

The effects of nitrogen and phosphorus enrichment on algal community development: Artificial mini-reefs on the Belize Barrier Reef sedimentary lagoon

Mark M. Littler^{*}, Diane S. Littler, Barrett L. Brooks

Department of Botany, P.O. Box 37012, National Museum of Natural History #166, Smithsonian Institution, Washington, DC 20013-7012, USA

ARTICLE INFO

Article history:

Received 16 January 2009

Received in revised form 11 November 2009

Accepted 12 November 2009

Keywords:

Algae
Belize
Colonization
Reef lagoon
Nitrogen
Phosphorus

ABSTRACT

The experiments to compare DIN and SRP enrichment effects on algal community development were conducted within a lagoonal rubble/sand reef apron west of the back reef flat at Carrie Bow Cay, Belize. Macroalgae dominate (23 taxa, 57% cover), ambient dissolved inorganic nutrient concentrations are above the levels documented for release of macroalgal growth (means of 2.06 μM DIN = dissolved inorganic nitrogen and 0.12 μM SRP = soluble reactive phosphorus) and grazing is negligible (1.7% *Acanthophora spicifera* consumed 6 h^{-1}). Mini-reef diffusers containing slow-release fertilizers significantly ($P < 0.05$) increased DIN in the experimental DIN and SRP + DIN treatments by 2- and 3-fold (means of 4.64 and 6.41 μM), respectively; while SRP was increased significantly ($P < 0.05$) in the SRP and SRP + DIN treatments by 5- and 8-fold to means of 0.69 and 0.94 μM , relative to the control treatments. SRP and SRP + DIN treatments favored colonization by Cyanobacteria such as *Spirulina* sp. on the mini-reef/diffusers, with an opposite detrimental effect on reef-building crustose coralline algae. The Cyanobacteria bloom further inhibited the long-term settlement and colonization of crustose coralline algae. Conversely, the DIN and control mini-reef treatments showed low Cyanobacteria cover and became colonized by abundant reef-building coralline algae, consisting mostly of *Hydrolithon boergesenii* and crust stages of *Amphiroa fragilissima*. After 1 year, the competitively overgrowing macrophytes *A. spicifera*, *Palisada papillosa*, *Padina sanctae-crucis* and *Spyridia filamentosa* conspicuously dominated all of the treatment- and control-replicates, in accordance with the nutrient-replete ambient waters and negligible herbivory within this habitat. As predicted, nutrient additions to the mini-reef diffusers resulted in significant elevations of tissue nutrients in the most-abundant colonizer and habitat dominant, *A. spicifera*. Although, the resultant decreases of both the C:N and C:P molar ratios corresponded to the elevated DIN and SRP treatments, this uptake was entirely superfluous, since control population colonization and growth matched that of the experimental nutrient treatments.

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1. Introduction

The productivity of benthic macroalgae is often nutrient-limited in tropical coral-reef ecosystems (Lapointe, 1987; Lapointe et al., 1987). Excessive water-column nutrient inputs to tropical reefs increase harmful algal interference with coral colonies, decreasing irradiance, reducing gas and nutrient exchanges, lowering productivity and ultimately causing die-offs. Nutrient/herbivory models, similar to the relative dominance model (RDM) for coral reefs (Littler and Littler, 2007), are receiving considerable attention [compare Fig. 7 in Littler and Littler, 1984 with the very similar Fig. 2a in Bellwood et al., 2004]. Understanding the effects of herbivore inclusion/exclusion-cage experiments (e.g., Lapointe

et al., 2004; Bellwood et al., 2006) and feeding-preference studies (top-down control), in addition to much-needed data from nutrient-enrichment manipulations (bottom-up control), on lagoonal and coral-reef ecosystems is central to the elucidation of mechanisms that determine relative dominances, phase shifts and stable states.

Within healthy sedimentary lagoon environments, water-column nutrient concentrations become slightly elevated, herbivory is minimal and most of the available space is occupied by a restricted diversity of plant life (Macintyre et al., 1987). Water-column dissolved nutrients in combination with ample current flow are generally sufficient to allow substantial productivity of epilithic, epiphytic and other non-rhizomatous algal species within these habitats. However, the extreme nutrient constraints typically observed in the healthy coral-reef ecosystems upstream (Littler et al., 2006a, 2006b) are not as common in sedimentary lagoonal habitats, which are characterized by greatly elevated pore-water nutrient availability. For example, organic detritus rarely develops to high levels on coral-dominated reefs because of

^{*} Corresponding author at: Smithsonian Institution – Botany, P.O. Box 37012, NMNH #166, Washington, DC 20013-7012, USA. Tel.: +1 202 633 0956; fax: +1 202 786 2563.

E-mail address: littlerm@si.edu (M.M. Littler).

intense herbivory and export; whereas, sedimentary back-reef environments contain decomposing organic materials within extensive sediment layers that substantially elevate pore-water nutrient concentrations. These pore-water nutrients can leach or be biologically pumped upward to enrich the overlying water column where they are then accessible to nearby macroalgae (Larned, 1998). Because nutrient uptake kinetics in algae are mostly concentration dependent (Lapointe, 1997), growth rates of epilithic algal communities on lagoon rubble should be less nutrient limited than those on upstream coral reefs.

