Variable responses within epiphytic and benthic microalgal communities to nutrient enrichment

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Abstract

We evaluated how changes in nutrient supply altered the composition of epiphytic and benthic microalgal communities in a *Thalassia testudinum* (turtle grass) bed in Florida Bay. We established study plots at four sites in the bay and added nitrogen (N) and phosphorus (P) to the sediments in a factorial design. After 18, 24, and 30 months of fertilization we measured the pigment concentrations in the epiphytic and benthic microalgal assemblages using high performance liquid chromatography. Overall, the epiphytic assemblage was P-limited in the eastern portion of the bay, but each phototrophic group displayed unique spatial and temporal responses to N and P addition. Epiphytic chlorophyll *a*, an indicator of total microalgal load, and epiphytic fucoxanthin, an indicator of diatoms, increased in response to P addition at one eastern bay site, decreased at another eastern bay site, and were not affected by P or N addition at two western bay sites. Epiphytic zeaxanthin, an indicator of the cyanobacterial/coralline red algae complex, and epiphytic chlorophyll *b*, an indicator of green algae, generally increased in response to P addition at both eastern bay sites but did not respond to P or N addition in the western bay. Benthic chlorophyll *a*, chlorophyll *b*, fucoxanthin, and zeaxanthin showed complex responses to N and P addition in the eastern bay, suggesting that the benthic assemblage is limited by both N and P. Benthic assemblages in the western bay were variable over time and displayed few responses to N or P addition. The contrasting nutrient limitation patterns between the epiphytic and benthic communities in the eastern bay suggest that altering nutrient input to the bay, as might occur during Everglades restoration, can shift microalgal community structure, which may subsequently alter food web support for upper trophic levels.

Introduction

Habitat management balances multiple ecological, social, and economic objectives (Arthur et al., 2004; Sklar et al., 2005) and often requires trade-offs (Brodziak et al., 2004; Pejchar et al., 2005), as management policies can benefit some community components and simultaneously negatively impact others. Understanding the links among ecosystem components and interpreting community-level responses to ecosystem changes can increase the overall success of management strategies by facilitating the prediction of indirect impacts of land-use projects and increasing the potential for positive community-level impacts.

Hydrological management in watersheds and coastal marshes can alter the supply of terrestrially-derived compounds, particularly nutrients such as nitrogen (N) and phosphorus (P), to nearshore communities (Valiela et al., 1997). In the context of habitat management, nutrient limitation within the primary producer community is often assumed to be uniform, but in coastal habitats, macro-producers such as seagrasses and macroalgae often show different responses to N and P enrichment (Fong et al., 1993; Udy & Dennison, 1997; Ferdie & Fourqurean, 2004; Armitage et al., 2005). Less is known about nutrient limitation within epiphytic or benthic microalgal communities, where biomass or production of typically diverse communities are usually represented by whole community estimates (Sullivan & Currin, 2000). Nitrogen and phosphorus enrichment has been associated with shifts towards cyanobacterial assemblages in benthic estuarine habitats (Pinckney et al., 1995; Armitage & Fong, 2004), particularly when diatoms are limited by silica (Rocha et al., 2002) or grazing pressure (Cuker, 1983). Green algae (Chlorophyta) are often palatable and limited by grazing but are also fast-growing and may respond rapidly to increased nutrient input (Valiela et al., 1997; Lotze et al., 2000). Epiphytic and benthic microalgal communities have distinct compositions and patterns of nutrient

limitation may vary between these assemblages. These communities often provide support for higher trophic levels (Moncreiff & Sullivan, 2001), and shifts in microalgal community composition can have important implications for food web dynamics (Armitage & Fong, 2004).

Implementation of the Comprehensive Everglades Restoration Plan in south Florida might change freshwater input and associated nutrient supply to Florida Bay, which is directly connected to the southern border of the Everglades. Previous work in the Bay and the Florida Keys has demonstrated that increased nutrient input can alter the relative composition of seagrass and macroalgal assemblages, although the degree of alteration depends on the nutrient availability status of the area (Fourqurean et al., 1995; Ferdie & Fourqurean, 2004; Armitage et al., 2005). The objective of this study was to further evaluate how increased nutrient supply might alter marine primary producer communities by focusing on nutrient enrichment responses within the epiphytic and benthic microalgal communities. We hypothesized that nutrient enrichment would shift microalgal community composition, increasing the abundance of faster growing groups including palatable green algae and less palatable cyanobacteria.

Methods

To evaluate the epiphytic and benthic microalgal responses to N and P enrichment over time within Everglades National Park in Florida Bay, we used a three-way ANOVA design, where the factors were P addition, N addition, and sampling date. In October 2002 we established four study sites (all depths < 2 m) as part of a long-term enrichment study (Armitage et al., 2005). The two eastern sites (Duck Key and Bob Allen Keys Long Term Ecological Research (LTER) sites, Fig. 1) were characterized by a sparse, short *Thalassia testudinum* Banks *ex* König canopy with some calcareous green macroalgae, primarily *Penicillus capitatus* Lamarck and *P. lamourouxii* Decaisne. These two sites occurred in an area of severe P-limitation (Fourqurean &

Zieman, 2002; Armitage et al., 2005). The two western sites (Nine Mile Bank, Sprigger Bank LTER site) were located in a region that may experience both N- and P-limitation but varied in their vegetation characteristics. Nine Mile Bank featured a dense, tall *T. testudinum* canopy with few macroalgae. Sprigger Bank was characterized by a dense and diverse macroalgal community mixed with the seagrasses *Syringodium filiforme* Kützing (manatee grass) and *T. testudinum*. At each site we established 24 0.25 m^2 study plots demarcated with a PVC frame secured to the benthos at one meter intervals.

We randomly assigned treatments [control (C), nitrogen only (N), phosphorus only (P), both nitrogen and phosphorus (NP)] to six plots per site (at the Sprigger Bank LTER site, n = 3 per treatment due to the loss of 12 plots from erosion and boat disturbance over the course of the study). Bimonthly fertilizer applications began in October 2002. Nitrogen was added in the form of slow release nitrogen fertilizer (Polyon™, Pursell Technologies Inc., 38-0-0) and phosphorus as granular phosphate rock (Multifos™, IMC Global, $Ca_3(PO_4)_2$, 18% P). Loading rates of 1.43 g N m^{-2} day^{-1} and 0.18 g P m^{-2} day^{-1} (molar N:P ratio 17.6:1) were selected based on potential sewage loading rates (MCSM, 2001) and previous studies in the region (Ferdie & Fourqurean, 2004; Armitage et al., 2005). We sprinkled the fertilizer evenly on the sediment surface and gently worked it into the sediment by hand. Sediment in the control plots was similarly disturbed but no fertilizer was added. Benthic fertilizer applications ensured accessibility of nutrients to both above-ground and benthic primary producers (Ferdie & Fourqurean, 2004; Mutchler et al., 2004; Armitage et al., 2005). The plots and experimental treatments in this study are the same plots sampled for a recent study (Armitage et al., 2005), but all samples collected for this experiment are independent of the previous study.

