# Ecology and nutrition of invasive *Caulerpa brachypus f. parvifolia* blooms on coral reefs off southeast Florida, U.S.A.

Brian E. Lapointe *, Bradley J. Bedford

*Center for Marine Ecosystem Health, Harbor Branch Oceanographic Institute, Florida Atlantic University, 5600 U.S. I North, Fort Pierce, FL 34946, USA*

### A B S T R A C T

Coral reefs off southeast Florida have experienced an unprecedented succession of invasive chlorophyte blooms over the past two decades, most recently the non-native *Caulerpa brachypus f. parvifolia*. To better understand the ecology and nutrition of the *C. brachypus* invasion, we monitored benthic cover, water column dissolved inorganic nutrients, tissue C:N:P ratios and stable nitrogen isotopes ($\delta^{15}$N) of *C. brachypus* and native chlorophytes (*Caulerpa racemosa*, *Caulerpa verticillata*, *Caulerpa mexicana*, *Codium isthmocladum*) quarterly at two reef sites – the Princess Anne (PA) and North Colonel's Ledge (NCL) – in 2003–2004. The PA site was influenced by stormwater discharges from the Lake Worth inlet whereas NCL was farther distant from these discharges. Between winter and spring of 2003, *C. brachypus* became the dominant benthic chlorophyte, expanding to >60% cover at both PA and NCL. Following cold temperatures (13 °C) associated with strong upwelling and high nitrate concentrations (21 μM) at NCL in July 2003, *C. brachypus* cover decreased, suggesting that upwelling can stress growth of this tropical alga. Mean ammonium (0.60 μM), nitrate (2.7 μM) and DIN (3.2 μM) concentrations were high for coral reef environments. Low mean C:N ratios of ~13 in *C. brachypus* at both PA and NCL indicated little, if any, N-limitation compared to higher C:N ratios (up to 24) and greater N-limitation in native chlorophytes. Despite a relatively high mean SRP concentration (0.21 μM), mean N:P ratios of ~39 in *C. brachypus* and other chlorophytes at PA and NCL suggested that these blooms were P-limited. Multiple lines of evidence support the hypothesis that land-based nutrient sources fueled the *C. brachypus* invasion. First, more persistent blooms of *C. brachypus* at PA compared to NCL correlated with significantly lower tissue C:P and higher $\delta^{15}$N values (wet season) at PA, the site most directly influenced by land-based stormwater runoff. Second, C:N, C:P, and $\delta^{15}$N values of *C. brachypus* correlated with seasonal patterns of rainfall and stormwater runoff. Third, $\delta^{15}$N values of *C. brachypus* and other chlorophytes decreased at NCL following strong upwelling in July 2003, confirming that upwelled nitrate was not the cause of the elevated $\delta^{15}$N values observed in these blooms. Lastly, the mean $\delta^{15}$N values of *C. brachypus* and other chlorophytes off southeast Florida (+4.9‰) were in the range of sewage nitrogen and significantly higher than values (+1.2‰) for reference chlorophytes in the Abacos, Bahamas, an area that experiences relatively little sewage input.

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## 1. Introduction

Invasions of native and non-native macroalgae have been recognized as a major driver of coastal ecosystem change worldwide (UNEP, 2005). Coral reefs in southeast Florida have experienced an unprecedented succession of macroalgal blooms and invasions involving both native and non-native chlorophytes over the past two decades. Spectacular blooms of unattached *Codium isthmocladum* first developed during summer months in 1989–1990 on deep reefs (24–43 m) off southern Palm Beach and

northern Broward counties (Lapointe, 1997). These initial blooms were followed by blooms of attached populations of *C. isthmocladum* as well as *Caulerpa verticillata* and *Caulerpa racemosa* in the late 1990s on reefs off northern Palm Beach County (Lapointe et al., 2005a,b). *Caulerpa brachypus f. parvifolia* (Harvey) Cribb was discovered invading these reef communities in May 2001 (Lapointe et al., 2005b). The discovery of *C. brachypus f. parvifolia* was the first report of this species and form in Florida's coastal waters. *C. brachypus f. parvifolia* was previously reported only from the western Pacific (Harvey, 1860; Cribb, 1958; Lewis, 1987; Silva et al., 1987, 1996; Huisman and Walker, 1990; Yoshida et al., 1990; Cribb, 1996; N'Yeurt et al., 1996; Phillips, 1997; Yoshida, 1998; Huang, 2000; Huisman, 2000; Phillips, 2002; Coppejans et al., 2005). In comparison, *C. brachypus* (Harvey) in the western

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* Corresponding author. Tel.: +1 772 465 2400x276; fax: +1 772 468 0757.
  *E-mail address:* blapoin1@hboi.fau.edu (B.E. Lapointe).

Atlantic/Caribbean has been reported only from the Greater and Lesser Antilles (Littler and Littler, 2000). Historically, neither form has ever been reported from Florida waters (Taylor, 1960; Dawes, 1974; Hanisak and Blair, 1988; Schneider and Searles, 1991; Littler and Littler, 2000; Dawes and Mathieson, 2008).

The unprecedented macroalgal blooms on southeast Florida's coral reefs over the past two decades have been related to increasing land-based nutrient inputs. Near-bottom seawater nutrient concentrations and $\delta^{15}N$, C:N:P ratios, alkaline phosphatase activity and nutrient-enrichment bioassays of summer *Codium* blooms in 1994–1995 supported the hypothesis that these blooms were related to increasing land-based nutrient pollution (Lapointe, 1997). Hughes et al. (1999) suggested that reduced herbivory from overfishing of grazers, die-off of the sea urchin *Diadema antillarum* in 1983 and nutrient enrichment from upwelling were more likely factors causing the extensive *Codium* blooms. However, herbivorous fishes (parrotfish, surgeonfish) are not targeted by fishermen in Florida and are a major trophic group in fish assemblages on reefs in northern Palm Beach County (reef.org/db/reports/geo/TWA/3301). *D. antillarum* was never abundant on these deep reefs, as is the case for the Caribbean in general, and their die-off could not explain these deep-reef bloom phenomena (Lapointe, 1997). Recent mapping of $\delta^{15}N$ in macroalgae at 21 stations over a network of shallow, mid-depth and deep reefs in Palm Beach and Broward counties demonstrated that nitrogen enrichment of these blooms resulted from land-based nutrient discharges, including sewage, and not from transient, summertime upwelling of nitrate (Lapointe et al., 2005b).

Species in the genus *Caulerpa* are not usually abundant on oligotrophic coral reefs and have long been considered "indicator species" of nutrient enrichment from natural and/or anthropogenic sources. Ollivier (1929) first suggested that expansion of *Caulerpa prolifera* off the French Riviera resulted from increasing domestic sewage pollution. Several studies have noted the importance of high nutrient availability to the success of invasive *Caulerpa taxifolia* in the Mediterranean. Bioassays of *C. taxifolia* off the coast of Spain indicated a lack of severe nutrient-limitation year-round (Delgado et al., 1996) and areas of explosive growth have been centered adjacent to urban stormwater and sewage discharges (Chisholm et al., 1997; Jaubert et al., 2003). Since the introduction of the non-native *C. taxifolia* at Monaco in 1984 (Meinesz and Boudouresque, 1996; Jousson et al., 1998), this alga has colonized some 131 km$^2$ (Meinesz, 2001), although Jaubert et al. (2003) have suggested that estimates of the distribution and abundance of *C. taxifolia* have been overestimated by a factor of 10-fold. Regardless, the invasion has resulted in extensive, monospecific stands of *C. taxifolia* that can impact native seagrasses such as *Posidonia oceanica* (de Ville and Verlaque, 1995) and *Cymodocea nodosa* (Ceccherelli and Cinelli, 1997). In the wider Caribbean region, localized blooms of *Caulerpa* spp. have also been noted around nutrient enriched habitats. For example, the diminutive *C. verticillata* is normally found in mangrove habitats (Littler and Littler, 2000) but forms thick mats on the vertical walls of sewage impacted canal systems in the Florida Keys (Lapointe et al., 1994).

There are several interesting parallels between the *C. brachypus* f. *parvifolia* (hereafter referred to as *C. brachypus*) invasion in southeast Florida and that of *C. taxifolia* in the Mediterranean. Both areas are subtropical, contain overlapping ranges of tropical and temperate species, and have widespread human activity on their watersheds. In addition, both invaders are tropical species capable of explosive growth when irradiance, temperature, and nutrient availability are favorable. In his seminal work on biological invasions, Elton (1958) emphasized the importance of human-mediated vectors, especially physical transport. Since then, humans have been well recognized as the primary vector in the global epidemic of biotic invasions in aquatic ecosystems (Carlton

and Geller, 1993). However, less is known about how human alteration of chemical factors, such as increasing anthropogenic land-based nutrient pollution (NRC, 2000), can facilitate biological invasions and decreases in biological diversity.

