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Variable responses within epiphytic and benthic microalgal communities to nutrient enrichment

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5 Variable responses within epiphytic and benthic microalgal communities to nutrient enrichment

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19 This paper has not been submitted elsewhere in identical or similar form, nor will it be during the

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21

22 **Key words:** Chemotaxonomy, HPLC, Florida Bay, microphytobenthos, seagrass, subtropical

23 estuaries

24 **Abstract**

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

45 **Introduction**

46 Habitat management balances multiple ecological, social, and economic objectives (Arthur et
47 al., 2004; Sklar et al., 2005) and often requires trade-offs (Brodziak et al., 2004; Pejchar et al.,
48 2005), as management policies can benefit some community components and simultaneously
49 negatively impact others. Understanding the links among ecosystem components and interpreting
50 community-level responses to ecosystem changes can increase the overall success of
51 management strategies by facilitating the prediction of indirect impacts of land-use projects and
52 increasing the potential for positive community-level impacts.

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

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

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

82 **Methods**

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

91 Zieman, 2002; Armitage et al., 2005). The two western sites (Nine Mile Bank, Sprigger Bank
92 LTER site) were located in a region that may experience both N- and P-limitation but varied in
93 their vegetation characteristics. Nine Mile Bank featured a dense, tall *T. testudinum* canopy with
94 few macroalgae. Sprigger Bank was characterized by a dense and diverse macroalgal community
95 mixed with the seagrasses *Syringodium filiforme* Kützing (manatee grass) and *T. testudinum*. At
96 each site we established 24 0.25 m² study plots demarcated with a PVC frame secured to the
97 benthos at one meter intervals.

98 We randomly assigned treatments [control (C), nitrogen only (N), phosphorus only (P), both
99 nitrogen and phosphorus (NP)] to six plots per site (at the Sprigger Bank LTER site, n = 3 per
100 treatment due to the loss of 12 plots from erosion and boat disturbance over the course of the
101 study). Bimonthly fertilizer applications began in October 2002. Nitrogen was added in the form
102 of slow release nitrogen fertilizer (Polyon™, Pursell Technologies Inc., 38-0-0) and phosphorus
103 as granular phosphate rock (Multifos™, IMC Global, Ca₃(PO₄)₂, 18% P). Loading rates of 1.43 g
104 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
105 sewage loading rates (MCSM, 2001) and previous studies in the region (Ferdie & Fourqurean,
106 2004; Armitage et al., 2005). We sprinkled the fertilizer evenly on the sediment surface and
107 gently worked it into the sediment by hand. Sediment in the control plots was similarly disturbed
108 but no fertilizer was added. Benthic fertilizer applications ensured accessibility of nutrients to
109 both above-ground and benthic primary producers (Ferdie & Fourqurean, 2004; Mutchler et al.,
110 2004; Armitage et al., 2005). The plots and experimental treatments in this study are the same
111 plots sampled for a recent study (Armitage et al., 2005), but all samples collected for this
112 experiment are independent of the previous study.

113 In February 2004, August 2004, and February 2005, we collected one *T. testudinum* short-
114 shoot from each plot and removed the epiphytes by gently scraping the seagrass leaves with a
115 razor blade. At Sprigger Bank, *T. testudinum* was not present in all plots. Leaf morphometrics
116 were measured to calculate two-sided leaf area. We measured shoot density in each plot and
117 calculated leaf area index (LAI = cm² seagrass leaf m⁻² habitat). We also collected a 2.5 cm
118 diameter, 1 cm deep core haphazardly located within each plot. Due to logistical constraints,
119 sediment cores were collected only on the two 2004 sampling dates. Epiphytes and sediments
120 were freeze dried and stored at -20° in the dark until further analysis.