In February 2004, August 2004, and February 2005, we collected one *T. testudinum* short-shoot from each plot and removed the epiphytes by gently scraping the seagrass leaves with a razor blade. At Sprigger Bank, *T. testudinum* was not present in all plots. Leaf morphometrics were measured to calculate two-sided leaf area. We measured shoot density in each plot and calculated leaf area index (LAI = cm^2 seagrass leaf m^{-2} habitat). We also collected a 2.5 cm diameter, 1 cm deep core haphazardly located within each plot. Due to logistical constraints, sediment cores were collected only on the two 2004 sampling dates. Epiphytes and sediments were freeze dried and stored at -20º in the dark until further analysis.

We determined the relative abundance of major phototrophic groups with high performance liquid chromatography (HPLC), which measures the relative concentrations of taxa-specific indicator pigments (chlorophyll *a*, chlorophyll *b*, fucoxanthin, and zeaxanthin) (Pinckney et al., 1995). Pigments were extracted from freeze-dried epiphytes and sediments with 90% acetone for at least 12 hours at -20ºC. An ion-pairing solution (1.00 M ammonium acetate) was added to the filtered extracts at a ratio of 4 parts extract: 1 part ammonium acetate just prior to injection. Extracts (250 µl) were injected into a Hewlett Packard 1090 HPLC equipped with a monomeric reverse-phase C_{18} column (Rainin-Microsorb-MV, 100 x 4.6 mm, 3 µm) and a polymeric reverse-phase C_{18} column (Vydac, 201TP, 250 x 4.6 mm, 5 µm) in series and a photodiode array detector set at 440 nm. Solvents and flow rates followed Pinckney et al. (1999) and the column temperature was 40ºC. Pigments were identified based on retention times and comparisons with pure standards extracted from phytoplankton cultures in 90% acetone (chlorophyll *a*, chlorophyll *b*) or 100% ethanol (fucoxanthin, zeaxanthin) obtained from DHI Water & Environment (Denmark). Epiphyte load is represented as µg pigment cm^{-2} of seagrass leaf; benthic load is µg pigment cm^{-2} of sediment. Microalgal biomass is represented as the average pigment

136 concentration (mg) m^{-2} of habitat. Epiphytic biomass is (μg pigment cm^{-2} seagrass
137 leaf)*(LAI)/1000, and benthic biomass is (μg pigment cm^{-2} sediment)*10.

138 In February 2005 we collected one additional *T. testudinum* leaf from each plot, removed the
139 epiphytes, and preserved them in 6% Lugol's solution. We qualitatively verified composition of
140 the microalgal assemblages by examining the cells at 100x under a light microscope and noting
141 the cell types present.

142 All data were tested for normality and variances for homoscedasticity using the F_{max} test and
143 log transformed if necessary to conform to the assumptions of ANOVA. We performed a three-
144 way ANOVA with Type III Sums of Squares for unequal sample size within each site separately
145 for epiphyte and benthic pigment concentrations. The three fixed factors were date (3 dates for
146 epiphytes, 2 dates for benthic pigments), P addition (±P), and N addition (±N). Dependent
147 variables were epiphyte loads, represented by μg chlorophyll *a*, fucoxanthin, zeaxanthin, and
148 chlorophyll *b* cm^{-2} of seagrass leaf or cm^{-2} of sediment. *T. testudinum* was not present in all plots
149 at Sprigger Bank ($n \leq 2$), resulting in insufficient replication for ANOVA, and so means and
150 standard errors are reported for epiphytic loads at that site.

151 **Results**

152 Qualitative microscopic examination indicated that the most common components of the
153 epiphytic microalgal community were diatoms and calcareous red algae. Hence, we interpreted
154 fucoxanthin concentration to primarily represent diatom abundance, though a few dinoflagellate
155 cysts were also noted at most sites (excepting Nine Mile Bank). Diatom species lists for this
156 region are presented in Frankovich et al. (this issue). At Sprigger Bank, part of the fucoxanthin
157 signal came from brown algae (e.g., *Cladosiphon occidentalis* Kylin), particularly in the
158 February samplings. The zeaxanthin signal represented a red algal/cyanobacterial complex. Most

of the red algae were encrusting calcareous forms (e.g., *Melobesia membranacea* (Esper) Lamouroux, *Hydrolithon farinosum* (J.V. Lamouroux) Penrose & Y.M. Chamberlain), though uncalcified forms (e.g., *Polysiphonia binneyi* Harvey, *Ceramium brevizonatum* var. *caraibicum* H.E. Petersen & Børgesen in Børgesen) were also present. The cyanobacteria were primarily *Lyngbya* spp. and unidentified sheathed filaments. The chlorophyll *b* signal represented green microalgae (e.g., *Ulvella lens* P. Crouan & H. Crouan).

Comparisons of epiphytic and benthic microalgal biomass (as represented by mg chlorophyll *a* m^{-2} habitat) in control plots suggest that benthic microalgal biomass was 6-10 times higher than epiphytic biomass at all sites (Fig. 2). Epiphytic biomass (mg m^{-2} habitat) was higher at Nine Mile and Sprigger Banks than at the other sites (Fig. 2a). Benthic biomass was lowest at Duck and similar between the other three sites (Fig. 2b).

At Duck Key, each pigment responded differently to date and nutrient addition treatments. In the epiphyte community, a significant interaction between date and P addition for chlorophyll *a* ($df = 2$, $F = 16.336$, $p < 0.001$) was caused by a large increase in response to P addition in February 2004 but not on any other date (Fig. 3a). A significant P*N interaction ($df = 1$, $F = 8.412$, $p = 0.005$) was driven by consistently lower chlorophyll *a* concentrations in plots that received both N and P compared to P only treatments. A significant interaction between date and P addition for epiphytic fucoxanthin ($df = 2$, $F = 14.307$, $p < 0.001$) was driven by a P-induced increase in February 2004, a P-induced decrease in August 2004, and no nutrient effects in February 2005 (Fig. 3b). N addition did not affect epiphytic fucoxanthin. A significant interaction between date and P addition for epiphytic zeaxanthin ($df = 2$, $F = 15.860$, $p < 0.001$) was caused by a larger response to P addition in the February samplings than in August (Fig. 3c). Epiphytic zeaxanthin was not affected by N addition. A significant date*P interaction for

epiphytic chlorophyll *b* ($df = 2$, $F = 14.100$, $p < 0.001$) was driven by the largest response to P addition occurring in February 2004 and the smallest response in February 2005 (Fig. 3d). A significant interaction between N and P addition ($df = 1$, $F = 4.532$, $p = 0.037$) was caused by lower chlorophyll *b* concentration in NP than in P only treatments.

