The Importance of Organic Phosphorus in Promoting Cyanobacterial Blooms in Florida Bay: Competition Between Bacteria and Phytoplankton

Joseph N. Boyer, Susan K. Dailey, Patrick J. Gibson, Matthew T. Rogers, Danielle-Mir-Gonzalez

Southeast Environmental Research Center, OE-148, Florida International University, Miami, FL 33199, 305-348-4076 (O), 305-348-4096 (fax), boyerj@fiu.edu

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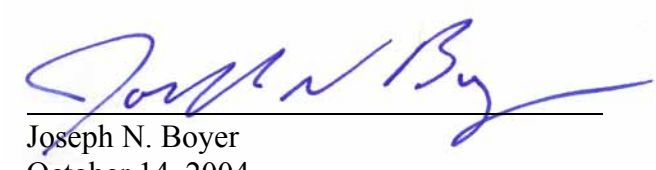

Joseph N. Boyer
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Abstract

The clear, shallow, oligotrophic waters of Florida Bay are characterized by typically low phytoplankton biomass, yet periodic cyanobacteria blooms occur. We hypothesized that allochthonous DOM was providing a subsidy to the system in the form of organic nutrients. Water from four bay sites was incunbated under natural light and dark conditions with either DOM (>1 kD) or inorganic nutrient (N+P) enrichments. Samples were analyzed for bacterial numbers (DAPI epifluorescent microscopy), bacterial production (^{3}H-thymidine uptake), phytoplankton production (PAM fluorometry), CHLA, nutrients, and alkaline phosphatase (AP) activity. Inorganic nutrient additions resulted in an ephemeral bloom characterized initially as cyanobacterial and brown algal guilds but changed to green and/or brown guilds by day six. DOM influence on overall phytoplankton concentrations was slow and relatively little, but it yielded a different algal community structure by the end of the experiment. Because of sustained AP activity, the DIN:TP ratio decreased 10 fold in the N+P treatments as the system progressed towards N limitation. This ratio did not significantly change for DOM treatments. We believe that the composition of nutrient inputs is a key factor in bloom initiation but that the alleviation of N and P limitation by enzymatic activity on the DOM pool alters their composition and facilitates their persistence. In addition, these experiments indicated that both autotrophic and heterotrophic microbial populations in Florida Bay are co-limited by organic and inorganic nutrient availability. Both treatments of organic and inorganic enrichment revealed significant and positive response in bioavailability (BDOC). Potential BDOC ranged from 1.1 – 55.1 %, with the most labile forms occurring in Whipray basin. Thus, bioavailability of organic materials to heterotrophic and autotrophic communities are tightly coupled. This has major implications in the fate of DOC from the Everglades and Florida Bay ecosystems and corresponding carbon budget models and implicate bioavailablity as a key factor controlling the onset and persistence of microbial blooms.

Executive Summary

Water quality and bioavailability of materials entering Florida Bay is one concern of managers and continuing development of the Comprehensive Everglades Restoration Plan to restore freshwater from to the Everglades and the Greater Florida Bay ecosystem. To better understand microbial and biogeochemical cycling in Florida Bay we designed a two part approach to first determine the coupling between nutrients and microbes across the hydroscape of Florida Bay and second to quantify bioavailability of dissolved organic material and microbial contributions to this pool. For this second part we specifically addressed the role of both autotrophic and heterotrophic microbial community components.

To address our first research priority, we collected a microbial-biogeochemical dataset consisting of C, N, and P dissolved and total constituents, physical parameters including temperature, salinity, turbidity, dissolved oxygen, bacteria numbers and production, chlorophyll *a* (CHLA), cyanobacterial, green and brown CHLA, and quantum yield. Our two-year data set revealed biogeochemical dynamics in Florida Bay are best explained by microbial activity and total phosphorus availability. We examined the data spatially and temporally across 28 sites in Florida Bay that we monitored monthly for water column biogeochemistry with parameters of bacterial and algal energetics and total and dissolved nutrients.

Principal component analysis revealed a strong coupling of heterotrophic and autotrophic communities with total phosphorus dynamics throughout the study period. Five principal components explained 65% of the variability of all parameters over the entire study period. Algal and bacterial dynamics varied with total phosphorus and explained the majority of the variability in the biogeochemical data set across Florida Bay for the period from January 2001 to December 2002. The individual spatial analyses of each of the three groups of brown, green and cyanobacteria show that very low concentrations of algae are present in the northeast corner of Florida Bay. This portion of the Bay is where inorganic nitrogen components are highest Bay-wide. This suggests that there is little inorganic N limitation in the algal populations that we were observing over the study period. This finding was only evident through the combined analysis of all sites and all parameter across the entire study period.

Cyanobacteria blooms observed in Florida Bay in recent years have been a growing concern of the federal and state and municipal government, public and private organizations in South Florida. Sources of bloom formation have been postulated to result from increases in nutrient concentrations, changes in species composition and even seagrass die-off events in Florida Bay. We observed the highest concentration of cyanobacteria in the central Bay where we also observed the highest concentrations of green algae. Brown algae were in the highest concentration in the western Bay where we expected to see the cyanobacteria population blooms that had been described prior to this study. These data suggest that cyanobacterial blooms may be more associated with the organic nutrients of TON and TOC introduced into central Florida Bay from mainland Everglades.

Our second research hypothesis addressed how the potential availability of organic carbon (BDOC) affected the cyanobacterial population and specifically the role of organic phosphorus availability in the system. Bioavailability assays of two times the ambient dissolved organic matter from Taylor River were fed to light and dark bottles concurrent with control and N+P treatments. We conducted the assays with water from four sites in Florida Bay in the eastern and western Bay.

These experiments indicated that both autotrophic and heterotrophic microbial populations in Florida Bay are co-limited by organic and inorganic nutrient availability. Both treatments of organic and inorganic enrichment revealed significant and positive response in %BDOC. Potential

bioavailability ranged from 1.1 – 55.1 %, with the most labile forms occurring in Whipray basin. Thus, bioavailability of organic materials to heterotrophic and autotrophic communities are tightly coupled. This has major implications in the fate of DOC from the Everglades and Florida Bay ecosystems and corresponding carbon budget models. These experiments implicate bioavailable nutrients as the key factor controlling the onset and persistence of microbial blooms and that %BDOC increases with increased availability of DOC.

Bioavailability of total organic carbon varied between sites yet was within the range of reported coastal values across the eastern coast of the United States. Western sites responded in greater increments across both treatments than the eastern central sites where ambient DOC concentrations are highest. Bioavailability was highest in the wet season when DOC is most likely to be transported to Florida Bay from the mangroves and freshwater sloughs of mainland Everglades. The DOC produced during the wet season may be "fresher" from the new biomass produced during the wet season and there may be greater leaching rates from both and freshwater marsh and mangrove zone.

Throughout these experiments several results helped elucidate the coupling between bacterial and algal dynamics in Florida Bay and their dependence on total and dissolved constituents. We saw that there was a response in both heterotrophic and autotrophic response parameters from our bioavailability assays. Both bacterial and cyanobacterial alkaline phosphatase production is likely one of the key processes facilitating large population growth of autotrophic communities. These communities will lead to increased heterotrophic microbial activity by exuding highly labile carbon and a cycling of bioavailable nutrients. This implies that once initiated a diverse microbial bloom can persist by autochthonous resource cycling. The controls have the very same P limited conditions that occur in Florida Bay where very little DOC is bioavailable to the microbial organisms in this system. The mineralization of DOC becomes increasingly important in determining the overall production of carbon in the estuary. The positive feedback of higher availability of DOC which lead to higher bioavailability was shown through two lines of data in this project. First we saw the significant positive effect of the 2XDOC treatment on %BDOC, but also we saw significantly higher %BDOC with the onset of the wet season. We expect events where massive transport of water occurs to have similarly high %BDOC.

Cyanobacteria was not a large fraction of the overall CHLA contribution in any of the experiments except for one time at one site. This occurrence was in Whipray basin, at the ecotone of the mangrove-Bay coastal exchange zone, and was the same site where we saw the extreme differences between ambient BDOC. However N+P showed the only notable effect on the cyanobacterial population, with a mid experiment maxima. The endpoint CHLA concentration from cynanobacteria was the same as the DOC and ambient treatments. Brown algae had a greater response than cyanobacteria and was the principal contributor to the CHLA concentration in the water column. Surprisingly, alkaline phosphatase activity, an index inorganic phosphorus availability attained from microbial ectoenzyme activity, showed that brown algae, and not cyanobacteria or green algae contributed most to the explaining trends. Bacteria also responded to the DOC additions with greater production and bacteria numbers at the end of the incubation period at several of the study sites.

The combination of treatments that we tried in these experiments helped further our understanding of the mechanisms that induce changes in the algal community in Florida Bay. We saw a shift in the algal community in cyanobacteria and green algae to brown algae with the introduction of greater concentrations of organic carbon into the water column. With N+P addition, we observed a mid experiment increase in concentration of cyanobacteria and green algae that was greater than the

response seen with the inorganic nutrient additions. The response was most seen in the brown algae. Thus, in none of our experiments were we able to induce a bloom affect such as those described in Florida Bay. However, we were able to stimulate growth of brown algae, which include diatoms, with the DOC treatment, although blooms of this sort have not been previously described in Florida Bay. These brief blooms of all algal groups and dynamic trends in bacterial numbers and TP suggest that they are tightly coupled, as seen in the 2 year microbial, nutrient and physical factor model in this report.

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Purpose

Florida Bay is a wedge-shaped estuary separated from the Straits of Florida by the Florida Keys which form a semi-permeable barrier. Carbonate mud banks act to compartmentalize Florida Bay into a network of shallow basins, restricting circulation among them. Tidal advection from the Gulf of Mexico is quickly attenuated by the western mud banks leaving most of central and northeastern Florida Bay unaffected (Turney and Perkins 1972; Holmquist et al. 1989). Freshwater inputs to Florida Bay are contributed to by flow from through the Taylor Slough basin and rainfall The Taylor Slough is significantly affected by water management activities which can influence salinities in the eastern Bay (Boyer and Jones 1999). Groundwater inputs are generally not considered to be important (Corbett et al. 1999). Another potentially important source of freshwater to the Bay is overland flow along the north-central boundary (Fig. 1; Jones and Boyer 1999). These episodic inflows are very high in dissolved organic carbon (DOC) making this region a potentially important source for the Bay (Fig. 2).

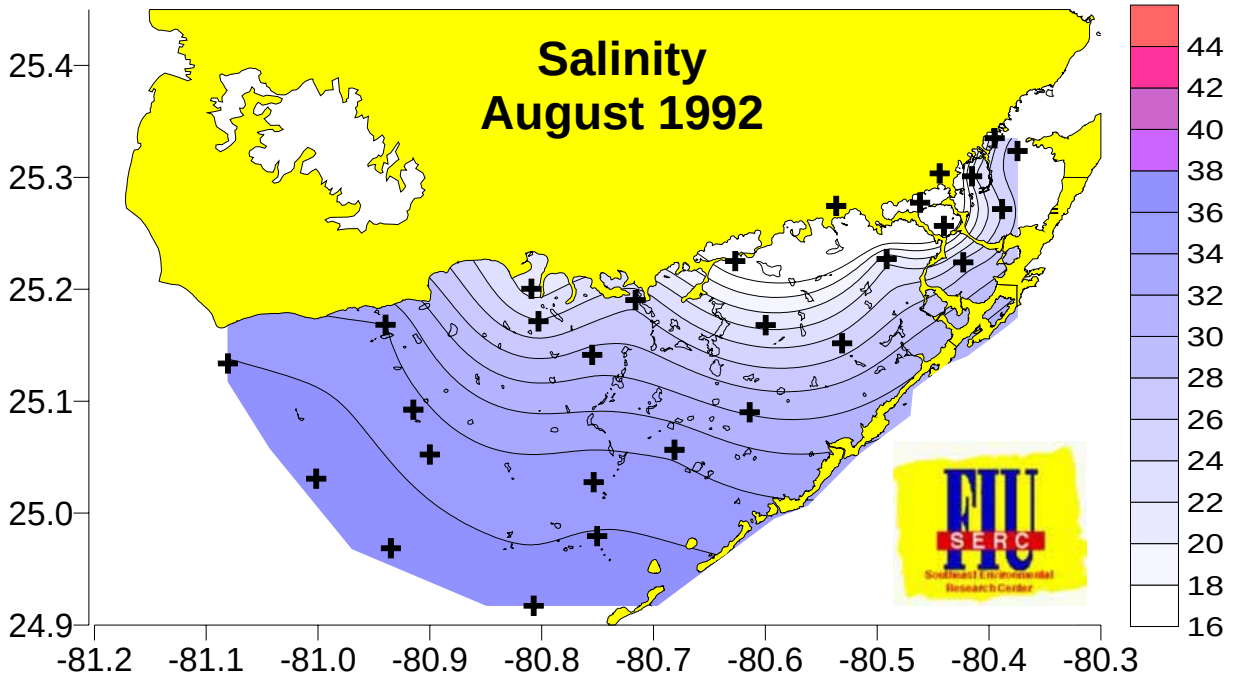

Figure 1: Median salinity across Florida Bay during 1992.