121 We determined the relative abundance of major phototrophic groups with high performance
122 liquid chromatography (HPLC), which measures the relative concentrations of taxa-specific
123 indicator pigments (chlorophyll *a*, chlorophyll *b*, fucoxanthin, and zeaxanthin) (Pinckney et al.,
124 1995). Pigments were extracted from freeze-dried epiphytes and sediments with 90% acetone for
125 at least 12 hours at -20°C. An ion-pairing solution (1.00 M ammonium acetate) was added to the
126 filtered extracts at a ratio of 4 parts extract: 1 part ammonium acetate just prior to injection.
127 Extracts (250 µl) were injected into a Hewlett Packard 1090 HPLC equipped with a monomeric
128 reverse-phase C₁₈ column (Rainin-Microsorb-MV, 100 x 4.6 mm, 3 µm) and a polymeric
129 reverse-phase C₁₈ column (Vydac, 201TP, 250 x 4.6 mm, 5 µm) in series and a photodiode array
130 detector set at 440 nm. Solvents and flow rates followed Pinckney et al. (1999) and the column
131 temperature was 40°C. Pigments were identified based on retention times and comparisons with
132 pure standards extracted from phytoplankton cultures in 90% acetone (chlorophyll *a*, chlorophyll
133 *b*) or 100% ethanol (fucoxanthin, zeaxanthin) obtained from DHI Water & Environment
134 (Denmark). Epiphyte load is represented as µg pigment cm⁻² of seagrass leaf; benthic load is µg
135 pigment cm⁻² of sediment. Microalgal biomass is represented as the average pigment

136 concentration (mg m^{-2} of habitat. Epiphytic biomass is ($\mu\text{g pigment cm}^{-2}$ seagrass
137 leaf)*(LAI)/1000, and benthic biomass is ($\mu\text{g pigment cm}^{-2}$ 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 ($\pm\text{P}$), and N addition ($\pm\text{N}$). Dependent
147 variables were epiphyte loads, represented by $\mu\text{g chlorophyll } a$, 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

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

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

170 At Duck Key, each pigment responded differently to date and nutrient addition treatments. In
171 the epiphyte community, a significant interaction between date and P addition for chlorophyll *a*
172 (df = 2, F = 16.336, p < 0.001) was caused by a large increase in response to P addition in
173 February 2004 but not on any other date (Fig. 3a). A significant P*N interaction (df = 1, F =
174 8.412, p = 0.005) was driven by consistently lower chlorophyll *a* concentrations in plots that
175 received both N and P compared to P only treatments. A significant interaction between date and
176 P addition for epiphytic fucoxanthin (df = 2, F = 14.307, p < 0.001) was driven by a P-induced
177 increase in February 2004, a P-induced decrease in August 2004, and no nutrient effects in
178 February 2005 (Fig. 3b). N addition did not affect epiphytic fucoxanthin. A significant
179 interaction between date and P addition for epiphytic zeaxanthin (df = 2, F = 15.860, p < 0.001)
180 was caused by a larger response to P addition in the February samplings than in August (Fig. 3c).
181 Epiphytic zeaxanthin was not affected by N addition. A significant date*P interaction for

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

186 The Duck Key benthic microalgal community responded differently to nutrient addition than
187 the epiphyte assemblage. Both P ($df = 1$, $F = 17.564$, $p < 0.001$) and N ($df = 1$, $F = 9.921$, $p =$
188 0.003) had significant and additive effects on benthic chlorophyll *a* concentration, with no date
189 effect and no interactions between factors (Fig. 3e). Fucoxanthin was significantly affected by all
190 three factors with no interactions (Date $df = 1$, $F = 5.449$, $p = 0.025$; P $df = 1$, $F = 11.007$, $p =$
191 0.002 ; N $df = 1$, $F = 8.306$, $p = 0.006$). Benthic fucoxanthin was higher in August than in
192 February and was increased by both N and P addition (Fig. 3f). Zeaxanthin increased in response
193 to P addition ($df = 1$, $F = 36.509$, $p < 0.001$) but was not affected by date or N addition, with no
194 interactions between factors (Fig. 3g). Likewise, chlorophyll *b* increased in response to P
195 addition ($df = 1$, $F = 6.589$, $p = 0.014$) but was not affected by date or N addition, with no
196 interactions between factors (Fig. 3h).