The Duck Key benthic microalgal community responded differently to nutrient addition than the epiphyte assemblage. Both P ($df = 1$, $F = 17.564$, $p < 0.001$) and N ($df = 1$, $F = 9.921$, $p = 0.003$) had significant and additive effects on benthic chlorophyll *a* concentration, with no date effect and no interactions between factors (Fig. 3e). Fucoxanthin was significantly affected by all three factors with no interactions (Date $df = 1$, $F = 5.449$, $p = 0.025$; P $df = 1$, $F = 11.007$, $p = 0.002$; N $df = 1$, $F = 8.306$, $p = 0.006$). Benthic fucoxanthin was higher in August than in February and was increased by both N and P addition (Fig. 3f). Zeaxanthin increased in response to P addition ($df = 1$, $F = 36.509$, $p < 0.001$) but was not affected by date or N addition, with no interactions between factors (Fig. 3g). Likewise, chlorophyll *b* increased in response to P addition ($df = 1$, $F = 6.589$, $p = 0.014$) but was not affected by date or N addition, with no interactions between factors (Fig. 3h).

The Bob Allen epiphyte assemblage was variable over time and generally responded to P but not N addition. Epiphytic chlorophyll *a* was significantly affected by date ($df = 2$, $F = 5.058$, $p = 0.009$) and P addition ($df = 1$, $F = 25.779$, $p < 0.001$) but was not affected by N addition, with no interactions between factors. Chlorophyll *a* was lower in February 2004 than on the other dates and decreased in response to P addition on all dates (Fig. 4a). Epiphytic fucoxanthin was significantly affected by date ($df = 2$, $F = 17.516$, $p < 0.001$) and P addition ($df = 1$, $F = 27.746$, $p < 0.001$) but was not affected by N addition, with no interactions between factors. Fucoxanthin was lower in February 2004 than on the other dates and decreased in response to P addition on

all dates (Fig. 4b). Zeaxanthin significantly increased in response to P addition ($df = 1$, $F = 5.533$, $p = 0.022$) but was not affected by date or N addition, with no interactions between factors (Fig. 4c). A significant date*P interaction for epiphytic chlorophyll *b* ($df = 2$, $F = 6.821$, $p = 0.002$) was driven by a larger increase in response to P in August than in February (Fig. 4d). Chlorophyll *b* was not affected by N addition.

The Bob Allen benthic microalgal community exhibited complex responses to date and nutrient addition treatments. A significant date*N interaction for benthic chlorophyll *a* ($df = 1$, $F = 5.463$, $p = 0.025$) was caused by a stronger response to N addition in August than in February (Fig. 4e). A significant P*N interaction ($df = 1$, $F = 6.408$, $p = 0.015$) was driven by an increase in chlorophyll *a* in response to P addition only when N was also added. Benthic fucoxanthin was significantly higher in August than in February ($df = 1$, $F = 21.022$, $p < 0.001$). A significant P*N interaction ($df = 1$, $F = 4.842$, $p = 0.034$) was driven by an increase in fucoxanthin in response to P addition only when N was also added (Fig. 4f). Benthic zeaxanthin increased in response to N addition ($df = 1$, $F = 6.197$, $p = 0.017$). A significant date*P interaction ($df = 1$, $F = 4.293$, $p = 0.045$) was driven by a stronger zeaxanthin response to P addition in August than in February (Fig. 4g). Benthic chlorophyll *b* concentration was not significantly affected by date or nutrient treatment (Fig. 4h).

The benthic and epiphytic communities at Nine Mile Bank were variable over time but largely unaffected by nutrient addition treatments (Fig. 5). The exception was epiphytic zeaxanthin, where a significant date*P interaction ($df = 2$, $F = 3.250$, $p = 0.046$) was driven by a strong increase in response to P in February 2005, a weak P response in February 2004, and no P response in August 2004 (Fig. 5c). Date significantly affected epiphytic chlorophyll *a* ($df = 2$, $F = 20.274$, $p < 0.001$), fucoxanthin ($df = 2$, $F = 22.449$, $p < 0.001$), and chlorophyll *b* ($df = 2$, $F =$

58.830, $p < 0.001$). The concentrations of each of these pigments were higher in August than in the February samplings (Figs. 5a, b, d). Date significantly affected benthic chlorophyll *a* ($df = 1$, $F = 27.425$, $p < 0.001$) and benthic fucoxanthin ($df = 1$, $F = 23.726$, $p < 0.001$). The concentrations of both of these pigments were higher in August than in February but were not significantly affected by nutrient treatment (Figs. 5e, f). Benthic zeaxanthin and chlorophyll *b* were unaffected by date or nutrient treatments (Figs. 5g, h).

The benthic and epiphytic communities at Sprigger Bank were generally unaffected by nutrient treatments. No statistical analyses were performed for the epiphyte community at this site due to insufficient replication ($n \leq 2$), but mean estimates of chlorophyll *a*, fucoxanthin, and zeaxanthin were higher on the February dates than in August and did not appear to be affected by nutrient addition (Figs. 6a-c). Mean chlorophyll *b* was higher in August than in February but did not appear to be affected by N or P addition (Fig. 6d). A significant date*P interaction for benthic zeaxanthin ($df = 1$, $F = 4.758$, $p = 0.044$) was driven by a P-induced decrease in February and no P effect in August (Fig. 6g). None of the other benthic pigments were significantly affected by date or nutrient treatments (Figs. 6e, f, h).

Discussion

Spatial, temporal, and taxa-specific variability in microalgal responses to nutrient enrichment demonstrated that the primary producer components of the Florida Bay ecosystem do not respond uniformly to changes in nutrient input. In general, nutrient responses were stronger in the eastern bay, corresponding with previous studies documenting severe nutrient limitation in seagrass (Armitage et al., 2005) and phytoplankton (Fourqurean et al., 1993) in that region. However, each microalgal group displayed a unique spatial pattern in response to N and P enrichment. Epiphytic chlorophyll *a* and fucoxanthin responded to P addition differently at each

site, with a P-induced increase at one site, a decrease at another site, and no P response at two western sites. In contrast, epiphytic zeaxanthin and chlorophyll *b* were consistently higher in P addition treatments in the eastern bay. In the benthos, both N and P impacted chlorophyll *a*, fucoxanthin, and zeaxanthin concentrations, though nutrient addition effects were generally complex. These taxa-specific patterns are consistent with previous work documenting within-community variability in nutrient limitation patterns in a variety of habitats, including salt marshes (Sundareshwar et al., 2003), freshwater wetlands (Havens et al., 1999), and marine seagrass beds (Ferdie & Fourqurean, 2004).