It is generally known and accepted that low-nutrient levels can saturate growth rates of macroalgae (see Lapointe, 1997, 1999). However, controversy has arisen (e.g., Hughes et al., 1999) as to the efficacy of universal threshold concentration values for DIN ($1.0 \mu\text{M}$ dissolved inorganic nitrogen) and SRP ($0.1 \mu\text{M}$ soluble reactive phosphorus) releasing macroalgal nutrient limitation for potential deleterious overgrowth of coral reefs, as well as the relative importance of each in regulating algal growth in tropical waters (Smith, 1984; Howarth, 1988). These very low-nutrient thresholds were originally hypothesized (Bell, 1992; Lapointe et al., 1997; Bell et al., 2007) regarding the lowered resiliencies for transitions from coral domination toward fleshy algal states. Such low-nutrient threshold concentrations are correlated with (Lapointe et al., 1993) harmful macroalgal overgrowth of seagrass and coral-reef communities along natural nutrient gradients on the Belize Barrier Reef, as well as being corroborated by *in situ* experimental nutrient-enrichment studies (e.g., Larkum and Koop, 1997; Thacker et al., 2001; Belliveau and Paul, 2002) on other coral reefs and associated lagoonal patch reefs (McClanahan et al., 2002, 2003). The above *in situ* experiments, carried out in geographically separated areas characterized by nutrient levels only marginally above $0.1 \mu\text{M}$ SRP and $1.0 \mu\text{M}$ DIN and documenting minimal algal stimulation following nutrient enrichment alone, serve to emphasize the low-nutrient concentrations involved (Littler and Littler, 2007).

Comparative studies contrasting nutrient limitation in back-reef sedimentary environments vs. coral reefs are few. Moreover, whether DIN or SRP are the most limiting to algal colonization and growth has been experimentally addressed only rarely (e.g., McClanahan et al., 2003). Historically, DIN is considered to be the main nutrient limiting productivity in tropical waters (Parsons et al., 1977). However, geochemical models (Smith, 1984) and *in situ* macroalgal bioassays (Lapointe, 1985, 1987; Littler et al., 1991; Lapointe et al., 1992) suggest that SRP, rather than DIN, is often the primary growth-limiting nutrient in carbonate-rich systems, such as the site studied here. In well-oxygenated tropical reef systems, SRP is often low due to its reaction with carbonate fluoroapatite (Gulbrandsen and Robertson, 1973). Conversely, in siliciclastic environments (Hanisak, 1990; Lapointe et al., 1992), DIN is most often the limiting nutrient.

The present study was designed to address the comparative ecological effects, in respect to DIN and/or SRP additions, regarding algal community development on artificial mini-reefs within a tropical back-reef sedimentary lagoon. The 12-mo study included *in situ* nutrient-enrichment recruitment/colonization experiments on mini-reef diffusers, as well as standing stock measurements, current assessments, seawater nutrient/tissue analyses and herbivory assays.

2. Materials and methods

2.1. Study area

The 2.0 m deep study site ($\text{N}16^{\circ}48'11''$, $\text{W}88^{\circ}04'50''$) west of the Belize Barrier Reef back-reef flat is located on the back-reef sedimentary lagoon behind Carrie Bow Cay (Fig. 1) – not far (~ 230 m west) from upstream oligotrophic coral-reef sites that were studied earlier (Lewis, 1986; Littler et al., 2006a, 2006b; Littler and Littler, 2007). Proximity to shelter has long been recognized (Randall, 1965; Ogden et al., 1973) as an important

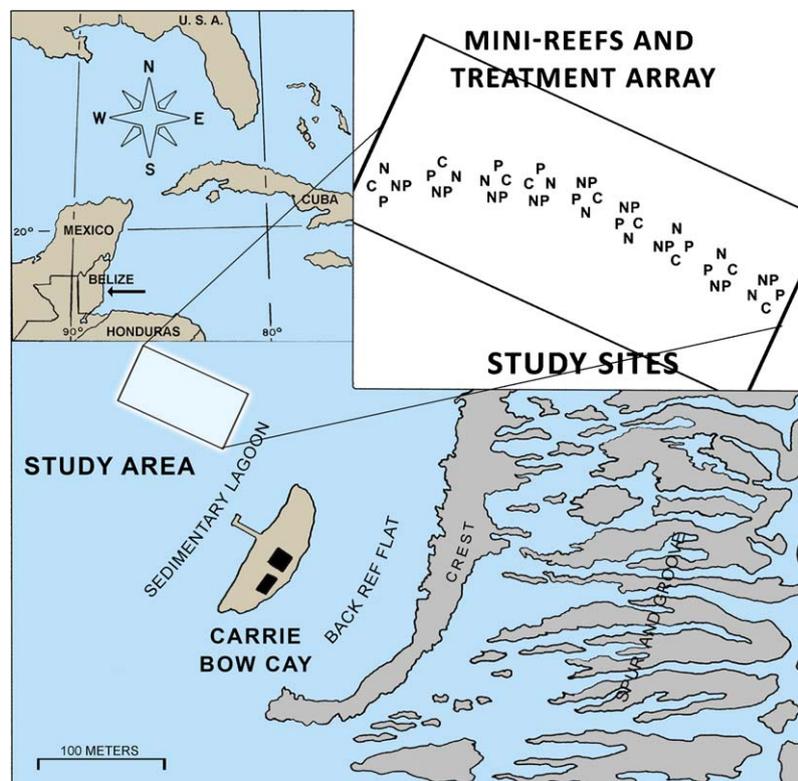


Fig. 1. Location of the study area, transects, herbivory assays, current studies and randomized 36-diffuser array on the CBC sedimentary lagoon.

factor determining herbivore foraging ranges. Consequently, the sedimentary lagoonal site is avoided by herbivorous fishes because of the lack of structural shelter from carnivorous fishes (e.g., barracudas, sharks, jacks, snappers) and birds (e.g., ospreys, cormorants, pelicans), which forage daily throughout this habitat (personal observations).

