g., Atkinson and Grigg, 1984) to describe mechanisms where either the actions of predators or resource availability regulate the structure of coral-reef communities. These factors provide a useful perspective to assess human activities that affect the interactive mechanisms controlling stable states and phase shifts (e.g., eutrophication, destructive overfishing) among the dominant functional groups on healthy tropical reefs (see Littler et al., in press, Relative Dominance Model, RDM). Top–down control by abundant populations of large mobile herbivores has been documented repeatedly for nearly half a century (i.e., since the time of the classic exclusion study by Stephenson and Searles, 1960), in strong support of the prominent role of herbivory on coral reefs. Many workers (reviewed in Steneck, 1989; McCook, 1999; Bellwood et al., 2004) have verified that decreasing

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herbivory without changing nutrient inputs (assumed in most grazing studies) usually results in rapid increases of fleshy algae on coral reefs. However, the importance of nutrients (Smith et al., 2001; Littler et al., in press) has been less frequently supported and often disputed (e.g., Miller et al., 1999; Lesser, 2004). This discrepancy is largely due to insufficient experimental data and fewer advocates as a function of the greater logistical difficulties in conducting manipulative nutrient enrichment studies.

Several short-term (<4 months) studies manipulating both nutrients and herbivory (Thacker et al., 2001; Belliveau and Paul, 2002; Miller et al., 1999) did not show algal stimulation following nutrient enrichment (counter to the RDM), although early recruitment data were obtained. In most of these in-situ studies, the nutrient component was either unnatural (see review by Worm et al., 2000), or the duration was too short for competition/dominance interactions to occur (e.g., Miller et al., 1999; Thacker et al., 2001; McClanahan et al., 2002; Belliveau and Paul, 2002). Also, adequate nutrient analyses sometimes were lacking (e.g., no NH_4^+ data, high detection limits, no repeated monitoring) or, most importantly, ambient nutrient background concentrations already exceeded critical levels for ample algal growth (e.g., Larkum and Koop, 1997; Thacker et al., 2001; Belliveau and Paul, 2002).

A sophisticated nutrient enrichment experiment on a large scale (ENCORE Program; Larkum and Koop, 1997) produced useful, but less than ideal, results because: (1) ambient nutrient concentrations within the lagoon at One Tree Island, Australia were already well above levels that are more than sufficient to sustain the surrounding luxuriant frondose macroalgal indicator communities (Bell, 1992; Lapointe, 1997), while being inhibitory to several reef-building corals (e.g., Høegh-Guldberg et al., 1997) and (2) the experimental organisms were isolated and monitored for weight gains, precluding natural encroachment, overgrowth or other key competitive interactions central to the RDM. Interestingly, all increases in nutrient levels adversely affected coral reproduction (Koop et al., 2001). Other laboratory and field experiments (e.g., Pastorok and Bilyard, 1985; Tomascik and Sander, 1987; Muscatine et al., 1989; Stambler et al., 1991; Jokiel et al., 1994) also concluded that corals are negatively affected by increased levels of nutrients. However, eutrophication effects are often species specific to different degrees and on varying scales, and several authors (e.g., Atkinson et al., 1995; Grigg, 1995; Steven and Broadbent, 1997; McCook, 1999; Bongiorni et al., 2003) have indicated no substantial detrimental responses of some coral species to elevated nutrients.

Using experimental in-situ approaches on appropriately healthy (i.e., low nutrient) coral-dominated reefs, Smith et al. (2001) and Littler et al. (in press) provided the most relevant evidence demonstrating the importance of both nutrient and herbivory interactions, and the present study builds on these findings. Additionally, highly diverse large-scale aquarium systems of healthy coral-reef communities (i.e., mesocosms), which operated for decades (Small and Adey, 2001), clearly have demonstrated that minute increases in nitrogen and phosphorus reduce coral growth (sometimes causing substantial die-backs). Furthermore, such self-contained systems require an abundance of fish and invertebrate grazers, in concert with continuous nutrient removal by scrubbers or protein skimmers, to maintain a rich coral and algal diversity.

The decline of herbivorous fishes and other keystone grazers has been identified as the leading cause of harmful macroalgal blooms on the reefs of southeast Florida and Jamaica (Hughes et al., 1999; see also Jackson et al., 2001; Pandolfi et al., 2003). However, the compelling information summarized in Lapointe et al. (2005) that documents the broad scale and escalating rate of anthropogenic nutrient pollution and its consequences to Florida's coral reefs is seldom discussed (Szmant and Forrester, 1996; Precht and Miller, 2006). The intriguing explanation (Jackson et al., 2001) invoking large keystone carnivore and herbivore losses for the decline of coastal ecosystems, including the emergence of harmful macroalgal blooms, has been questioned by Boesch et al. (2001). The mitigating factor is timing, since the large carnivores and herbivores were gone centuries ago, yet the decline of reefs has accelerated dramatically within the past several decades.