To better understand the ecology, nutrition and bloom dynamics of the non-native *C. brachypus* relative to native chlorophytes, we performed a 20-month field study with quarterly (winter, spring, summer, fall) sampling at two reef sites with different exposures to land-based nutrient discharges from the Lake Worth Inlet, Palm Beach County, FL (Fig. 1). The study was part of a multi-faceted Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) project that included: (1) digital underwater video imagery of benthic transects at the Princess Anne (PA) and North Colonel's Ledge (NCL) to determine spatial and temporal variation in native versus non-native chlorophyte abundance, including *C. brachypus*, *C. racemosa*, *Caulerpa prolifera*, *C. verticillata*, *Caulerpa mexicana*, and *C. isthmocladum*; (2) quarterly collection and analysis of macroalgal tissue samples from the two reef sites for C:N:P and stable nitrogen isotope ($\delta^{15}N$) values to assess spatial and temporal variation in nutritional status (i.e. type and degree of N vs. P limitation), and to quantify the relative contributions of various natural and anthropogenic nitrogen sources; and (3) quarterly collection of near-bottom seawater samples from PA and NCL for determination of water column dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite) and soluble reactive phosphorus (SRP) concentrations to characterize spatial and temporal variation in nutrient availability.

## 2. Materials and methods

### 2.1. Study sites and approach

The study area in northern Palm Beach County (Fig. 1) is located at the extreme northern end of the Florida Reef Tract, which includes the only barrier-bank coral reef ecosystem within the continental United States (Jaap and Hallock, 1990). Representatives of tropical reef biota occur as far north as Jupiter Island near Stuart, FL, and become increasingly important southward towards the Florida Keys. Although fossil reef structures off Palm Beach County support growth of hermatypic corals, octocorals, and sponges of the same species that occur in Florida Keys, corals are not presently building reefs in the northern region (Lighty et al., 1978).

We hypothesized that discharges from the Lake Worth Inlet in Palm Beach County would result in significantly greater nutrient enrichment of the invasive chlorophyte community at the PA reef site, located just north of the inlet, compared to the more northern site at NCL, located some 9 km north of PA and further downstream from inlet discharges (Fig. 1).

### 2.2. Benthic video transects

Seasonal patterns in abundance (% cover) of the various HAB species were quantified using underwater digital video imagery taken along replicate ($n$ = 3) 50 m belt transects at the PA (28 msw) and NCL (26 msw) reef sites (Fig. 1). A SCUBA diver swam slowly along the transects recording high-resolution video imagery using a Sony TRV 900$^{TM}$ digital camcorder in an Amphibico Navigator$^{TM}$ underwater housing held ~0.3 m above the benthos to allow identification of targeted chlorophytes and quantification of benthic cover. A second diver collected voucher specimens for taxonomic identification in the laboratory. To quantify reef macroalgae, ten evenly spaced benthic quadrats (~0.4 m$^2$ ea.) per transect were obtained by frame-grabbing still images from the transect video. Using ten randomized points projected onto the video quadrats using a high resolution color monitor, two separate
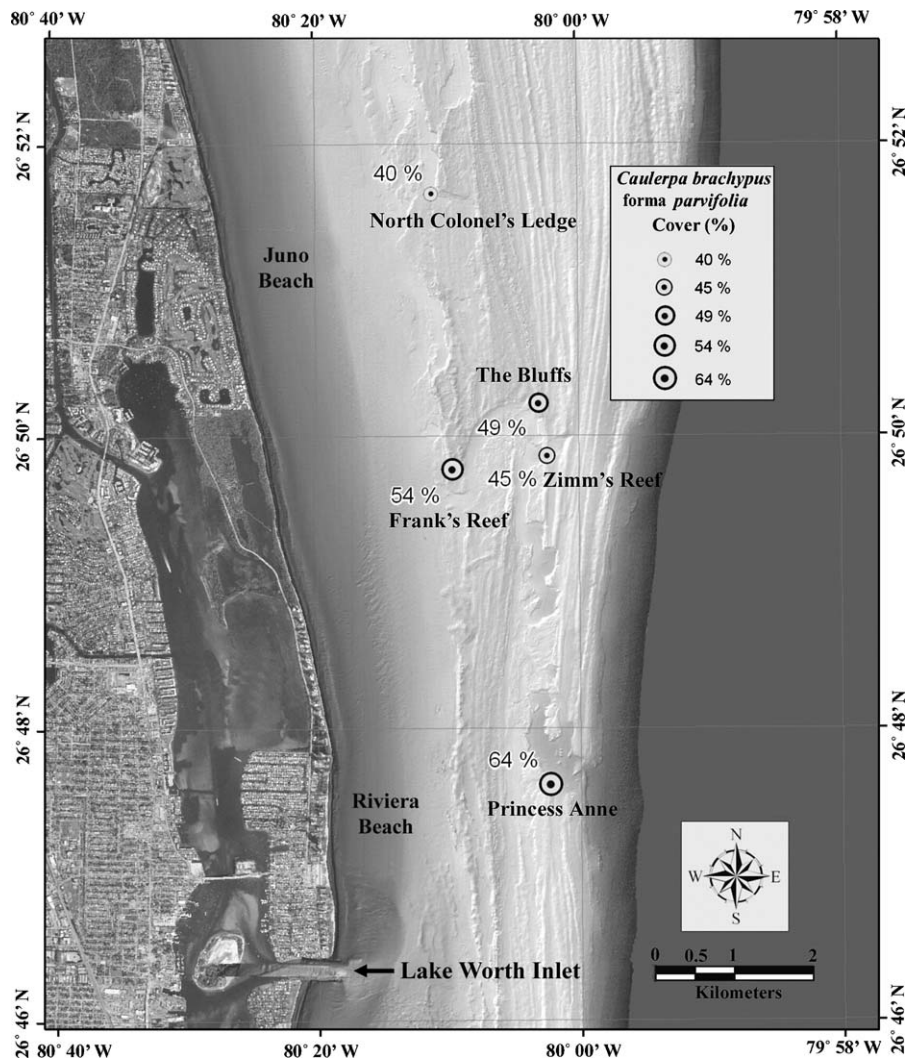
**Fig. 1.** LAADS image of reef formations in northern Palm Beach County showing ECOHAB monitoring sites at Princess Anne (PA) and North Colonel's Ledge (NCL). Values represent *Caulerpa brachypus f. parvifolia* benthic cover (%) at PA, NCL, and nearby reef sites in August 2004, prior to hurricanes Frances and Jeanne. Note decreasing %cover of *C. brachypus* with increasing distance north of the Lake Worth inlet.

scorers used the random point count method to estimate benthic cover (%) of target species. This method has been used successfully to quantify macroalgal cover on reefs in southeast Florida (Lapointe et al., 2006).

### 2.3. Sampling macroalgae for tissue C:N:P and $\delta^{15}N$

Five composite samples (each sample containing 5–8 different algal thalli) of *C. brachypus* and other chlorophytes were collected quarterly by SCUBA divers from the study sites off northern Palm Beach County. Immediately following collection, macroalgae were sorted, cleaned of visible epiphytes and sediments, and rinsed briefly (~3 s) in deionized water to remove salt and debris. The cleaned, composite samples were dried in a Fisher Scientific, Isotemp™ oven at 60 °C for 48 h. The dried macroalgae was ground to a fine powder using a mortar and pestle, and was stored in 7 ml, plastic, screw-top, scintillation vials. Macroalgae samples ($n = 5$) were subsequently analyzed for %C, %N, and %P at Nutrient Analytical Services, Chesapeake Biological Laboratory, University of Maryland System, Solomons, MD (NAS-CBL). Tissue C and N were measured on an Exeter Analytical, Inc. (EAI) CE-440 Elemental Analyzer; P was measured following the methodology of Asplia et al. (1976) using a Technicon Autoanalyzer II interfaced with a Labtronics, Inc. DP500 software data collection system

(D'Elia et al., 1997). The dried, powdered macroalgae ($n = 5$) were also analyzed for stable nitrogen isotope ratios ($\delta^{15}N$) at Isotope Services, Inc., Los Alamos, NM, with a Carlo-Erba N/A 1500 Elemental Analyzer and a VG Isomass mass spectrometer using Dumas combustion. Macroalgae analyzed for $\delta^{15}N$ included not only the native and non-native chlorophytes collected from northern Palm Beach County, but also reference specimens collected from coral reefs offshore Green Turtle Cay in the Abacos, Bahamas. We hypothesized that, because the human population and sewage inputs on Green Turtle Cay (population of ~500) are much lower than in southeast Florida (population of ~5.4 million), the $\delta^{15}N$ values of the Bahamian samples would be significantly lower than those from Florida (Lapointe et al., 2005b). The standard used for stable nitrogen isotope analysis was $\delta^{15}N$ in air. $\delta^{15}N$ values (‰) were calculated as $[(R_{sample}/R_{standard}) - 1] \times 10^3$; where $R = {}^{15}N/{}^{14}N$.

### 2.4. Seawater nutrient analyses

To quantify water column nutrient concentrations, SCUBA divers collected replicate ($n = 2$) water samples from PA and NCL at quarterly intervals for DIN and SRP analyses. Water samples were collected from near-bottom (~10 cm above the benthos) into clean, screw-top, HDPE bottles. Water samples were held on ice in

a cooler for transport to the HBOI lab within 6 h. In the laboratory, 100 ml aliquots were filtered through 25 mm Whatman GF/F syringe filters and frozen ($< -20\,°C$) until analysis. Within 28 days of collection, the samples were analyzed for ammonium, nitrate + nitrite, and SRP at NAS-CBL. Nitrate + nitrite and SRP were analyzed using a Technicon Auto Analyzer II; ammonium was analyzed using a Technicon TRAACS 800. Detection limits for these analyses were: ammonium, 0.21 μM; nitrate and nitrite, 0.05 μM; SRP, 0.02 μM (D'Elia et al., 1997). We used a modified f-ratio (nitrate + nitrite/DIN) to gauge the relative importance of ammonium versus nitrate as a nitrogen source and the relative importance of upwelling (McCarthy et al., 1975; Harrison et al., 1987). The methods of collection, handling, and processing of water samples for low-level nutrient analyses followed a quality assurance/quality control protocol designed to prevent problems associated with sample contamination and excessive holding times, and to provide accurate and reliable data.