The Belize Barrier Reef complex is the largest continuous coral-reef 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. Within back-reef sedimentary habitats, such as the one studied here (Fig. 1), assemblages of seagrasses, corals and macroalgae have the same general taxonomic composition along the entire barrier-reef tract (Burke, 1982; Macintyre et al., 1987, personal observations). Carrie Bow Cay (CBC) reef habitats and surrounding environments comprise well-developed, typical, barrier-reef systems remote from major human influences. Offshore Secchi disc depths in excess of 40 m are not unusual, indicating Jerlov Type I oceanic waters. Most importantly, nutrient levels above the tipping-point concentrations noted (Bell, 1992; Bell et al., 2007) to potentially enable macroalgal overgrowth (i.e., $>0.1 \mu\text{M}$ SRP and $>1.0 \mu\text{M}$ DIN) have seldom been recorded (Lapointe et al., 1987, 1993; Littler et al., 2006a, 2006b) from the barrier coral reefs of this system. However, a previous study at Glover's Reef (offshore of the Belize Barrier Reef) reported anomalously high background SRP concentrations of $0.35\text{--}0.77 \mu\text{M}$ (McClanahan et al., 2002) for lagoon patch-reef habitats. Those values are at least 20-fold higher than the typical SRP concentrations previously reported for this atoll ($\sim 0.05 \mu\text{M}$; Lapointe, 2004), for the Belize Barrier Reef ($\sim 0.04 \mu\text{M}$; Littler et al., 2006a) and for Caribbean offshore waters in general (see Rajendran et al., 1991; Lapointe, 2004). The explanation, as pointed out by Lapointe (2004), is that the nutrient analyses (Hach Kit) were probably inaccurate.

The topography, geology and general biology of CBC are well known due to over a quarter century of study (see Ruetzler and Macintyre, 1982). Herbivory has been extensively studied throughout many of the CBC reef habitats (Hay, 1981; Littler et al., 1983a, 1986, 1987, 1989, 1995, 2006a; Lewis and Wainwright, 1985; Lewis, 1986; Lewis et al., 1987; Macintyre et al., 1987; Reinthal and Macintyre, 1994), including near the site studied here. The CBC study site (Fig. 1), located 2.0 m deep on the back-reef sedimentary lagoon of the northwest side of the island ($16^{\circ}48'N$, $88^{\circ}05'W$), is representative of such systems reported for much of the Belize Barrier Reef tract (James et al., 1976; Macintyre et al., 1987), as well as commonly found elsewhere throughout the Caribbean (Burke, 1982; personal observations).

2.2. Approach

A combination of environmental, survey/inventory and bioassay data are essential to characterize and monitor the ambient nutrient/herbivory environments and antecedent nutrient history of any given study area (Littler and Littler, 2007). The present 12-mo investigation included: (1) characterization of environmental parameters (i.e., nearby natural populations, current speeds, water-column nutrients, tissue C:N:P analyses and herbivory bioassays), (2) controlled manipulations of nutrient types and concentrations in an area having negligible herbivory and (3) distribution and abundance of dominant algal functional groups colonizing artificial mini-reef nutrient diffusers (similar to those used by Littler et al., 2006a).

2.3. Water-column nutrient levels

To characterize the nutrient environments of the study site, three replicate water samples were collected at 5-d intervals 3-cm

Table 1

Documentation of ample DIN=dissolved inorganic nitrogen and SRP=soluble reactive phosphorus concentrations remaining inside the diffusers after 12 mo. Although, some of the ($N=4$) sample replicates were lost, all experimental nutrient treatments remained dramatically elevated (500 to 50,000 times) relative to controls.

Treatments	DIN levels ($\mu\text{M} \pm \text{S.E.}$)	SRP levels ($\mu\text{M} \pm \text{S.E.}$)
C ($N=2$)	34 ± 30	0.4 ± 0.2
N ($N=3$)	$16,783 \pm 7041$	–
P ($N=1$)	–	7441
N+P ($N=4$)	$21,389 \pm 6791$	$19,918 \pm 8243$

from the bases of the control and experimental mini-reefs on 3 separate days, immediately filtered through combusted Gelman 0.45 mm GF/F filters, placed in a refrigerator and frozen until analysis. Dissolved inorganic nitrogen ($\text{DIN} = \text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-$) and soluble reactive phosphorus ($\text{SRP} = \text{PO}_4^{3-}$) concentrations were analyzed, by the methods outlined in D'Elia et al. (1997; Nutrient Analytical Services, Chesapeake Biological Laboratory, Solomons, MD). SRP and NO_3^- were measured with a Technicon Autoanalyzer II; whereas, NH_4^+ and NO_2^- were determined 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. At the end of the 12-mo experiment, the contents of the mini-reef diffusers were sampled and analyzed to show ample long-term nutrient availability (Table 1).

2.4. Macroalgal tissue-nutrient levels (C:N and C:P ratios)

Collections of the habitat dominant and most-abundant colonizing macroalga, *Acanthophora spicifera*, were cleaned, dried and analyzed for tissue C, N and P contents ($N=6$ for controls, 10 for experimentals). Such data are useful to assess the long-term nutrient history within the various treatments, since antecedent nutrient exposures will have been recorded within the plants themselves. Tissue samples were analyzed by the Nutrient Analytical Services Laboratory, Chesapeake Biology Laboratory. The resultant C:N and C:P molar ratios were compared between treatments, as well as with the range of values previously reported (Lapointe et al., 1992, 1997, 2005a; Larned, 1998; Furman and Heck, 2008) for a wide variety of macrophyte taxa throughout a spectrum of systems ranging from oligotrophic to eutrophic.

2.5. Current speeds

To further characterize the study site, current speeds were measured on three separate occasions under typical non-storm wind and wave conditions ($N=20 \text{ d}^{-1}$) by fluorescent dye injected next to the nutrient diffusers on the bottom and timing the movement over a downstream horizontal distance of 2.0 m.

