Co-occurrence of dinoflagellate and cyanobacterial harmful algal blooms in southwest Florida coastal waters: dual nutrient (N and P) input controls

Hans W. Paerl[1],*, Jennifer J. Joyner[1], Alan R. Joyner[1], Karen Arthur[2], Valerie Paul[2], Judith M. O'Neil[3], Cynthia A. Heil[4]

[1]**Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell Street, Morehead City, North Carolina 28557, USA**

[2]**Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949, USA**

[3]**Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, Maryland 21613, USA**

[4]**Fish and Wildlife Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Avenue SE, St. Petersburg, Florida 33701-5020, USA**

ABSTRACT: During July 2006, 2 distinctly different harmful algal blooms (HABs), one dominated by the pelagic red tide dinoflagellates *Karenia* spp. and the other by the benthic cyanobacterium *Lyngbya majuscula*, occurred simultaneously in the coastal embayments surrounding Sanibel and Captiva Islands, Florida, USA. The co-occurring HABs were investigated using *in situ* bioassays with additions of nitrogen (N) and phosphorus (P) alone and in combination to assess nutrient controls of these 'dueling' toxin-producing species. Photosynthetic, biomass (chlorophyll *a*), and (in *L. majuscula*) nitrogen fixation responses to nutrient enrichment were examined over 4 d. Primary productivity in *Karenia* spp. was consistently stimulated by N additions, while P additions failed to show stimulation. When added in combination with N, P did not lead to additional stimulation above N alone. Similar patterns of chlorophyll *a* stimulation were observed. These patterns were observed at 2 d, after which the cells fell out of suspension. Nutrient stimulation of *L. majuscula* metabolic activities as well as biomass production was smaller and much slower, relative to controls, than responses observed in *Karenia* spp. After the demise of *Karenia* spp., *L. majuscula* was able to continue utilizing subsequent nutrient additions, and it responded most strongly to the N+P additions after 4 d. This study confirms previous estuarine and coastal studies that indicated that when non-N_2-fixing HABs co-occur with N_2-fixing cyanobacterial HAB species, both N and P inputs need to be carefully considered and, in all likelihood, controlled.

KEY WORDS: *Lyngbya* · *Karenia* · Charlotte Harbor · Sanibel and Captiva Islands · Florida · Blooms · Nutrients

INTRODUCTION

Increasing amounts of anthropogenic N and P enrichment have accompanied accelerating agricultural and industrial development and urbanization in Florida's coastal watersheds, embayments and keys (Turner et al. 2006, Walsh et al. 2006, Heil et al. 2007, Vargo et al. 2008). Nutrient over-enrichment has been linked to accelerating primary production, or eutrophication, and increased algal bloom frequencies of both harmful and non-harmful phytoplankon species in many coastal regions (Paerl 1987, 1988, Nixon 1995, Boesch et al. 2001, Cloern 2001, Elmgren & Larsson 2001), including those in Florida (Tomasko et al. 1996, Lapointe 1997, Turner et al. 2006). In Florida and other subtropical regions, increases in nutrient loading have been linked with both diatom (Turner et al. 2006) and cyanobacterial (Glibert et al. 2004) blooms, although blooms of planktonic dinoflagellates and benthic cyanobacteria are also common: the most notorious

species include the Florida red tide dinoflagellate *Karenia brevis* (Steidinger et al. 1998, Steidinger 2002, Vargo et al. 2008) and, among the cyanobacteria, the planktonic genus *Trichodesmium* and the benthic genus *Lyngbya*. While the origin of these bloom-forming taxa (i.e. offshore, nearshore, estuarine) can vary substantially, there is good agreement that when these bloom species encounter nutrient-enriched conditions, growth and bloom intensity of many of these taxa may be enhanced (Paerl 1988, Lapointe 1997, Elmgren & Larsson 2001, Paerl & Fulton 2006). Accordingly, there is a great deal of concern about coastal nutrient enrichment associated with rapid rates of coastal development in Florida and elsewhere.

During July 2006, 2 taxonomically and ecologically distinct harmful algal blooms (HABs), one comprised of planktonic Florida red tide dinoflagellates *Karenia* spp. and the other of the benthic filamentous cyanobacterium *Lyngbya majuscula*, co-occurred in the coastal embayments of Sanibel and Captiva Islands, on Florida's southwest coast (see Fig. 1). Both genera have been implicated in regional water quality, fisheries habitat, and human health problems (Steidinger et al. 1998, O'Neil & Dennison 2005, Heil et al. 2007, Paul et al. 2007). Blooms of *Karenia* spp. are initiated from 18 to 64 km offshore in the southwest Gulf, and are transported into coastal embayments by currents and tides during the bloom maintenance stage (Steidinger et al. 1998, Walsh et al. 2006). *Lyngbya* spp. are largely found attached to seagrasses and other benthic substrates, including sand, rocks, oysters, and other benthic flora and fauna. While these species have previously been reported as individual blooms in Florida and other coastal habitats, this was one of the first opportunities to assess their nutrient requirements simultaneously in the same water mass, using *in situ* nutrient addition bioassays. Despite their contrasting habitat preferences, these HAB species co-occurred, in large part because *Karenia* spp. was transported into shallow, subtidal waters in which *L. majuscula* flourished as benthic mats and epiphytes. Due to their close proximity, it was likely that both *Karenia* spp. and *L. majuscula* derived their nutrients from similar anthropogenic and natural origins. This enabled us to contemporaneously examine nutrient limitation among these HABs.