Because coral reefs reach their peak development in the most oligotrophic of warm oceanic waters, they would be predicted to be sensitive to increases in the concentrations of dissolved inorganic nitrogen ($DIN = NH_4^+ + NO_3^- + NO_2^-$) and soluble reactive phosphorus ($SRP = PO_4^{3-}$) associated with cultural eutrophication. Nutrient enrichment of coral reefs has complex direct and indirect effects that, over sufficient time, can degrade resiliencies and result in alternative states dominated by fleshy, non-calcifying macroalgae (Hatcher, 1984; Knowlton, 1992; Lapointe et al., 1993, 1997; Lapointe, 1997; NRC, 2000; Bellwood et al., 2004). Very low universal nutrient thresholds have been hypothesized (NTH, Bell, 1992; Lapointe et al., 1997) regarding the lowered resiliencies

for transitions from coral domination toward fleshy algal states.

Therefore, further understanding of the complex effects of nutrient enrichment (bottom–up), in addition to the wealth of data from herbivore exclusion-cage experiments (top–down), is central to the elucidation of mechanisms that mediate relative dominances/stable states on coral reefs. The pressing challenge is to rigorously conduct large-scale in-situ manipulations in extreme low-nutrient coral-dominated settings (e.g., Smith et al., 2001; Littler et al., in press), in conjunction with staged competitive bouts among the major functional groups, to determine how herbivore/nutrient interactions affect relative dominances over sufficient periods of time. The present study, by employing a completely randomized, interspersed, independent (Hurlbert, 1984) design that combines different levels and kinds of nutrient enrichment (utilizing long-established functional groups of reef organisms growing on 0.5-liter porous clay-pot mini-reefs), partly addresses this need and provides new insights into the design of nutrient studies for coral reefs.

2. Methods

2.1. Study area

The Belize Barrier Reef complex is the largest coral-reef tract in the western hemisphere (over 250 km in length and from 10 to 32 km wide), consisting of an almost unbroken barrier reef containing hundreds of patch reefs and mangrove islands. Carrie Bow Cay (CBC, Fig. 1) reef habitats and surrounding environs comprise a well-developed, representative, barrier-reef system that thus far has remained beyond major mainland influences. Offshore Secchi-disc depths in excess of 43 m are typical, indicating Jerlov (1976) Type I oceanic waters. Most importantly, nutrient levels above the NTH concentrations (Bell, 1992) that potentially enable macroalgal overgrowth (i.e., >0.1 μM SRP and >1.0 μM DIN) have seldom been recorded (Lapointe et al., 1987, Table 1) from coral reefs of this system.

The Study Site (Fig. 1), located 35 m shoreward of the intertidal and spatially complex reef crest on the

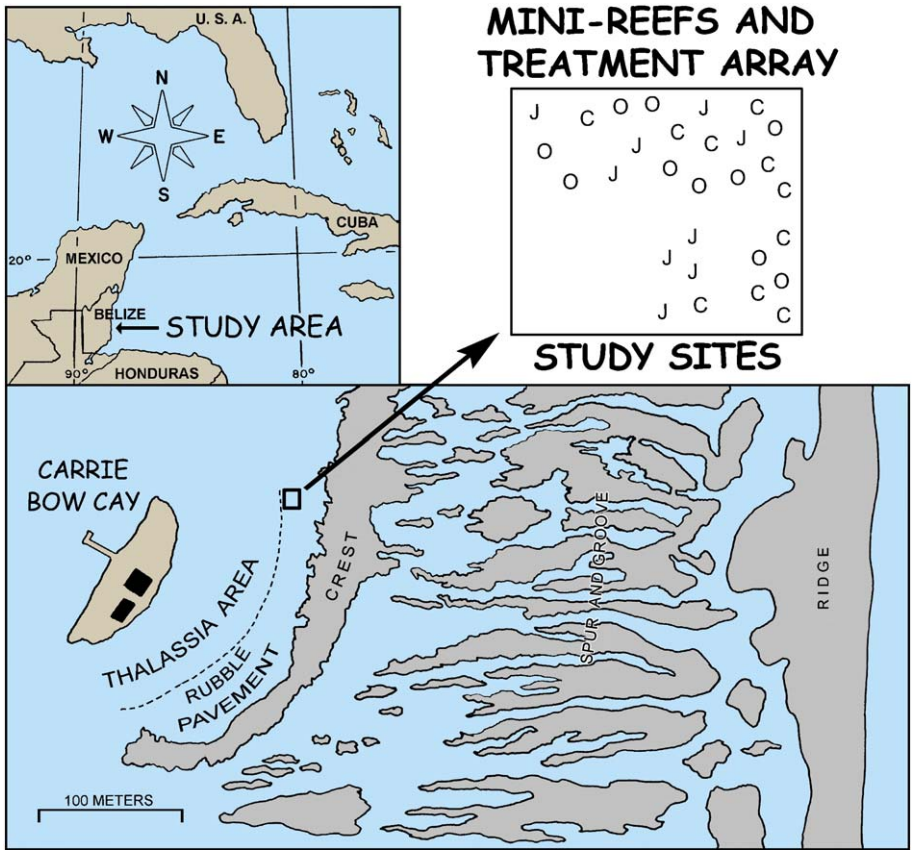

Fig. 1. Location of the study site and randomized 30-diffuser array on the CBC back-reef flat (C=Controls, O=Osmocote, J=Tree Food Stakes).