Phytoplankton biomass in eastern Florida Bay is strongly P-limited (Fourqurean et al. 1993; Phlips and Badylak 1996; Boyer et al. 1997; Lavrentyev et al. 1998), however other resources (e.g. light, N, Si) have been shown to partially control productivity in the central and western Bay (Lavrentyev et al. 1998; Brand 1999). Dissolved inorganic P concentrations (measured as soluble reactive P, SRP) are extremely low (~30 nM; eastern Bay), while dissolved inorganic nitrogen concentrations (DIN; mostly ammonium) can reach 100 μM in the central Bay (Boyer et al. 1997; Boyer et al. 1999).

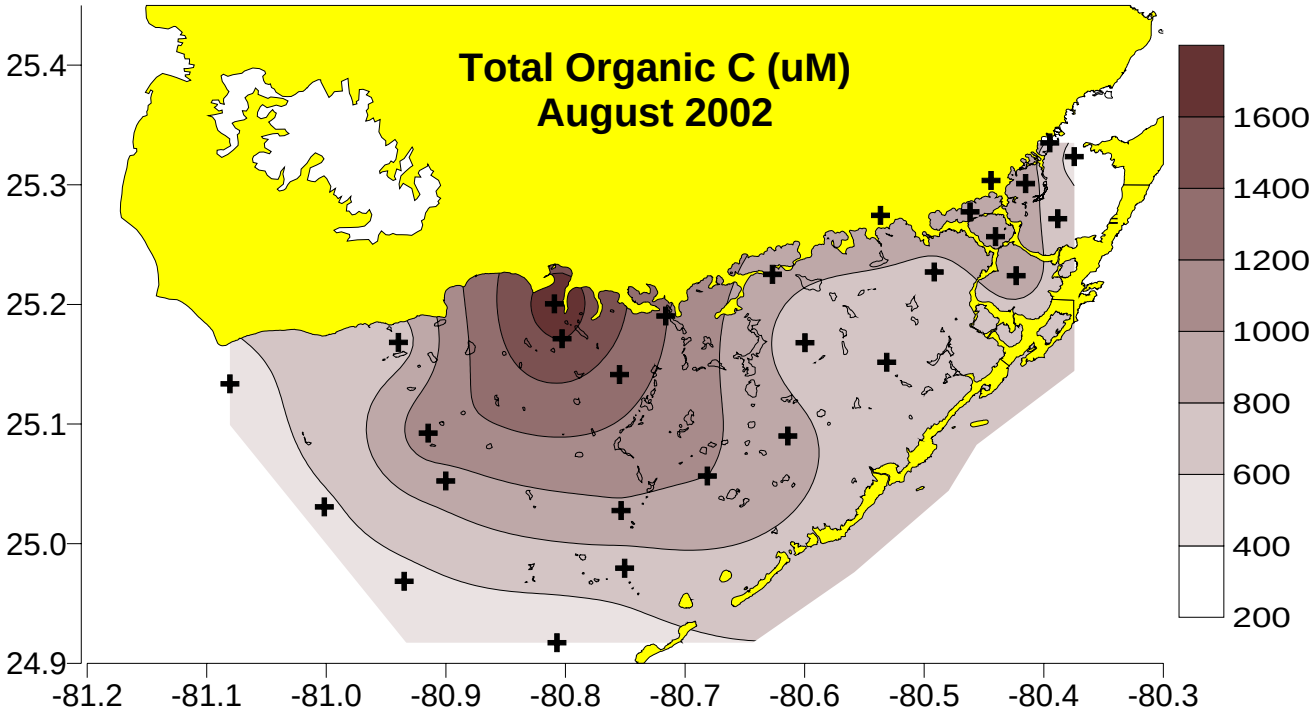

Figure 2: Total Organic Carbon across Florida Bay in August 2002.

The occurrence of extensive phytoplankton blooms in central Florida Bay has precipitated a significant amount of public, political, and scientific concern. Most vexing is the fact that these blooms are often dominated by cyanobacteria, specifically *Synechococcus elongatus* (Phlips and Badylak 1996; Steidinger et al. 1996; Phlips et al. 1999). Marine *Synechococcus* is a group of small unicellular planktonic photoautotrophs ca. 0.6 x 1.6 µm in diameter. The ability to fix atmospheric N_2 may give *Synechococcus* a selective advantage in areas of both low N concentrations as well as low dissolved N:P ratios (Phlips et al. 1989). In the ocean gyres where N concentrations are very low, N_2 fixation by cyanobacteria may provide a significant source of N to the system. In freshwater lakes and rivers, where P concentrations may be high due to external loading, N_2 fixation fuels the massive floating mats of cyanobacteria - the bane of managers and landowners. Neither of the above conditions apply to Florida Bay which necessitates a new conceptual model.

The connection between phytoplankton productivity and nutrient loading inputs to estuaries has been demonstrated repeatedly (Boynton et al., 1982). However, data from eastern Florida Bay show that while nutrient loading increases with flow through the Taylor Slough, inflowing nutrient concentrations actually decline (Boyer and Jones 1999). This pattern does not follow the typical eutrophication model because the limiting nutrient actually becomes more dilute as its loading rate increases. In addition, the concentration of incoming SRP is slightly below the ambient concentrations of the Bay meaning that the P load concentration may already be at or below the kinetic threshold for phytoplankton uptake.

Most nutrient and phytoplankton studies to date have been primarily concerned with the amounts of DIN and DIP as these fractions are directly available for phytoplankton uptake. However, a significant portion of the dissolved organic carbon pool (DOC) may be remineralized by microbes to inorganic constituents depending upon ambient nutrient status, C:N:P ratio of the source material (Tezuka, 1990), and chemical bioavailability (Benner et al. 1986; Amon and Benner 1996). Bacterial utilization of DOC, and subsequent grazing on these bacteria by protists and

microzooplankton (the microbial loop), is an important alternative pathway in many aquatic food webs (Azam et al. 1983). The microbial loop may also play a significant role in nutrient remineralization when C:N and C:P ratios are low. Conversely, when nutrient concentrations are limiting, bacterioplankton compete with phytoplankton for inorganic nutrients (Caron 1994). Therefore, the bioavailability and C:N:P ratio of the dissolved pool can determine whether the microbial loop is a source or sink for dissolved nutrients.

Little is known about the microbial loop in Florida Bay as no measurements of bacterial productivity have ever been published. Bugden et al. (1998) measured heterotrophic potential (^{14}C-glucose uptake) and direct counts of bacteria and found the central Bay to have highest activity and biomass, followed by the western Bay. They also found significant correlations between DOC and heterotrophic activity and bacterial counts. Lavrentyev et al. (1998) found lowest numbers of bacteria in the east and highest levels in the central Bay.

In Florida Bay, 92% of the total phosphorus pool (TP) is in the form of dissolved organic phosphorus (DOP; Boyer et al. 1997). This is a result of the extremely low SRP loading concentrations and because of abiotic binding of SRP to carbonate sediments (Kitano et al. 1978). DOP then becomes a very important P fraction to those organisms which can access it. Alkaline phosphatase (AP) is an inducible ectoenzyme produced in response to low SRP concentrations by bacteria, cyanobacteria, and some algae which hydrolyzes organic P to PO_4^{3-} (Chrost 1990). AP activity is usually inversely related to SRP concentration (Smith and Kalff 1981) and is inhibited by high SRP concentrations (Ammerman and Azam 1991). We have been measuring AP activity at 28 sites in Florida Bay for the past 10 years (Jones and Boyer 1999) and have found that AP activity is positively correlated with DOC concentrations, not with SRP or chlorophyll *a* (CHLA) as expected (Boyer et al. 1997). Both AP activity and DOC concentrations are three times higher in the central Bay than the eastern Bay. We believe that the microbial community in the eastern Bay is co-limited by P and DOC as it serves no purpose for microorganisms to express AP when there is no enzyme substrate present. Unfortunately, neither DOP bioavailability nor its contribution to primary production is known for this ecosystem.

Our understanding of bacterial and algal coupling dynamics and nutrient mineralization is incomplete, largely because no one experiment addresses TOC uptake by both trophic levels. In addition, the quality/lability of DOC is a function of its chemical characteristics, molecular weight, elemental ratio (C:N:P:other), and age. Stimulation of estuarine AP activity and CHLA by the addition of DOC has been demonstrated in microcosms (Carlsson and Graneli 1993). Previous studies have also demonstrated the importance of DOP in phytoplankton metabolism (see review by Cembella et al. 1984) but recent kinetic experiments by Bentzen et al. (1992) showed that the bacterial fraction dominated P uptake (as ATP), while small nanoplankton (1-12 μm) obtained some P and phytoplankton >12 μm took up very little. These results lead us to believe that AP activity is the main pathway by which both bacteria and phytoplankton in Florida Bay obtain P for growth and reproduction.

Looking at the relative differences in N:P ratio across the Bay, we notice that the western Bay exhibits more N limited conditions than other areas (Fig. 3). It is important to note that the DIN:SRP ratio is not that of organismal biomass as used by Redfield (1958) but of bulk dissolved fractions remaining in the water column. Nutrient ratios are the result of both biotic uptake and regeneration as well as abiotic processes such as adsorption and desorption. According to the DIN:SRP ratio, all of Florida Bay should be P limited. However we have shown strong evidence that much of the DOP is accessible to microorganisms via the AP enzyme system. If we assume all TP is accessible by AP then the DIN:TP ratio becomes a more valid indicator of water column nutrient limitation (Fig. 4).

The area of Florida Bay where DOP bioavailability becomes most important is the central Bay where most of the cyanobacterial blooms have occurred.

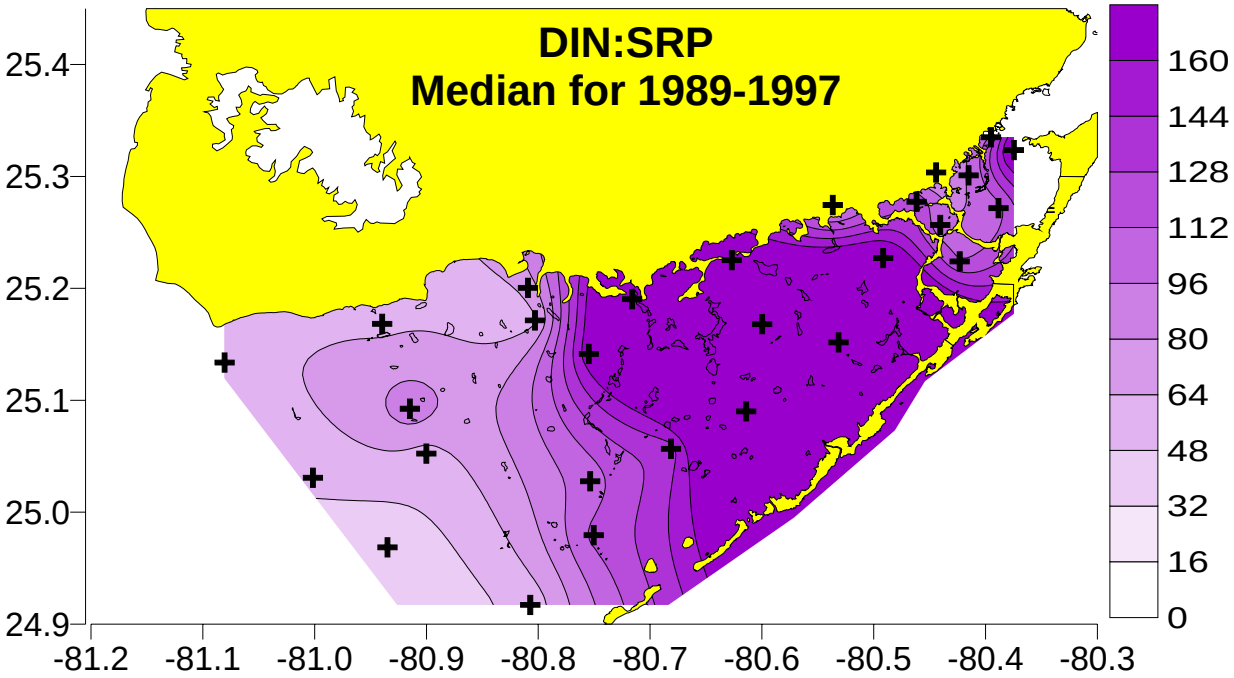

Figure 3: Dissolved Organic Nitrogen to Soluble Reactive Phosphorus ratio median value 1989 to 1997.