197 The Bob Allen epiphyte assemblage was variable over time and generally responded to P but
198 not N addition. Epiphytic chlorophyll *a* was significantly affected by date ($df = 2$, $F = 5.058$, $p =$
199 0.009) and P addition ($df = 1$, $F = 25.779$, $p < 0.001$) but was not affected by N addition, with no
200 interactions between factors. Chlorophyll *a* was lower in February 2004 than on the other dates
201 and decreased in response to P addition on all dates (Fig. 4a). Epiphytic fucoxanthin was
202 significantly affected by date ($df = 2$, $F = 17.516$, $p < 0.001$) and P addition ($df = 1$, $F = 27.746$,
203 $p < 0.001$) but was not affected by N addition, with no interactions between factors. Fucoxanthin
204 was lower in February 2004 than on the other dates and decreased in response to P addition on

205 all dates (Fig. 4b). Zeaxanthin significantly increased in response to P addition ($df = 1$, $F =$
206 5.533 , $p = 0.022$) but was not affected by date or N addition, with no interactions between factors
207 (Fig. 4c). A significant date*P interaction for epiphytic chlorophyll *b* ($df = 2$, $F = 6.821$, $p =$
208 0.002) was driven by a larger increase in response to P in August than in February (Fig. 4d).
209 Chlorophyll *b* was not affected by N addition.

210 The Bob Allen benthic microalgal community exhibited complex responses to date and
211 nutrient addition treatments. A significant date*N interaction for benthic chlorophyll *a* ($df = 1$, F
212 $= 5.463$, $p = 0.025$) was caused by a stronger response to N addition in August than in February
213 (Fig. 4e). A significant P*N interaction ($df = 1$, $F = 6.408$, $p = 0.015$) was driven by an increase
214 in chlorophyll *a* in response to P addition only when N was also added. Benthic fucoxanthin was
215 significantly higher in August than in February ($df = 1$, $F = 21.022$, $p < 0.001$). A significant
216 P*N interaction ($df = 1$, $F = 4.842$, $p = 0.034$) was driven by an increase in fucoxanthin in
217 response to P addition only when N was also added (Fig. 4f). Benthic zeaxanthin increased in
218 response to N addition ($df = 1$, $F = 6.197$, $p = 0.017$). A significant date*P interaction ($df = 1$, F
219 $= 4.293$, $p = 0.045$) was driven by a stronger zeaxanthin response to P addition in August than in
220 February (Fig. 4g). Benthic chlorophyll *b* concentration was not significantly affected by date or
221 nutrient treatment (Fig. 4h).

222 The benthic and epiphytic communities at Nine Mile Bank were variable over time but
223 largely unaffected by nutrient addition treatments (Fig. 5). The exception was epiphytic
224 zeaxanthin, where a significant date*P interaction ($df = 2$, $F = 3.250$, $p = 0.046$) was driven by a
225 strong increase in response to P in February 2005, a weak P response in February 2004, and no P
226 response in August 2004 (Fig. 5c). Date significantly affected epiphytic chlorophyll *a* ($df = 2$, F
227 $= 20.274$, $p < 0.001$), fucoxanthin ($df = 2$, $F = 22.449$, $p < 0.001$), and chlorophyll *b* ($df = 2$, $F =$

228 58.830, $p < 0.001$). The concentrations of each of these pigments were higher in August than in
229 the February samplings (Figs. 5a, b, d). Date significantly affected benthic chlorophyll *a* ($df = 1$,
230 $F = 27.425$, $p < 0.001$) and benthic fucoxanthin ($df = 1$, $F = 23.726$, $p < 0.001$). The
231 concentrations of both of these pigments were higher in August than in February but were not
232 significantly affected by nutrient treatment (Figs. 5e, f). Benthic zeaxanthin and chlorophyll *b*
233 were unaffected by date or nutrient treatments (Figs. 5g, h).