We utilized an *in situ* bioassay technique (Paerl & Bowles 1987, Paerl et al. 1987), previously applied to benthic and planktonic microalgae, to examine photosynthetic and (in the case of *Lyngbya majuscula*) nitrogen fixation responses together and individually to dissolved inorganic nitrogen (DIN) as nitrate, and phosphorus as phosphate, enrichments. This was done to determine which nutrient(s) were limiting and to what extent these HABs may have been competing for

similar nutrient resources. The bioassays were designed to address one central question: Should we be concerned about and hence control only 1 nutrient or both N and P?

MATERIALS AND METHODS

Water column and benthic HAB samples were collected by boat at Redfish Pass (Fig. 1), a tidal inlet between North Captiva and Captiva Islands (26° 32′ 50.76″ N, 82° 11′ 30.18″ W) on 11 July 2006. Water was collected during an incoming tide. Water column samples, which were discolored by a bloom of *Karenia* spp. (Fig. 2), were collected in 0.1 N HCl pre-cleaned 20 l Nalgene polyethylene carboys that were thoroughly rinsed with sample water prior to filling. At the same time and at the same location, *Lyngbya majuscula*, which occurred as benthic tuft-like mats (Fig. 2), was collected by snorkelers, rinsed with ambient seawater and placed in darkened coolers containing ambient seawater. Both water column and benthic *L. majuscula* samples were transported to the marine laboratory at the Sanibel-Captiva Conservation Foundation, Sanibel Island, Florida, where they were processed for *in situ* bioassays within ~2 h of collection. Care was taken to maintain water temperatures similar to those at the sampling locations and to avoid potentially photo-inhibitory irradiance levels when the samples were brought back to the laboratory.

***In situ* bioassays.** Bioassays were conducted in quintuplicate in 3 l clear polycarbonate wide-mouth jars, which were pre-cleaned with 0.1 N HCl and then rinsed twice with deionized water (DI), ensuring nutrient-free conditions prior to adding sample water. One set of jars contained only ambient seawater which was dominated by *Karenia* spp., and 1 set of jars contained the same ambient seawater and *Lyngbya majuscula*. Both sets received a full range of nutrient treatments (Table 1). The concentrations and ratios of each species reflected what was in the environment as we did not concentrate the *Karenia* spp. (i.e. used whole water samples), and the *L. majuscula* represented what was covering the benthos. Tufts of *L. majuscula* that displaced 10 ml were added to 3 l of water collected at the same site (Figs. 2 & 3), taking care to use pre-cleaned (rinsed with 0.1 N HCl and DI) 4 l graduated cylinders and forceps to transfer sample water and *L. majuscula* tufts, respectively.

All nutrient additions were made with reagent grade (Fisher) chemicals. Nitrogen was added as potassium nitrate (KNO_3), while P was added as monobasic potassium phosphate (K_2HPO_4). Nutrient stock solutions were made up with 18 MΩ DI in polyethylene (Nalgene) bottles, which were sterilized. Nutrients were
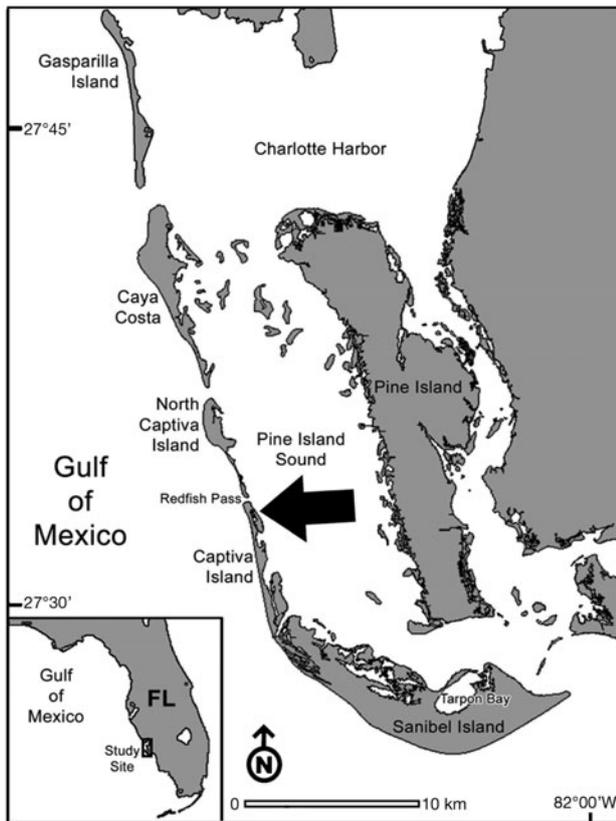
Fig. 1. The Sanibel-Captiva region, Florida, USA, showing location near Redfish Pass (black arrow) where samples were collected. Samples were incubated *in situ* at the Sanibel-Captiva Conservation Foundation Marine Laboratory near Tarpon Bay, Sanibel Island

dispensed to the bioassay containers from stock solutions using sterile pipettes pre-cleaned with DI. Nutrient additions of nitrate and phosphate were made every evening at the concentrations shown in Table 1 to allow time for nutrient uptake before the following morning's primary productivity response measurements were conducted. Determinations of nutrient additions and ratios used in bioassay treatments were based on allochthonous and internally regenerated nutrient inputs and ratios representative of this region (Montgomery et al. 1991, Tomasko et al. 1996, Vargo et al. 2008).