### 2.5. Statistical analyses

Statistical analyses were performed using the generalized linear model procedure (GLM; Type III sum of squares) and Tukey's HSD (THSD) post hoc test in SPSS 11 for Mac (www.spss.com); differences were considered significant at $p \leq 0.05$. The GLM ANOVA was used to assess differences between sites (df = 1) and to determine if significant differences occurred among samplings (df = 6) and among species (df = 2). Where differences were determined by GLM to be significant among samplings or species, THSD tests were then performed to examine the source(s) of the difference.

## 3. Results

### 3.1. Benthic transect surveys

During the initial sampling in February 2003, C. verticillata dominated benthic cover at both PA ($62 \pm 4\%$; $n = 6$) and NCL ($58 \pm 3\%$; $n = 6$; Fig. 2) and C. brachypus (Fig. 3A) was at its lowest cover ($p < 0.001$, THSD) of the study at both sites (PA: $19 \pm 4\%$; NCL: $9 \pm 1\%$; $n = 6$ site$^{-1}$). By April 2003, C. verticillata was replaced by spectacular blooms of C. brachypus (Fig. 3A) at PA ($72 \pm 5\%$; $n = 6$) and NCL ($63 \pm 10\%$; $n = 6$; Fig. 2), which formed widespread mats 5–15 cm thick on the reef surface and smothered benthic organisms, including hard corals, octocorals, and sponges (Fig. 3B). C. brachypus continued to dominate cover at PA throughout the study, with peak cover

occurring from October 2003 through February 2004 ($p \leq 0.014$, THSD). In comparison, C. brachypus cover at NCL peaked in July 2003 ($p < 0.001$, THSD) coincident with a strong upwelling event and near-bottom temperatures of $\sim13\,°C$, declining thereafter (Fig. 2). At PA, however, temperatures decreased to only $\sim19\,°C$ and C. brachypus remained abundant but declined significantly in 2004 ($p < 0.001$, THSD; Fig. 2). In August 2004, C. brachypus cover at PA remained similar to May 2004 values whereas, at NCL, the bloom re-emerged to levels similar to those in February 2004. Overall, C. brachypus cover was higher at PA compared to NCL ($p \leq 0.017$, GLM) during all samplings except April 2003, when cover at the two sites was similar, and in July 2003 when cover was highest at NCL ($p < 0.001$, GLM; Fig. 2).

Significant secondary blooms also occurred during the study. Blooms of C. mexicana and C. racemosa (Fig. 3C) formed at PA in 2003 and 2004 but generally accounted for < 10% cover (Fig. 2). At NCL, localized blooms of C. prolifera (up to 10% cover in May 2004; $n = 6$) and C. racemosa (up to 30% cover in August 2004; $n = 6$) also occurred (Fig. 2). Due to C. racemosa's relatively large size (up to 25 cm high), the August 2004 bloom of this species resulted in dramatic overgrowth of the benthic community at NCL (Fig. 3D). In May 2004, an extensive bloom of the filamentous cyanobacterium Lyngbya sp. ($61 \pm 11\%$ cover; $n = 6$) occurred at NCL, which blanketed the benthos, resulting in an apparent corresponding decline in C. brachypus cover.

### 3.2. Tissue $\delta^{15}N$

The mean $\delta^{15}N$ values of C. brachypus at PA ($+4.7 \pm 0.9‰$) and NCL ($+4.1 \pm 1.5‰$) were similar during the study (samplings pooled; $n = 35$ site$^{-1}$; Table 1), averaging $+4.4 \pm 1.3‰$ and ranging from $+3.7‰$ to $+5.4‰$ at PA and $+1.9‰$ to $+6.2‰$ at NCL. February and April 2003 $\delta^{15}N$ values were lower ($p \leq 0.011$, GLM) at PA compared to NCL whereas, in July and October 2003, $\delta^{15}N$ values were higher ($p \leq 0.004$, GLM) at PA than at NCL ($n = 5$ site$^{-1}$ sampling$^{-1}$; Figs. 4 and 5).

Seasonal patterns in $\delta^{15}N$ differed between the sites. At PA (Fig. 4), $\delta^{15}N$ values were lowest among samplings ($p \leq 0.033$, THSD) in October 2003, February and August 2004. In comparison, $\delta^{15}N$ values at NCL (Fig. 5) were lowest among samplings ($p \leq 0.011$, THSD) in July and October 2003 (upwelling, wet season) and highest ($p \leq 0.013$, THSD) in February and April 2003 and May 2004 (dry season). $\delta^{15}N$ values at PA were consistently $>+3‰$ throughout the study whereas, at NCL, values $<+3‰$ occurred in both July and October 2003.

Interspecies comparisons showed that $\delta^{15}N$ values of C. brachypus were similar to native chlorophytes at PA and NCL during the study (Table 2; Figs. 4 and 5). However, a comparison of $\delta^{15}N$ values in chlorophytes (C. brachypus, C. racemosa, C. verticillata, C. isthmocladum) from PA and NCL with Caulerpa species from the Abaco Barrier Reef, Bahamas, showed that $\delta^{15}N$ values of the Floridian macroalgae were $\sim4$-fold higher ($p < 0.001$; THSD) than those of the Bahamian macroalgae ($+1.2 \pm 0.5‰$; $n = 32$; Fig. 6).

### 3.3. Tissue C:N, C:P, and N:P ratios

Overall, C:N ratios of C. brachypus were similar at PA ($13 \pm 3$) and NCL ($13 \pm 4$), averaging $13 \pm 3$ (samplings pooled, $n = 35$ site$^{-1}$; Table 1) and ranging from 10 to 17 at PA and 10 to 19 at NCL. C:N ratios remained similar during all samplings except April 2003, when C:N ratios were lower ($p \leq 0.034$, GLM) at PA than at NCL (Figs. 4 and 5; $n = 5$ site$^{-1}$).

C:N ratios of C. brachypus varied seasonally. The lowest ($p \leq 0.006$, THSD) C:N ratios at both sites occurred in August 2004 (wet season). However, the highest ($p \leq 0.037$, THSD; $n = 5$ site$^{-1}$ sampling$^{-1}$) values at PA (Fig. 4) were during the July
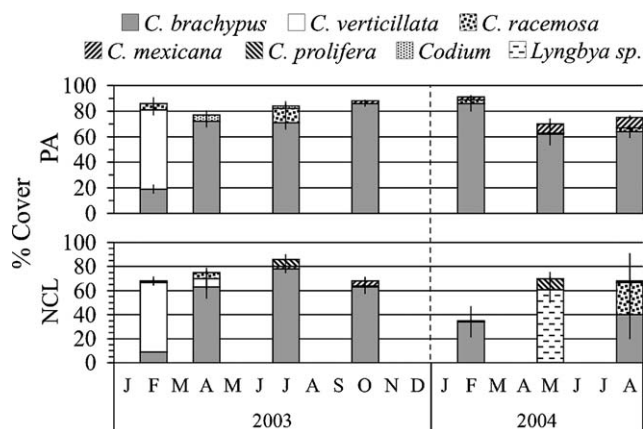