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

243 **Discussion**

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

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

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

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

282 Taxonomic groups within microalgal assemblages have shown different nutrient limitation
283 patterns in a wide range of habitats including coral reef turf communities (Miller et al., 1999),
284 marine microalgal mats (Pinckney et al., 1995), and planktonic assemblages (Kononen, 2001).
285 Nitrogen-fixing cyanobacteria are particularly able to increase in response to P addition in both
286 epiphytic (Neckles et al., 1994) and benthic assemblages (Pinckney et al., 1995; Armitage &
287 Fong, 2004). Our study generally concurred with these previous studies in that cyanobacteria
288 were part of the zeaxanthin signature that increased in P addition treatments, particularly in the
289 eastern bay. However, our microscopic examinations of the epiphytic assemblages suggest that
290 coralline red algae were a major component of the epiphytic zeaxanthin signal. The relative
291 dominance model (Littler & Littler, 1984) predicts that crustose coralline algae will dominate in
292 high nutrient, high herbivory conditions. There is some evidence for this pattern on coral reefs
293 (Smith et al., 2001), but little is known about epiphytic coralline algal responses to nutrient
294 enrichment. The strong zeaxanthin responses to P enrichment that we observed suggest an
295 increase in epiphytic coralline reds in enriched conditions, as predicted by the relative dominance
296 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;

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

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

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

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

346 The complex patterns of nutrient limitation within and between the epiphytic and benthic
347 microalgal communities illustrate the importance of using experimental manipulations to aid in
348 the prediction of ecosystem responses to alterations. This study contributes to a growing body of
349 work in the region (Ferdie & Fourqurean, 2004; Armitage et al., 2005; Gil et al., this issue)
350 revealing that the potential impacts of nutrient enrichment are not uniform among closely
351 associated primary producers. Varying nutrient responses within the primary producer
352 assemblage in Florida Bay suggest that increased freshwater flow and associated nutrient input
353 during Everglades restoration efforts may cause shifts in microalgal community composition and
354 cascade up to higher trophic levels by modifying food web support (Sullivan & Currin, 2000;
355 Armitage & Fong, 2004). Consideration of strategies that will minimize nutrient input during
356 restoration will lessen the indirect impacts of Everglades management on the Florida Bay faunal
357 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 ($\mu\text{g cm}^{-2}$ seagrass leaf) and benthic ($\mu\text{g cm}^{-2}$ 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 ($\mu\text{g cm}^{-2}$ seagrass leaf) and benthic ($\mu\text{g cm}^{-2}$ 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 ($\mu\text{g cm}^{-2}$ seagrass leaf) and benthic ($\mu\text{g cm}^{-2}$ 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 ($\mu\text{g cm}^{-2}$ seagrass leaf) and benthic ($\mu\text{g cm}^{-2}$ 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