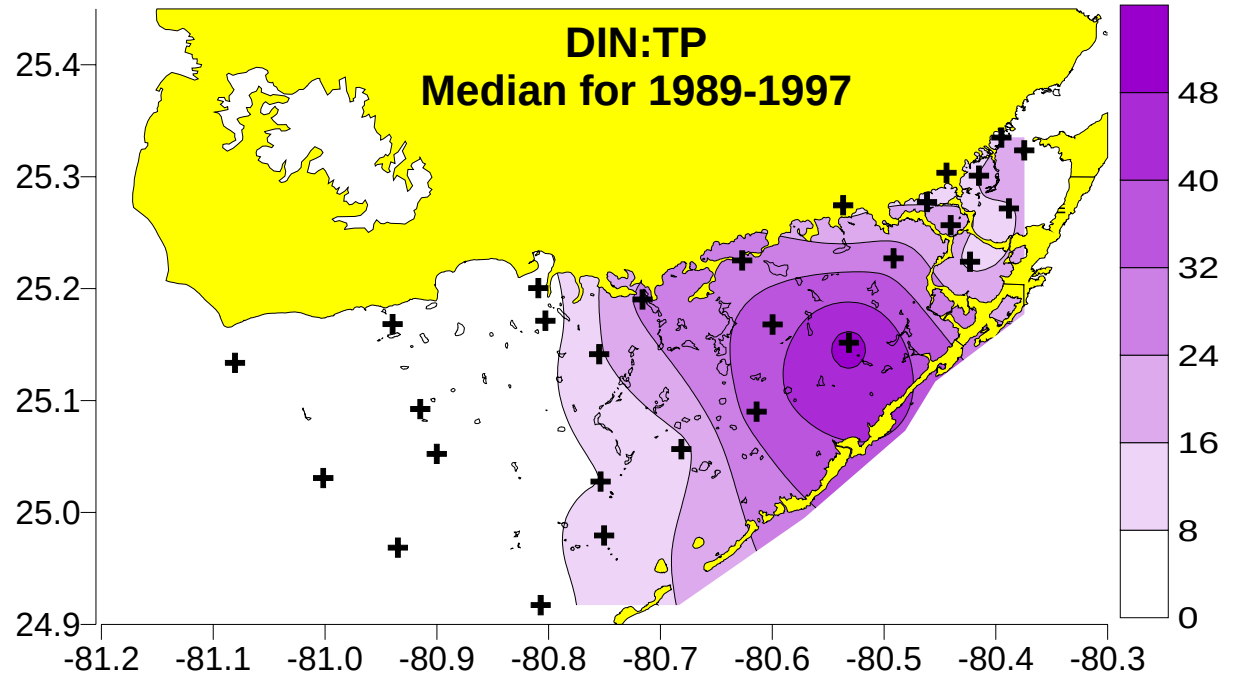

Figure 4: Dissolved Inorganic Nitrogen to Total Phosphorus ratio median from 1989 to 1998.

In addition to DOC concentration and AP activity, DOC bioavailability may also help to explain the prevalence of cyanobacteria over eukaryotic algae in blooms in the central Bay. Cyanobacteria have much higher cell-specific AP production than do eukaryotic algae (Giraudet et al. 1997). This means that when P is limiting, cyanobacteria have an enhanced ability to hydrolyze DOP for their own uptake. We also propose that the bioavailability of DOC provides a C source that may be important in promoting bacterial and/or cyanobacterial production. The observation that the cyanobacteria blooms usually begin in the central Bay where episodic DOC inputs occur adds support to this idea. Therefore we proposed that:

> Research Hypothesis 1: Spatial patterns in primary production of bacteria and phytoplankton in Florida Bay are a product of inorganic N and organic P availability.
>
> Research Hypothesis 2: Under P limited conditions and high DOC concentrations, cyanobacteria are favored over eukaryotic algae by their greater cell-specific production of alkaline phosphatase.

We used a two tiered approach to elucidate a mechanism for cyanobacterial bloom development. First we determined the relative contribution of bacteria to the microbial community. We hypothesized that spatial patterns in primary production of bacteria and phytoplankton in Florida Bay are a product of inorganic N and organic P availability. We used a 2 year segment of a running 14 year database of nutrients and phytoplankton biomass (chlorophyll *a*) combined with new measurements of primary production, bacterial production, bacterial biomass, and enzyme assays to develop statistical models of substrate and nutrient competition among the major components of the phyto- and bacterioplankton community.

Second, we tested the hypothesis that both the local source of labile DOC and the enhanced alkaline phosphatase activity of cyanobacteria favor the initiation and dominance of cyanobacterial blooms in central Florida Bay. We conducted quarterly sampling of ambient water from central Bay zones and incubated samples under four treatments: ambient light incubation, dark incubation, nitrogen and phosphorus enrichment, and DOC amendments. From these treatments we expected to quantify: 1) the effect of DOC on phytoplankton community structure; 2) the bacterial contribution to ambient community production; 3) the bacterial contribution to DOC amended community production; and 4) the effect of bioavailability of ambient and amended DOC on community structure. This was the first attempt to determine the actual mechanism which promotes the onset and persistence of cyanobacteria blooms in Florida Bay.

Data and research are addressed in two sections in this report that each address one of the two hypotheses presented in the proposed research.

<u>Research Hypothesis 1</u>: Spatial patterns in primary production of bacteria and phytoplankton in Florida Bay are a product of inorganic N and organic P availability.

Study Sites

We quantified microbial characteristics at the 28 Florida Bay monitoring sites from the South Florida Water Quality Monitoring Network program (Fig. 5). All study sites are within the boundary of Everglades National Park (ENP). Florida Bay is an ecotone with the largest section of contiguous flow wetland in the Everglades and its waters flow into Florida Bay and eventually the Gulf of Mexico and the Atlantic Ocean.

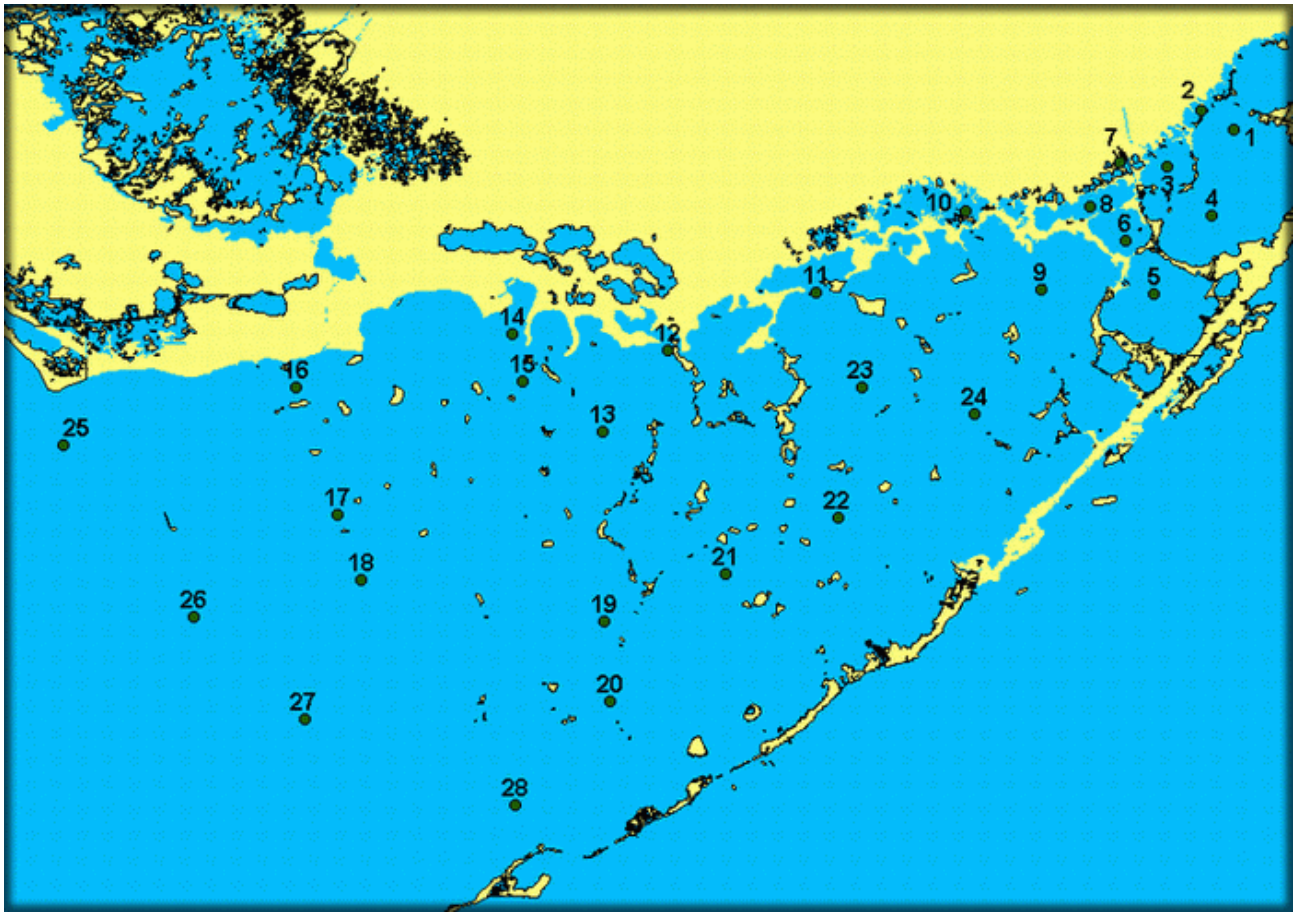

Figure 5: Map showing all 28 Florida Bay sites where microbial and nutrient characteristics were quantified.

Methods

Nutrient Analyses

All nutrient analyses were conducted using standard methodology by the Southeast Environmental Research Center. Sample water was submitted unfiltered for TOC, DOC, TN, and TP analysis in sample rinsed 120ml HDPE bottles. TOC was measured by direct injection onto hot platinum catalyst in a Shimadzu TOC-5000 after first acidifying to pH<2 and purging with CO_2-free air. TN was measured using an ANTEK 7000N Nitrogen Analyzer using O_2 as carrier gas (Frankovich and Jones 1998). TP was determined using a dry ashing, acid hydrolysis technique (Solarzano and Sharp 1980). Dissolved organic carbon was measured in the same way as TOC, only this water was filtered through a Whatman GF/F immediately after sampling then analyzed using the TOC method described. Sample water was filtered by hand through a sample rinsed 25mm GF/F into acetone-washed and sample rinsed 60ml HDPE bottles for analysis of SRP, NO_x, NO_2, NH_4, and $Si(OH)_4$. These parameters were obtained by flow injection analysis (Alpkem model RFA 300). The filters from the dissolved constituents were placed in 2ml plastic centrifuge tubes with 90% acetone for CHLA analysis. These tubes were kept at –20°C for a minimum of 4 days to complete extraction and were analyzed within 28 days. The extracts were analyzed using a Gilford Fluoro IV Spectrophotometer (excitation = 435nm, emission = 667nm) and compared to a standard curve of pure CHLA (Sigma).

Three N parameters were not measured directly but rather calculated by difference. NO_3 was calculated as $NO_x - NO_2$; DIN was calculated as $NO_x + NH_4$; and TON was defined as TN – DIN. We used an alkaline phosphatase activity (AP) assay experiment to determine the production of extracellular enzymes to mineralize phosphate from organic material (Hashimoto et al., 1985). We incubated duplicate samples of 3ml aliquots for 2 hours after adding o-methylfluorescein phosphate to whole water. We determined initial and 2 hour readings using Gilford Fluoro IV Spectrophotometer (excitation = 430nm, emission = 507nm) and used the change over the incubation period to determine AP activity (μM h-1).

Microbial Analyses

Pulse amplitude modulated (PAM) fluorometry was used to quantify the phytoplankton community structure and photosynthetic parameters quantum yield (QY) and maximum electron transport rate (ETR_{max}). Sample water was allowed to sit at 4°C overnight prior to analysis to ensure complete reduction of photosystem II. A 3ml aliquot was first run in the PAM fluorometer to determine the range of fluorescence for the individual sample and gain was adjusted accordingly. Next we ran a 3ml aliquot of the sample that we filter sterilized though a 0.2μm filter to determine the blank (Z off) for plankton free water. Another 3ml of sample were run again as whole water to determine CHLA concentrations for blue, green, and brown algal groups, QY, and ETR_{max}, from a productivity-irradiance curve run across 10 light intervals.

Bacterial counts (BACT) were determined through epifluorescent microscopy using DAPI staining technique (Coleman 1980; Porter and Feig 1980). Sample water was collected and fixed with Formalin buffered with phosphate solution to a final concentration of 2%. Samples were incubated at a final concentration of 25μg ml^{-1} DAPI (Molecular probes©) in a filtration tower for 20 minutes prior to filtration onto a 0.2μm black polycarbonate filter. The filter was mounted onto a slide with low fluorescent immersion oil and examined under a 100W Hg epifluorescent bulb by counting 10 sampling fields of a known size per slide, with a minimum of 300 cells per slide counted.

A final value of cells ml^{-1} was obtained with a formula using the sample volume counted and the percentage of effective filter area counted.

Bacteria production (BP) was determined using ^{3}H-thymidine incorporation incubations (Bell, 1993). We ran triplicates of each sample with a 4% final concentration formalin blank for each. With each ^{3}H-thymidine incubation experiment we ran a blank sample for specific activity of the ^{3}H-thymidine. We converted disintegrations per minute (dpm) from the liquid scintillation counter using then following equation:

$$\mu g\ C\ l^{-1}\ h^{-1} = (\text{moles thymidine}\ l^{-1}\ h^{-1}) * (\text{cells mole}^{-1}) * (\text{carbon cell}^{-1})$$

Where picomoles of thymidine incorporated were calculated through using the actual activity of the ^{3}H thymidine (dpm) versus the bacteria activity from live - killed (dpm).