2.6. Herbivory bioassay

Herbivory in the study site was assayed according to the methods of Littler and Littler (2007) using the major colonizer, community dominant and highly palatable cosmopolitan alga *A. spicifera* (Lewis and Wainwright, 1985). This ubiquitously abundant red alga is a preferred food item by both parrotfishes and surgeonfishes (Lewis and Wainwright, 1985; Littler et al., 2006a), as well as by sea urchins (Littler et al., 1983b). The alga was cut into 7.0 cm lengths and attached to dead coral-rubble fragments by thin rubber bands. Replicates (28) were placed haphazardly along the north and south borders of the diffuser array (Fig. 1) for 6 h at mid-day, collected and again measured. Percent eaten was determined for the re-measured algal segments and the results

(% lost) were analyzed using one-way ANOVA followed by the Bonferroni (Dunn) *t*-test (SAS, 2003, v. 9.1; SAS Inst., Inc., Cary, NC). A >50% loss per 6 h (<6 h half-life) indicates a healthy level of herbivory (Littler and Littler, 2007). Herbivore abundances also were enumerated by counting numbers of individuals (by species), from mid-morning to mid-afternoon throughout a typical day for weather on either side of two random replicated standardized transect lines (20-m long) deployed along the north and south margins of the mini-reef diffuser array (Fig. 1).

2.7. Standing stocks of dominant organisms

To characterize the natural biota of the study site, two replicate transects also running west to east along the north and south edges of the arrays of mini-reefs were established. Quantitative samples were obtained by scoring (perpendicular to the substrate) point intercepts centered at every 0.1 m mark. Due to the patchy nature of the rubble substrate and attached biota, uniformly spaced point arrays produced a more representative sampling than would clustered (i.e., randomized) hit-or-miss clumped arrays (see Discussion in Littler and Littler, 1985). Simultaneously, voucher specimens of dominant coralline algae, macrophytes and turf microalgae were taken for taxonomic purposes.

2.8. Controlled nutrient manipulations

We chose porous terra-cotta clay flowerpots as the experimental nutrient diffusers to provide gradual release, realistic gradients and uniform dispersion (Littler et al., 1989, 2006a, 2006b), following their previously successful application for this purpose (see also Chapman and Craigie, 1977; Hatcher and Larkum, 1983). Within the study site (Fig. 1), 36, independent, terra-cotta, clay-pot, nutrient diffusers (1.18-liter volume, 14.0-cm high, 14.0-cm mouth diameter, 584 cm² of total outside surface area) were each cemented upside down to a single solid concrete block using hydraulic cement to completely seal the rims. These incipient “mini-reefs” were placed in randomized groups of four in the field at ~1.5 m distances from each other, a sufficient distance to avoid cross contamination (Miller et al., 1999; Littler et al., 2006a). Harrell’s custom slow-release fertilizers (0.45-l of each containing 42% N (as ammonium nitrate), as well as 46% P (as calcium phosphate) were poured into the three experimental diffusers (randomly selected for SRP, DIN or SRP + DIN treatment) in each of the 9 four-unit clustered groups (*N* = 9) and the holes were then stoppered. The internal slow-release fertilizer levels were analyzed following 12 mo to confirm that ample nutrients were present throughout the study. The remaining nine ambient-nutrient diffusers (natural controls, one in each four-pot cluster) were filled with seawater and stoppered. Consequently, the nine four-pot diffuser clusters (one ambient nutrients and three elevated nutrients in each group of four) provided nine experimental arrays that included four randomly selected independent nutrient treatments exposed in a nutrient-replete lagoonal study site with a near absence of herbivory. This design yielded the following four combinations of experimental conditions: (1) ambient nutrients, (2) elevated DIN, (3) elevated SRP and (4) elevated DIN + SRP.

2.9. Colonization studies

Comparisons were made between treatments to detect changes in the relative abundances of the benthic groups that recruited and colonized during a 12-mo period. Each of the three experimental nutrient sources [i.e., utilizing the 1.18-liter unglazed clay-pot diffusers containing (Harrell’s, Llc.) slow-release N (42-0-0), P (0-46-0) and N + P (combined) polymer-coated fertilizers] and the

controls were analyzed separately for percent-cover changes of species comprising the following three functional-groups: blue-green algae turfs, crustose coralline algae and mixed fleshy macroalgae. Corals were not present in the surrounding sand/rubble habitat and did not colonize during the 12-mo study. Detailed field estimates were made using magnifying lenses, followed by taking macro-images of the top, east, west, north and south sides (108-cm², 9 cm × 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-cm² plots enhanced the resolution and, in conjunction with the field notes, facilitated discrimination of microscopic turf species and crusts.

For each category, a repeated-measures ANOVA (SAS, 2003, v. 9.1; SAS Inst., Inc., Cary, NC) was conducted by employing a completely randomized, interspersed, independent (Hurlbert, 1984) design that combines different levels and kinds of nutrient enrichment. Zero cover at the initial set-up census was used as the baseline and the subsequent biotic censuses were analyzed as the responses (dependent variable) to test for significant effects and possible interactions of the main treatments (i.e., independent variables = enrichment, time). To test the null hypothesis that the percent cover differences of functional groups under elevated (DIN, SRP, DIN + SRP) vs. ambient nutrients (controls) were not statistically different (at $\alpha = P > 0.05$), we used one-way ANOVA followed by Bonferroni (Dunn) *t*-test, *a posteriori*, multiple classification analysis (SAS, 2003, v. 9.1). All percent cover data were arcsine transformed prior to analysis.

3. Results

3.1. Water-column nutrient levels

The ambient DIN and SRP concentrations next to the bases of the non-enriched control diffusers were consistently above the 1.0 μM DIN and at the 0.1 μM SRP tipping-point levels for release of macroalgal growth (means = 2.06 μM DIN and 0.12 μM SRP; Figs. 2 and 3), indicating that strictly oligotrophic conditions do not prevail on this sedimentary back-reef lagoon system. Both the DIN and the DIN + SRP nutrient diffusers filled with slow-release fertilizers produced the desired results (Figs. 2 and 3), significantly

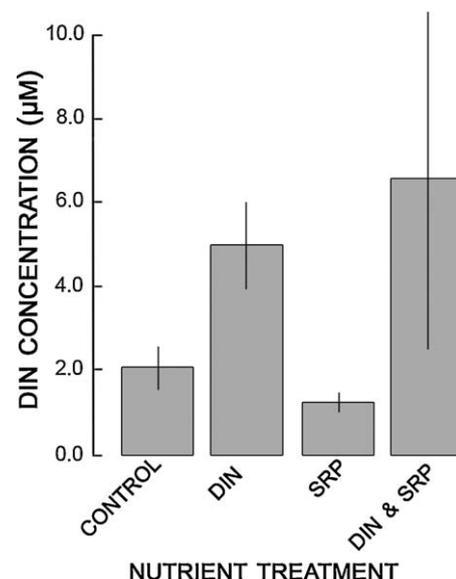


Fig. 2. Mean (\pm S.D., *N* = 9) DIN concentrations adjacent to the four treatments after the addition of DIN and DIN + SRP. All experimental DIN and DIN + SRP enrichment means were significantly ($P < 0.05$) elevated relative to the controls.