Table 1. *Lyngbya majuscula* and *Karenia* spp. Treatments and corresponding nutrient additions for the bioassay incubations

Treatment	Nutrient additions
Control	None
N	10 µM N-KNO$_3$
P	5 µM P-K$_2$HPO$_4$
N + P	10 µM N-KNO$_3$, 5 µM P-K$_2$HPO$_4$

Following nutrient additions, bioassay vessels were placed in a floating 'corral' that was suspended off the dock at the Sanibel-Captiva Conservation Foundation Marine Laboratory (Fig. 3). Irradiance, temperature and wave conditions were similar to those at the sampling location. A layer of neutral density screening was placed over the corral to reduce incident radiation by ~50%, thereby minimizing photoinhibition during the bioassay incubation period of up to 4 d. Subsamples were collected from each bioassay at the beginning (T_0), Day 2 (T_2, 48 h), and for *Lyngbya majuscula* only, on Day 4 (T_4, 96 h) after the start of the bioassay.

Algal response parameters included the following: photosynthetic activity, determined with the ^{14}C technique adapted for bioassays (Paerl et al. 1987); nitrogen fixation activity (nitrogenase activity), assessed using the acetylene reduction assay (Paerl 1998); and for *Karenia* spp., growth based on cell counts and changes in chlorophyll *a* (chl *a*) content (Paerl et al. 1987). Although chl *a* in *Lyngbya majuscula* does not provide a proxy for growth, it is an indicator of health or robustness of nutrient stores. Photosynthetic and nitrogenase activity were determined on a per-unit biomass (g dry wt) basis. A wet weight was taken of all of the *L. majuscula* tufts, and at least 10 tufts were later dried to calculate a wet weight to dry weight conversion based on the linear relationship between wet and dry weights. Wet weights were converted to dry weights using the formula $D = 0.3273 \times W - 0.0036$, where W is wet weight (g) and D is dry weight (g).

Samples to measure photosynthetic CO_2 fixation were collected at T_0 and from each bioassay container during subsampling periods. For planktonic samples, a 20 ml subsample was withdrawn from each container using a 20 ml syringe and dispensed into a 20 ml glass liquid scintillation vial, which served as the incubation vessel. In the case of *Lyngbya majuscula*, subsamples of a consistent weight (0.527 ± 0.105 g wet wt; wet weights were converted to dry weights following the experiment using the formula in the previous paragraph) were taken from the bioassay containers and placed in the 20 ml scintillation vials with 20 ml bioassay incubation water. Each scintillation vial received 0.3 ml NaH^{14}CO$_3$ (10.6 µCi ml^{-1}; specific activity, 58 µCi µmol^{-1}). The scintillation vials were then incubated for ~4 h during mid-day in the bioassay incubation corrals and covered with 1 layer of neutral density screening. One subsample per treatment was placed in a 20 ml darkened vial to account for dark CO_2 fixation and/or abiotic uptake. Following the incubation, the samples were filtered onto 25 mm Whatman GF/F filters. The filters were fumed for 4 h with concentrated HCl to remove unincorporated ^{14}C and then dried. Once dry, the filters were placed in 7 ml scintillation vials and transported to the Institute of Marine Sciences (Morehead City, North Carolina), where 5 ml of scintil-
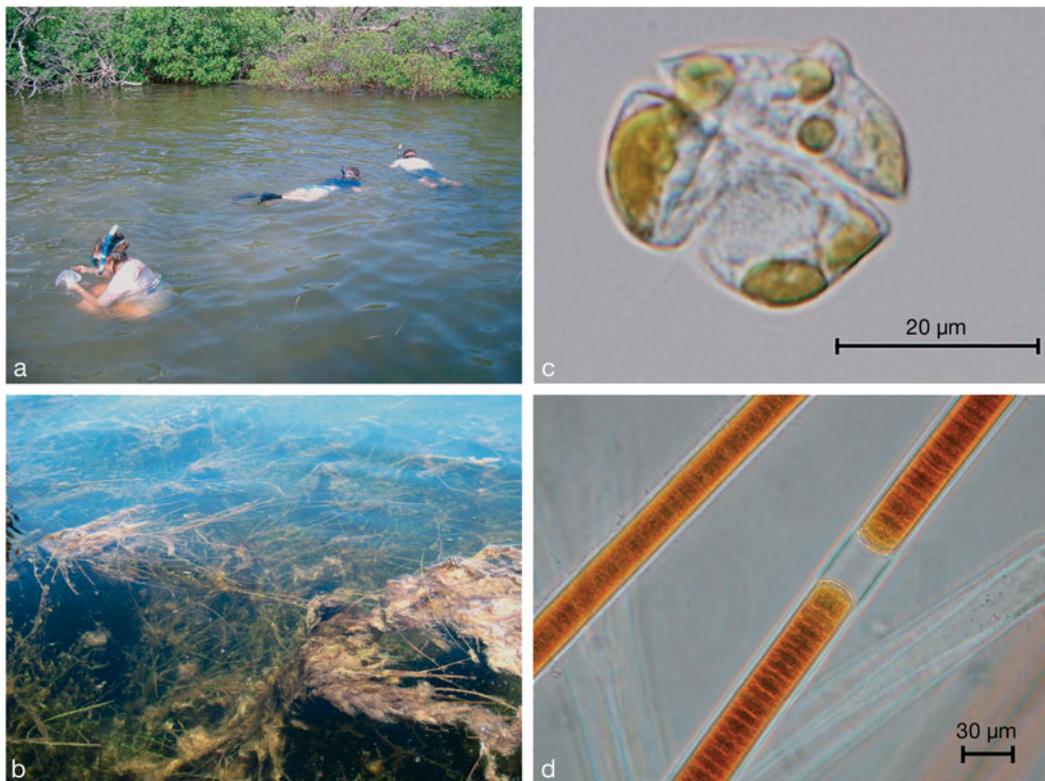
Fig. 2. *Karenia* spp. and *Lyngbya majuscula*. Habitats near Redfish Pass. (a) Collection of *L. majuscula* in waters containing co-occurring blooms of *Karenia* spp. (the waters being sampled were discolored due to this red tide). (b) *L. majuscula* smothering a seagrass bed. Also shown are photomicrographs of (c) *K. brevis* and (d) *L. majuscula*