**Fig. 2.** Benthic macroalgal cover at Princess Anne (PA) and North Colonel's Ledge (NCL). Values represent means ± 1 S.D. ($n = 6$ site$^{-1}$ sampling$^{-1}$).
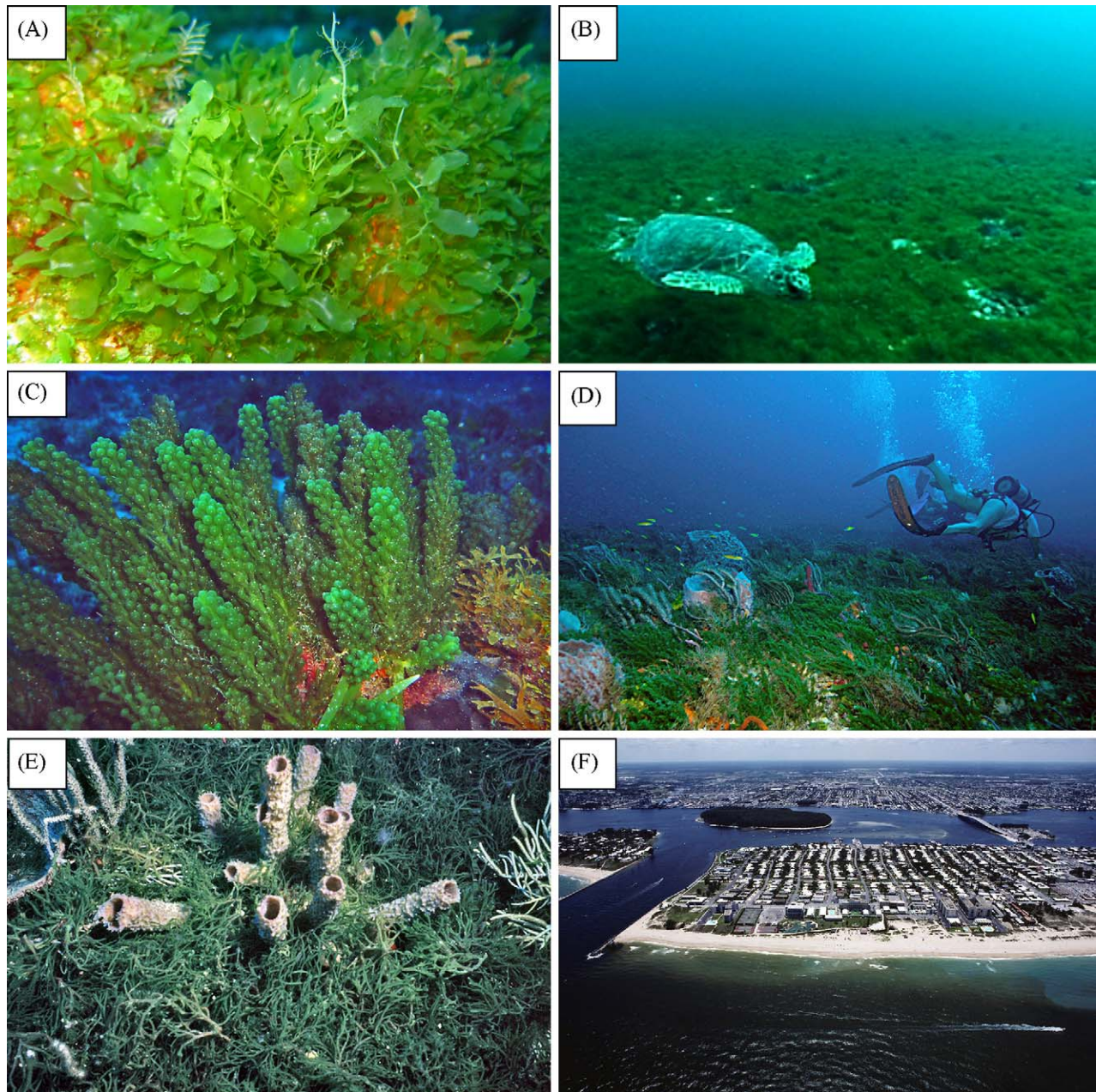
**Fig. 3.** Images: (A) *Caulerpa brachypus f. parvifolia*; (B) *Caulerpa brachypus* cover at PA, February 2004; (C) *Caulerpa racemosa*; (D) *Caulerpa racemosa* cover at NCL, August 2004; (E) *Codium isthmocladum*; (F) discolored water, indicative of terrestrial runoff, discharging northward from Lake Worth Inlet, Palm Beach County, FL.

2003 upwelling event but, at NCL (Fig. 5), were in April 2003 (dry season).

Among species, *C. brachypus* C:N ratios were consistently lower than those of the native chlorophytes ($p \leq 0.022$, THSD) at both sites (samplings pooled; Table 1), a pattern that held for most individual samplings (Table 2). An exception to this pattern occurred at both sites during the July 2003 upwelling event, when *C. brachypus* had the highest C:N among species ($p \leq 0.023$, THSD; Figs. 4 and 5; Table 2).

Overall, *C. brachypus* C:P ratios were similar at PA ($468 \pm 98$) and NCL ($484 \pm 122$), averaging $476 \pm 110$ (samplings pooled, $n = 35$ site$^{-1}$; Table 1) and ranging from 396 to 600 at PA and 401 to 673 at NCL. However, when all five chlorophyte species were pooled overall, mean C:P ratios were lower ($p = 0.043$, GLM; $n = 105$ site$^{-1}$) at PA ($497 \pm 127$) compared to NCL ($544 \pm 201$; Table 1).

C:P ratios of *C. brachypus* were similar between sites during all samplings except October 2003 (wet season) when C:P was lower ($p = 0.043$, GLM; $n = 5$ site$^{-1}$) at PA than at NCL (Figs. 4 and 5). The highest C:P ratios ($p \leq 0.038$, THSD; $n = 5$ site$^{-1}$ sampling$^{-1}$) of *C. brachypus* occurred at both sites in October 2003 and May 2004; values were similar among remaining samplings (Figs. 4 and 5).

Among species, overall (samplings pooled) *C. brachypus* C:P ratios were lower ($p \leq 0.019$, THSD) than those of *C. mexicana* and *C. verticillata* but similar to the other native chlorophytes (Table 1). Sampling-by-sampling, *C. brachypus* C:P ratios were generally lower than those of native chlorophytes species, but the differences were often not significant (Table 2).

Overall (samplings pooled), N:P ratios of *C. brachypus* were similar at PA ($38 \pm 9$) and NCL ($39 \pm 13$), averaging $39 \pm 11$ (samplings pooled, $n = 35$ site$^{-1}$; Table 1) and ranging from 23 to 44 at PA and 24 to 58 at NCL; similarity between sites extended to

**Table 1**
Pooled macroalgae tissue %C, %N, and %P; C:N, C:P, and N:P molar ratios; and $\delta^{15}$N values in *Caulerpa brachypus, Caulerpa racemosa, Caulerpa verticillata, Caulerpa mexicana,* and *Codium isthmocladum* at the Princess Anne (PA) and N. Colonel's Ledge (NCL) sites. Values represent means ± 1 S.D.

| Site | Species | $n$ | %C | %N | %P | C:N | C:P | N:P | $\delta^{15}$N (‰) |
|------|---------|-----|----|----|----|-----|-----|-----|------|
| PA | *C. brachypus* | 35 | 19.8 ± 3.6 | 1.9 ± 0.6 | 0.11 ± 0.02 | 13 ± 3 | 468 ± 98 | 38 ± 9 | 4.7 ± 0.9 |
| | *C. mexicana* | 5 | 24.1 ± 2.5 | 1.8 ± 0.3 | 0.10 ± 0.01 | 15 ± 2 | 637 ± 43 | 40 ± 4 | 4.1 ± 0.6 |
| | *C. racemosa* | 35 | 12.6 ± 2.9 | 1.0 ± 0.4 | 0.08 ± 0.02 | 16 ± 4 | 457 ± 149 | 31 ± 12 | 5.0 ± 1.6 |
| | *C. verticillata* | 5 | 15.5 ± 1.3 | 1.0 ± 0.2 | 0.07 ± 0.01 | 18 ± 2 | 615 ± 65 | 36 ± 6 | 5.8 ± 0.7 |
| | *Codium* | 25 | 11.9 ± 1.9 | 1.0 ± 0.2 | 0.06 ± 0.01 | 15 ± 2 | 545 ± 109 | 38 ± 6 | 5.2 ± 1.5 |
| | PA mean | 105 | 15.5 ± 4.9 | 1.3 ± 0.6 | 0.08 ± 0.03 | 14 ± 3 | 497 ± 127 | 36 ± 10 | 4.9 ± 1.2 |
| NCL | *C. brachypus* | 35 | 19.5 ± 3.7 | 1.9 ± 0.7 | 0.11 ± 0.03 | 13 ± 4 | 484 ± 122 | 39 ± 13 | 4.1 ± 1.5 |
| | *C. mexicana* | 5 | 26.3 ± 2.4 | 2.0 ± 0.4 | 0.08 ± 0.01 | 16 ± 2 | 878 ± 97 | 57 ± 10 | 4.9 ± 0.8 |
| | *C. racemosa* | 35 | 16.7 ± 7.6 | 1.2 ± 0.5 | 0.08 ± 0.02 | 16 ± 3 | 556 ± 260 | 34 ± 13 | 4.8 ± 1.8 |
| | *C. verticillata* | 5 | 16.0 ± 2.1 | 09 ± 0.3 | 0.07 ± 0.03 | 21 ± 3 | 669 ± 247 | 33 ± 18 | 4.5 ± 0.5 |
| | *Codium* | 25 | 12.3 ± 2.0 | 1.0 ± 0.2 | 0.06 ± 0.01 | 15 ± 5 | 521 ± 118 | 36 ± 9 | 6.4 ± 2.3 |
| | NCL mean | 105 | 17.0 ± 6.0 | 1.4 ± 0.6 | 0.08 ± 0.03 | 15 ± 4 | 544 ± 201 | 37 ± 13 | 4.9 ± 1.9 |
| | Grand mean | 210 | 16.3 ± 5.5 | 1.4 ± 0.6 | 0.08 ± 0.03 | 15 ± 4 | 521 ± 169 | 37 ± 12 | 4.9 ± 1.6 |

individual samplings as well (Figs. 4 and 5). Among samplings, N:P ratios at PA were lowest ($p \leq 0.019$, THSD; $n = 5$ sampling$^{-1}$) in July 2003 (Fig. 4), while N:P ratios at NCL were lowest ($p \leq 0.041$, THSD) in April 2003 (Fig. 5).

Among species, *C. racemosa* generally had lower N:P ratios relative to other species, including *C. brachypus,* at both sites but the differences were often not significant; *C. brachypus* showed no other consistent N:P trend relative to other species (Tables 1, 2).