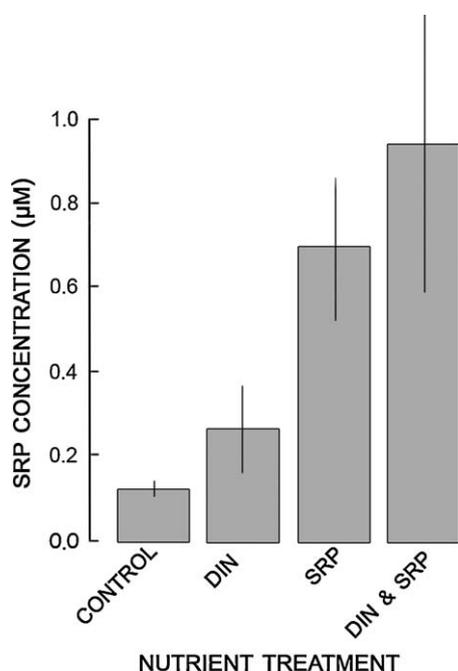


Fig. 3. Mean (\pm S.D., $N = 9$) SRP concentrations adjacent to the four treatments after the addition of SRP and SRP + DIN. All experimental SRP and SRP + DIN enrichment means were significantly ($P < 0.05$) elevated relative to the controls.

increasing DIN by 2–3-fold to means of 4.64 and 6.41 μM , respectively; while SRP was significantly increased in the SRP and SRP + DIN treatments by 5- and 8-fold to means of 0.69 and 0.94 μM , respectively (all differences significant at $P < 0.05$).

3.2. Tissue-nutrient levels

Tissue molar ratios of C:P (mean = 358 ± 132 S.E.) and C:N (mean = 15 ± 3 S.E.) were lowered significantly ($P < 0.05$, Bonferroni)

for the dominant colonizing *A. spicifera* from the controls (mean for C:P = 610 ± 130 S.E., mean for C:N = 19 ± 2 S.E., Table 3).

3.3. Current speeds

Predominant westerly current speeds were reasonably constant, ranging from 4.0 to 10.0 cm s^{-1} (mean = 6.8 ± 0.6 S.E., $N = 60$) in the study site. Tidal effects are minor and the currents are unidirectional, driven by the consistent pumping action of offshore waves breaking over the barrier-reef crest and slowly flowing southwestward through the study site (Fig. 1).

3.4. Herbivory bioassays

Grazing throughout this structurally simple (2-dimensional) study site proved to be negligible (insignificant at $P > 0.32$) with only one out of 28 assay samples being half eaten during one of the 6-h test periods. This yields a 1.7% half-life 6 h^{-1} , which is far below the $>50\%$ 6-h half-life threshold value suggested (Littler and Littler, 2007) to be characteristic of healthy coral-dominated reefs. Visual transects (two 20-m long \times 10-m wide) revealed only one juvenile *Acanthurus bahianus* in the study area.

3.5. Standing stocks of dominant organisms

The study site was dominated by 57% cover of fleshy macroalgae (23 taxa, Table 2), as would be predicted by the herbivory bioassay. *A. spicifera* with 17% cover was predominant, followed by *Hypnea spinella* (13%), *Dictyota cervicornis* (9%), *D. pulchella* (8%), *Palisada papillosa* (5%) and *Padina sanctae-crucis* (5.0%). Bare rubble rock (11% cover) and sand (32%) comprised the remainder.

3.6. Colonization studies

Turf algae (mostly Cyanobacteria = Cyanophyta) were the earliest colonizers (Figs. 4 and 5), rapidly appearing on the

Table 2

List of the predominant benthic organisms and their natural abundances (% cover, for two 12-m long transects) on the adjacent rubble substrate communities on the north and south borders of the diffuser array (tr = trace).

Algal Taxa	Percent Cover		
	North Transect	South Transect	Means
<i>Acanthophora spicifera</i> (M. Vahl) Børgesen	10.8	22.5	16.6
<i>Hypnea spinella</i> (C. Agardh) Kützing	8.3	17.5	12.9
<i>Dictyota cervicornis</i> Kützing	10.8	6.7	8.8
<i>Dictyota pulchella</i> Hörnig & Schnetter	10.0	5.0	7.5
<i>Palisada papillosa</i> (C. Agardh) K.W. Nam	4.2	5.8	5.0
<i>Padina sanctae-crucis</i> Børgesen	7.5	2.5	5.0
<i>Laurencia intricata</i> J.V. Lamouroux	0	0.8	0.4
<i>Amphiroa fragilissima</i> (Linnaeus) J.V. Lamouroux	0	0.8	0.4
<i>Aglaothamnion</i> sp.		tr	tr
<i>Bryopsis pinnata</i> J.V. Lamouroux		tr	tr
<i>Centroceras clavulatum</i> (C. Agardh) Montagne		tr	tr
<i>Ceramium</i> sp.		tr	tr
<i>Ceramium virgatum</i> Roth		tr	tr
<i>Coelothrix irregularis</i> (Harvey) Børgesen		tr	tr
<i>Dictyosphaera</i> stage of <i>Padina</i>		tr	tr
<i>Digenea symplex</i> (Wulfen) C. Agardh		tr	tr
<i>Gelidiella trinitatensis</i> W.R. Taylor		tr	tr
<i>Haematocelis</i> sp.		tr	tr
<i>Jamia capillacea</i> Harvey		tr	tr
<i>Liagora</i> spp.		tr	tr
<i>Polysiphonia</i> sp.		tr	tr
<i>Spirocoleus</i> cf. <i>fragilis</i> (Meneghini) P.C. Silva		tr	tr
<i>Trichosolen</i> sp.		tr	tr
Total Biota			56.6
Bare rock			11.2
Sand			32.2