Cells $mole^{-1}$ was determined by multiplying the thymidine conversion factor of 2×10^{18} cells $mole^{-1}$ by the moles of thymidine $l^{-1}\ h^{-1}$. For the amount of carbon per cell we used the 20 fg conversion rate used in coastal waters. Our observations of water from these Florida Bay sites over the past two years have shown bacteria numbers that are consistently lower than those found in estuaries or other coastal systems but can be highly variable across sampling months. Thus we used a mid-value conversion factor to multiply the number of cells by to estimate the change in C content (Bell 1993).

Statistical Analyses

In order to assess the underlying patterns in the distribution of the measured parameters, we followed the objective analysis procedure of Boyer et al. (1997). Briefly, principal component analysis (PCA) was used to extract composite variables (principal components) from the original data (Overland and Preisendorfer 1982). Data were standardized (Z-scores) prior to analysis to reduce artifacts of magnitude. The PCA solution was rotated (using VARIMAX) in order to facilitate the interpretation of the principal components and the factor scores saved for each data record. The purpose of this analysis was to collapse the number of variables into a few groups, which could then be analyzed in more detail.

Results

Statistical Analyses

Overall, microbial components were coupled across Florida Bay for the study period from January 2001 to December 2002. PCA identified five composite variables (hereafter called PCI, PCII, etc.) that passed the rule N for significance at $P<0.05$ (Overland and Preisendorfer 1982). The factor loadings, as correlations between the original variables and the principal components (Table 1), indicated five separate modes of variation in the data. These five principal components accounted for 60.7% of the total variance of the original variables.

PCI was composed of CHLA, BACT, TP, BP, and turbidity and provided the greatest contribution to variability in the dataset. This grouping inferred that there was a strong coupling between phytoplankton biomass and P availability. It also suggested that some of the CHLA in the water column may come from sediment resuspension.

PCII included the inorganic N forms of NH_4^+, NO_2^- and NO_3^-. This was an expected grouping and is common in water quality monitoring, possibly from the tight microbial coupling in inorganic nitrogen mineralization and remineralization. What this told us was that higher concentrations of DIN occurred in lower salinity waters. Both increased precipitation and high canal inputs during the wet season may be important in driving this relationship.

PCIII included salinity, temperature and DO, where DO was negatively correlated with salinity and temperature, an association based on the physical nature of gas solubility. This relationship is routinely observed in South Florida estuaries (Boyer et al. 1997).

PCIV had high factor loadings for TON, APA, TOC and was inversely reated to salinity. When salinity is low there is high TOC and TON that flushes out of the mangrove estuaries into the Bay. Higher AP activity suggests that the microbial community is able to respond to the immediate flushing of organics to harvest soluble reactive phosphorus. As in a previous study (Boyer et al. 1997), APA was not inversely related to SRP concentration as expected, but was related to the dissolved organic carbon (DOC) pool. We believe that this may be due to C substrate limitation of bacteria in these systems (unpublished results).

PCV revealed SRP and QY contributed significantly to explaining variability across the dataset. Overall, these five factors explained 65% of the variability in the data set over the study period from January 2001 to December 2002. Thus, in summary, organic phosphorus, along with both bacteria and algal community structure and function, is associated with most of the variability across time and space in Florida Bay biogeochemistry and microbial dynamics.

Table 1: Principal component analysis for the entire Florida Bay microbial, nutrient, and physical dataset from January 2001 through January 2003. The five Principal components accounted for 65.0% of the variance.

Parameter	PCI1	PCII	PCIII	PCIV	PCV
BACT	**0.667**	0.069	0.140	0.078	-0.092
BP	**0.610**	-0.098	-0.062	0.053	0.280
QY	0.127	-0.056	-0.239	-0.174	**0.570**
NO_3^-	-0.083	**0.753**	-0.100	0.081	0.127
NO_2^-	0.036	**0.818**	0.019	-0.205	0.100
NH_4^+	0.071	**0.778**	0.060	0.059	-0.103
TON	-0.004	-0.243	0.109	**0.601**	0.229
TP	**0.631**	-0.070	0.130	-0.074	0.137
SRP	-0.046	0.194	0.152	0.183	**0.650**
APA	0.117	0.052	-0.036	**0.721**	-0.058
CHLA	**0.734**	0.013	-0.056	0.086	-0.090
TOC	0.124	0.083	-0.005	**0.727**	-0.053
Salinity	0.159	**-0.437**	**0.492**	**-0.531**	0.066
Temperature	0.021	0.001	**0.784**	0.213	-0.198
DO	-0.156	-0.006	**-0.878**	0.161	-0.037
Turbidity	**0.532**	0.165	-0.474	-0.102	-0.262
% Variance explained	22.8	16.0	11.7	7.9	6.6

Spatial Characterization

With the connection of nutrients and particularly total phosphorus clearly established with algae and bacteria, we addressed the spatial relationships of each of the dissolved and total nutrients and microbial characteristics through GIS analyses. We graphed monthly results of nutrient and microbial analyses over the two-year period using Arc GIS (ESRI© 2003) spatial analyses of kriging with a nearest neighbor analyses of 12 of the 28 data points. We did the analyses with a smallest data area unit of 100 m^2. These data maps reveal contour plots by concentrations where highest concentrations are the darkest areas and the lighter areas are the lowest concentrations. Missing months of data for BACT were during the months of transition in counting dye from SYBR Green (Molecular Probes©) to DAPI. We produced a map for each month over the study period from January 2001 to December 2002.

Nitrite+Nitrate (NO_x) - The nitrogen components of nitrate and nitrite combined in the water column were low across Florida Bay compared to other estuarine systems. The highest concentrations that we observed during the study period were in the eastern Bay and in Feb 2001 in the western-most Florida Bay site (Figure 6a). NO_x concentrations in 2002 (Figure 6b) were lower than those in 2001 and higher concentrations were observed that originated from the southern central Everglades terrestrial margin.

Nitrate - Spatial analyses of nitrate concentrations across Florida Bay revealed a distribution of highest concentration observed in the eastern sites of Florida Bay (Figure 7a and Figure 7b). Over the study period concentrations of nitrate in November 2001 were higher in the furthest most eastern portion of Florida Bay. Outlying observations from this general observation for monthly spatial analyses were in April and May 2001, deep into the dry season and the highest concentrations in the Bay at this time were derived from the western study sites. These high concentrations of nitrate may be from the extreme concentration of nutrients in the mangrove creeks during the dry season when no freshwater flushing occurs. With the exception of April and May 2001, the highest concentrations each month were found consistently in the central-eastern Bay study sites and primarily the northeastern study sites. These results suggest that freshwater Everglades outflow from the Taylor Sough and Panhandle are the derivation of this nitrogen constituent.

Ammonium - The highest contribution of inorganic nitrogen in Florida Bay was from ammonium. Spatial distribution of ammonium revealed a pattern of highest concentrations in the central and eastern study sites of Florida Bay (Figure 8a and Figure 8b). The western region of Florida Bay and the margin with the Gulf of Mexico revealed consistently low concentrations of NH_4^+, indicating that the GOM was not a source for this nitrogen component.

Total Organic Nitrogen - The TON in Florida Bay over the study period was highest in the central and eastern study sites of Florida Bay (Figure 9a and Figure 9b). This higher TON is likely associated with the higher TOC concentrations and general organic matter distribution that we observe concurrently in the Bay. The spatial distribution of the organic portion of nitrogen differs from the inorganic components of nitrogen and is associated with the central study sites of Florida Bay. Highest concentrations are found along the land margins in the central and eastern portion of the Bay where McCormick Creek and then further to the east, Taylor Sough and Taylor river empty freshwater into Florida Bay.

Total Nitrogen - Highest Total Nitrogen (TN) concentrations (Figure 10a and Figure 10b) in Florida Bay over the study period were observed in the central and eastern study sites in the Bay. Total organic nitrogen in Florida Bay was the greatest contribution to the total nitrogen pool in Florida Bay and the similarities in their spatial analyses were revealed.

Total Phosphorus - Spatial analyses of total phosphorus in Florida Bay revealed the highest concentrations of TP in the western study sites in Florida Bay during the dry season of 2001 (Figure 11a). Monthly spatial analyses observed throughout 2002 (Figure 11b) did not reveal the same pattern of highest concentrations in the western study sites of Florida Bay, although the high concentrations that were observed were not as widespread as those we observed in 2001. During the wet season, TP concentrations across Florida Bay were generally lower than those observed during the dry season. This phenomenon may be associated with the dilution factor provide by the increased freshwater flow during the wet season.

Total Organic Phosphorus - Spatial analyses of the total organic phosphorus pool are very similar to those we observed in our spatial analyses of total phosphorus. The highest concentrations of total organic phosphorus (TOP) were observed during the dry season in 2001 (Figure 12a). Another trend in 2002 (Figure 12b) of highest concentrations in the western Bay was revealed through these GIS analyses. The TOP in the latter case appears to be derived from the Whitewater Bay and Shark River Slough drainage basin of the Everglades.

Soluble Reactive Phosphorus - Spatial distribution of the biologically available fraction of inorganic phosphorus revealed the trend of the very highest concentrations in the eastern region of Florida Bay (figure 13a and Figure 13b). The concentration of SRP over the study period was low relative to other estuarine sites along the east coast, so "highest concentrations' must be viewed in relative to the larger, less available pools of TP. The variability between months was higher than that seen in most of the other inorganic constituents and may be more associated with biological interactions that are measured on a different time scale.

Total Organic Carbon - Spatial distribution of TOC in Florida Bay across the study period revealed consistently higher concentrations in central Bay (Figure 14a and Figure 14b). The highest concentration we observed from all of the study sites over space and time was in August 2002 in this same central region of the Bay. Spatial analyses also showed that the highest concentrations were at the land-estuary margins, suggesting that the source of TOC derived from the freshwater Everglades and estuaries from the mainland.

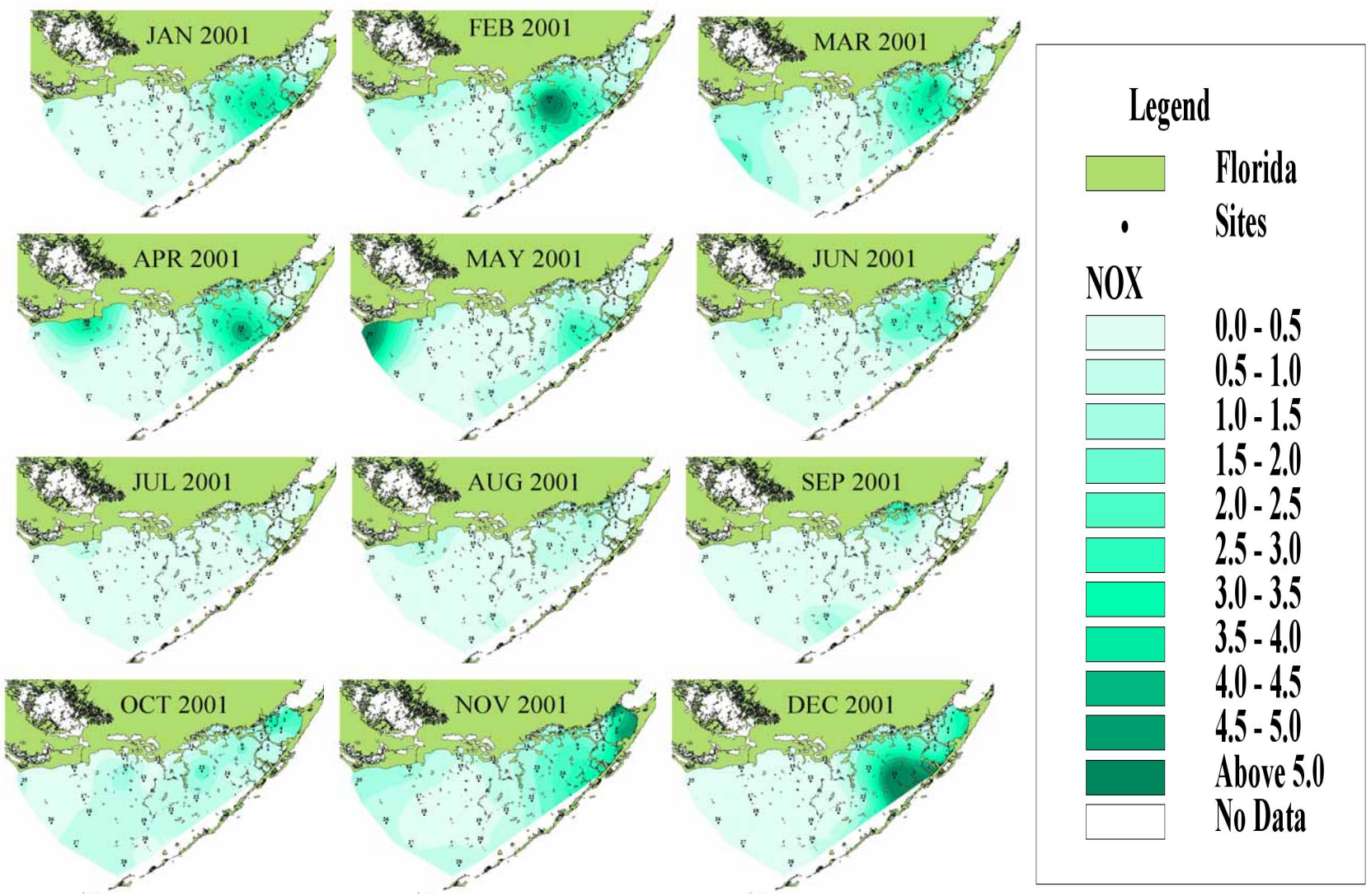

Figure 6a: Nitrate and Nitrite concentrations (μM) presented across Florida Bay from January 2001 to December 2001.