Fig. 3. Bioassay preparation and deployment at Sanibel Island. (a) Clear polycarbonate vessels containing *Lyngbya majuscula* mats. (b,c) *In situ* incubation corrals containing bioassay vessels. Note layer of neutral density screening used to attenuate direct sunlight and minimize photoinhibition

lation cocktail (Cytoscint; ICN) was added to each vial. Vials were sealed and gently shaken, and allowed to sit for 24 h before being counted. Counts (Beckman Coulter LS 6500 liquid scintillation counter) were converted to disintegrations min^{-1} using a quench curve based on calibrated (^{14}C) hexadecane (NEN). The dissolved inorganic carbon content of overlying water was measured by infrared gas absorption analysis (LiCor Model Li6252). Rates of total CO_2 fixation (primary productivity) were calculated using the equation provided in Paerl et al. (1999).

Nitrogenase activity was measured using the acetylene reduction technique (Paerl 1998). On T_0 (prior to the addition of nutrients), T_2, and T_4, pre-weighed tufts of *Lyngbya majuscula* (0.5 g wet wt, 0.16 ± 0 g dry wt) were subsampled from the bioassay and placed into 167 ml glass serum vials, 100 ml bioassay incubation water was added, and the vials were capped with rubber stoppers. From each vial, 20 ml of gas were removed, resulting in a slight vacuum, then 20 ml acetylene gas was bubbled into each of the bottles. Controls consisted of a set of 3 bottles containing only DI, a set containing control treatment water and *L. majuscula* and no acetylene gas, and a set containing control treatment water and acetylene gas. After ~4 h incubations in the corrals (covered by 1 layer of neutral density screening), the vials were vigorously shaken for 20 s, and then 4 ml gas from the headspace of each vial was transferred to evacuated 3 ml Vacutainers (Becton Dickinson) for storage and travel back to the Institute of Marine Sciences. Ethylene concentrations were quantified using a Shimadzu model GC9A gas chromatograph with flame ionization detector and a 2 m Poropak-T column at 80°C (Paerl 1998). The DI control values were subtracted from each measurement to account for any ambient ethylene (Paerl 1998).

Samples for *Karenia* spp. identification and enumeration were taken from the overlying water in the *Lyngbya majuscula* bioassay at T_4 and chl *a* samples were collected at T_0 and T_2. Samples for identification were immediately preserved with Lugol's preservative and counted within 4 wk of the experiment. *Karenia* spp. was identified to the species level where possible according to Steidinger (2008). For the *L. majuscula* chl *a* values, 0.5 g wet wt was frozen in individual aluminum foil packets. For the phytoplankton and *Karenia* spp. chl *a* analyses, 50 ml bioassay incubation water was filtered onto Whatman GF/F filters. The filters were blotted dry, wrapped in individual aluminum foil packets and frozen at –20°C in a freezer on site for transportation on blue ice to the laboratory for analysis. In the laboratory, chl *a* samples were extracted overnight at –20°C using 90:10 acetone:water mix and the concentration was determined by narrow band-pass fluorometry (Welschmeyer 1994) using a Turner TD-700 fluorometer.

Water nutrient analysis samples were collected at 39 h after the T_0 dosing. Bioassay water (100 ml) was gently filtered through a pre-combusted Whatman 25 mm GF/F filter, and the filtrate was frozen at –20°C within 6 h of collection. Filters were also frozen for transport. At the Institute of Marine Sciences, cellular C and N were analyzed by fuming the filters with concentrated HCl to remove excess inorganic C, and then analyzed on a Perkin Elmer Series II CHNS/O 2400 analyzer (Peierls et al. 2003). Total dissolved nitrogen (TDN), $NO_2^- + NO_3^-$, NH_4^+, and PO_4^{3-} were measured from the filtrate on a Lachat Quick-chem 8000 auto-analyzer using standard protocols (Lachat Quik-chem methods 31-107-04-3-B, 31-107-04-1-C, 31-107-06-1-B, and 31-115-01-3-C, respectively).

Statistical analyses. All results are presented as mean ± SE. Statistical analyses were conducted using Sigma Stat V3.11 (Systat Software) and consisted of a 1-way ANOVA after determining that the data were normally distributed and displayed homogeneity of variances. Where a significant result was obtained for the ANOVA, a Tukey's post hoc pairwise comparison was done to assess differences between treatment groups.