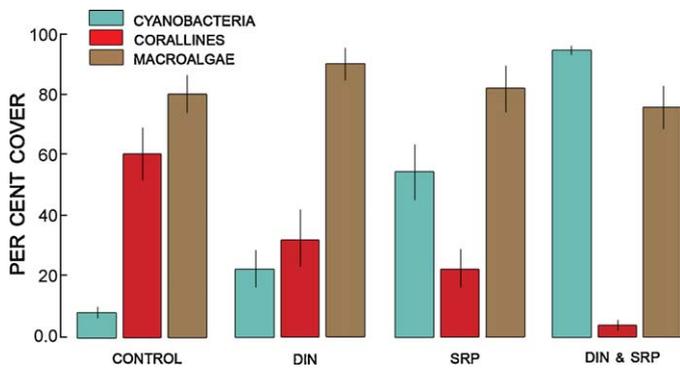


Fig. 4. The development of turf algae (dominated by the cyanophyte *Spirulina*, light shading), crustose coralline algae (mostly *Hydrolithon* and *Amphiroa* crusts, medium shading) and fleshy macroalgae (*Acanthophora*, *Palisada*, *Padina* and *Spyridia*, dark shading, cf. Table 2) on terra-cotta mini-reef diffusers after 12-mo exposure to DIN, SRP, DIN + SRP and ambient (control) nutrients.

phosphorus-enriched diffusers after only 3 d. *Spirulina* sp. was a dominant occupier of space showing significant ($P < 0.05$) stimulation under the SRP and DIN treatments, relative to the controls, and was particularly enhanced ($P < 0.05$) under SRP + DIN combined at the 12-mo assessment (Figs. 4 and 5).

Concomitantly, beneficial reef-building crustose coralline algae were inhibited in the presence of Cyanobacteria, under high SRP (Fig. 4), and this resulted in a pronounced divergence from the successional pathways shown by the controls. Coralline algae, consisting mostly of *Hydrolithon boergesenii* and crusts of *Amphiroa fragilissima*, were significantly ($P < 0.05$) inhibited in both the DIN and SRP treatments, but especially under SRP (Fig. 5).

Fleshy macroalgal blooms were the most-abundant colonizers (Fig. 5), dominated by *A. spicifera*, *P. sanctae-crucis*, *Spyridia filamentosa*, *Dictyota* spp. and *Palisada (Laurencia)* spp. (similar to the surrounding natural macrophyte community, Table 2), but were not significantly ($P > 0.05$) enhanced or inhibited by the addition of nutrients (Fig. 4), relative to the controls.

4. Discussion

Nutrient manipulative studies have used window-screen baggies (Thacker et al., 2001), plastic pipes with holes (Flothmann and Werner, 1992; Furman and Heck, 2008), internally inoculated plastic bags (Lapointe, 1985, 1987; Miller and Hay, 1996), drilled hollow concrete blocks (Miller et al., 1999) and broadcast spreading (McClanahan et al., 2002; 1.0 kg of Scott's slow-release high-P fertilizer per 0.36 m² plot) to disperse various chemical nutrients. We selected porous terra-cotta clay flowerpots as the slow-release nutrient diffusers to provide gradual release, realistic gradients and uniform dispersion, based on our own experiments (Littler et al., 1989, 2006a, 2006b) and following other successful applications for this purpose (see Chapman and Craigie, 1977; Hatcher and Larkum, 1983).

As in the case of the majority of reef studies that have manipulated nutrients (e.g., Larkum and Koop, 1997; Miller et al., 1999; Thacker et al., 2001; Belliveau and Paul, 2002; McClanahan et al., 2002, 2003), the present findings cannot be used to test the RDM (Fig. 1 in Littler and Littler, 2007)—because the ambient nutrient concentrations (as determined next to the non-enriched control diffusers) were already at or above the suggested 0.1 μM SRP and 1.0 μM DIN tipping-point levels for release of macroalgal growth. We further suggest (as did Lapointe et al., 2004) that *in situ* enrichment studies in such nutrient-replete systems should not show macroalgal stimulation, because the concentrations conducive to macroalgal growth have already been exceeded. This was definitely the case in the present study (supported by the tissue

analyses, Table 3), as well as in relatively short-term (<4 mo) fertilization studies in the Florida Keys (Miller et al., 1999; Furman and Heck, 2008), Guam (Thacker et al., 2001) and Glovers Atoll (McClanahan et al., 2002), which were mostly carried out in nutrient-sufficient study areas characterized by minimal coral cover and abundant algae. However, as in the present study, most of these studies showed nutrient stimulation of algal turf species—primarily Cyanobacteria (see also Littler et al., 2006b). Many marine Cyanobacteria fix atmospheric nitrogen and, therefore, are stimulated by phosphorus enrichment (Kuffner and Paul, 2001). The Cyanobacteria bloom we recorded (Fig. 4) likely inhibited the settlement, recruitment and expansion of the beneficial reef-building crustose coralline algal group.