Table 1
Nutrient data from the CBC back-reef study site (ranges and means ±1 S.D., $N=10$)

Nutrients	DIN levels (μM)	SRP levels (μM)
Control	UD to 0.61	UD to 0.09
	(0.38±0.09)	(0.04±0.02)
Tree Food Stakes	2.0 to 9.5	0.18 to 0.70
	(4.1±0.73)	(0.38±0.03)
Osmocote	1.9 to 7.8	0.12 to 0.88
	(3.9±0.87)	(0.30±0.06)

DIN=dissolved inorganic nitrogen, SRP=soluble reactive phosphorus, UD=undetectable (not used in means). All experimental means increased significantly ($P<0.05$) relative to Controls.

northeast side of CBC (16°48′N, 88°05′W), is not unlike the back-reef algal–coral systems found throughout much of the Belizean barrier tract (Burke, 1982, personal observations). The community composition and zonational patterns of the CBC region are also representative of much of the entire barrier reef platform (Burke, 1982; Littler et al., 1989, 1995, in press).

The experimental site is located in the structurally homogeneous algal/coral-dominated rubble–pavement zone that does not support damselfish or other potentially confounding organisms. Based on earlier transects (Littler et al., 1989, in press), this site is rarely frequented by herbivorous fishes because of the lack of both large- and small-scale structural shelter from predatory carnivorous fishes (e.g., barracudas, sharks, jacks, snappers) and birds (e.g., ospreys, herons, cormorants, pelicans), which forage daily on the back-reef flat (personal observations). Furthermore, bite scars of fishes were not observed (Figs. 4, 5) on any of the randomly distributed mini-reefs (Fig. 1) prior to, or during, the study.

A previous project (Littler et al., 1989) established a compact experimental system (about 3 m by 8 m, Fig. 1) on the reef flat at CBC that was never used (until now). Over a decade earlier, the open ends of 36 terra-cotta clay-pot nutrient diffusers (0.5 l) had been glued haphazardly to the scraped reef substrate at about 0.5 m intervals using underwater epoxy cement to fully cover the interior bottoms. The taxa from the four functional groups colonizing the mini-reefs are listed in Table 2, along with their initial abundances. We utilized these overgrown clay-pot diffusers (i.e., hollow mini-reefs, Figs. 4, 5), that had developed uniform biotic cover (Table 2) indiscernible from the natural surrounding reef-flat community, for testing hypotheses concerning the advantages/disadvantages of the two most commonly used coated nutrient sources for enrichment studies (i.e., Tree Food Stakes and Osmocote slow-release fertilizers). The present methods (detailed below) replicated the effects of nutrients (bottom–up) on established competitive interactions (each mini-reef contained in excess of 100% biotic cover); this enabled a shorter experimental time-frame to be used.

2.2. Sampling methods

To characterize the nutrient environment of CBC, water samples were collected from the study site (Fig. 1) in clean 100-ml polyethylene bottles. Each sample was taken as three separate replicates (to increase coverage) and pooled (to reduce analytical costs). Samples were obtained 3 cm from the bases of the overgrown clay-pot diffusers (see description below, Figs. 4, 5) 3 days following the addition of fertilizer ($N=20$ separate samples of three pooled replicates each) during midday. At the same time, an additional 10 concurrent samples were taken 3 cm from the bases of the non-enriched (Control) mini-reefs (additional samples were taken from inside 3 separate mini-reefs, near the sealed bottoms) to compare both natural and enriched levels of nutrients. We prefer porous terra-cotta flowerpots as nutrient diffusers to provide gradual release, realistic

Table 2
List of the predominant algal and coral groups and taxa and their initial abundances (% cover) on the mini-reefs

Algal and coral groups and taxa	Mean percent cover
Corallines and other calcified algae	89.0
Porolithon pachydermum (Fosl.) Fosl.	85.5
Hydrolithon boergesenii (Fosl.) Fosl.	
Amphiroa rigida var. *antillana* Børg.	
Halimeda opuntia (L.) Lamour.	0.5
Jania capillacea Harvey	1.0
Jania adhaerens Lamour.	1.0
Peyssonnelia sp.	
Neogoniolithon strictum (Fosl.) Setch. et Mason	1.0
Fleshy algae forming mixed turfs	38.3
Caulerpa racemosa (Forssk.) J. Ag.	
Centroceras clavulatum (C. Ag.) Montagne	
Coelothrix irregularis (Harvey) Børg.	
Dictyota pulchella Lamour.	5.2
Dictyota sp.	0.2
Digenea simplex (Wulfen) C. Ag.	
Gelidiopsis/Gelidiella/Gelidium turf	32.9
Heterosiphonia spp.	
Laurencia papillosa (C. Ag.) Greville	
Lobophora variegata (Lamour.) Womersley	
Neomeris annulata Dickie020.	
Padina sanctae-crucis Børg.	
Turbinaria turbinata (L.) Kuntze	
Blue-green algae (Cyanobacteria)	6.1
Corals	1.1
Porites astreoides	0.9
Siderastrea radians	0.2