RESULTS

Both *Karenia brevis* and *Lyngbya majuscula* were dominant in planktonic and benthic environments, respectively, at the time of collection and appeared to thrive in bioassay containers for at least 48 h as assessed at the T_2 time point. Three different species of *Karenia* were identified in the bloom and a fourth category, *Karenia* sp., for cells that could not be identified to species level due to cell orientation in samples. These cells were most likely *K. brevis* or *K. mikimotoi*. The *Karenia* spp. portion of the bloom was dominated by *K. brevis* (74%), followed by *K. mikimotoi* (17%), *Karenia* sp. (identification to be confirmed) (8%), and *K. seliformis* (1%). Total *Karenia* spp. cell counts at T_4 were highly variable, making it difficult to ascertain a nutrient treatment effect, although the highest average *Karenia* spp. cell counts were observed in the N+P treatment (Fig. 4). The incubation comprising *Karenia* spp. alone was terminated after 3 d (following 72 h incubation) because the dinoflagellates began to fall out of suspension. The *L. majuscula* bioassay was continued for 4 d (T_4).

During the bioassays, the HAB species took up all soluble N and P nutrients in the water column of unenriched control samples, and treatments with nutrient additions had sufficient concentrations to ensure that the organisms were not N- or P-limited as there were excess nutrients in the water column 39 h after nutrient additions (Fig. 5). At T_2, both species appeared to be

healthy, as they were photosynthetically fixing CO_2 (Fig. 6), and *Lyngbya majuscula* was fixing nitrogen (data not shown). At T_2, *Karenia* spp. in both the *L. majuscula* incubation water and the bioassays of *Karenia* spp. incubated alone exhibited nitrogen stimulation of primary productivity as CO_2 fixation (p = 0.008 and p < 0.001, respectively; Fig. 6A) and a similar trend was observed in biomass as chl *a* concentration, although this was not statistically significant (p = 0.074 and p = 0.598, respectively; Fig. 6B). In contrast to what was observed for *Karenia* spp. at T_2, *L. majuscula* did not show any nutrient treatment effects in terms of productivity or in terms of chl *a* concentration relative to controls (p = 0.963 and p = 0.061, respectively; Fig. 6), but at T_4 after the *Karenia* spp. were observed to fall out of suspension, productivity in the *L. majuscula* bioassay increased in the N+P treatment from an average of 19.6 µg C g^{-1} h^{-1} at T_2 to 33.6 µg C g^{-1} h^{-1} at T_4 (Fig. 7). When N and P were administered together, this treatment yielded maximum productivity values at T_4 (p = 0.053), which suggested that while the *Karenia* spp. was in suspension, it effectively utilized N and P in the presence of *L. majuscula*. After the apparent settling out of suspension of *Karenia* spp., *L. majuscula* continued to utilize subsequent nutrient additions.

Nitrogen fixation activity (nitrogenase activity) was confined to samples and treatments that contained *Lyngbya majuscula*, confirming earlier studies that members of this filamentous, non-heterocystous cyanobacterial genus are capable of N_2 fixation (Paerl et al. 1991, Phlips et al. 1991). Rates of nitrogenase activity, measured as acetylene (C_2H_2) reduction, exhibited very high variability, ranging from 13.4 to 617.2 nmol C_2H_4 mg^{-1} *L. majuscula* dry wt h^{-1}. The highest values for nitrogenase activity occurred on the initial day of sample collection (T_0). At T_0, values were 71.9 nmol C_2H_4 mg^{-1} *L. majuscula* dry wt h^{-1} during the day and 353.4 nmol C_2H_4 mg^{-1} *L. majuscula* dry wt h^{-1} at night.

DISCUSSION

This opportunistic study of co-occurring HAB species demonstrates that the pelagic non-nitrogen-fixing dinoflagellates *Karenia* spp. are stimulated by N additions and probably dominate the uptake of latent nutrients over the benthic cyanobacterium *Lyngbya majuscula*. After the demise of the dinoflagellates in the bioassays there was an increase in *L. majuscula* productivity in the treatment where both N and P were added. The observed N and P co-stimulation of *L. majuscula* primary production demonstrates the potential for these nutrients to individually and jointly enhance growth of this benthic HAB, an observation that has also recently been made for *L. majuscula* populations in Moreton Bay, a subtropical estuarine and coastal embayment in Australia (Albert et al. 2005, Ahern et al. 2007). These findings further illustrate the metabolic flexibility of the genus *Lyngbya*, freshwater and marine species which are capable of at least partially satisfying their N requirements through N_2 fixation (Paerl et al. 1991, Phlips et al. 1991, Olson et al. 1999, Lundgren et al. 2003, Joyner et al. 2008).

Even though members of this genus are capable of diazotrophy, they are also capable of growth on com-
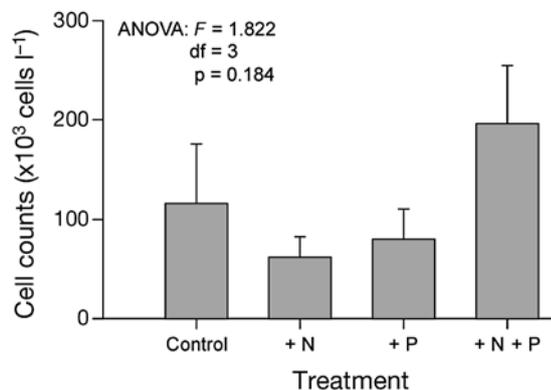