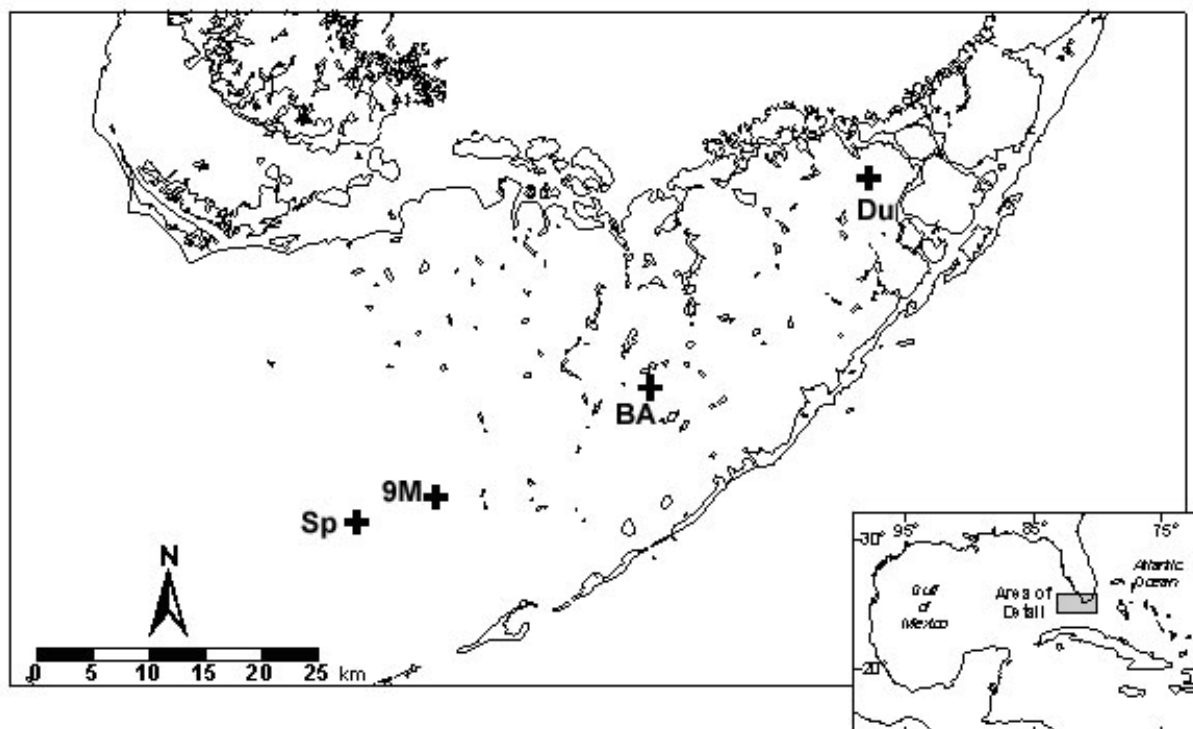
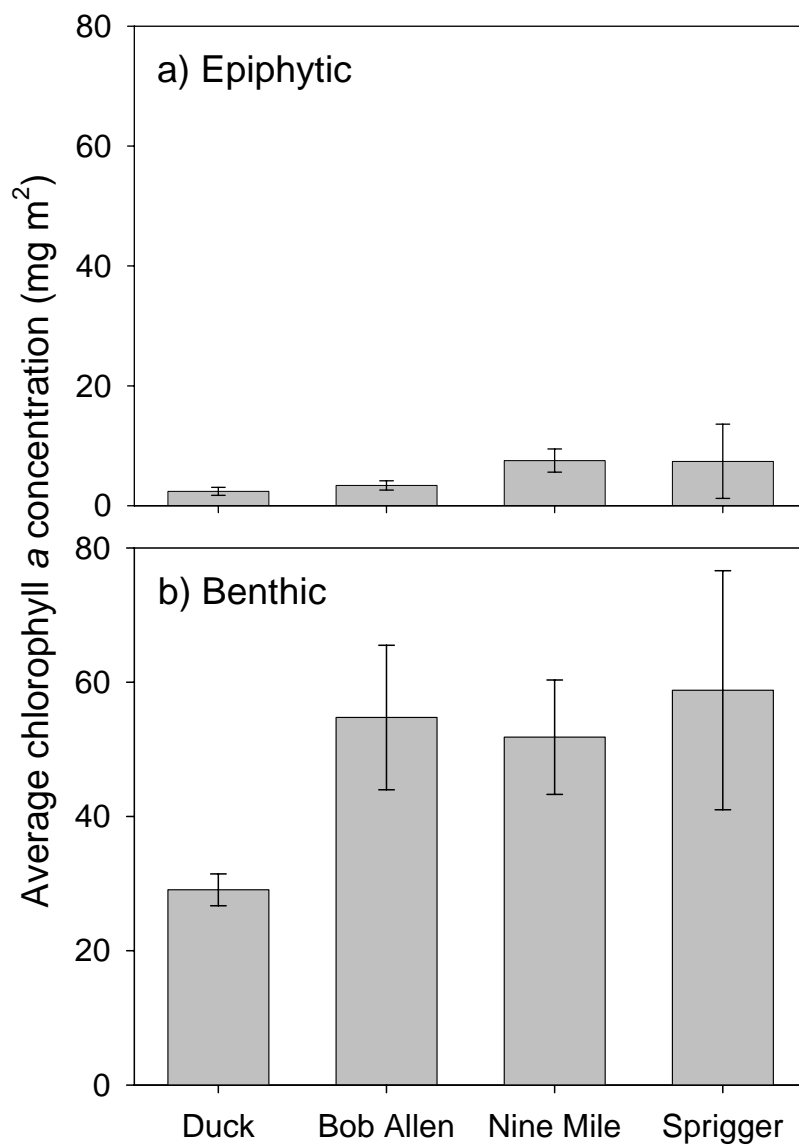