gradients and uniform application (Littler et al., 1989, in press), following their introduction for this purpose by Chapman and Craigie (1977). Even though partly coralline/coral encrusted, the fertilized mini-reefs were effective in significantly elevating adjacent water-column nutrients (see Table 1).

The seawater samples were immediately filtered through Gelman 0.45 μm GF/F filters, placed in a cooler of ice and frozen in the laboratory until analysis. Dissolved inorganic nitrogen and soluble reactive phosphorus concentrations were determined by the Nutrient Analytical Services Laboratory, Chesapeake Biological Laboratory, Solomons, MD. SRP and NO_3^- were measured with a Technicon Autoanalyzer II; whereas NH_4^+ and NO_2^- were analyzed using a Technicon TRAACS 800. The detection limits for NH_4^+, NO_3^- plus NO_2^- and SRP were 0.21, 0.01 and 0.02 μM, respectively (Table 1).

The back-reef study site ranged from 0.4 to 0.6 m deep. The current speeds were measured initially under typical non-storm wind and wave conditions and 16 days later by fluorescent dye injected next to the nutrient diffusers on the bottom and by timing the movement over a horizontal distance of 2.0 m.

A thorough analysis of 33 in-situ nutrient enrichment studies by Worm et al. (2000), as well as their own experimental data, led the foregoing authors to recommend coated slow-release fertilizer pellets or tree stakes, because these facilitate a controlled application. A decade earlier more than 36 independent, unglazed clay pots (0.5-liter volume) had been glued upside down to the sealed calcium carbonate reef substrate at about 0.5 m distances from each other using marine epoxy-putty to completely seal the interior bottoms and rims. We utilized 30 of these shallow-water "mini-reefs", each dominated by the same algal indicator groups (coralline algae, mixed fleshy algal turfs and blue-green algae, see Table 2 for complete taxonomic list and initial abundances), which had overgrown the unused terra-cotta diffusers remaining from the earlier project (Littler et al., 1989). Ten of these mini-reefs were randomly chosen as Controls, 10 as Osmocote enriched and 10 as Tree Food Stakes enriched (Fig. 1).

A measured amount (0.45 l volume) of Osmocote (Sierra Chemical Co., California, USA—available at most garden stores) slow-release fertilizer containing 18% N (as ammonium nitrate and ammonium phosphate) and 6% P (as ammonium phosphate and calcium phosphate) was poured into each of 10 mini-reefs (each randomly selected for treatment) until nearly full, and the holes were then stoppered. Another 10 were each selected at random and pulsed with an equal amount (0.45 l) of crushed slow-release Tree Food Stakes/ Jobe's Tree Spikes (Domestic Fertilizer, Inc., Paris, Kentucky, USA—also available at most garden stores) containing 15% N and 7% P (as ammonium phosphate and ammonium sulfate, see Fig. 2 for complete contents). The remaining 10 mini-reefs (Controls) were flushed and filled with ambient seawater and stoppered.

Abundances of each established functional group were determined at both the time of initial set-up and then again 16 days later. This period was adequate for demonstration of mortality effects since recruitment, settlement, colonization and competition had already occurred. Detailed field estimates were made using magnifying lenses, followed by taking macro-images of the top, east, west, north and south sides (108 cm^2, 9×12 cm framer) of each mini-reef. The images were scored for percent cover of predominant taxa (see details in Littler and Littler, 1985). The increased magnification afforded by macro-photography of the 108-cm^2 photo-plots enhanced the resolution and, in conjunction with the field notes, facilitated discrimination of microscopic turf species and crusts (Table 2).

Comparisons were made between treatments to detect changes in the relative abundances of the benthic groups that decreased or persisted on the 30 mini-reefs over the 16-day study period. Each of the two experimental nutrient treatments and the Controls were analyzed separately for the percent cover growth response by the three dominant functional-group categories, blue-green algae, coralline algae and mixed fleshy algal turfs (corals and frondose macroalgae were present, but only in trace amounts, on the mini-reefs).