Fig. 4. *Karenia* spp. Cell counts in bioassay of *Karenia* spp. incubated alone at T_2 (48 h incubation). Error bars represent +1 SE
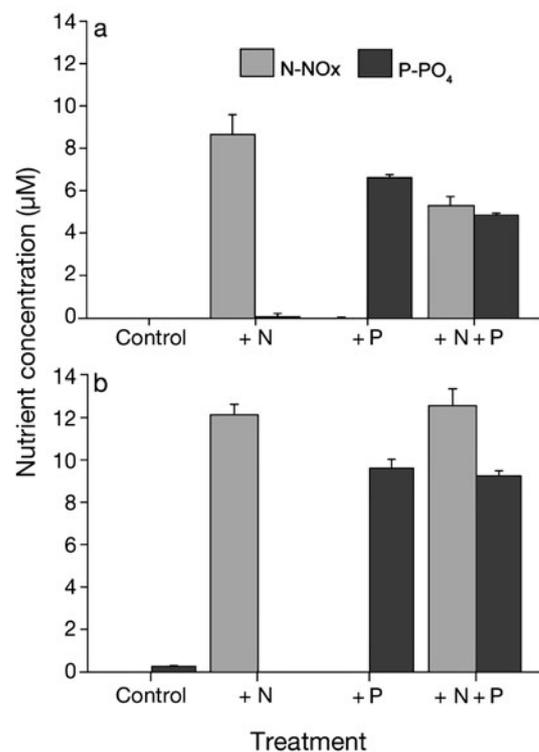

Fig. 5. *Karenia* spp. and *Lyngbya majuscula*. Nitrate and phosphate concentrations in water of each bioassay treatment (a: *L. majuscula*; b: *Karenia* spp.) at T_2 (48 h incubation). Error bars represent +1 SE
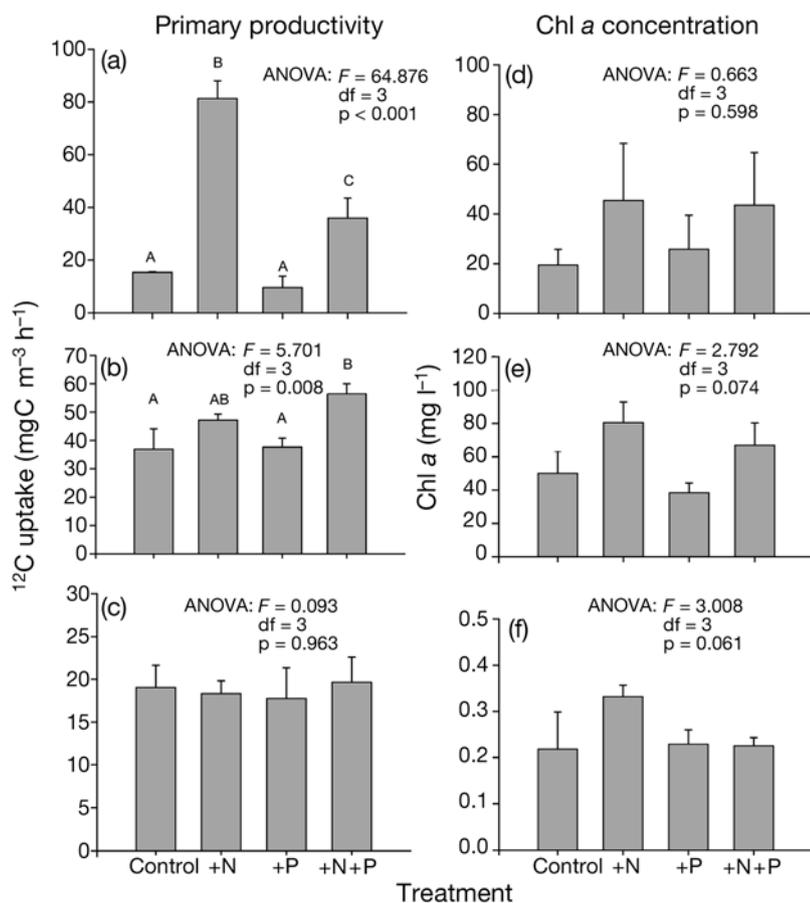
Fig. 6. *Karenia* spp. and *Lyngbya majuscula*. Mean (a–c) primary productivity and (d–f) chl *a* concentrations taken at T_2 (48 h incubation) for (a,d) *Karenia* spp. incubated alone, (b,e) *L. majuscula* bioassay incubation water containing *Karenia* spp., and (c,f) *L. majuscula*. Error bars represent +1 SE. Letters above error bars represent statistically different groups based on Tukey's post hoc pairwise comparisons
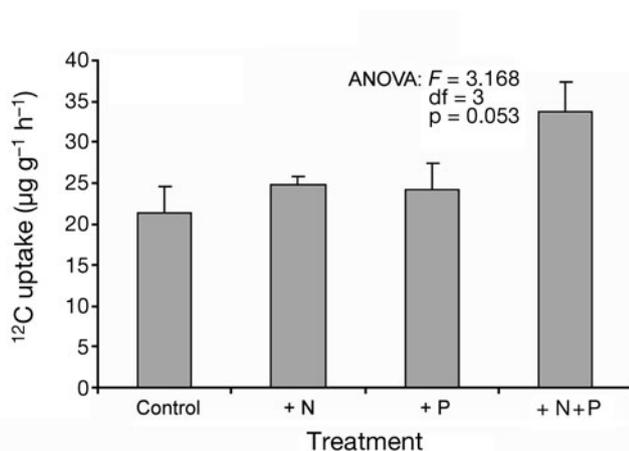