### 3.4. Water column nutrient concentrations

Mean near-bottom ammonium concentrations were similar between PA (0.54 ± 0.20 μM) and NCL (0.64 ± 0.12 μM), averaging 0.60 ± 0.2 μM overall (sites and samplings pooled; $n = 28 = 2$ site$^{-1}$ sampling$^{-1}$) and ranging from undetectable (<0.2 μM) to 0.90 μM at PA and undetectable to 0.80 μM at NCL. The similarity between sites extended to individual samplings (Fig. 7). Among samplings, the highest ($p = 0.027$, GLM) ammonium concentrations at PA occurred in February 2004 whereas the lowest at both PA and NCL were in May 2004 (Fig. 7).

Mean nitrate concentrations were statistically similar between PA (1.66 ± 2.33 μM) and NCL (3.69 ± 7.10 μM), averaging 2.7 ± 5.5 μM overall (sites and samplings pooled; $n = 28 = 2$ site$^{-1}$ sampling$^{-1}$) and ranging from 0.25 μM to 7.3 μM at PA and 0.33 μM to 21.05 μM at NCL. High nitrate concentrations associated with the July 2003 upwelling skewed overall means upward at both sites. When July 2003 nitrate data were excluded, nitrate averaged 0.8 ± 0.4 μM overall ($n = 24$). In July 2003 and August 2004, nitrate was lower ($p \leq 0.014$, GLM) at PA relative to NCL whereas, in February 2004, nitrate was higher ($p = 0.017$, GLM) at PA than at NCL (Fig. 7).

At both sites, April 2003 and May 2004 (dry season) nitrate concentrations were lowest ($p \leq 0.018$; THSD) among samplings, whereas July 2003 (upwelling) nitrate concentrations were highest ($p < 0.001$, THSD). The July 2003 upwelling was stronger at NCL, compared to PA, as evidenced by a lower temperature and ~3-fold higher nitrate concentrations at NCL (13 °C; 21.1 ± 0.2 μM) relative to PA (19 °C; 7.3 ± 0.1 μM; Fig. 7).

Mean DIN concentrations were similar at PA (2.2 ± 2.5 μM) and NCL (4.3 ± 7.4 μM), averaging 3.2 ± 5.5 μM overall (sites and samplings pooled; $n = 28 = 2$ site$^{-1}$ sampling$^{-1}$) and ranging from
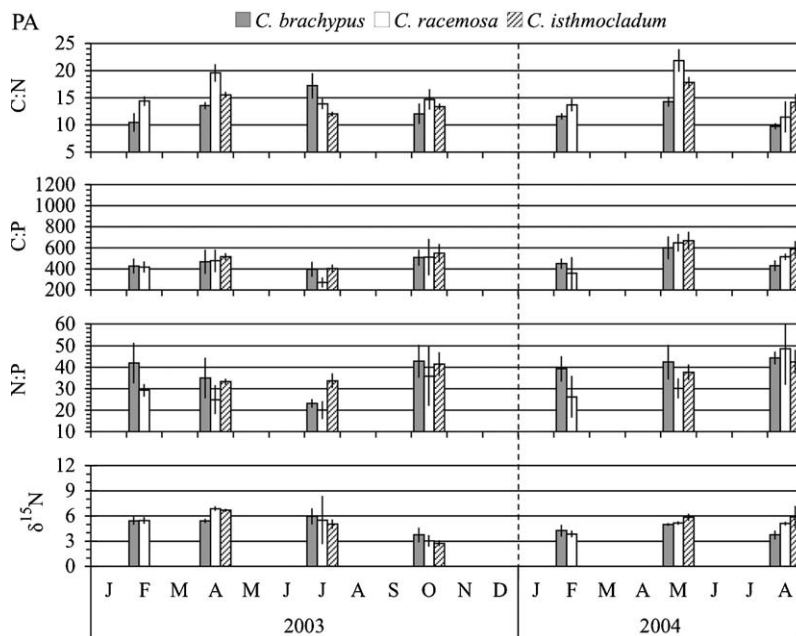


**Fig. 4.** CNP ratios and $\delta^{15}$N values of *Caulerpa brachypus, Caulepa racemosa,* and *Codium isthmocladum* at the Princess Anne (PA) site. Values represent means ± 1 S.D. ($n = 5$ species$^{-1}$ sampling$^{-1}$).
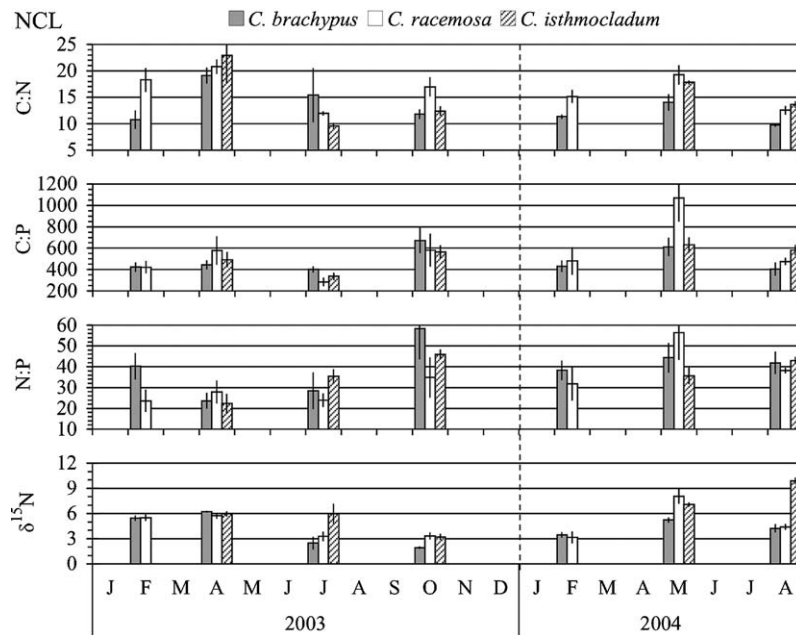
**Fig. 5.** CNP ratios and $\delta^{15}$N values of *Caulerpa brachypus*, *Caulerpa racemosa*, and *Codium isthmocladum* at the N. Colonel's Ledge (NCL) site. Values represent means ± 1 S.D. ($n$ = 5 species$^{-1}$ sampling$^{-1}$).

0.4 μM to 8.0 μM at PA and 0.5 μM to 21.6 μM at NCL. When July 2003 upwelling data were excluded, overall DIN averaged 1.3 ± 0.6 μM ($n$ = 24). DIN was lower at PA compared to NCL in February 2003 ($p \leq 0.044$, GLM) and July 2003 ($p < 0.001$, GLM), otherwise sites were similar (Fig. 7).

Among samplings, DIN at both sites was highest ($p < 0.001$, THSD) in July 2003. DIN was lowest at PA in May 2004 and at NCL in April 2003, but differences were only significant relative to July 2003. DIN approached or exceeded 1.0 μM during all samplings at both sites, with the exception of May 2004 (Fig. 7).

The mean $f$-ratios (nitrate:DIN ratios) were similar at PA (0.6 ± 0.2) and NCL (0.6 ± 0.2), averaging 0.6 ± 0.2 ($n$ = 28) overall (sites and samplings pooled; $n$ = 28 = 2 site$^{-1}$ sampling$^{-1}$) and ranging from 0.4 to 0.9 at PA and 0.4 to 1.0 at NCL. This indicates a slight (~10%) dominance of the DIN pool by nitrate over ammonium during the period of study. The $f$-ratios at both sites were highest ($p \leq 0.024$, THSD; $n$ = 2 site$^{-1}$ sampling$^{-1}$) during the July 2003 upwelling event, when nitrate comprised ≥90% of the DIN pool. Among the other samplings, $f$-ratios were lower and clustered around an overall mean of 0.56, indicating a DIN pool comprised of ammonium and nitrate in near equal concentrations (Fig. 7).

Mean SRP concentrations at PA (0.15 ± 0.13 μM) and NCL (0.27 ± μM) averaged 0.21 ± 0.31 μM overall (sites and samplings pooled; $n$ = 28 = 2 site$^{-1}$ sampling$^{-1}$) and ranged from 0.7 μM to 0.46 μM at PA and 0.05 μM to 1.26 μM at NCL. When the high concentrations associated with the July 2003 upwelling were excluded, SRP averaged 0.10 ± 0.05 μM overall ($n$ = 24). SRP differed between sites only in July 2003 when SRP at PA was lower ($p < 0.001$, GLM) than NCL (Fig. 7). SRP concentrations at both sites were highest ($p < 0.001$, THSD) in July 2003, followed by February 2003; the lowest SRP concentrations occurred in April 2003 and May 2004 (dry season) at PA, and in October 2003 at NCL (Fig. 7). SRP concentrations approached or exceeded 0.1 μM four times at PA and five times at NCL, out of the seven samplings (Fig. 7).

DIN:SRP ratios were similar at PA (13 ± 4) and NCL (16 ± 7), averaging 7.4 ± 3.7 (sites and samplings pooled; $n$ = 28 = 2 site$^{-1}$ sampling$^{-1}$) and ranging from 8 to 18 at PA and 4 to 30 at NCL. There was little seasonal variation in DIN:SRP ratios; significant ($p = 0.048$, THSD; Fig. 7) differences occurred only at PA, where July 2003 and February 2004 DIN:SRP ratios were higher than those in February 2003 and May 2004.