Nutrient studies are logistically difficult and, because the growth/inhibition responses are relatively slow (i.e., chronic), require sufficiently long time periods with more emphasis on multifaceted approaches (e.g., Littler et al., 2006a). Optimally, research should test the long-term competitive interactions of functional indicator groups on healthy coral-dominated reefs and lagoons, in addition to precisely monitoring water-column and tissue-nutrient levels. The ambient dissolved inorganic nutrient concentrations next to the bases of the non-enriched control mini-reef diffusers were consistently above (mean = 2.06 μM DIN) or just at (0.12 μM SRP) suggested tipping-point levels for release of macroalgal growth. Nutrient additions to the mini-reef diffusers resulted in significant elevations of tissue nutrients in the dominant colonizer *A. spicifera* (Table 3). As expected, all tissue nutrient ratios showed levels characteristic of their particular nutrient-enhanced treatments. Interestingly, the resultant significant ($P < 0.05$) decreases of both the C:N and C:P ratios clearly implicate superfluous (luxury) uptake, since control population colonization and growth matched that of the experimental nutrient treatments (Figs. 4 and 5).

The present research adds to the wealth of *in situ* experimental findings (e.g., Larkum and Koop, 1997; Thacker et al., 2001; Belliveau and Paul, 2002; McClanahan et al., 2002, 2003) documenting the absence of stimulatory bottom-up effects on fleshy macroalgae. The low ambient levels present over such a wide spectrum of locations are important in supporting the concept of low-nutrient thresholds (Bell, 1992; Lapointe et al., 1993; Bell et al., 2007), or tipping-points, for potential fleshy algal overgrowth.

Harmful macroalgal blooms (HAB's = excessive algal biomass) on coral reefs have long been attributed to nutrient enrichment and eutrophication (e.g., Littler, 1973; Banner, 1974; Johannes, 1975; Smith et al., 1981; Lapointe, 1997; Lapointe et al., 2005a, 2005b; Vroom et al., 2005). However, some reef scientists recently have countered that such changes in benthic community structure are dependant on natural stochastic events (Precht and Miller, 2007; Littler et al., 2009), overfishing of herbivorous fish stocks (Hughes, 1994; Pandolfi et al., 2003) and/or loss of keystone grazers such as the long-spined sea urchin *Diadema antillarum* (Jackson et al., 2001). These last conclusions are supported by the numerous grazer-reduction experiments in nutrient-replete systems but not in strictly **oligotrophic** environments that, for the most part, have reported an expansion of low-growing fleshy algal forms, algal turfs and/or filamentous forms (<3 cm high) rather than macroalgal blooms (as predicted in the RDM; see Lapointe, 1999). These include case studies in the Red Sea (Vine, 1974), Fiji (Littler and Littler, 1997), Belize (Littler et al., 2006a, 2006b), the Great Barrier Reef (Sammarco, 1983) and St. Croix (Carpenter, 1988). Conversely, the combination of exceeded tipping points in the present study for both nutrients and herbivory led ultimately to dominance by undesirable frondose macroalgal blooms under all experimental and control conditions (see also Smith et al., 2001; Littler et al., 2006a). Similar control of reef systems by interactions