Nutrient limitation patterns were markedly different between the epiphytic and benthic communities, especially in the two eastern bay sites (Duck Key, Bob Allen Keys). In particular, N addition had more positive effects on benthic pigments than on epiphytic pigments at both sites. Positive effects of N addition were detected for benthic chlorophyll *a* and fucoxanthin at both sites and for benthic zeaxanthin at Bob Allen Keys. In contrast, N addition had negative effects on epiphytic chlorophyll *a* and chlorophyll *b* at Duck Key and no effects on epiphytic pigments at Bob Allen Keys. These patterns suggest that N limitation may be stronger in the benthos than in the epiphytes in the eastern bay. *Thalassia testudinum* tissue N content is generally high in Florida Bay (Fourqurean & Zieman, 2002), suggesting high N availability in this habitat. N-limitation in an N-replete environment may occur through microbial transformations such as denitrification that increase the loss of N (Ferdie & Fourqurean, 2004). In addition, P has a high affinity for carbonate sediments such as those in our study (de Kanel & Morse, 1978), but the rhizosphere of seagrass beds can actively dissolve carbonate sediments (Burdige & Zimmerman, 2002) and make P more available for uptake (Jensen et al., 1998). Such processes may increase the bioavailability of P relative to N in the sediments and explain why

there was a tendency toward more N-limitation in the benthic than in the epiphytic microalgal community. Alternatively, species-specific patterns of nutrient limitation have been documented within microalgal communities in freshwater and marine systems (Tilman, 1977; Coleman & Burkholder, 1994). Little is known about how similar the epiphytic and benthic microalgal communities are in Florida Bay, but the contrasting nutrient limitation patterns that we observed suggest that they are taxonomically distinct from each other. Coralline algae in particular were unlikely to be present in the benthic algal community, as they require firmer substrate for growth (T.A. Frankovich, pers. obs.).

Taxonomic groups within microalgal assemblages have shown different nutrient limitation patterns in a wide range of habitats including coral reef turf communities (Miller et al., 1999), marine microalgal mats (Pinckney et al., 1995), and planktonic assemblages (Kononen, 2001). Nitrogen-fixing cyanobacteria are particularly able to increase in response to P addition in both epiphytic (Neckles et al., 1994) and benthic assemblages (Pinckney et al., 1995; Armitage & Fong, 2004). Our study generally concurred with these previous studies in that cyanobacteria were part of the zeaxanthin signature that increased in P addition treatments, particularly in the eastern bay. However, our microscopic examinations of the epiphytic assemblages suggest that coralline red algae were a major component of the epiphytic zeaxanthin signal. The relative dominance model (Littler & Littler, 1984) predicts that crustose coralline algae will dominate in high nutrient, high herbivory conditions. There is some evidence for this pattern on coral reefs (Smith et al., 2001), but little is known about epiphytic coralline algal responses to nutrient enrichment. The strong zeaxanthin responses to P enrichment that we observed suggest an increase in epiphytic coralline reds in enriched conditions, as predicted by the relative dominance model. Because the zeaxanthin signal represented a cyanobacterial-red algal complex and

297 zeaxanthin is a relatively minor pigment in red algae relative to water soluble pigments such as
298 phycoerythrin (van den Hoek et al., 1995) that were not detected with our HPLC protocol,
299 further microscopic examination and cell enumeration is necessary to document the extent of
300 independent cyanobacterial and coralline red algal responses to N and P enrichment.
301 Blooms of green macroalgae are often associated with N enrichment in marine habitats
302 (Valiela et al., 1997; Kamer et al., 2001). In contrast, we detected little chlorophyll *b* response to
303 N addition, but the strong P-induced increases we observed are consistent with the P-limited
304 nature of the benthic community in eastern Florida Bay (Armitage et al., 2005). Despite
305 substantial increases in green algal load following P addition, the concentration of chlorophyll *b*
306 was relatively low, even in enriched treatments, suggesting that the contribution of green algae to
307 the total epiphytic biomass was small. Green algae are often highly palatable and recruitment and
308 growth may be controlled by grazers (Gacia et al., 1999; Lotze et al., 2000). Grazer density was
309 higher in P-enriched treatments in another study in Florida Bay (Gil et al., this issue), suggesting
310 that grazers could have potentially limited green algal responses to the nutrient treatments.
311 We did not detect consistent responses of diatoms as a group to nutrient enrichment. In fact,
312 the site with the strongest fucoxanthin response to nutrient treatments, Bob Allen Keys, exhibited
313 a decrease following P addition. Increased *T. testudinum* productivity and corresponding reduced
314 leaf turnover period or high grazer abundance at that site may explain this pattern, which has
315 been previously observed in this region (Ferdie & Fourqurean, 2004; Armitage et al., 2005). In
316 addition, diatom responses to increased nutrients can be variable. In temperate benthic
317 microalgal communities, nutrient addition can stimulate diatom growth (Sundbäck & Snoeijs,
318 1991), though that response may vary with sediment type (Armitage & Fong, 2004). Nutrients
319 may cause shifts within diatom guilds, altering species composition (Sundbäck & Snoeijs, 1991;

Coleman & Burkholder, 1994) and masking group-level responses to enrichment. Alternatively, intense grazing pressure in enriched treatments, as with green algae, may limit epiphytic and benthic diatom responses to nutrients (Cuker, 1983; Neckles et al., 1994).

The shifts in epiphytic and benthic community composition that we observed in P-enriched treatments in the eastern bay may alter support for upper trophic levels in Florida Bay. Green algae, which are generally palatable (Gacia et al., 1999; Lotze et al., 2000), increased in P addition treatments in the eastern bay, but our microscopic examinations of the epiphytic cells suggest that green algae were always rare relative to coralline red algae, diatoms, and cyanobacterial filaments. Fucoxanthin was abundant relative to the other pigments we measured, and diatoms are an important food source for epiphyte grazers (Sullivan & Currin, 2000), but the P-induced increase in coralline algae and cyanobacteria may have decreased the accessibility of diatoms to grazers by creating a more complex algal matrix with increased resistance to herbivory (Klumpp et al., 1992; Geddes & Trexler, 2003).

Our estimates of microalgal biomass (mg chlorophyll *a* m^{-2} habitat) suggest that benthic microalgal productivity may be higher than epiphytic production in Florida Bay. We did not directly test extraction efficiencies, and the use of acetone to extract pigments from carbonate sediments may underestimate benthic microalgal biomass (Louda et al., 2000). In addition, water-soluble pigments such as phycoerythrin that were not detected with our HPLC protocol are more abundant in red algae than chlorophyll *a* (van den Hoek et al., 1995), suggesting that we underestimated the biomass of the epiphytic microalgal community as well. Although our estimation of the difference between benthic and epiphytic productivity is not an absolute value, few comparisons between epiphytic and benthic productivity within habitats exist in subtropical estuaries. One notable exception found that epiphytic production was about three times higher

than benthic production in *Halodule wrightii* Ascherson beds in the nutrient-enriched northern Gulf of Mexico (Moncreiff et al., 1992), which contrasts with the patterns we observed in oligotrophic Florida Bay.