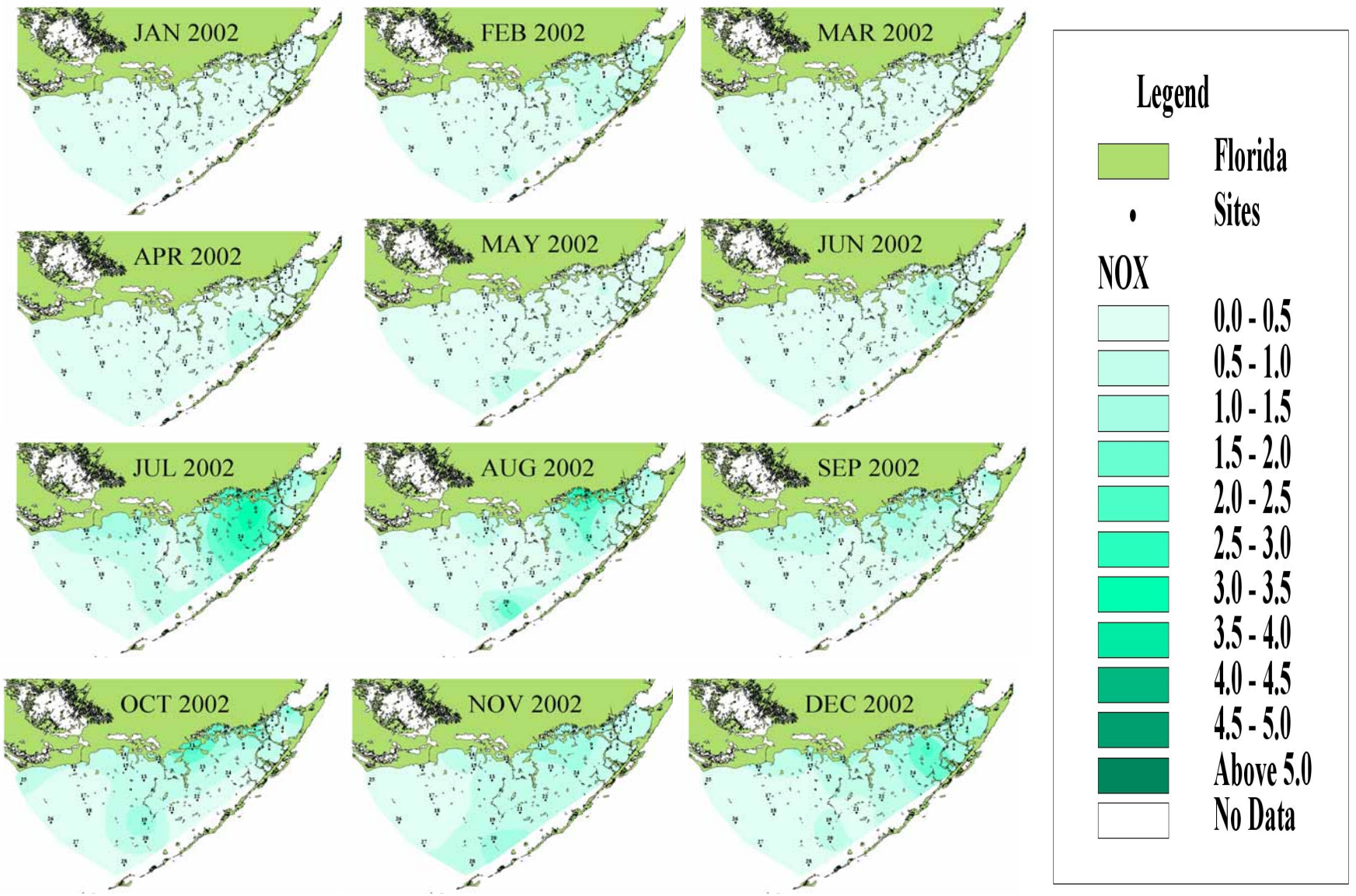

Figure 6b: Nitrate and Nitrite concentrations (μM) presented across Florida Bay from January 2002 to December 2002.

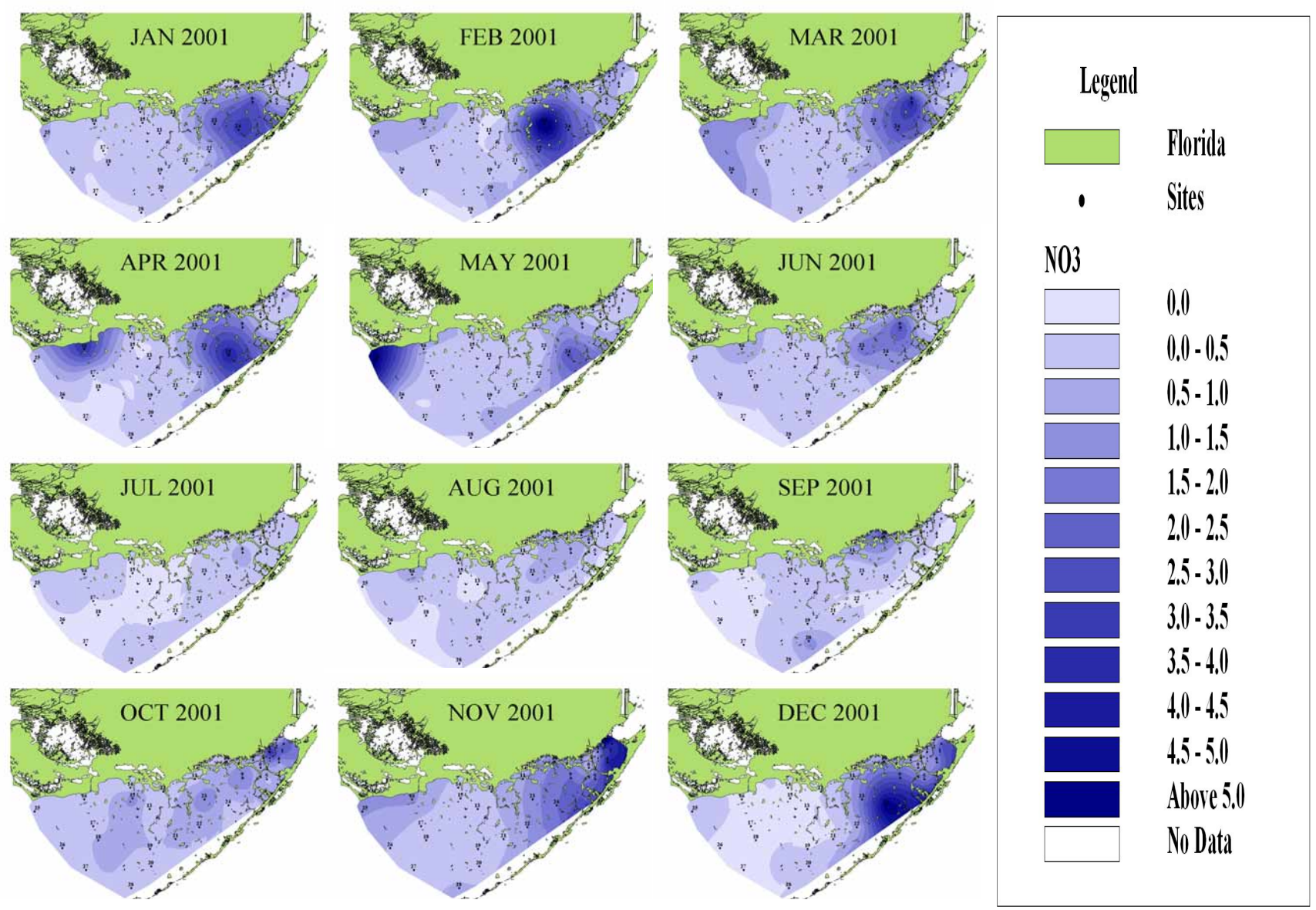

Figure 7a: Nitrate concentrations (μM) presented across Florida Bay by month from January 2001 to December 2001.

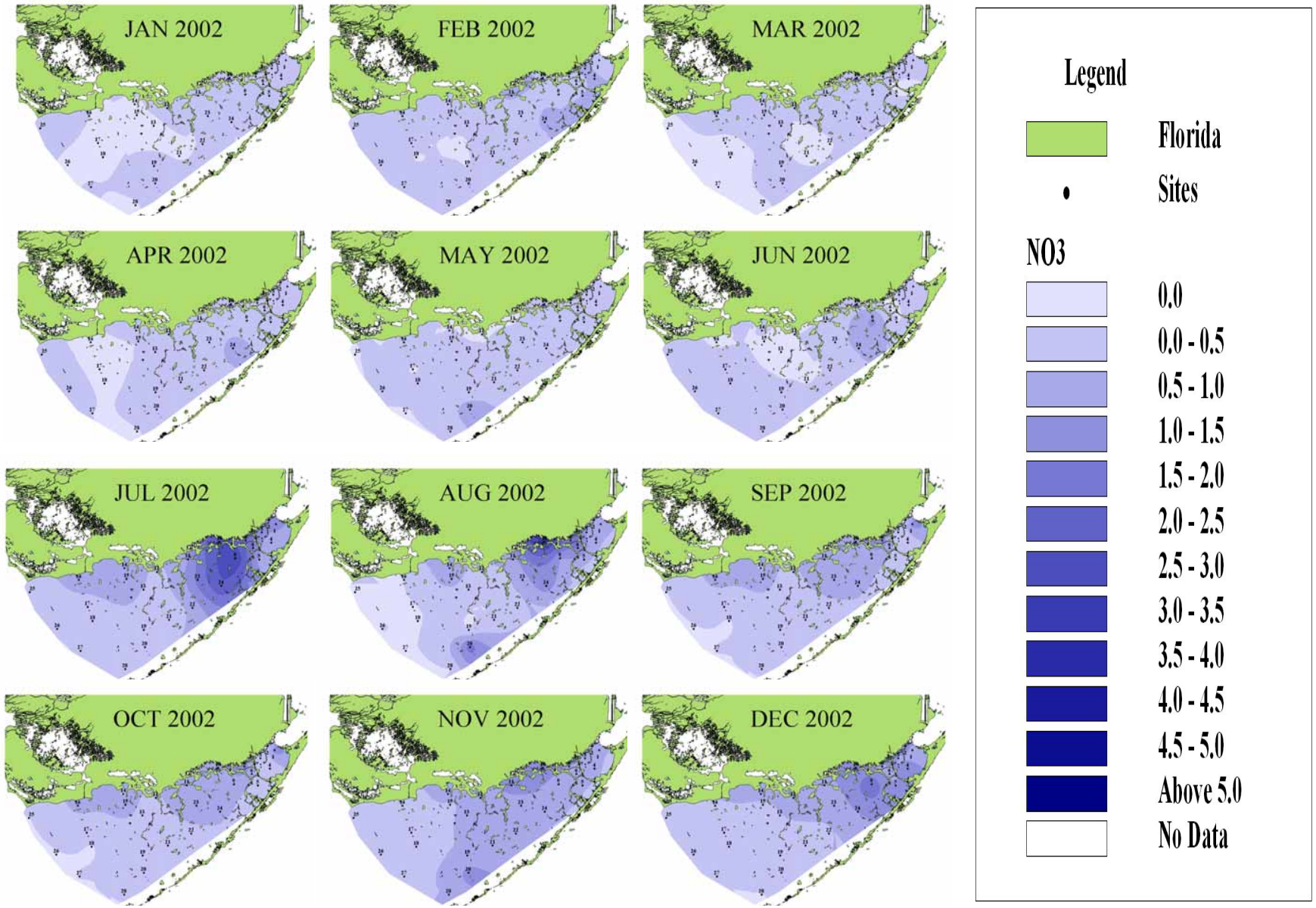

Figure 7b: Nitrate concentrations (μM) presented across Florida Bay by month from January 2002 to December 2002.