Fig. 7. *Lyngbya majuscula*. Primary productivity represented as ^{12}C uptake in *L. majuscula* bioassay at T_4 (96 h incubation) after daily additions of nitrate (N), phosphate (P) and both N and P. Error bars represent +1 SE (n = 5)

bined N, when it is available (Ahern et al. 2007). Freshwater studies on the benthic/ planktonic species *Lyngbya birgei* and *L. wollei* have shown that these potential diazotrophs exhibit opportunism when they encounter N-enriched conditions, by effectively utilizing available N sources to stimulate growth and bloom potential (Beer et al. 1986, Yin et al. 1997, Cowell & Dawes 2004). For example, riverine, lake, and spring environments that are experiencing N enrichment from urban, agricultural, and industrial sources have shown a dramatic upsurge in *L. birgei* and *L. wollei* blooms that can accumulate as nuisance surface scums fouling beaches, choking macrophyte beds, and promoting bottom-water hypoxia (Speziale & Dyck 1992, Cowell & Botts 1994). Similarly, in estuarine and coastal marine environments experiencing anthropogenic N and P enrichment, opportunistic *Lyngbya* species have become increasingly widespread and dominant as benthic epilithic and epiphytic blooms (Lapointe 1997, Paul et al. 2005, Paerl & Fulton 2006).

Nutrient stimulation of *Lyngbya majuscula* primary productivity was consistently lower and much slower, relative to controls, than responses observed in *Karenia* spp. In part, the less profound stimulatory response in *L. majuscula* may have been a reflection of the inherently slower growth rates and doubling times for this benthic cyanobacterium (and filamentous cyanobacteria in general), when compared to published doubling times for diatoms, cryptophytes, and other representative eukaryotic phytoplankton taxa (Pinckney et al. 1999, Reynolds 2006). *Karenia brevis* has a maximum growth rate of ~0.3 d^{-1} (Shanley & Vargo 1993, Magaña & Villareal 2006), which, although slow compared with other microalgae, is still faster than *Lyngbya* spp. (Kuffner & Paul 2001). Similar relative differences in comparative growth responses (cyanobacteria vs. eukaryotes) have been observed in estuarine nutrient addition bioassays (Paerl 1987, Pinckney et al. 1997).

Rates of CO_2 fixation observed in bioassays were much higher than typically reported for the Charlotte Harbor estuary (e.g. 9.3 ± 4 mg C m^{-3} h^{-1}, Turner et al. 2006), although they fall within the range reported for coastal *Karenia brevis* blooms (19.2 to 127.3 mg C m^{-3} h^{-1}, Heil et al. 2004). Primary productivity in water col-

umn samples dominated by *Karenia* spp. was strongly and consistently stimulated by N additions, while P additions failed to show stimulation (Fig. 6), which is consistent with earlier observations of N limitation of phytoplankton in bioassays in this estuary (McPherson et al. 1990, Montgomery et al. 1991, Heil et al. 2007). When added in combination with N, P did not lead to additional stimulation above that with N alone (Fig. 6). Similar patterns of chl *a* stimulation were observed in water column samples, with maximum stimulation taking place in response to the higher level of N enrichment (Fig. 6). Overall, *Karenia* spp. primary productivity and biomass production were controlled by N availability, as has been reported for other *Karenia* spp. blooms (Heil et al. 2001).

Expanding agricultural, urban, and industrial activities in the coastal zone are responsible for both increased N and P enrichment (Boesch et al. 2001), although in southwest Florida, these patterns are complicated by natural P enrichment resulting from the Meiocene Hawthorn phosphatic deposits in central west Florida. Phosphorus enrichment is capable of selectively stimulating N_2-fixing cyanobacterial blooms in coastal regions (cf. Elmgren & Larsson 2001, Paerl & Fulton 2006), which has also been hypothesized for areas dominated by benthic *Lyngbya* spp. populations (Elmetri & Bell 2004). The observed N and P costimulation of coastal *L. majuscula* populations in Florida and elsewhere (Ahern et al. 2007) raises concern that P over-enrichment in these waters plays a role in expansion of this HAB in addition to the well-established link between N loading and coastal eutrophication (Ryther & Dunstan 1971, Paerl 1997, Boesch et al. 2001).

This region of southwest coastal Florida lies at the transition zone between strong N limitation (from Tampa to Sanibel) and potential P limitation due to DIN inputs from the Caloosahatchee River and dissolved organic nitrogen (DON) inputs from the Everglades (Heil et al. 2007). Heil et al. (2007) speculated that the occurrence of *Karenia* spp. blooms in this transition region may be due to its ability to utilize DON in a DIN-limited environment. The ability of *Lyngbya* spp. to 'switch hit' between utilizing exogenous N compounds (when available) and the vast reservoir of dissolved atmospheric N_2 when exogenous combined N supplies are unavailable and P is sufficient to support the N_2 fixing process suggests that *L. majuscula* may be utilizing a similar strategy, only relying on utilization of gaseous N via N_2 fixation rather than DON uptake.

In addition to clearly benefiting from dissolved inorganic P inputs to sustain its N_2-fixing potential, benthic HABs like *Lyngbya majuscula* may also benefit from particulate P inputs, originating from either riverine (terrigenous) or oceanic sources, or possibly dissolved organic P, which is present in significant concentrations in this region (Heil et al. 2007). These P sources, while perhaps not immediately bioavailable, may accumulate in the benthic mats and tufts that *L. majuscula* produces (Fig. 2) or be available via enzymatic hydrolysis (Al-Shehri 2006). Over time scales longer than the bioassay period (<1 wk), particulate (and perhaps dissolved organic) P sources may at least in part be solubilized and converted to dissolved inorganic P by low oxygen microenvironments known to periodically occur in these microphytobenthic environments (Sundbäck et al. 1991, Paerl & Pinckney 1996, Watkinson et al. 2005). In this manner, mats and tufts can 'trap' potentially biologically available sources of P to support long-term growth and N_2 fixation needs.