Figure 2



Duck Key

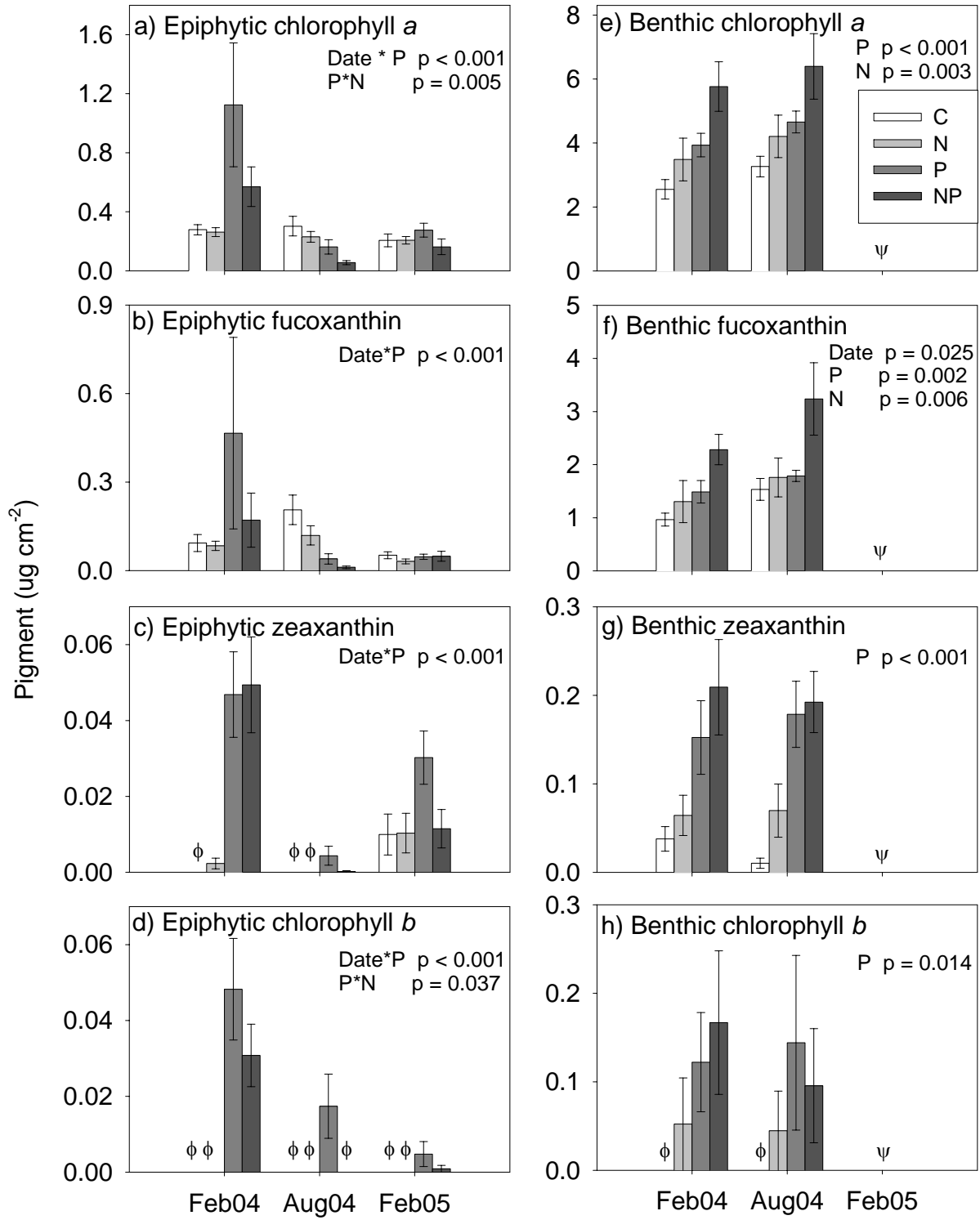