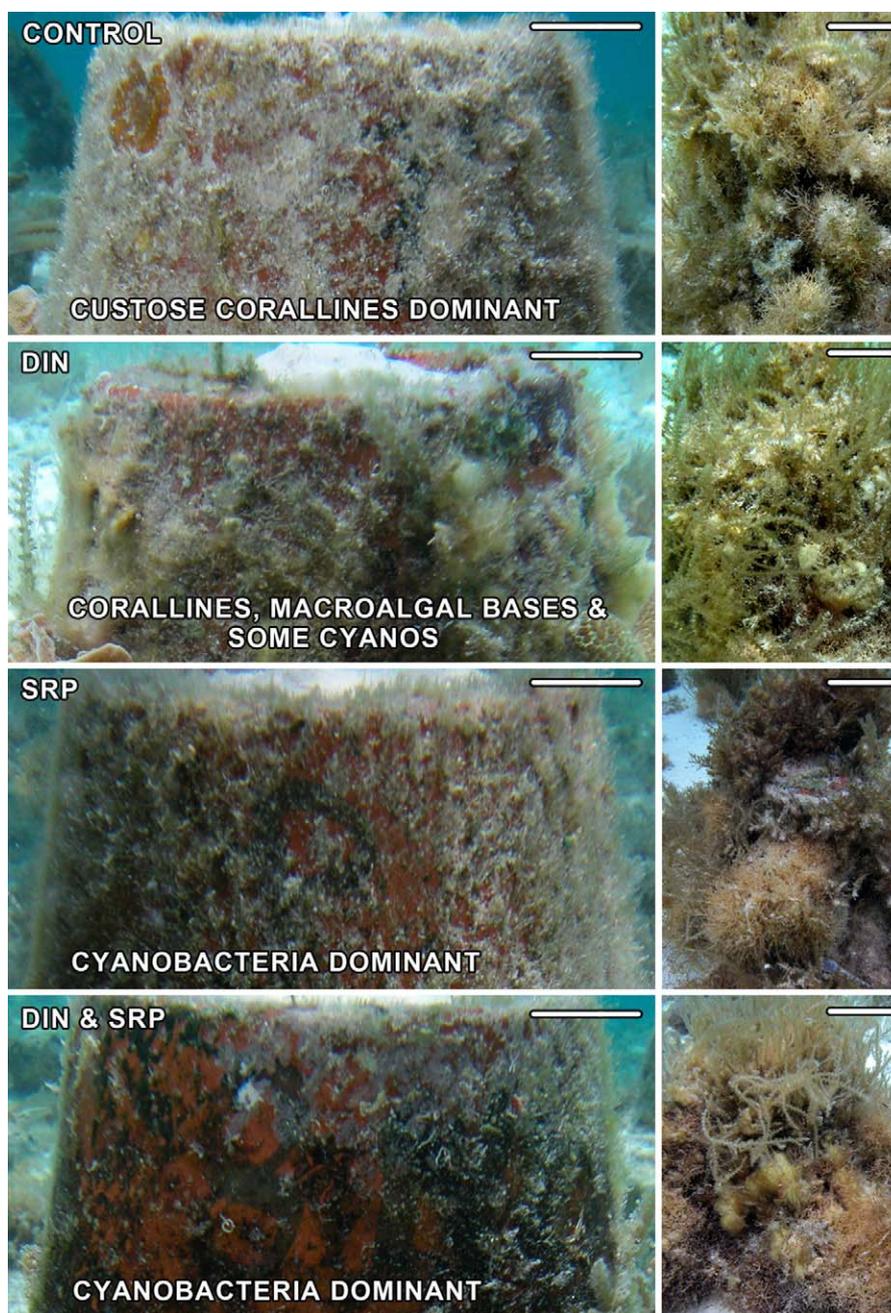


Fig. 5. Photoquadrat examples of fleshy macroalgal overstory communities (right side images, scale bars = 4 cm) and predominant understory populations (left side larger images—following careful removal of fleshy macroalgae, scale bars = 2 cm) after 12-mo exposure to ambient (CONTROL), DIN, SRP and DIN + SRP nutrients. Cyanobacteria (dominant *Spirulina*) is the black crust-like turf.

of both nutrients and grazing have been shown by correlative/statistical studies over large regional scales (Burkepile and Hay, 2007; Mora, 2008).

Cyanobacteria blooms (blue-green algae) were significantly stimulated by nutrient enrichment at this back-reef site (Fig. 4),

Table 3

Tissue C:N and C:P molar ratios (\pm S.E.) for *A. spicifera* colonizing the various mini-reefs after 12 mo. All experimental N and P means ($N=10$) were significantly ($P < 0.05$, Bonferroni) reduced relative to the controls ($N=6$).

Treatments	C:N	C:P
Control	18.9 \pm 0.6	610.5 \pm 130.4
+N	14.8 \pm 2.7	–
+P	–	357.5 \pm 131.9

consistent with earlier findings (Miller et al., 1999; Littler et al., 2006a, 2006b). However, Miller et al. (1999), by dismissing the blue-green algae as bacteria, concluded that coral-reef algae are not nutrient-limited. Others consider blue-green algae to be among the most prevalent primary producers (often as large distinctive colonies) on coral reefs (Littler et al., 2006b), and include them as important components of coral-reef communities. This group is often considered harmful to coral-reef systems and has increased in abundance and importance (Kuffner and Paul, 2001) in association with world-wide declines in coral-reef health. Many blue-green algal species can fix atmospheric nitrogen and, hypothetically, should be among the first to bloom when SRP concentrations are elevated or when SRP:DIN ratios increase in general (shown by Kuffner and Paul, 2001). Interestingly, the

dominant cyanobacterium *Spirulina* lacks heterocysts that fix atmospheric nitrogen, suggesting why it may have responded so positively to additions of both DIN and SRP (Figs. 4 and 5).

As mentioned above, an important result of the present research (Fig. 4) is that crustose coralline algae were inhibited under the high SRP (and DIN) treatments and this resulted in a pronounced divergence in the successional pathways. Our interpretation is that the nutrient-induced Cyanobacteria bloom secondarily inhibited the long-term settlement and colonization of crustose coralline algae (Fig. 5) and not nutrients (in agreement with Furman and Heck, 2008). Unpalatable epiphytic Cyanobacteria have been documented (Fong et al., 2006) to maintain shifts toward their macroalgal hosts' dominance on coral reefs. Ultimately, a diversity of large fleshy macroalgae settled and colonized abundantly on all of these low-herbivory treatments, indicating nutrient-replete conditions, even though control nutrient levels, particularly SRP, remained low by most standards. Using experimental approaches on appropriately oligotrophic coral-dominated reefs, Smith et al. (2001) and Littler et al. (2006a) provided the most relevant experimental evidence testing and supporting both the nutrient and herbivory tenets of the RDM. These findings were taken a step further in developing useful assessment protocols and standards for coral-reef management and conservation (Littler and Littler, 2007).

Many workers (reviewed in Steneck, 1989; McCook, 1999; Bellwood et al., 2004) have established that decreasing herbivory, without variations in nutrient inputs (although only assumed to remain constant in most grazing studies), results in rapid increases of HAB's (excessive algal biomass) on tropical reefs. However, the reverse of this, "that increasing nutrients without changing herbivory also results in increases of HAB's", has been widely disputed. As mentioned, a major reason is that many accessible tropical-reef systems already exceed critical tipping points enabling fast-growing fleshy-algal blooms to occur quite rapidly (acute effects) when herbivory is reduced. Conversely, when nutrients are severely limiting, their additive effects are slower to materialize, hypothetically because of physiological stasis and the fact that the indirect competitive interactions set in motion (chronic effects) take place over much longer time scales. For this reason, more studies are needed that include *in situ* enrichment experiments testing **longer-term** competitive interactions of functional indicator groups on oligotrophic coral-dominated reefs (e.g., Smith et al., 2001; Littler et al., 2006a).

5. Conclusions

While many coral-reef scientists agree that both nutrients and herbivory are important, and the details of their relative roles depend on the situation (see Lapointe, 1999; Bellwood et al., 2004), the coral-reef research community still needs a broader biological perspective to further the recognition of the role played by chronic nutrient enrichment in the coral-reef health/resilience paradigm. The problem is that bottom-up experimental research is logistically difficult and requires more emphasis on multifaceted approaches carried out over sufficiently long time periods. These include proven oceanographic methods (e.g., sensitive 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). Such methods present logistical difficulties that have resulted in limited numbers of studies, 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 both factors (see Mora, 2008). Both scientists and managers are

beginning to consider more broadly the complex role that escalating nutrient enrichment plays in the regulation of HAB's on marine ecosystems.

Acknowledgements

Support for this study came from the Smithsonian Institution Scholarly Studies Program, Caribbean Coral Reef Ecosystems Program (CCRE Contribution No. 869), the Smithsonian Marine Station at Ft. Pierce (SMSFP Contribution No. 808) and the National Museum of Natural History.[SS]

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