**Table 2**

Interspecies tissue C:N, C:P, N:P and $\delta^{15}$N rankings, from low-to-high (left-to-right), of *Caulerpa brachypus* (B), *Caulerpa racemosa* (R), *Caulerpa verticillata* (V), *Caulerpa mexicana* (M), and *Codium isthmocladum* (C). Inequalities indicate significant differences; $p$ values indicate level of significance (Tukey's HSD test).

| Site | Date | C:N | | C:P | | N:P | | $\delta^{15}$N | |
|------|------|-----|---|-----|---|-----|---|------|---|
| PA | February '03 | B < R < V | $p \leq 0.006$ | R, B < V | $p = 0.001$ | R < B | $p = 0.029$ | B, R, V | $p \geq 0.528$ |
| | April '03 | B < C < R | $p \leq 0.028$ | B, R, C | $p \geq 0.731$ | R, C, B | $p \geq 0.084$ | B < C, R | $p < 0.001$ |
| | July '03 | C, R < B | $p \leq 0.010$ | R < B, C | $p \leq 0.009$ | R, B < C | $p \leq 0.001$ | R, C, B | $p \geq 0.248$ |
| | October '03 | B, C, R | $p \geq 0.051$ | B, R, C | $p \geq 0.844$ | R, C, B | $p \geq 0.513$ | C, R, B | $p \geq 0.091$ |
| | February '04 | B < R, M | $p \leq 0.041$ | R, B < M | $p \leq 0.043$ | R < B, M | $p \leq 0.030$ | R, M, B | $p \geq 0.563$ |
| | May '04 | B < C < R | $p \leq 0.005$ | B, R, C | $p \geq 0.523$ | R < B | $p = 0.014$ | B, R < C | $p \leq 0.009$ |
| | August '04 | B < C | $p = 0.009$ | B < C | $p = 0.001$ | C, B, R | $p \geq 0.627$ | B < C | $p = 0.004$ |
| NCL | February '03 | B < R, V | $p = 0001$ | R, B, V | $p \geq 0.053$ | R, V, B | $p \geq 0.089$ | V < B, R | $p \leq 0.009$ |
| | April '03 | B, R, C | $p \geq 0.203$ | B, C, R | $p \geq 0.090$ | C, B, R | $p \geq 0.199$ | R, C, B | $p \geq 0.057$ |
| | July '03 | C < B | $p = 0.023$ | R, C < B | $p \leq 0.033$ | R < C | $p = 0.022$ | B, R < C | $p \leq 0.001$ |
| | October '03 | B, C < R | $p < 0.001$ | C, R, B | $p \geq 0.362$ | R < B | $p = 0.009$ | B < C, R | $p < 0.001$ |
| | February '04 | B < R, M | $p \leq 0.001$ | B, R < M | $p < 0.001$ | R, B < M | $p \leq 0.009$ | R, B < M | $p \leq 0.016$ |
| | May '04 | B, C < R | $p \leq 0.003$ | B, C < R | $p = 0.001$ | C < R | $p = 0.008$ | B < C < R | $p < 0.001$ |
| | August '04 | B < R < C | $p < 0.001$ | B, R < C | $p \leq 0.017$ | R, B, C | $p \geq 0.108$ | B, R < C | $p < 0.001$ |
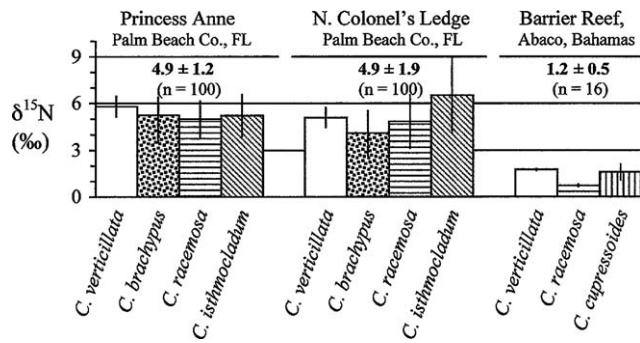
**Fig. 6.** A comparison of δ$^{15}$N (‰) values in *Caulerpa* spp. and *Codium isthmocladum* from Princess Anne and North Colonel's Ledge in Palm Beach County, Florida, U.S.A., with those from reference reef sites on the Abaco Barrier Reef near Green Turtle Cay, Abaco, Bahamas. Values represent means ± 1 S.D.

## 4. Discussion

### 4.1. Development of the C. brachypus f. parvifolia invasion

This study provides the first report of the invasion of southeast Florida's coral reefs by the non-native chlorophyte *C. brachypus f. parvifolia*. Although this Pacific native was first observed in May 2001 off northern Palm Beach County (Lapointe et al., 2005b), it did not form extensive blooms until the beginning of this study in winter and early spring of 2003. Based on the rapid increase in benthic cover we observed, *C. brachypus* clearly has the ability to undergo explosive growth during winter and early spring in these deep reef habitats off southeast Florida. In laboratory cultures, we have obtained growth rates of 0.1 doublings d$^{-1}$ under relatively low irradiance (50–200 μmol photons) and warm temperatures (22–29 °C), conditions that can occur in these deep reef habitats year-around. This contrasts with the seasonal growth pattern of *C. isthmocladum*, which forms blooms (Fig. 3E) primarily during late

spring and summer months on these reefs (Lapointe, 1997). *C. brachypus* not only overgrew the diminutive *C. verticillata* and other native benthic macroalgae during winter and spring of 2003, but also larger reef biota such as sponges and gorgonians (Fig. 3B). The *C. brachypus* bloom persisted, especially at PA, through the final sampling of this study in August 2004 but was subsequently removed by the "twin" hurricanes, Frances and Jeanne, which made landfall just north of the study area on September 4th and 24th, 2004, respectively (Lapointe et al., 2006).

The rapid northward spread of *C. brachypus* during the study appeared to be facilitated by vegetative propagation through fragmentation and dispersal northward via the Florida Current. Fragments of *C. brachypus*, which were observed by divers throughout the water column during the study, can potentially re-attach themselves and overgrow benthic biota. Fragmentation and vegetative growth is an important mechanism of dispersal for *C. taxifolia* in the Mediterranean Sea (Meinesz et al., 1993; Smith and Walters, 1999), where boat transport also plays an important anthropogenic role (Sant et al., 1996). Tidal currents also carried fragments of *C. brachypus* through the Jupiter and St. Lucie inlets into the Indian River Lagoon (IRL), but no populations of *C. brachypus* have been sustained within the IRL. Although not observed during the study, it is possible that this successful invasion also involved sexual reproduction. Pre-dawn mass-spawning of gametes has been observed for at least nine species of siphonous chlorophytes in the Caribbean, including *C. verticillata*, *C. mexicana*, and *C. racemosa* (Clifton, 1997).

Although *C. brachypus* dominated benthic cover at PA and NCL throughout most of the study, the blooms were significantly greater at the PA site. At PA, *C. brachypus* accounted for 60–80% reef cover between April 2003 and August 2004, compared to NCL where *C. brachypus* decreased to <40% cover following the strong upwelling event of July 2003. In addition, *C. brachypus* cover at three adjacent reef sites (The Bluffs, Zimm's Reef, Frank's Reef) surveyed between PA and NCL during August 2004 conformed to
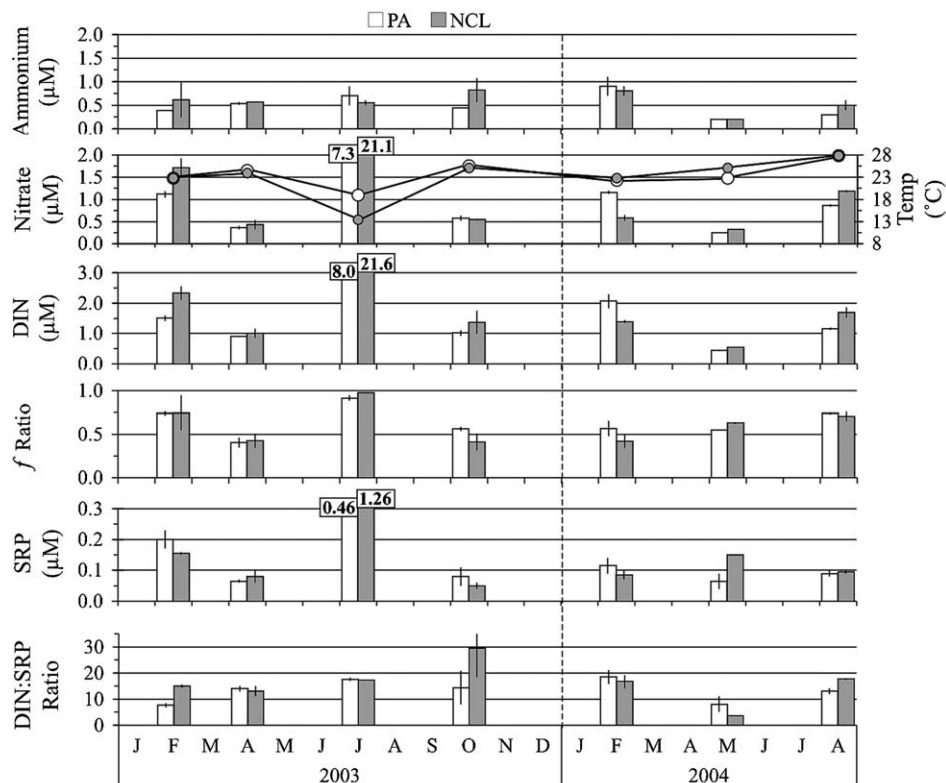