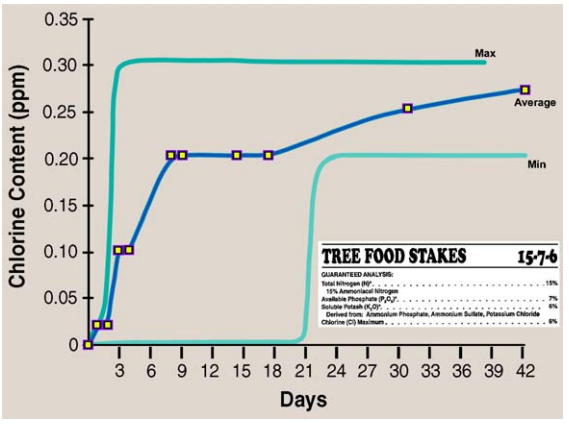

Fig. 2. Mean chlorine release levels over a 40-day period for 5 single Tree Food Stakes, each placed in separate 4.0-liter flasks of distilled water. The advertised contents are shown on the scanned label. Zero chlorine was detected in the Osmocote and distilled water Controls.

For each category, a repeated measures ANOVA (SAS, 2003, v. 9.1; SAS Inst., Inc., Cary, NC) was conducted using percent cover at the initial set-up census as the baseline and the subsequent censuses as the response (dependent variable) to test for significant reactions to the main effects (i.e., independent variables = enrichment, time) and possible interactions. To test the null hypothesis that the percent cover differences of functional groups under elevated (Tree Food Stakes vs. Osmocote) vs. ambient (Controls) nutrients were not statistically different (at alpha = $P > 0.05$), we used one-way ANOVA followed by Bonferroni (Dunn), a posteriori, multiple classification analysis (SAS, 2003, v. 9.1). All percent cover data were arcsine transformed prior to analysis.

The release pattern of free chlorine from Tree Food Stakes was documented over a 6-week period (Fig. 2). Five replicate Tree Food Stakes (113 g each), five 113-g Osmocote samples and five distilled-water Controls were each placed in 4000-ml Erlenmeyer flasks (15 total) filled with distilled water and stoppered. Samples were analyzed every few days for 40 days using Orthotolidine as the indicator for total chlorine (Bio-Guard 2^n1 Total Chlorine and pH Kit, Bio-Lab, Inc., Decatur, GA).

3. Results

3.1. Environmental data

The ambient DIN and SRP concentrations next to the bases of the non-enriched Control mini-reefs (Table 1) were almost undetectable (means ± S.D. = 0.38 ± 0.09 μM DIN and 0.04 ± 0.02 μM SRP), indicating healthy oligotrophic conditions. The same was true for samples taken from inside 3 of these mini-reefs, which were not significantly ($P > 0.05$) different from the outside ambient values. Conversely, both Tree Food

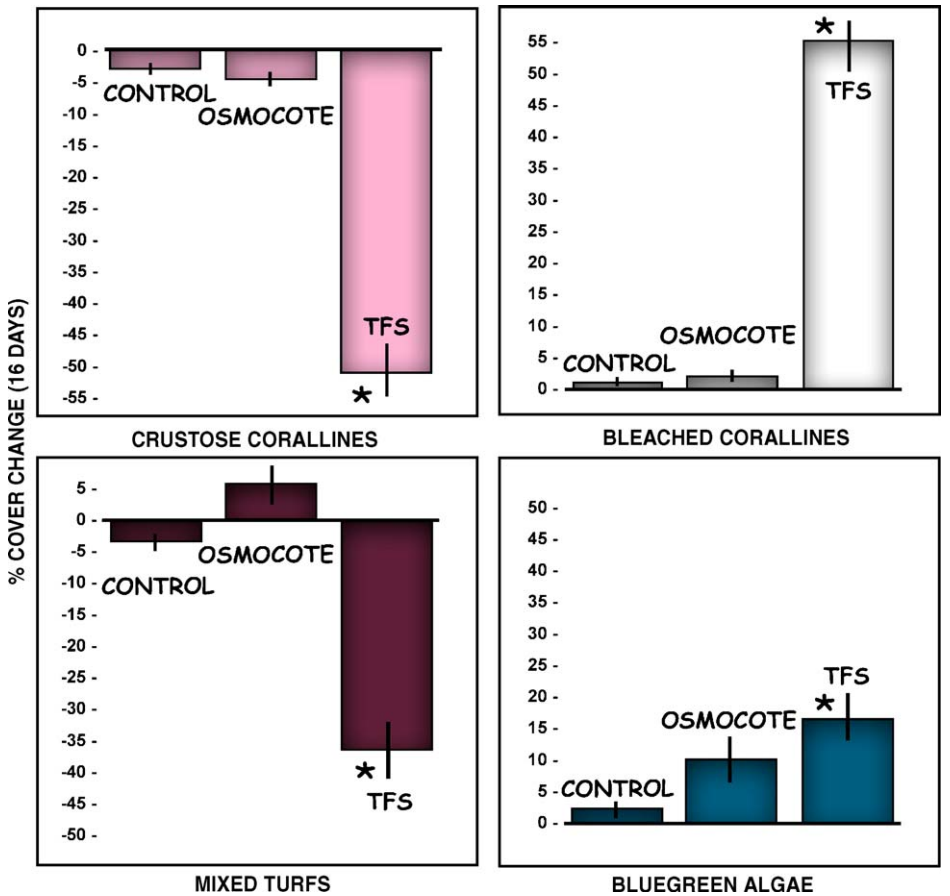

Fig. 3. Changes in established communities of dominant functional algal groups on artificial mini-reefs under exposure to the fertilizers Osmocote and Tree Food Stakes (TFS) versus unfertilized (Control) mini-reef communities for 16 days ($N = 10$). Error bars are ± 1 S.E. before arcsine transformation. Asterisks indicate statistically significant differences ($P < 0.05$) from the Controls.