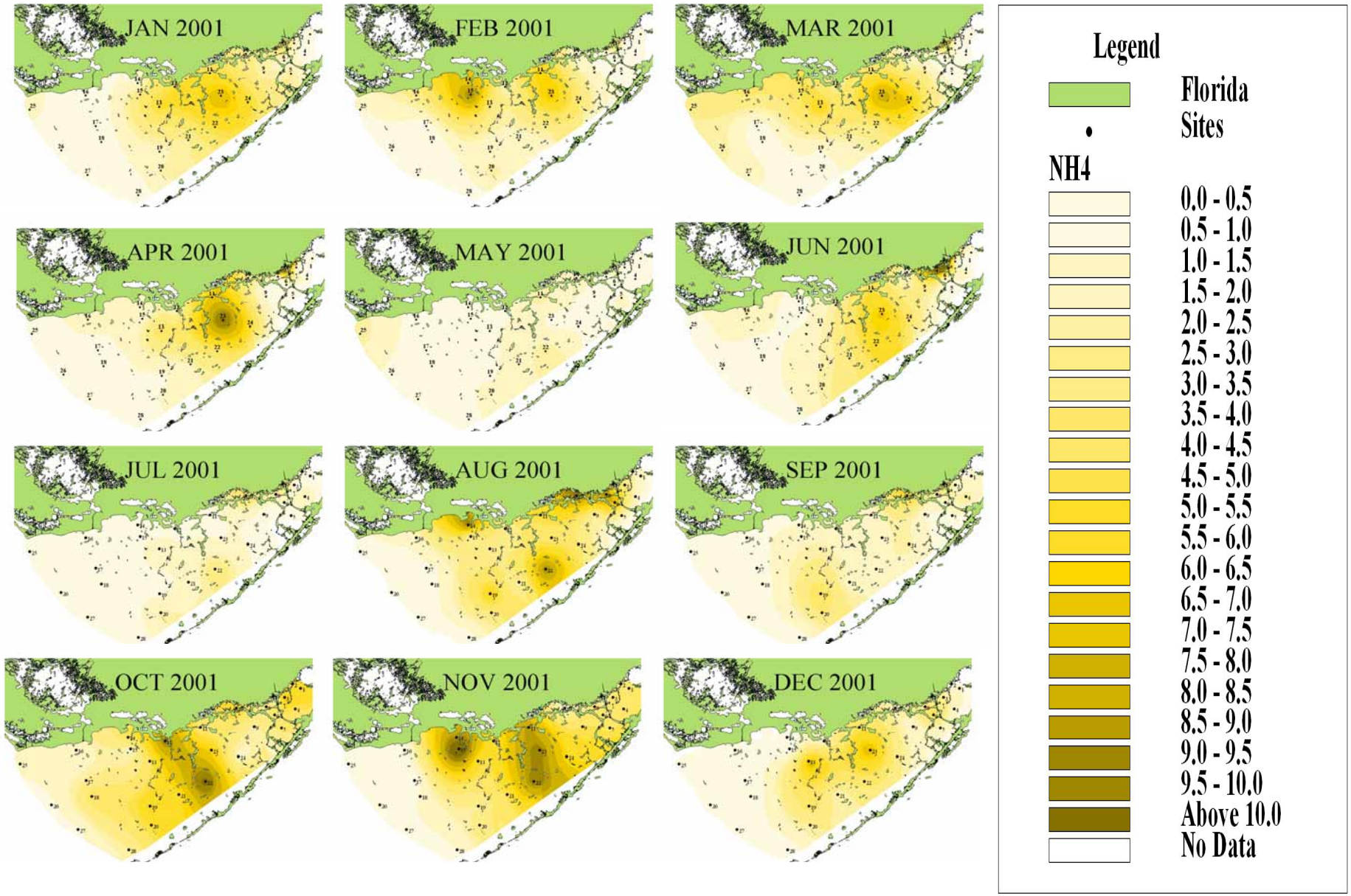

Figure 8a: Ammonium concentrations (μM) for Florida Bay from January 2001 to December 2001.

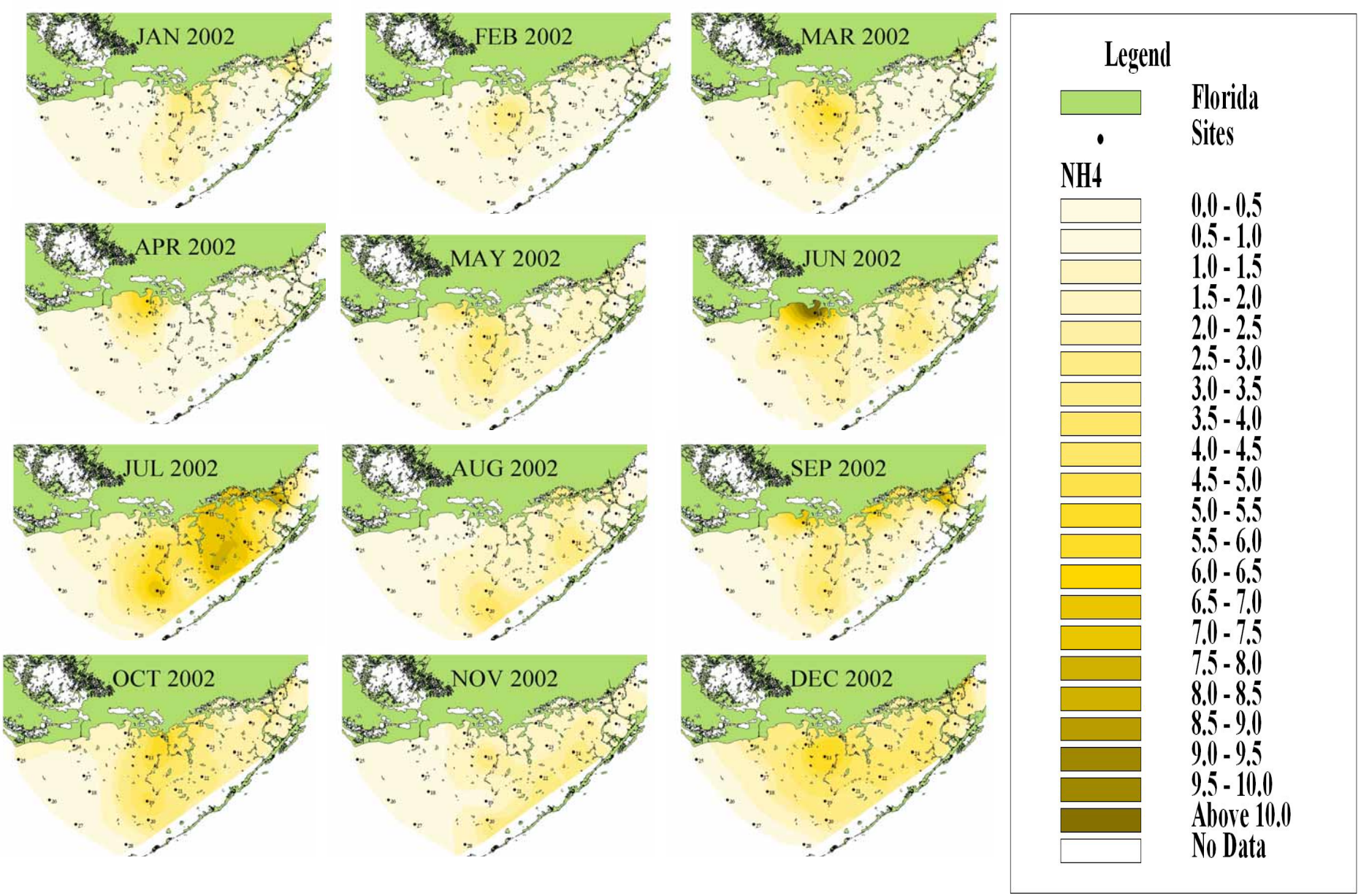

Figure 8b: Ammonium concentrations (μM) presented spatially across Florida Bay from January 2002 to December 2002.

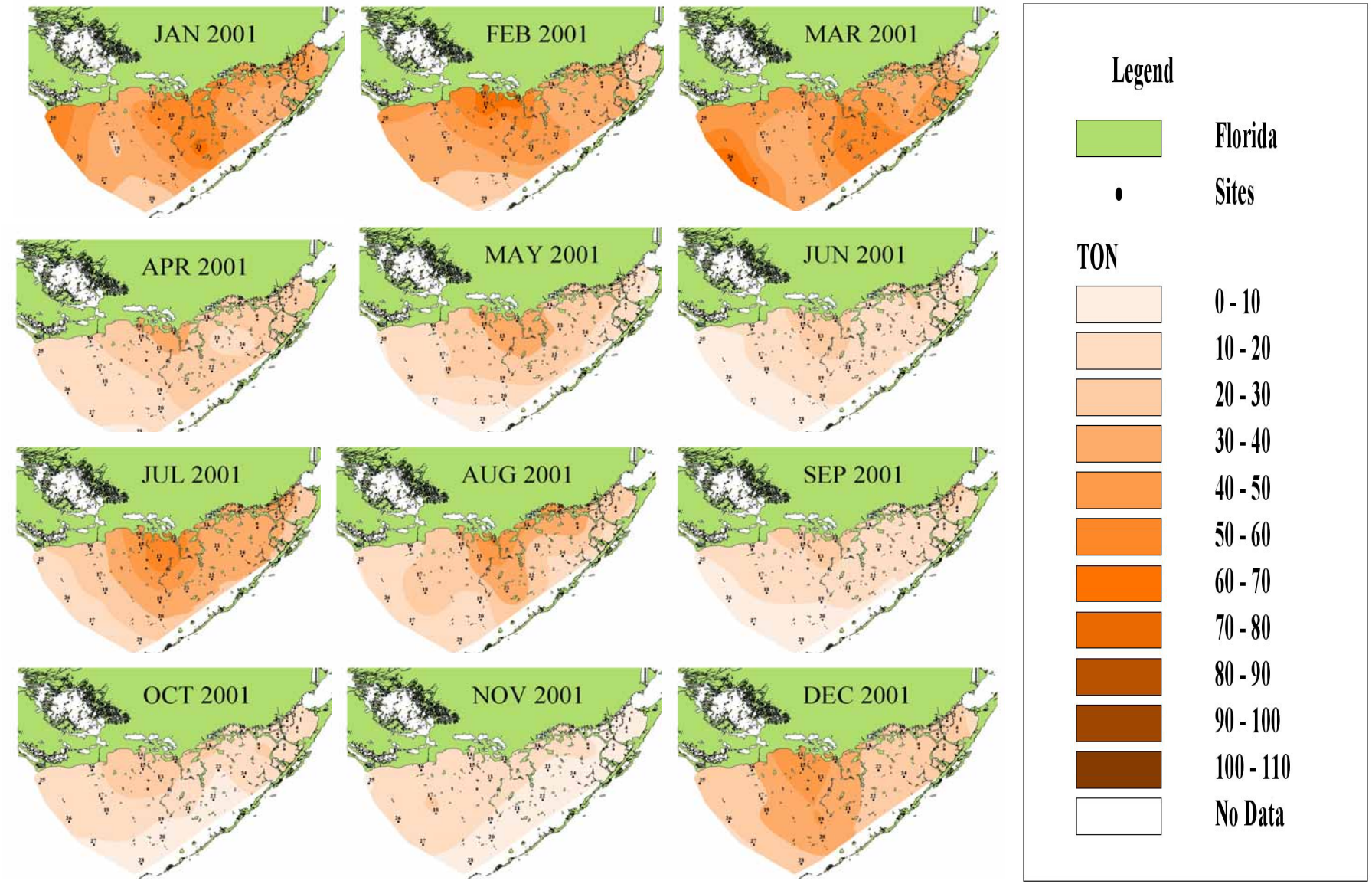

Figure 9a: Total Organic Nitrogen concentrations (μM) across Florida Bay from January 2001 to December 2001.