The complex patterns of nutrient limitation within and between the epiphytic and benthic microalgal communities illustrate the importance of using experimental manipulations to aid in the prediction of ecosystem responses to alterations. This study contributes to a growing body of work in the region (Ferdie & Fourqurean, 2004; Armitage et al., 2005; Gil et al., this issue) revealing that the potential impacts of nutrient enrichment are not uniform among closely associated primary producers. Varying nutrient responses within the primary producer assemblage in Florida Bay suggest that increased freshwater flow and associated nutrient input during Everglades restoration efforts may cause shifts in microalgal community composition and cascade up to higher trophic levels by modifying food web support (Sullivan & Currin, 2000; Armitage & Fong, 2004). Consideration of strategies that will minimize nutrient input during restoration will lessen the indirect impacts of Everglades management on the Florida Bay faunal community.

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Figure captions

Figure 1: Map of Florida Bay and study sites. Sp = Sprigger Bank, 9M = Nine Mile Bank, BA = Bob Allen Keys, Du = Duck Key.

Figure 2: Microalgal biomass, represented by chlorophyll *a* concentrations in control (unenriched) plots averaged over all sampling periods in the a) epiphytic and b) benthic communities. In all figures, bars represent standard error.

Figure 3: Responses of epiphytic (μg cm^{-2} seagrass leaf) and benthic (μg cm^{-2} sediment) pigments to nitrogen and phosphorus addition at Duck Key. ψ indicates no data collected and ϕ signifies that no pigment was detected. Significant p-values are depicted.

Figure 4: Responses of epiphytic (μg cm^{-2} seagrass leaf) and benthic (μg cm^{-2} sediment) pigments to nitrogen and phosphorus addition at Bob Allen Keys. ψ indicates no data collected and ϕ signifies that no pigment was detected. Significant p-values are depicted; NS indicates no significant effects.

Figure 5: Responses of epiphytic (μg cm^{-2} seagrass leaf) and benthic (μg cm^{-2} sediment) pigments to nitrogen and phosphorus addition at Nine Mile Bank. ψ indicates no data collected and ϕ signifies that no pigment was detected. Significant p-values are depicted; NS indicates no significant effects.

Figure 6: Responses of epiphytic (μg cm^{-2} seagrass leaf) and benthic (μg cm^{-2} sediment) pigments to nitrogen and phosphorus addition at Sprigger Bank. ψ indicates no data collected and ϕ signifies that no pigment was detected. Significant p-values are depicted; NS indicates no significant effects. No statistical analyses were performed for epiphytic pigments.

Figure 1

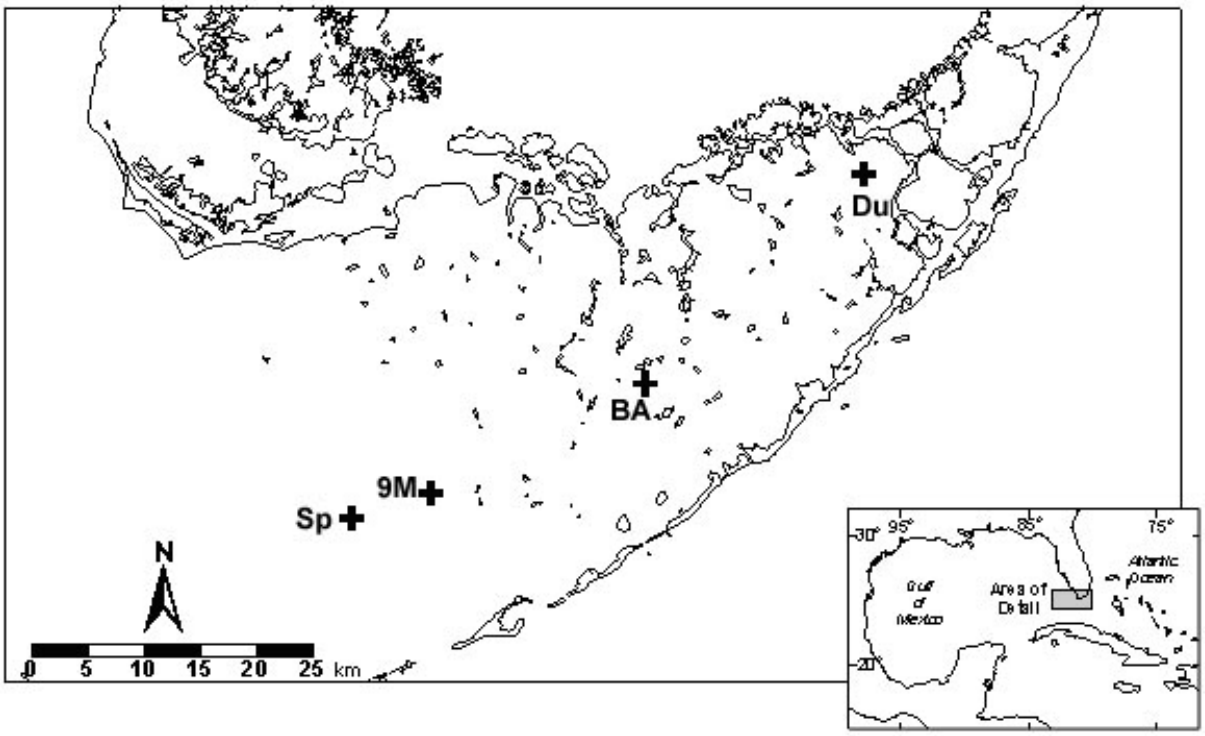

Du
BA
9M
Sp
N
0 5 10 15 20 25 km
30°
95°
85°
75°
Gulf of Mexico
Area of Detail
Atlantic Ocean
20°