Stakes and Osmocote mini-reefs filled with slow-release fertilizer showed significantly ($P<0.05$) elevated values (Table 1), increasing the adjacent water-column DIN by 10-fold to means of 4.1±0.72 and 3.9±0.87 μM and SRP by 10- and 8-fold to means of 0.38±0.03 and 0.30±0.06 μM, respectively.

Predominant current speeds were reasonably constant in a northwesterly direction (340° magnetic), ranging from 3.0 to 4.7 $cm \cdot s^{-1}$ (mean=3.6±0.5 S.D.) at the start of the study and 3.0 to 5.7 $cm \cdot s^{-1}$ (mean=4.9±0.8 S.D.) 16 days later. These currents are driven by the pumping action of offshore waves breaking over the reef crest and flowing through the study site (Fig. 1), exiting around the northern tip of CBC.

3.2. Biological data

Although terminated after only 16 days, the study of populations on the treated mini-reefs provided results (Fig. 3) that are compelling by comparing before and after photographs (Figs. 4, 5). A checklist of taxa that comprised the four functional groups colonizing the mini-reefs is given in Table 2, dominated by calcareous coralline algae. Tree Food Stakes fertilizer caused mortality (significant at $P<0.05$) of both fleshy turf algae and coralline algae (coralline bleaching also greatly increased >100-fold; Figs. 3, 5). Tree Food Stakes treatments showed 11-fold and 20-fold decreases, respectively, for these two functional indicator groups relative to the Control experiments; hypothetically, due to the chlorine content (Fig. 2). Most of the initial bleaching occurred late in the study (~day 10) and included the bases of fleshy turf algae as well as the prostrate coralline crusts. Conversely, blue-green algae (Cyanobacteria) were significantly (6 times, $P<0.05$) more abundant in the Tree Food Stakes treatments than in the Controls, but not in the Osmocote treatments ($P>0.05$, Fig. 3). The Osmocote-filled mini-

Fig. 4. Photographs of representative mini-reefs showing examples of minimal changes in dominant functional groups under Control (C=no fertilizer) and fertilized (O=Osmocote) conditions before and after 16-day exposure.

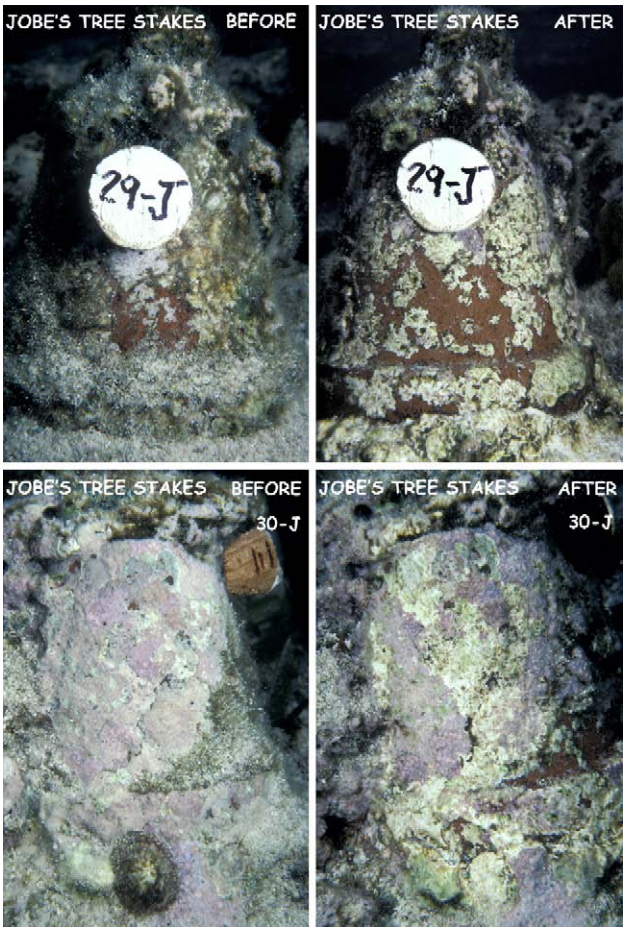

Fig. 5. Photographs of representative Tree Food Stakes (J) treated mini-reefs before and after 16-day exposure showing pronounced increases in bleaching. Note that in the bottom left sample (30-J, before), the limpet in the lower center part of the mini-reef had abandoned its home range (bottom right, 30-J) following exposure to Tree Food Stakes.

reefs showed no significant mortality ($P>0.05$) for any functional group (Figs. 3, 4), while healthy fleshy turf algae and blue-green algae had begun to show conspicuous increases; although only 16 days had elapsed. The absence of fish-scrape marks (e.g., Figs. 3, 4) indicated that herbivory was never a significant factor.