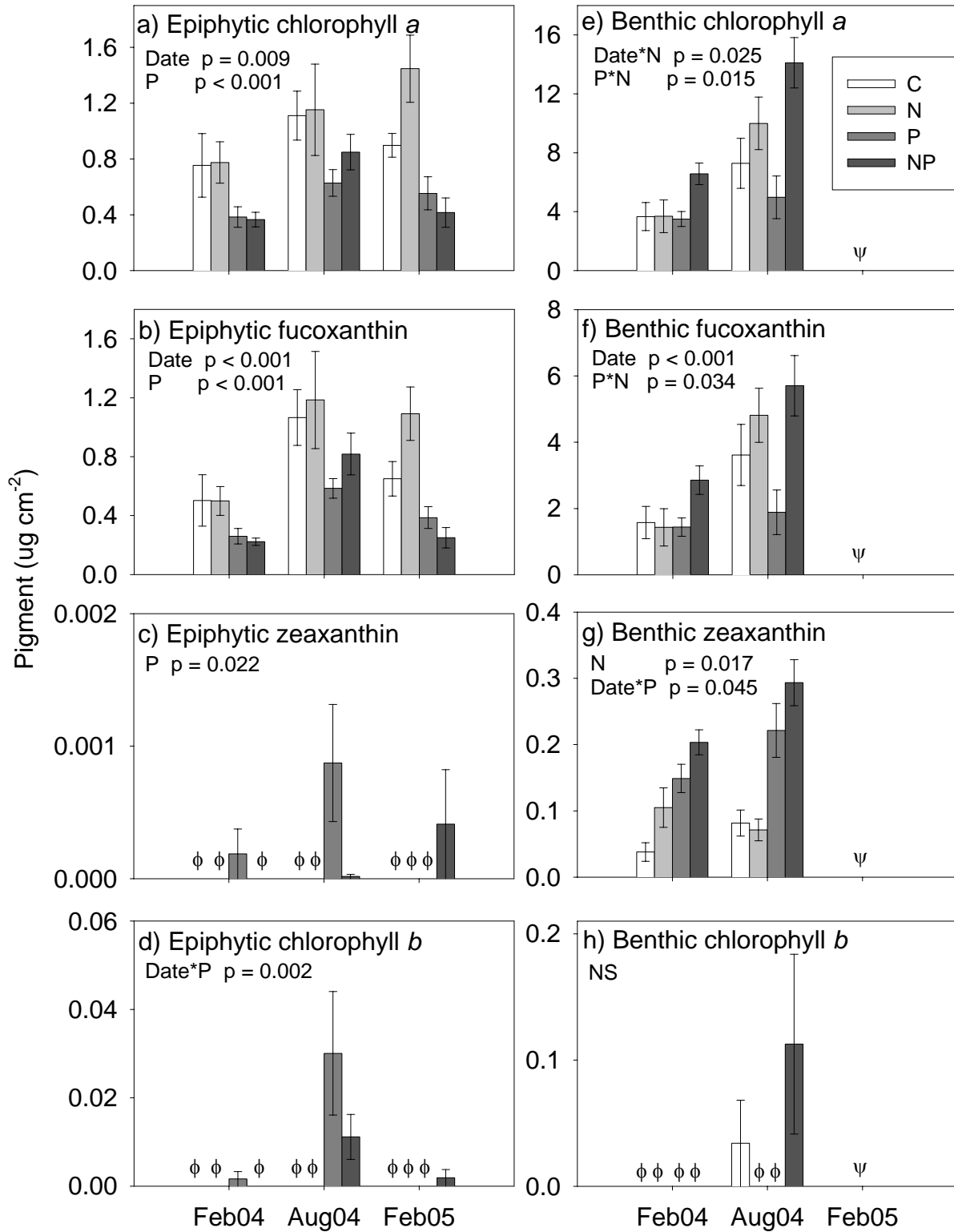