Figure 2

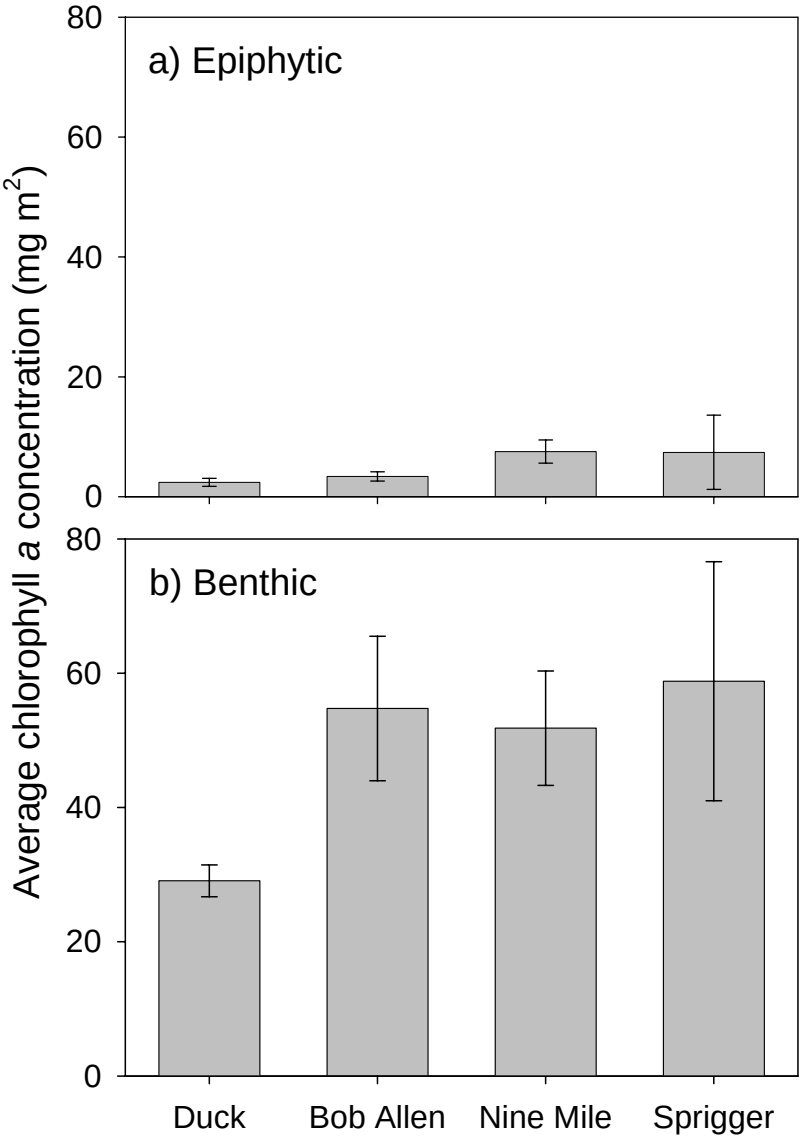

a) Epiphytic
b) Benthic
Average chlorophyll a concentration (mg m2)
0
20
40
60
80
Duck
Bob Allen
Nine Mile
Sprigger

Figure 3

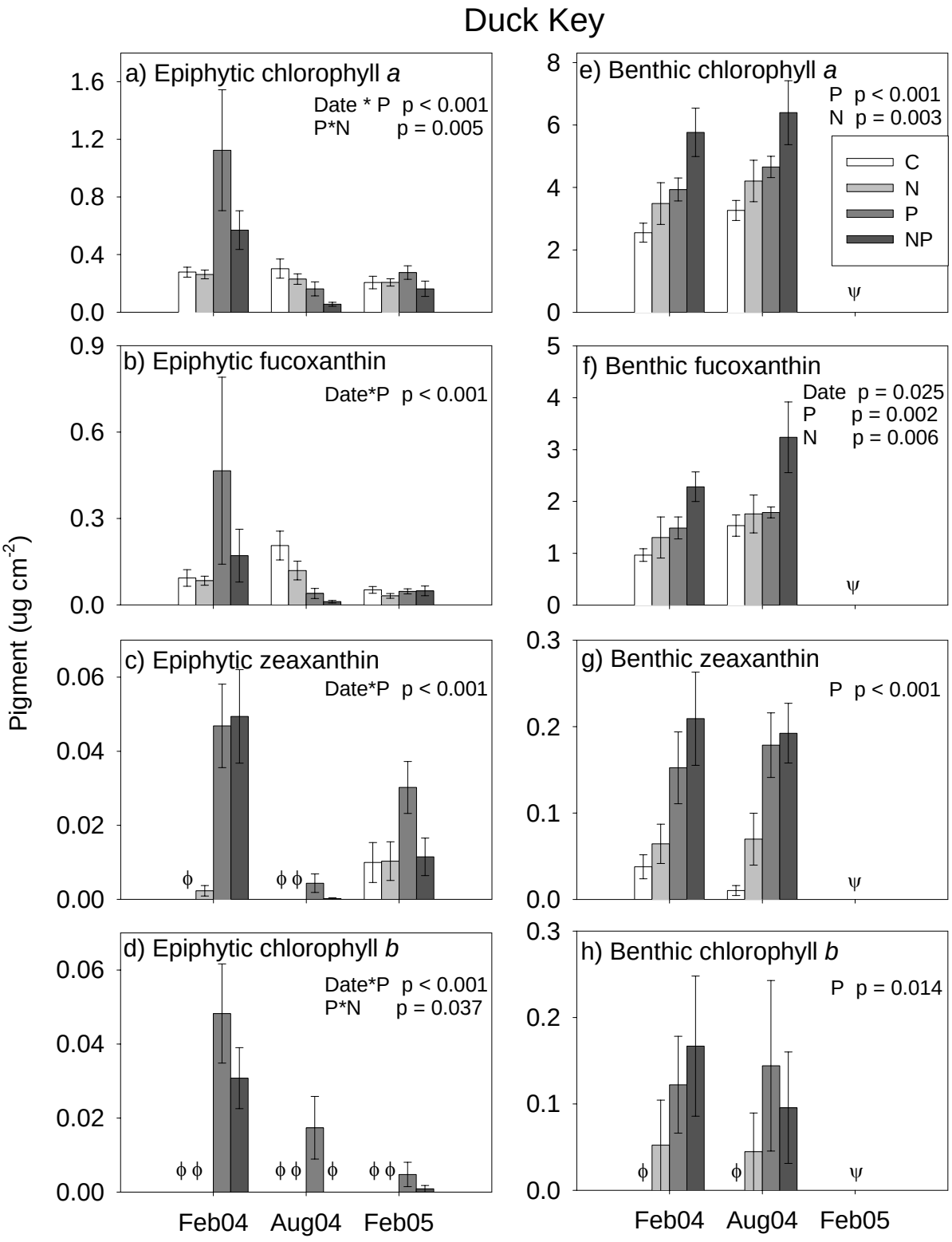

Duck Key
a) Epiphytic chlorophyll a
Date * P p < 0.001
P*N p = 0.005
e) Benthic chlorophyll a
P p < 0.001
N p = 0.003
C
N
P
NP
b) Epiphytic fucoxanthin
Date*P p < 0.001
f) Benthic fucoxanthin
Date p = 0.025
P p = 0.002
N p = 0.006
c) Epiphytic zeaxanthin
Date*P p < 0.001
g) Benthic zeaxanthin
P p < 0.001
d) Epiphytic chlorophyll b
Date*P p < 0.001
P*N p = 0.037
h) Benthic chlorophyll b
P p = 0.014
Pigment (ug cm-2)
Feb04
Aug04
Feb05