The release pattern of free chlorine from Tree Food Stakes is documented in Fig. 2 over a 40-day period. Levels to 0.2 ppm for chlorine consistently were present within 9 days, whereas both the Osmocote treatments and distilled water Controls contained no chlorine.

4. Discussion

Tree Food Stakes fertilizer caused mortality (significant at $P<0.05$) of both calcareous coralline algae (which decreased by a factor of 20) and fleshy turf algae (which showed an 11-fold decrease) relative to the Control and Osmocote treatments (Fig. 5). Most of the initial bleaching occurred midway in the study (conspicuous by day 10) and included the bases of fleshy turf algae as well as the prostrate coralline crusts. We posit that mortality on the Tree Food Stake treatments was caused by the chlorine, although some other chemicals could have been involved. The purpose of the 6% chlorine in Tree Food Stakes (Fig. 2) is to prevent populations of soil algae from assimilating the nutrients in a terrestrial situation, where soil constituents would neutralize chlorine within a very short distance. Others have used Tree Food Stakes to add nutrients to the sediment pore waters in seagrass beds (e.g., McGlathery, 1995), where the chlorine could be bound up quickly by organic sediments. Chlorine in a tropical-reef setting is not likely to be beneficial to healthy algal populations (Fig. 3).

An alternative hypothesis could be that because nutrients and herbivory are not independent and that the positive effects of nutrients on marine plant productivity and growth can actually make plants more palatable and susceptible to grazers (McGlathery, 1995; Boyer et al., 2004), herbivory may, therefore, have increased differentially on the interspersed Tree Food Stakes treatments. However, the notable absence of bite marks on any of the mini-reefs (e.g., Figs. 4 and 5) indicates that herbivory was not a significant factor.

Although the outcome remains the same (i.e., mortality), alternative possibilities to the harmful effects of free chlorine in Tree Food Stakes are that it could have been converted to non-biocidal forms or diluted in concrete-block diffusers (as "cinder" blocks) used by Miller et al. (1999), or in our terra-cotta mini-reefs, so as to be below lethal levels. However, chlorine concentrations would have been considerably higher in such low-volume containers than in the 8-fold larger flasks in which we documented substantial long-term release (Fig. 2).

Miller et al. (1999) have concluded "Results from an offshore reef in Key Largo, Florida show … negligible effects of nutrient enrichment—or effects that are opposite of predictions, … patterns observed for this reef did not confirm predictions of previously proposed models that frondose macroalgal or crustose algal abundance would be enhanced with nutrient enrichment or that dominance of filamentous turfs would be greater in unenriched conditions." The compromised Miller et al. (1999) study cannot be used to falsify (as claimed) the nutrient tenants of the Relative Dominance Model. The lack of stimulatory effects of nutrients they recorded for calcareous and fleshy algae was due to some lethal component (as documented herein, Fig. 5) in Tree Food Stakes fertilizer that must have differentially inhibited the initial colonizing algae on the inoculated settling plates in their nutrient treatments.

Blue-green algae (Cyanobacteria) were significantly elevated by Tree Food Stakes fertilizer in the present study (Fig. 3), consistent with the Miller et al. (1999) finding. Miller et al. (1999), by dismissing the blue-green algae as bacteria, concluded that coral-reef algae are not nutrient-limited. However, blue-green algae are among the most abundant primary producers (as distinctive colonies) on coral reefs and must be included as important components in any model of coral reef change. They survive in harsh environments, including chlorinated swimming pools, so it is not surprising that some can flourish under Tree Food Stakes fertilizer containing as much as 6% chlorine (Fig. 3).

Due to the escalating problems associated with eutrophication along tropical and subtropical shorelines, the ecological responses of corals and macroalgae to nutrient enrichment have been repeatedly cited (NRC, 1995, 2000) as priority areas in need of further research. Throughout the past decade, however, many coral reef biologists and managers have not recognized the severity of chronic nutrient enrichment and eutrophication problems facing coral reefs (see Risk, 1999). For example, although harmful macroalgal blooms on coral reefs have been attributed to nutrient enrichment and eutrophication (reviewed in Lapointe et al., 2005), some reef biologists have countered that such changes in benthic community structure result primarily from natural stochastic events (Precht and Miller, 2006) or overfishing of herbivorous fish stocks (Hughes, 1994; Pandolfi et al., 2003) and/or loss of keystone grazers, such as the sea urchin *Diadema antillarum* (Jackson et al., 2001). Fong et al. (2003) have questioned whether macroalgae in tropical systems are ever limited by nutrients. A similar interpretation was perpetuated in the frequently cited review article by Lesser (2004) who, unfortunately, relied on the flawed findings of Miller et al. (1999) in downplaying the role of nutrients.