Figure 4

Bob Allen Keys



Nine Mile Bank

Figure 5

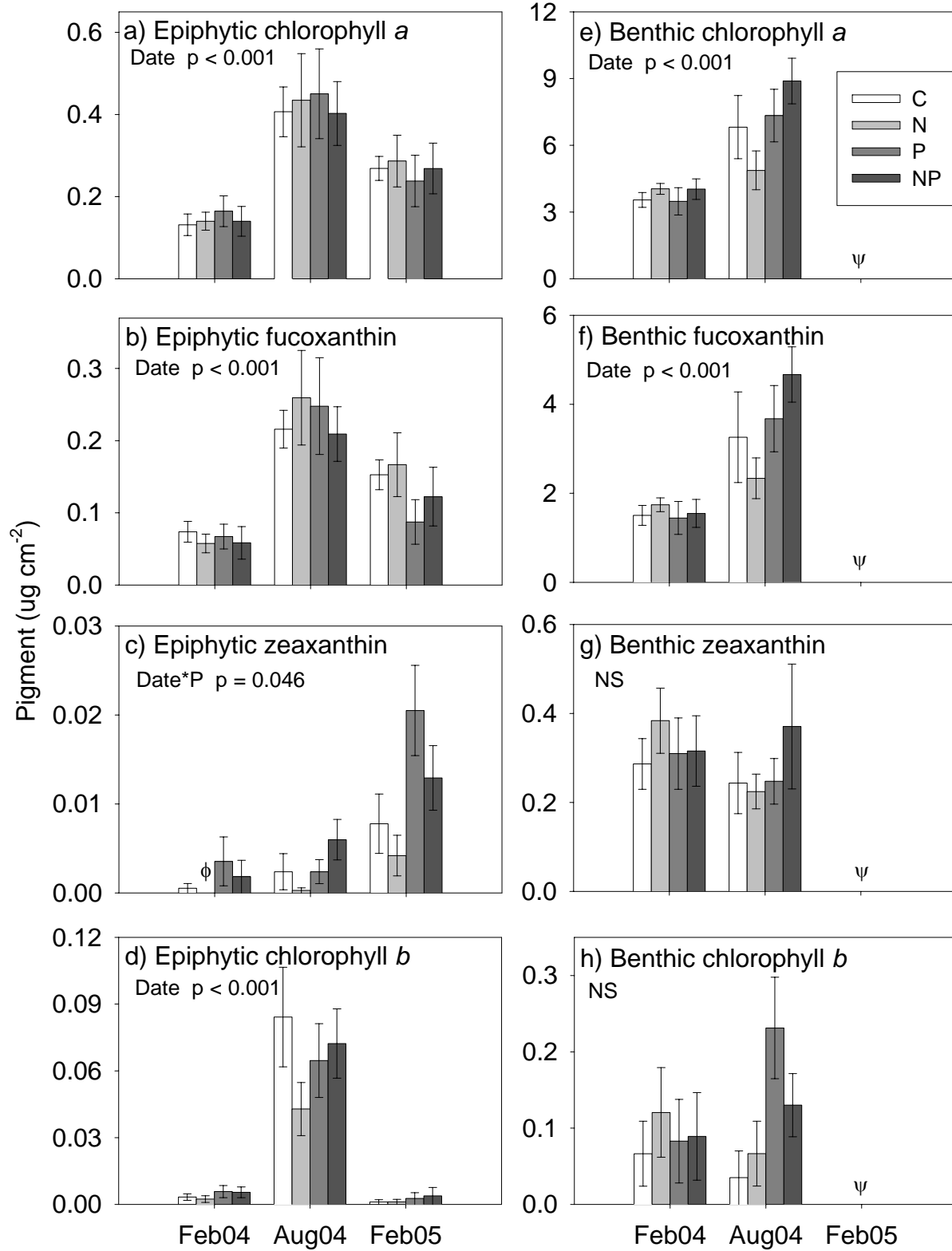


Figure 6

Sprigger Bank