Figure 4

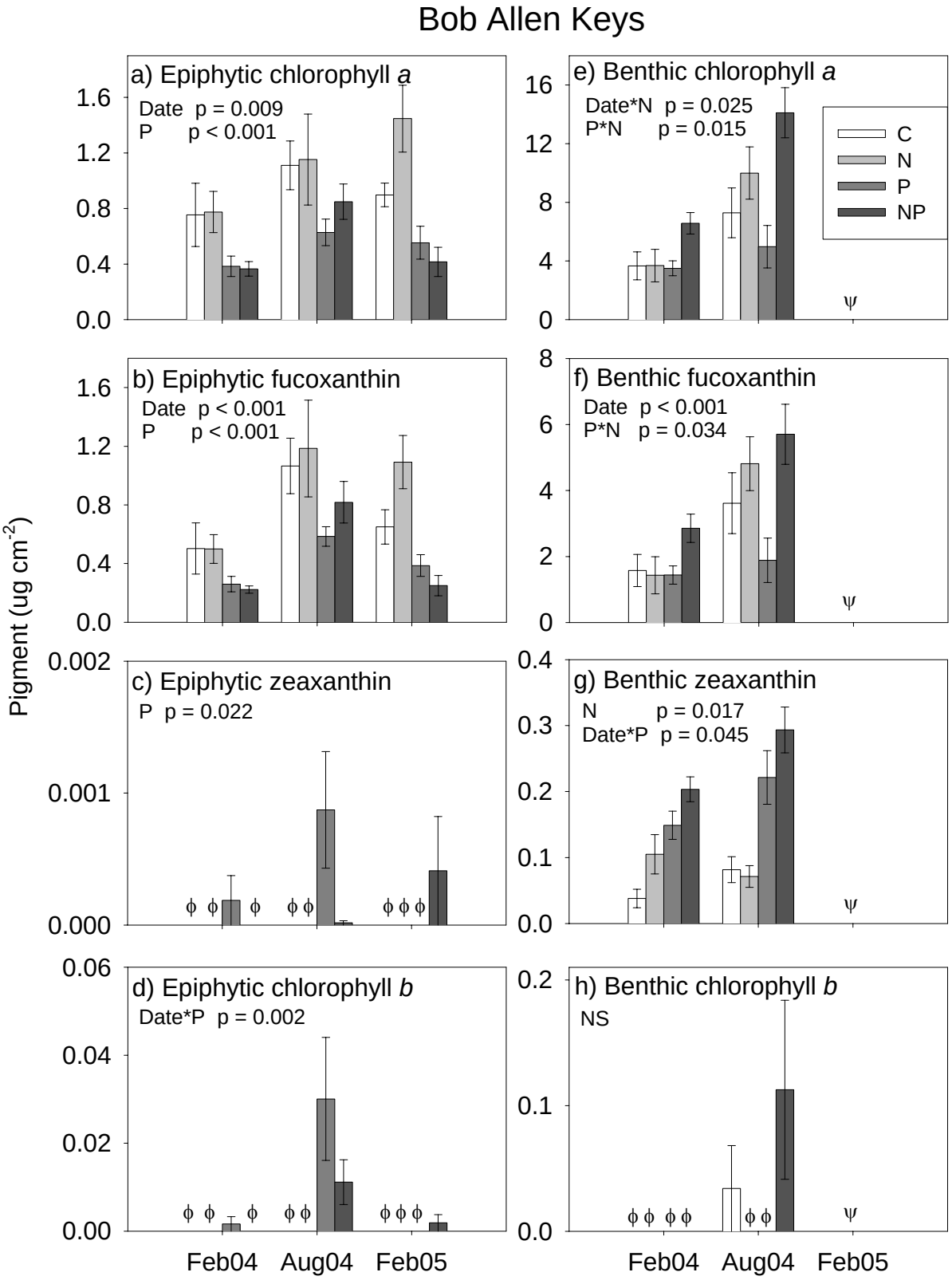

Bob Allen Keys
a) Epiphytic chlorophyll a
Date p = 0.009
P p < 0.001
e) Benthic chlorophyll a
Date*N p = 0.025
P*N p = 0.015
C
N
P
NP
b) Epiphytic fucoxanthin
Date p < 0.001
P p < 0.001
f) Benthic fucoxanthin
Date p < 0.001
P*N p = 0.034
c) Epiphytic zeaxanthin
P p = 0.022
g) Benthic zeaxanthin
N p = 0.017
Date*P p = 0.045
d) Epiphytic chlorophyll b
Date*P p = 0.002
h) Benthic chlorophyll b
NS
Pigment (ug cm-2)
Feb04
Aug04
Feb05