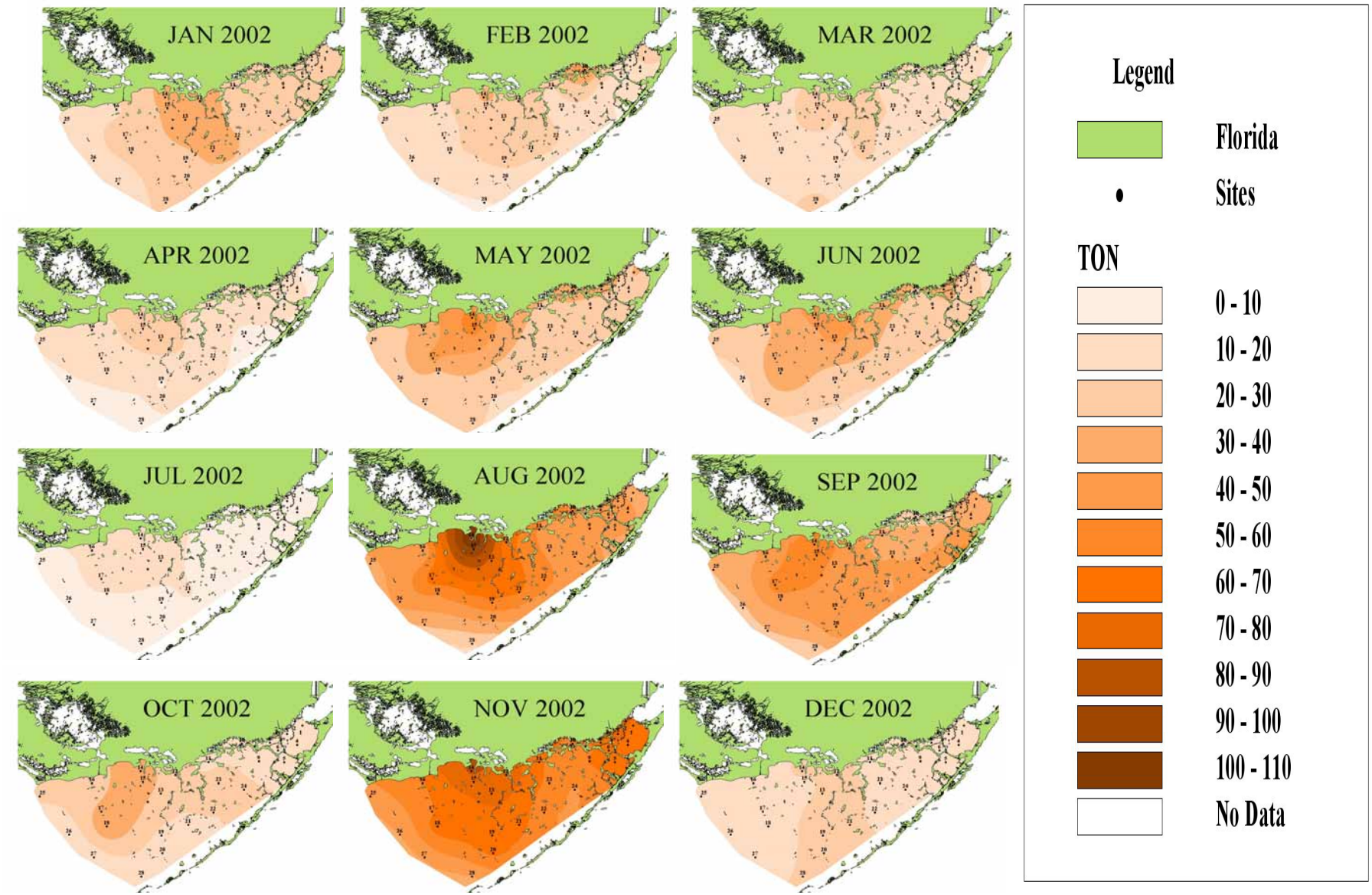

Figure 9b: Total Organic Nitrogen concentrations (μM) across Florida Bay for January 2002 to December 2002.

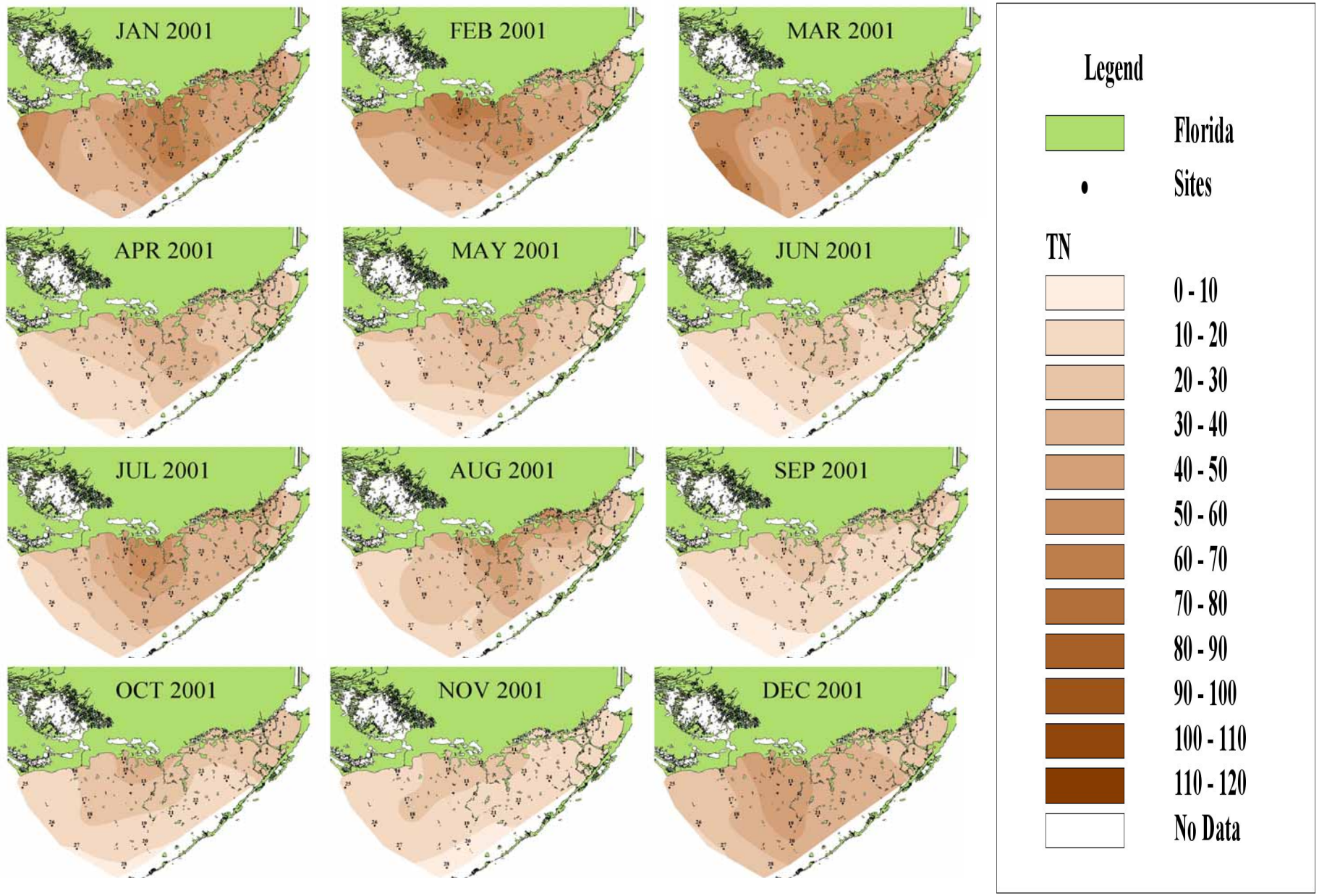

Figure 10a: Florida Bay Total Nitrogen concentrations (μM) for January 2001 to December 2001.

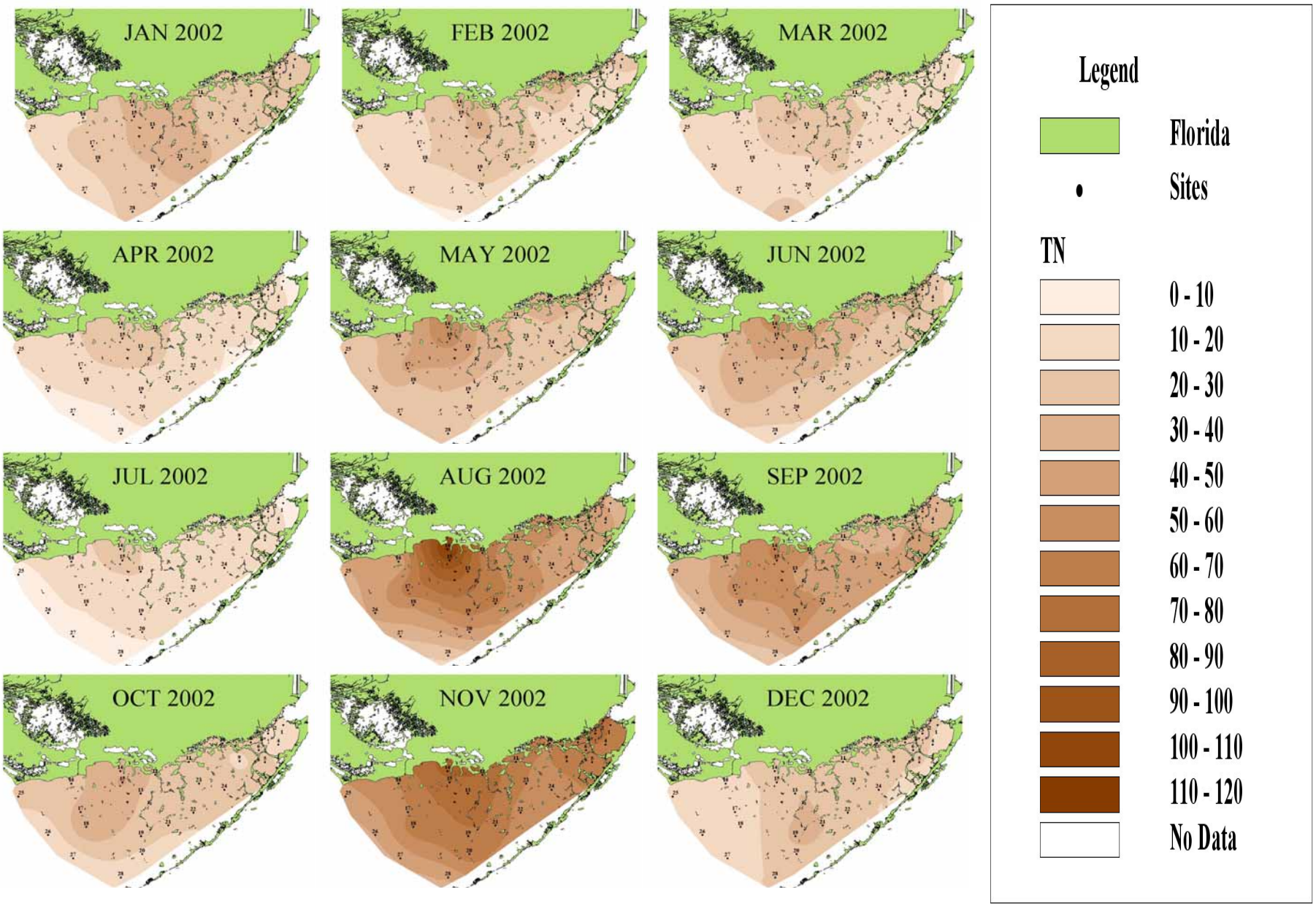

Figure 10b: Total Nitrogen concentrations (μM) presented spatially across Florida Bay for January 2002 to December 2002.

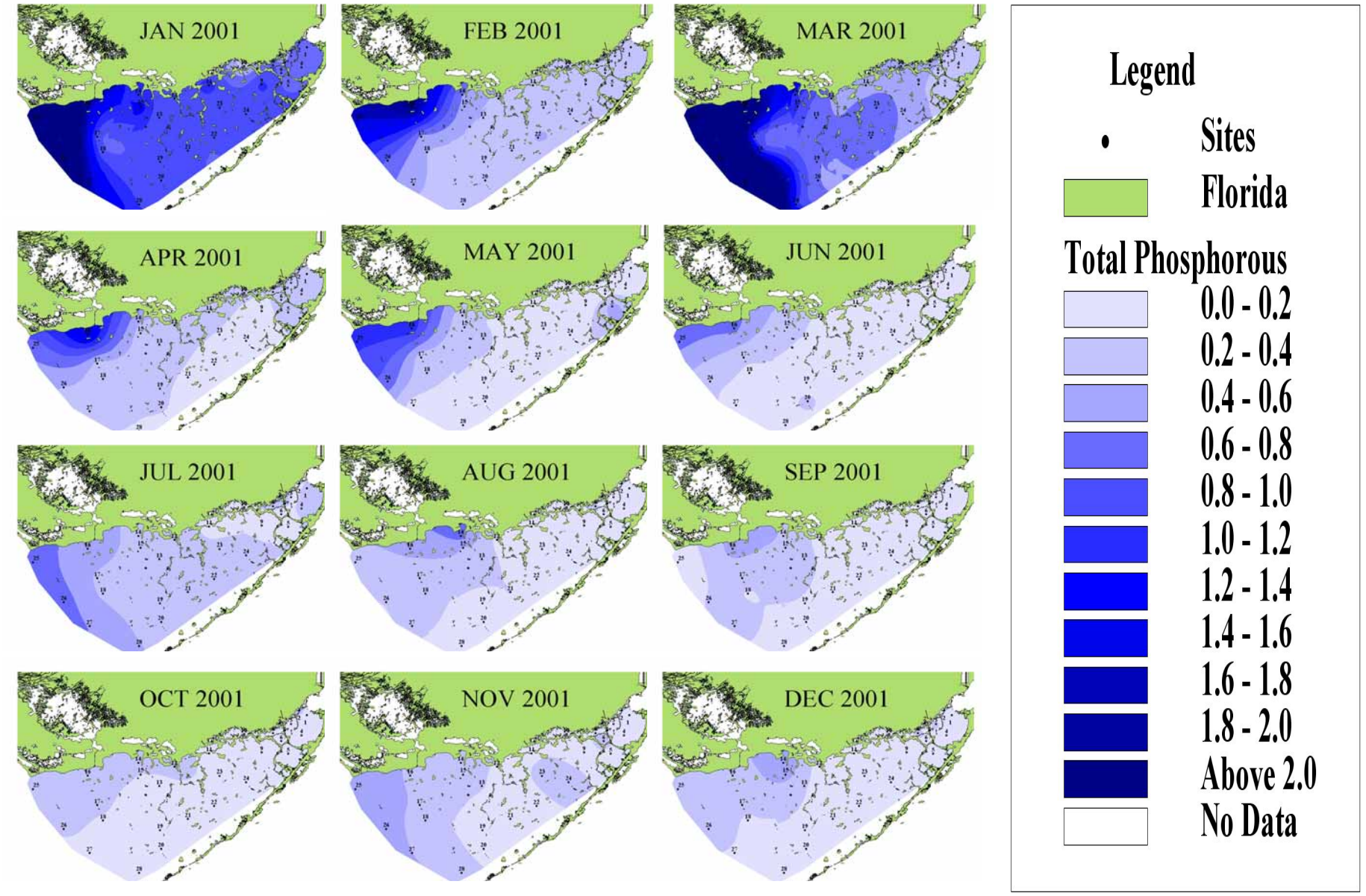

Figure 11a: Total Phosphorus concentrations (μM) across Florida Bay from January 2001 to December 2001.