**Fig. 7.** Near-bottom water column nutrients at the PA and NCL sites. Values represent means ± 1 S.D. (*n* = 2 site$^{-1}$ sampling$^{-1}$).

the decreasing south-to-north trend (Fig. 1). The decline in *C. brachypus* cover at NCL after July 2003 may have been initiated by cold temperature stress (~13 °C at NCL; 19 °C at PA) associated with the upwelling. Controlled experimental growth studies in our laboratory have shown that temperatures <15 °C can be lethal to *C. brachypus* and little growth occurs below 20 °C (unpublished data). *C. taxifolia* in the Mediterranean Sea does not grow below 12.5 °C, and below 10 °C this cold-hardy strain dies (Komatsu et al., 1997). In addition to *C. brachypus*, secondary blooms of *C. racemosa* and *C. mexicana* also developed during the study period, especially at NCL where *C. racemosa* accounted for >20% cover (Fig. 3D). *C. racemosa* also formed a secondary invasion in the Mediterranean Sea in 1994 (Ceccherelli and Piazzi, 2001).

### 4.2. Tissue $\delta^{15}N$ enrichment

The elevated $\delta^{15}N$ values of the chlorophytes measured at PA and NCL support previous research that linked these blooms to land-based nitrogen sources (Lapointe et al., 2005b). The overall mean value of ~5.0‰ in the present study is similar to the mean of +6.1‰ reported by Lapointe et al. (2005b) for chlorophytes on reefs in the same study area in 2001, and is within the range reported for macroalgae utilizing sewage nitrogen (Costanzo et al., 1999; Savage and Elmgren, 2004; Lapointe et al., 2004, 2005b; Risk et al., 2009). The significant $\delta^{15}N$ enrichment of chlorophytes at PA and NCL, compared to the Bahamas, supports the hypothesis that southeast Florida reefs are more enriched by sewage nitrogen than those of the Abaco Barrier Reef. The lower mean $\delta^{15}N$ value of chlorophytes from the Bahamian reefs (+1.2‰) is close to that of nitrogen-fixation (0‰; Owens, 1987; France et al., 1998), which can be a major source of nitrogen on remote coral reefs. Reefs in the Abacos are also influenced by atmospheric nitrogen deposition from airsheds within the United States, which can have $\delta^{15}N$ values ranging from −3 to +3‰ (Barile and Lapointe, 2005). These intrinsically low $\delta^{15}N$ nitrogen sources appear to supply a significant fraction of the nitrogen budget on Abacos reefs, which receive relatively little human sewage input compared to those of southeast Florida.

The spatial and temporal variability in $\delta^{15}N$ values of *C. brachypus* further links stormwater discharges with nitrogen enrichment and success of this non-native species. As shown in Fig. 8, canal discharges from the upland Florida watersheds were low in the dry season between February and April of 2003, a period when $\delta^{15}N$ values of *C. brachypus* were lower at PA than at NCL. Following the onset of the 2003 wet season between July and October of 2003 and discharges of stormwater runoff from Lake Worth inlet (Fig. 3F), $\delta^{15}N$ values of *C. brachypus* were higher at PA than NCL, reflecting a greater degree of sewage nitrogen enrichment. Similar increases in $\delta^{15}N$ of *C. isthmocladum* occurred on Jupiter Ledge (which can be influenced by discharges from the

Jupiter inlet) in the summer of 1995 following the onset of the wet season (Lapointe, 1997). The lowest $\delta^{15}N$ values of *C. brachypus* during the study occurred at NCL in July 2003 following nitrate enrichment associated with strong upwelling; this phenomenon was previously observed in 2001 (Lapointe et al., 2005b) and clearly demonstrates again that upwelled nitrate is not the source of elevated $\delta^{15}N$ values in these chlorophyte blooms, as suggested by Hughes et al. (1999). Other chlorophytes also had their lowest values of the study at both PA and NCL in October 2003 and February 2004. The lower $\delta^{15}N$ values during this period, when stormwater discharges continued despite reduced rainfall (Fig. 8), reflects the lower $\delta^{15}N$ source values of wet atmospheric deposition, fertilizers and topsoil compared to sewage nitrogen (Lapointe et al., 2005b; Risk et al., 2009). These results illustrate the importance of water management (irrespective of rainfall) to nitrogen loading of coastal waters and are consistent with previous observations. Studies in the Florida Keys (Lapointe et al., 2004) and Lee County in southwest Florida (Lapointe and Bedford, 2007) have both reported similar decreases in $\delta^{15}N$ values during periods when stormwater runoff and agricultural nitrogen inputs are high.

The greater $\delta^{15}N$ enrichment of *C. brachypus* at PA compared to NCL in July and October 2003 suggests that PA was significantly more impacted by land-based sewage discharges than was NCL. The increased $\delta^{15}N$ enrichment at PA compared to NCL could result from its closer proximity to stormwater discharges from the Lake Worth Inlet (~3 km southwest of the PA site) that would contain sewage nitrogen from septic tanks and other sources on the watershed (Fig. 3F). Discharges were clearly visible to survey divers during ebbing tides as a buoyant surface plume extending to ~5 m depth. Down-mixing of particulate organic matter and settling of flocculated *gelbstoff* from the lower-salinity surface plume would enhance transfer of surface water nutrients to the benthos (Chester, 2000). In addition, the water column at the PA site could be enriched by the far-field impacts of six upstream sewage outfalls between Dade County and Palm Beach County, which discharge ~4 billion l d$^{-1}$ of ammonium-rich, secondarily treated effluent into coral reef environments at 30 m depths. These discharges, which mix throughout the nearshore water column and are measurable for at least 66 km downstream (Wanninkhof et al., 2005), would result in cumulative enrichment over the many years that they have been operating. Uptake of ammonium by phytoplankton and recycling in reef communities by heterotrophic reef organisms, such as sponges, could increase nitrogen availability to associated benthic macroalgae (Corredor and Wilkinson, 1988), especially mat-forming species such as *C. brachypus*. Lastly, submarine groundwater discharge (SGD) is an important pathway for delivery of land-based nutrients, especially nitrogen, to reefs in northern Palm Beach County (Lapointe et al., 2005b; Swayze and Miller, 1984). SGD is of particular concern along this coastline where Class I injection wells are used to dispose of partially
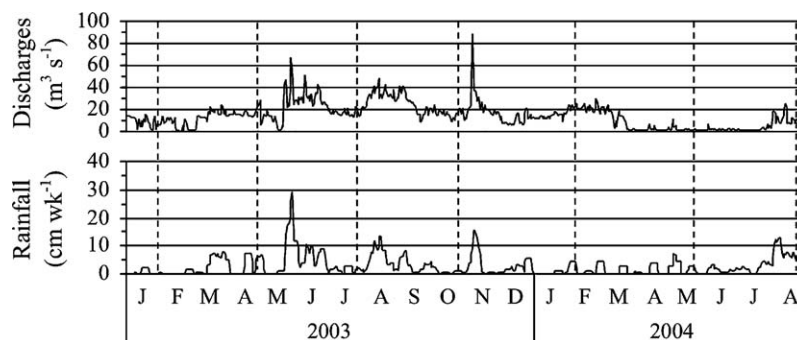


Fig. 8. Daily canal discharges (m³ s⁻¹) and 7-day cumulative rainfall (cm wk⁻¹) data for coastal Palm Beach County, FL, 2003–2004 (South Florida Water Management District, DBHYDRO database). Dashed lines indicate ECOHAB sampling dates.

treated, ammonium-rich sewage into groundwaters at depths of ~1000 m where they are subject to buoyant upward migration and seaward transport (USEPA, 2003).

### 4.3. Tissue C:N:P ratios

The nutrient-enriched status of the chlorophytes was best revealed by the relatively low C:N:P ratios in the algal tissue during the study. The mean C:N and C:P ratios of the chlorophytes at PA (14.5, 497) and NCL (15.3, 544) were well below the mean values reported for marine macrophytes (22, 700; Atkinson and Smith, 1983) and macroalgae on Caribbean reefs (23, 976; Lapointe et al., 1992), suggesting generally high availability of both nitrogen and phosphorus. These relatively low C:N and C:P ratios readily explain the explosive growth and blooms observed in several chlorophyte species. However, the mean N:P ratios of the chlorophytes at PA (36) and NCL (37) were similar to the mean N:P (35) of marine macrophytes (Atkinson and Smith, 1983) and approached the N:P (43) of Caribbean macroalgae (Lapointe et al., 1992); values suggesting phosphorus limitation of productivity.