Figure 5

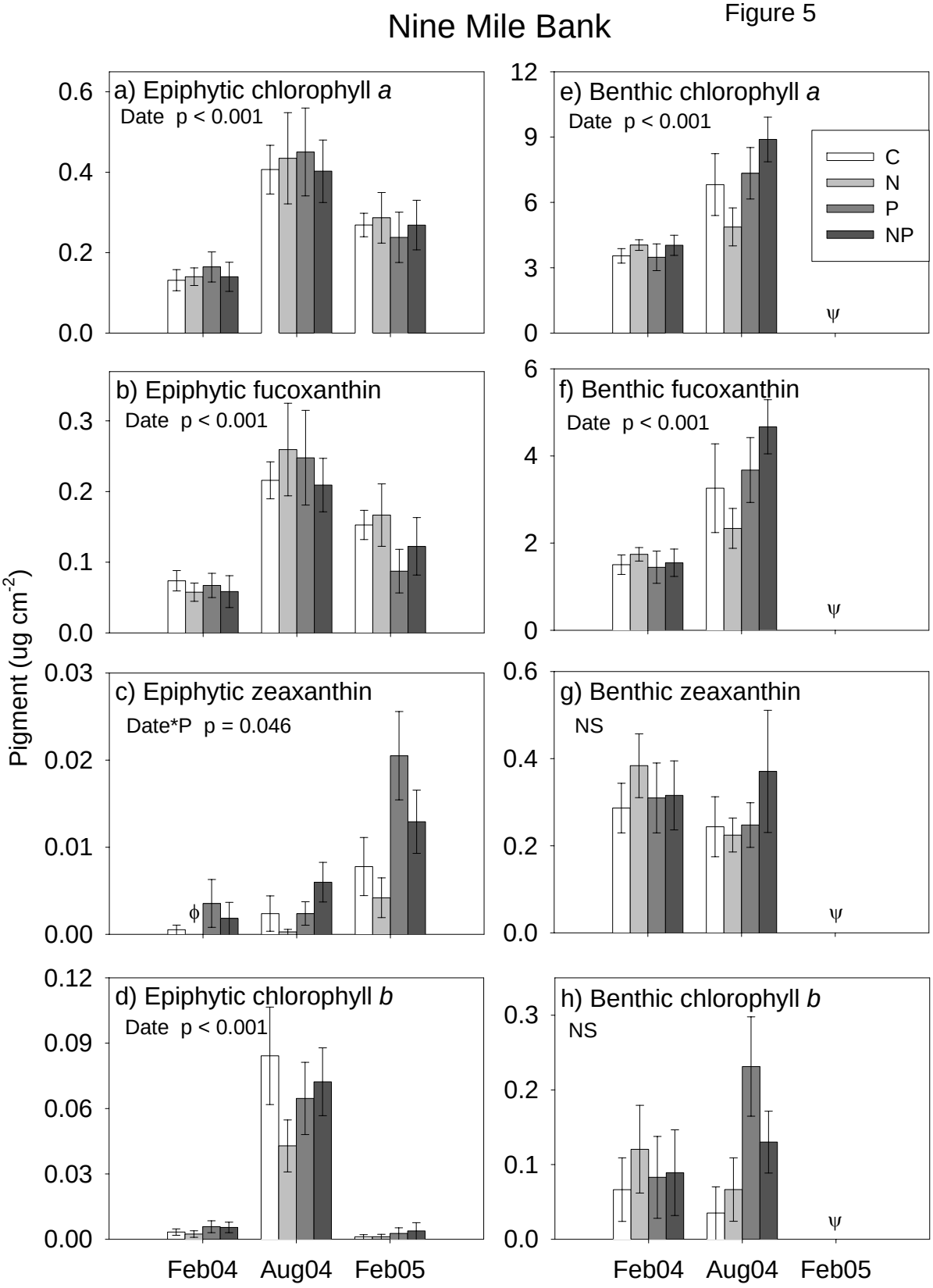
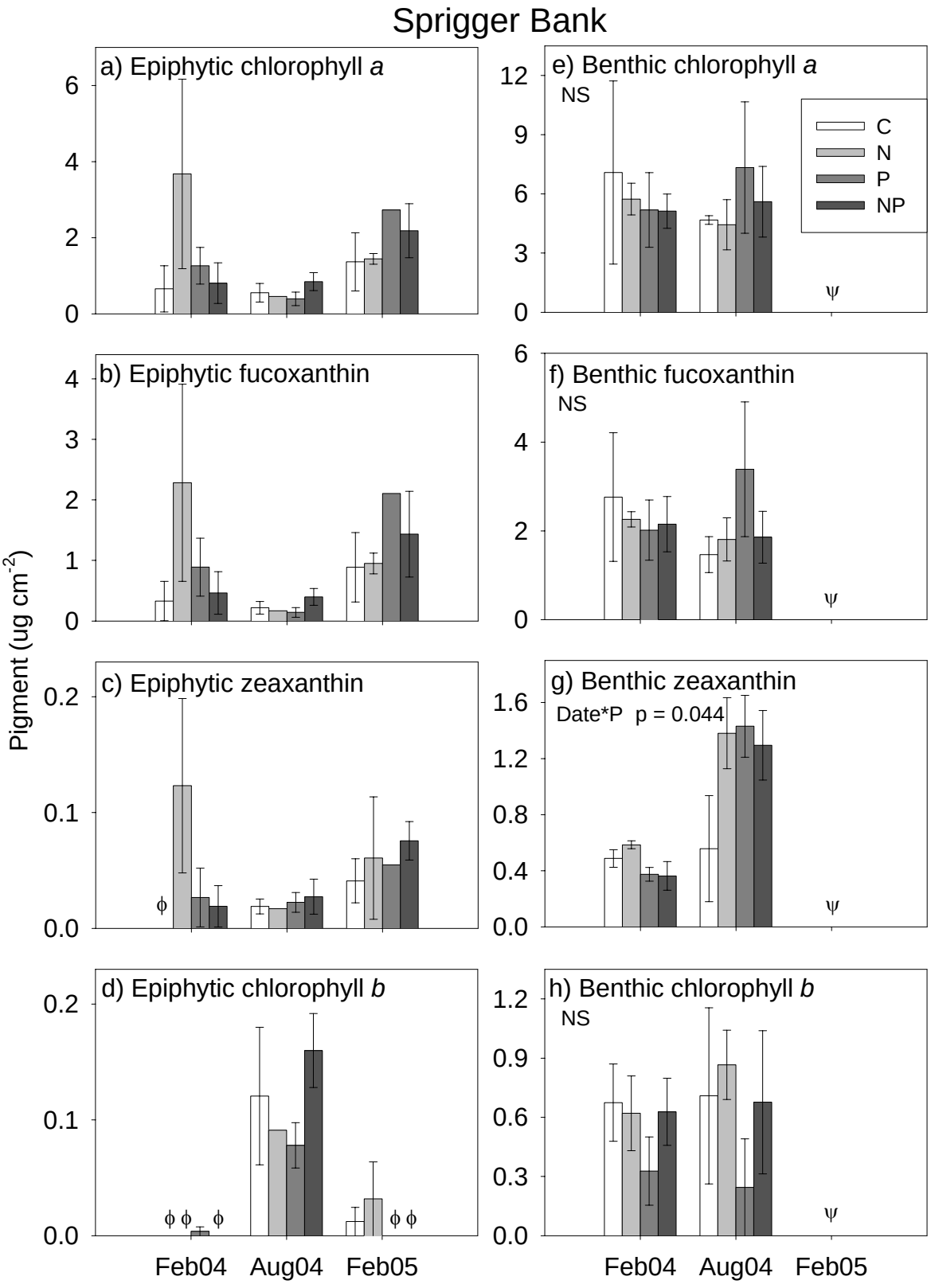

Nine Mile Bank
a) Epiphytic chlorophyll *a*
Date p < 0.001
e) Benthic chlorophyll *a*
Date p < 0.001
C
N
P
NP
b) Epiphytic fucoxanthin
Date p < 0.001
f) Benthic fucoxanthin
Date p < 0.001
c) Epiphytic zeaxanthin
Date*P p = 0.046
g) Benthic zeaxanthin
NS
d) Epiphytic chlorophyll *b*
Date p < 0.001
h) Benthic chlorophyll *b*
NS
Pigment (ug cm-2)
Feb04
Aug04
Feb05

Figure 6


Sprigger Bank
a) Epiphytic chlorophyll *a*
e) Benthic chlorophyll *a*
NS
C
N
P
NP
b) Epiphytic fucoxanthin
f) Benthic fucoxanthin
NS
c) Epiphytic zeaxanthin
g) Benthic zeaxanthin
Date*P p = 0.044
d) Epiphytic chlorophyll *b*
h) Benthic chlorophyll *b*
NS
Pigment (ug cm-2)
Feb04
Aug04
Feb05