In general, *Karenia* spp. demonstrated a strong need for additional exogenous N supplies, while *Lyngbya majuscula* revealed flexibility in meeting its N requirements, either via use of exogenous N or N_2 fixation, while benefiting from exogenous N and P inputs. These findings, while indicating differential limiting nutrient requirements, also demonstrate niche specificity and avoidance of competition for similar nitrogen resources to support growth and bloom formation among these HABs. It appears that based on its strong response to N inputs, *Karenia* spp. may be able to take advantage of episodic pulsed inputs of N from various sources such as rainfall, runoff events, and fish kills (Vargo et al. 2008, Walsh et al. in press) or from N_2 fixation-derived DON from nearby co-occurring cyanobacterial blooms such as *Trichodesmium* spp. (Lenes et al. 2001, Mulholland et al. 2004, 2006, Walsh et al. in press), or perhaps from *L. majuscula* in inshore environments.

Lyngbya majuscula, on the other hand, can also proceed in a more 'slow and steady' mode of satisfying its N needs, since it can at least partially supplement these needs through N_2 fixation. Conversely, *Karenia* spp. do not appear to have a problem accessing P to maintain their growth requirements, at least not during the time scale of bioassays (days to a week), while *L. majuscula* demonstrates a stronger demand for external P inputs (Kuffner & Paul 2001), most likely to support its N_2-fixing capabilities. Over time scales exceeding what we were able to capture with the bioassays, requirements for P for *L. majuscula* may be met by solubilization of particulate and organic P sources trapped in mats and tufts, as well as fluxes of P from the sediments (Watkinson et al. 2005). In any event, these HABs appear to be individually well-poised for taking advantage of nutrient inputs occurring as a result of anthropogenic activities and/or climatic perturbations such as storms and resultant runoff events.

Case for dual nutrient (N and P) controls of coastal eutrophication and HABs

The present study confirms results from a number of other estuarine and coastal studies, namely that when non-N$_2$-fixing HABs co-occur with N$_2$-fixing cyanobacterial HAB species, both N and P inputs need to be carefully considered and, in all likelihood, controlled. In this regard, it is important to consider potential scenarios like the one encountered in the present study, where a planktonic HAB may coincide with a benthic HAB, with each species having individualistic nutrient requirements, limitations, and specific physiological means for minimizing and/or circumventing these constraints. When tasked with the job of identifying and controlling nutrient inputs aimed at reducing eutrophication and HAB potentials along the freshwater–marine and estuarine–coastal continua, managers are continually challenged with having to make the choice of single versus dual nutrient input constraints. As this and other studies point out, it is becoming increasingly obvious that controls on both N and P input will be needed for effective long-term control of eutrophication, associated HAB outbreaks, and their ecological, biogeochemical, and human-health impacts (e.g. food-web alterations, hypoxia, toxicity, and loss of biodiversity and fisheries resources).

A parallel example can be drawn from the Baltic Sea region, where Elmgren & Larsson (2001) showed that effective control of eutrophication and HAB outbreaks must include the consideration of total amounts and ratios of N and P discharged to waters along the riverine–fjord–coastal–open sea continuum representing the Baltic Sea ecosystem. Similarly, Paerl et al. (2004) pointed out that single nutrient input reductions, including a P-detergent ban and improved wastewater treatment for P during the 1980s in North Carolina's Neuse River estuary, solved one problem (freshwater harmful cyanobacterial blooms), while exacerbating N-sensitive estuarine dinoflagellate blooms. In that case, parallel N input reductions were required in addition to P reductions to stem eutrophication and HAB potentials along the entire freshwater–marine continuum. Fisher et al. (1988) noted that high levels of anthropogenic N loading discharged to the upper reaches of Chesapeake Bay during the spring runoff period are a cause of seasonal P co-limitation, and at times exclusive P limitation. Using ship-based bioassays, Sylvan et al. (2006) demonstrated that excessive N loading, characterizing the past several decades of human activity in the Mississippi River Basin, has led to a periodic P limitation in the receiving coastal waters of the northern Gulf of Mexico, where excessive primary production has been identified as a causative agent for the expansion of bottom-water hypoxia (i.e. the 'Dead Zone'), which threatens the ecological integrity of this vitally important coastal ecosystem. In each case, both N and P inputs require reduction in order to counter accelerating eutrophication and minimize HAB outbreaks.

While evidence is mounting that dual nutrient (N and P) controls are likely to be the rule rather than the exception for managing coastal eutrophication, HAB occurrences, and proliferation, the good news is that controls of one nutrient often lead to parallel reductions in the other. Tertiary wastewater treatment is one example. Other examples include the creation of wetlands and riparian buffer zones for retaining and processing nutrients from storm water and agricultural (non-point source) runoff, and the use of flashboard risers and other structures to enhance retention and instream processing (i.e. denitrification) of nutrients that target both N and P.

Based on individual HAB responses as shown here and the need to take a basin-level watershed-receiving waters perspective in which nutrient–eutrophication–HAB interactions are considered along the entire freshwater-marine continuum, we can conclude that single nutrient input controls are largely incomplete and ineffective in addressing the negative impacts and ramifications of increasing levels of anthropogenic nutrient inputs associated with population growth in our coastal regions. A larger-scale vision is needed to address this problem. Understanding the ecophysiological complexities and capabilities of coinciding HABs that may originate in different segments of this continuum is proving to be an essential first step for creating the rationale and setting the thresholds for dual nutrient management strategies in the world's coastal waters.

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