The significantly lower mean C:N ratio of the non-native C. brachypus (13) compared to the pooled native chlorophytes (16) indicate that this non-native invader experienced the lowest degree of nitrogen limitation. The only exception to this pattern was the July 2003 upwelling period, when elevated C:N ratios occurred in C. brachypus. Critical C:N ratios, above which nitrogen limitation of growth develops, have been shown to be ~12–13 for both the chlorophyte Codium fragile (Hanisak, 1979) and the rhodophyte Gracilaria tikvahiae (Lapointe and Duke, 1984; Lapointe, 1987) in controlled enrichment experiments. Accordingly, the overall mean C:N of ~13 for C. brachypus during this study suggests that nitrogen was not a major limiting nutrient to its growth, unlike the other native chlorophyte species, which had higher C:N ratios ranging up to 24. Differences in nitrogen uptake kinetics of macroalgae are well known (D'Elia and DeBoer, 1978; Haines and Wheeler, 1978; Hanisak and Harlin, 1978) and can determine the relative rates of growth and outcomes of competitive interactions. Our finding of a lack of nitrogen limitation in C. brachypus is particularly significant considering its explosive growth and biomass production on these reefs, which would tend to elevate C:N ratios (Lapointe and Duke, 1984).

The uniformity of nitrogen availability to reefs in the study area was evidenced by the overall similar mean C:N ratios of pooled chlorophytes at PA (14) and NCL (15). However, significant seasonal variation in species-specific C:N ratios occurred. The highest C:N ratios occurred in the dry season (April 2003, May 2004) and the lowest in the wet season (July 2003, October 2003, August 2004), supporting previous conclusions that these blooms are linked to land-based runoff (Lapointe et al., 2005a,b). An exception to this pattern occurred during the July 2003 upwelling that was most pronounced at NCL, resulting in lower C:N ratios at NCL compared to PA in several chlorophyte species. Low temperatures can reduce growth and lead to accumulation of N, thereby lowering the C:N ratio, as has been shown experimentally in the rhodophyte G. tikvahiae (Lapointe et al., 1984). Interestingly, this temperature response did not occur in C. brachypus, whose highest C:N ratio of the entire study occurred during the July 2003 upwelling, possibly due to cold-temperature stress causing shutdown of active nitrate transport into the alga at a time when nitrate dominated the DIN pool (f-ratio $\geq$ 0.9). C. brachypus benthic cover also decreased at NCL following these low temperatures and elevated C:N ratios in July 2003, suggesting that upwelling had a longer-term negative effect on bloom development at NCL, where upwelling was strongest.

Unlike the C:N ratio, the pooled chlorophytes did have a significantly lower overall C:P ratio at PA (497) compared to NCL

(544). These results indicate a greater degree of phosphorus limitation at NCL, and helps explain the persistently higher cover of C. brachypus at the PA site, which is impacted episodically by stormwater and tidal discharges from Lake Worth Inlet (Fig. 3F). C. brachypus frequently had the lowest C:P ratios of all chlorophytes, further demonstrating its high affinity for growth-limiting nutrients. As was the case for C:N ratios, the highest C:P ratios occurred in the dry season (May 2004), when land-based discharges were minimal, in contrast to the lowest C:P ratios which occurred in summer (July 2003) when both upwelling and stormwater runoff co-occurred (Fig. 8). Comparative studies of C. isthmocladum from the wider Caribbean have shown the importance of lower C:P ratios and increased P availability to bloom development (Fig. 3E) on southeast Florida reefs: C:P ratios of C. isthmocladum blooms in southeast Florida averaged 425 compared to 985 in the more oligotrophic waters of the Caribbean that do not support blooms (Lapointe et al., 2005a). Significant P limitation of the chlorophyte blooms was also evidenced by the tissue N:P ratios at PA (36) and NCL (37) which, while lower than those in macroalgae from carbonate-rich waters of the Caribbean (43), are higher than N:P ratios from temperate, siliciclastic environments (15; Lapointe et al., 1992).

### 4.4. Water column nutrients

Results of this study showed that mean DIN and SRP concentrations at both PA and NCL were >~1 μM and 0.1 μM, respectively, values that are high for coral reefs and that can sustain the rapid growth of bloom-forming macroalgae (Lapointe, 1997). Although concentrations of ammonium, nitrate, DIN and SRP were not significantly different between the two study sites, seasonal differences in water column nutrients did occur. The lowest concentrations of ammonium, nitrate, DIN, and SRP occurred during the dry season (April 2003, May 2004) when land-based runoff was relatively low (Fig. 8). The highest nitrate and SRP concentrations (~21 μM and 1.3 μM, respectively) occurred at NCL during the July 2003 upwelling when temperatures decreased to ~13 °C and the f-ratio was 0.97. This upwelling event was stronger than the event of August 2001 when nitrate concentrations of 9 μM and temperatures of 19 °C were measured (Lapointe et al., 2005b). Upwellings along Florida's east coast are centered just north of the study area (Green, 1944) and typically occur during summer months when southerly winds (parallel to shore) and onshore movement of the Florida Current produce conditions favorable for upwelling (Smith, 1982); these events can persist for 7–14 days (Lee and Mayer, 1977).

Overall, the mean f-ratio (~0.6) we measured during the study was relatively low, indicating that upwelling was not a persistent feature on these reefs and pointing to the importance of ammonium as a nitrogen source supporting these blooms. Experimental nutrient uptake studies show that algae generally assimilate ammonium preferentially over nitrate. In controlled laboratory studies of C. fragile subsp. tomentosoides, ammonium uptake was ~7-fold greater than that of nitrate at temperatures of 20–25 °C, and the presence of ammonium inhibited nitrate uptake (Hanisak and Harlin, 1978). Similar preferences for ammonium over nitrate have been reported for rhodophytes (D'Elia and DeBoer, 1978) and natural phytoplankton communities (Conway, 1977). Because an average of 0.60 μM ammonium was present in the near-bottom waters during the study, it is unlikely that nitrate was a major DIN source to C. brachypus or the other chlorophyte species. In a seasonal upwelling system similar to that of southeast Florida, Fujita et al. (1989) reported that relatively low concentrations of ammonium (1.22 μM) supplied >100% of the nitrogen required for maximum growth of the chlorophyte Ulva rigida, even in the presence of much higher nitrate concentrations (10.8 μM).

This explains why episodic upwelling has not supported development of these chlorophyte blooms historically and why they have developed only over the past several decades, as background ammonium enrichment from land-based sources has increased (Lapointe et al., 2005a,b).

### 4.5. Summary and conclusions

Results of this study showed that invasion of coral reefs in southeast Florida by the non-native *C. brachypus* was facilitated by explosive growth of this alga in winter and spring, 2003. Studies along the northern coast of Yucatan, Mexico have also shown that maximum seasonal photosynthesis of several *Caulerpa* species occurred in the cool winter months when concentrations of DIN (9.1 μM) and SRP (0.5 μM) were seasonally elevated (Robledo and Freile-Pelegrin, 2005). Low mean tissue C:N ratios of ∼13 at the PA and NCL sites indicated that the massive biomass production by *C. brachypus* was not strongly nitrogen limited. Rather, mean N:P ratios of ∼37 at PA and NCL suggested the *C. brachypus* bloom was phosphorus-limited. *C. brachypus* had lower C:N ratios (and frequently lower C:P ratios) than native chlorophytes, indicating its superior competitive ability for sequestering growth-limiting nutrients. Because thick mats of macroalgae may attenuate nutrient flux from sediments to the overlying water column (McGlathery et al., 1997), the dense blooms of mat-forming *C. brachypus* could intercept SGD and/or recycled benthic nutrients before it is available to upright, native frondose macroalgae.

Multiple lines of evidence suggest the importance of land-based sources of nutrient enrichment to sustaining the *C. brachypus* blooms. First, more persistent blooms of *C. brachypus* at PA, compared to NCL, correlated with significantly lower tissue C:P and higher $\delta^{15}$N values at PA in the wet season, the site most directly influenced by stormwater runoff. Second, chlorophyte C:N, C:P, and $\delta^{15}$N values correlated with seasonal patterns in stormwater runoff. Third, the $\delta^{15}$N values of *C. brachypus* and other chlorophytes decreased following strong upwelling at NCL in July 2003, confirming that upwelling does not cause the widespread $\delta^{15}$N enrichment observed in these chlorophyte blooms. Lastly, the mean $\delta^{15}$N values of *C. brachypus* and other chlorophytes in southeast Florida (+4.9‰), where ∼4 billion l d$^{-1}$ of partially treated, ammonium-rich sewage are discharged directly into coastal waters, are several-fold higher than values (+1.2‰) for similar species of chlorophytes growing in the relatively clean coastal waters of the Abacos, Bahamas.

Non-native macroalgal invasions are expected to increase in coming years due to the anticipated increase in unintended introductions of non-native organisms as a side-effect of globalization (UNEP, 2005). Our results indicate that the spread of non-native macroalgae in oligotrophic coral reef regions can be facilitated by land-based nutrient pollution and eutrophication. This problem was recognized four decades ago in Kaneohe Bay, Hawaii, where the abundance of the non-native rhodophyte *Acanthophora spicifera* increased with proximity to a sewage outfall (Soegiarto, 1972; Johannes, 1975). Since then, the problem has worsened in Hawaii where at least five species of non-native macroalgae have spread and formed extensive blooms in bays and coastlines adjacent to urbanized watersheds (e.g. Waikiki, Kaneohe, Lahina; Russell, 1992; Rodgers and Cox, 1999; Smith et al., 2002). The role of land-based nutrient pollution in facilitating invasions of non-native macroalgae must be recognized if these phenomena are to be managed effectively in the future.

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