In-situ nutrient enrichment studies in systems with minimal coral cover and excessive algal overgrowth that had already exceeded critical nutrient concentrations (Larkum and Koop, 1997; Thacker et al., 2001; Belliveau and Paul, 2002) are important and serve to emphasize the universal low nutrient tipping-point concentrations (above 0.1 μM SRP and 1.0 μM DIN) involved. However, because nutrient levels already exceeded the tipping-point levels, their applicability also is doubtful in terms of testing the role of nutrient enrichment (i.e., in regard to falsifying the RDM).

Using (1) a longer-term (>6 months, macroalgae only appeared after 4 months) experimental approach on (2) an appropriately healthy (i.e., nutrients below the tipping points) coral-dominated reef and (3) a gradual, uniform-release, realistic-gradient, nutrient-dispersal system, Smith et al. (2001) provided the most relevant experimental evidence showing that both nutrient and herbivory factors act together to determine reef quality. These findings were further substantiated by the multi-faceted 24-month in-situ study of Littler et al. (in press). By avoiding the pitfalls of inappropriate study sites and sources of enrichment in future research, the potential to provide new insights into the roles of nutrients on coral reefs is greatly increased, and, thus, could improve our understanding of the ecology and sustainable management of these ecosystems.

Caging and feeding preference studies (top–down) have yielded quick, easy and important results, but unfortunately, relatively fewer coral-reef ecologists have employed the logistically more difficult technical methodologies required to adequately address the roles of bottom–up phenomena. These include established oceanographic methods (e.g., water-column nutrient/chlorophyll/turbidity analyses, tissue C:N:P ratios, stable isotopes), mariculture techniques (e.g., nutrient type/growth responses) and specialized physiological approaches (e.g., pulsed productivity bioassays, alkaline phosphatase assays). This logistical difficulty has led to disparate sample sizes, less information and fewer scientist advocates for bottom–up vs. top–down approaches. As more nutrient data are collected in regard to this incongruence, the more we will be able to say about the relative importance of each factor.

It is encouraging that the critical role of excess nutrients on coral reefs has begun to receive appropriate recognition in recent review papers (Scheffer et al., 2001; Hughes et al., 2003; Bellwood et al., 2004; Pandolfi et al., 2005). However, some scientists (e.g., Precht and Miller, 2006) continue to downplay declining resilience issues, instead emphasize important, but unmanageable, stochastic factors like upwellings, hurricanes and cold fronts, events from which coral reefs have recovered for millions of years (i.e., in the absence of humans). Also, nutrient/herbivory models are receiving considerable attention [cf. Fig. 7 in Littler and Littler (1984) with the very similar Fig. 2a in Bellwood et al. (2004)]. The coral-reef scientific community needs a broader biological perspective to further the recognition of the role played by chronic nutrient enrichment in the coral-reef health/resilience paradigm. Hopefully, the justifiable plea (Pandolfi et al., 2005) for scientists to "…stop arguing about the relative importance of different causes of coral reef decline…" will increase support for much-needed nutrient enrichment and eutrophication research.

To reiterate, bottom–up research is logistically difficult and requires more emphasis on multifaceted approaches carried out over sufficiently long time periods. These should include examining (by in-situ nutrient enrichment experiments) the resiliencies to shifts in competitive dominances of functional indicator groups on healthy oligotrophic coral-dominated reefs (e.g., Smith et al., 2001; Littler et al., in press), in addition to providing supporting data such as water-column nutrient levels, tissue C:N:P ratios (Lapointe et al., 1997; 2005) and assays of algal physiological responses (Lapointe et al., 1997; Littler et al., in press). We encourage both scientists and managers to consider more broadly the complex role that escalating nutrient enrichment plays in the regulation of harmful macroalgal blooms on coral-reef ecosystems.

Over half a decade ago, Risk (see "Paradise lost…" 1999) pointed out how both science and management are failing coral reef conservation, and we concur. Scientists, by advocating their own disciplines, have generated confusing arguments regarding the relative roles of herbivory vs. eutrophication vs. salinity vs. stochastic events etc., which have served to greatly "muddy the waters". Amidst this uncertainty, managers burdened by bureaucratic constraints have been forced to focus on piecemeal actions—developing/redeveloping management plans, reducing immediate threats, setting aside protected areas and enlarging jurisdictions; although some progress on eutrophication abatement recently has occurred in the Florida Keys (Causey and Andrews, 2005; Kruczynski, 2005) and the Great Barrier Reef (McCook personal communication). These otherwise reasonable efforts are not likely to be successful in the absence of vital long-term goals to improve the resilience of desirable coral/coralline communities—such as protecting/restoring herbivore stocks while simultaneously minimizing/eliminating anthropogenic nutrient enrichment.

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