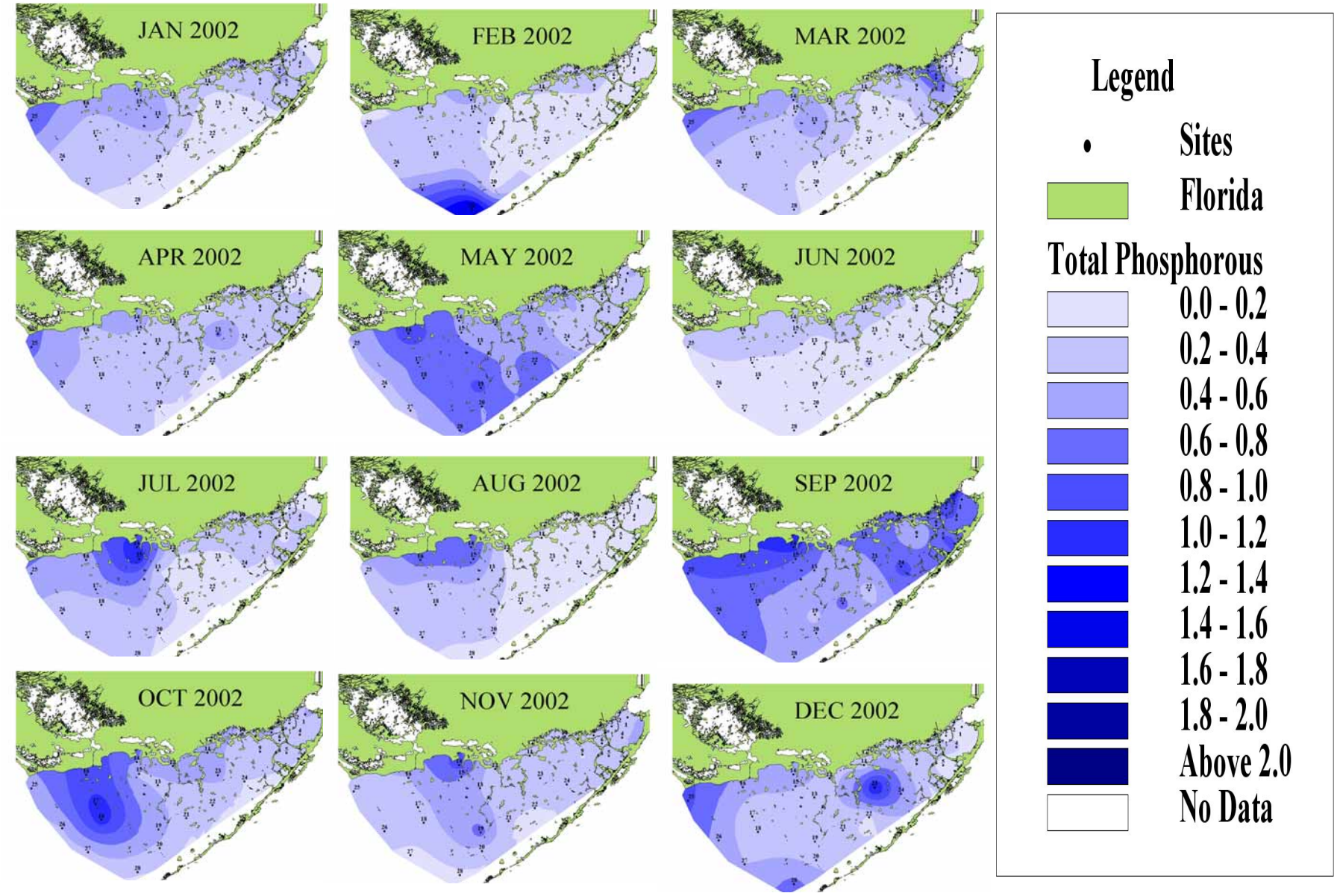

Figure 11b: Total Phosphorus concentrations (μM) across Florida Bay from January 2002 to December 2002.

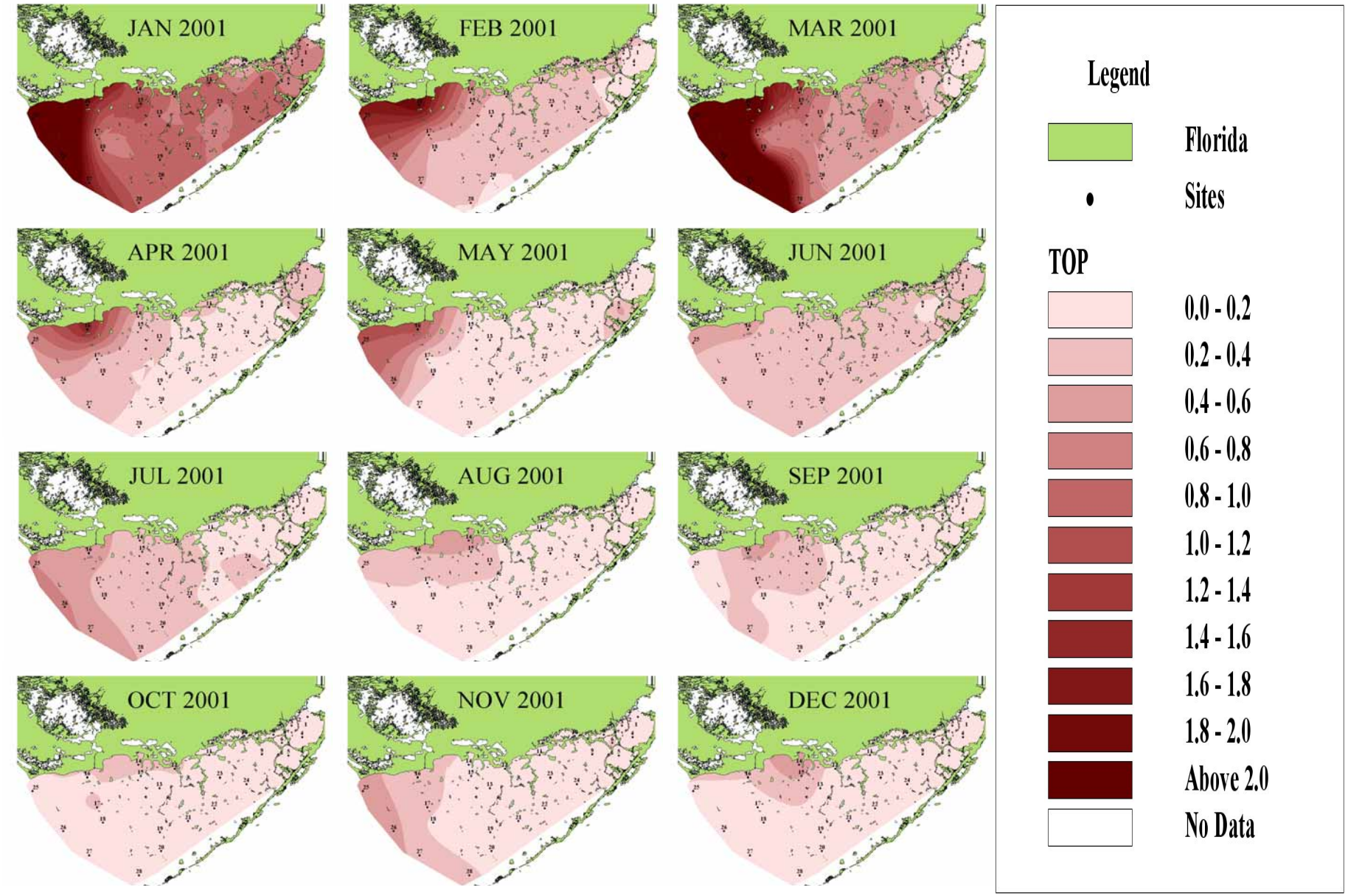

Figure 12a: Total Organic Phosphorus concentrations (μM) across Florida Bay from January 2001 to December 2001.

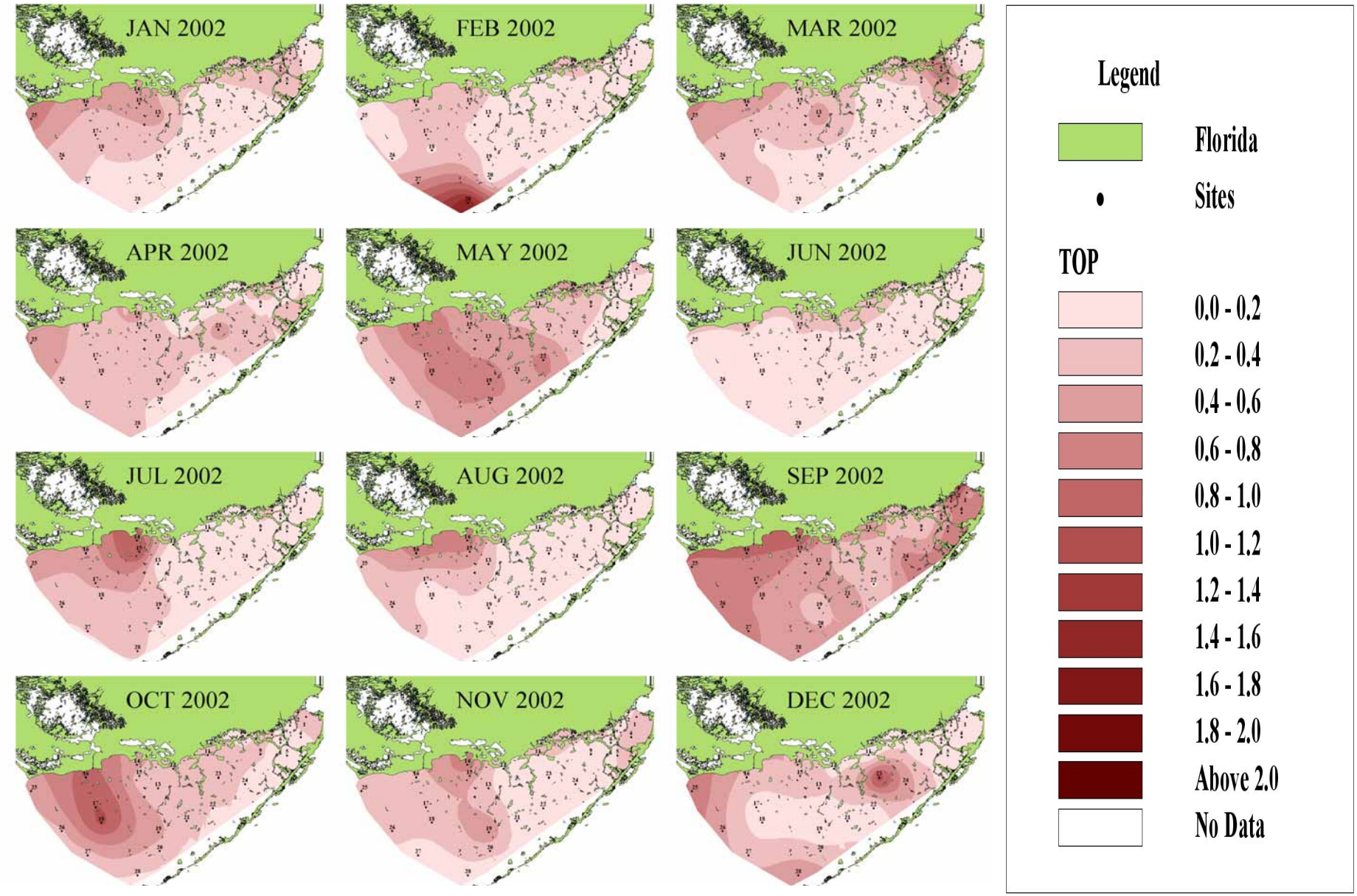

Figure 12b: Total Organic Phosphorus concentrations (μM) across Florida Bay for January 2002 to December 2002.

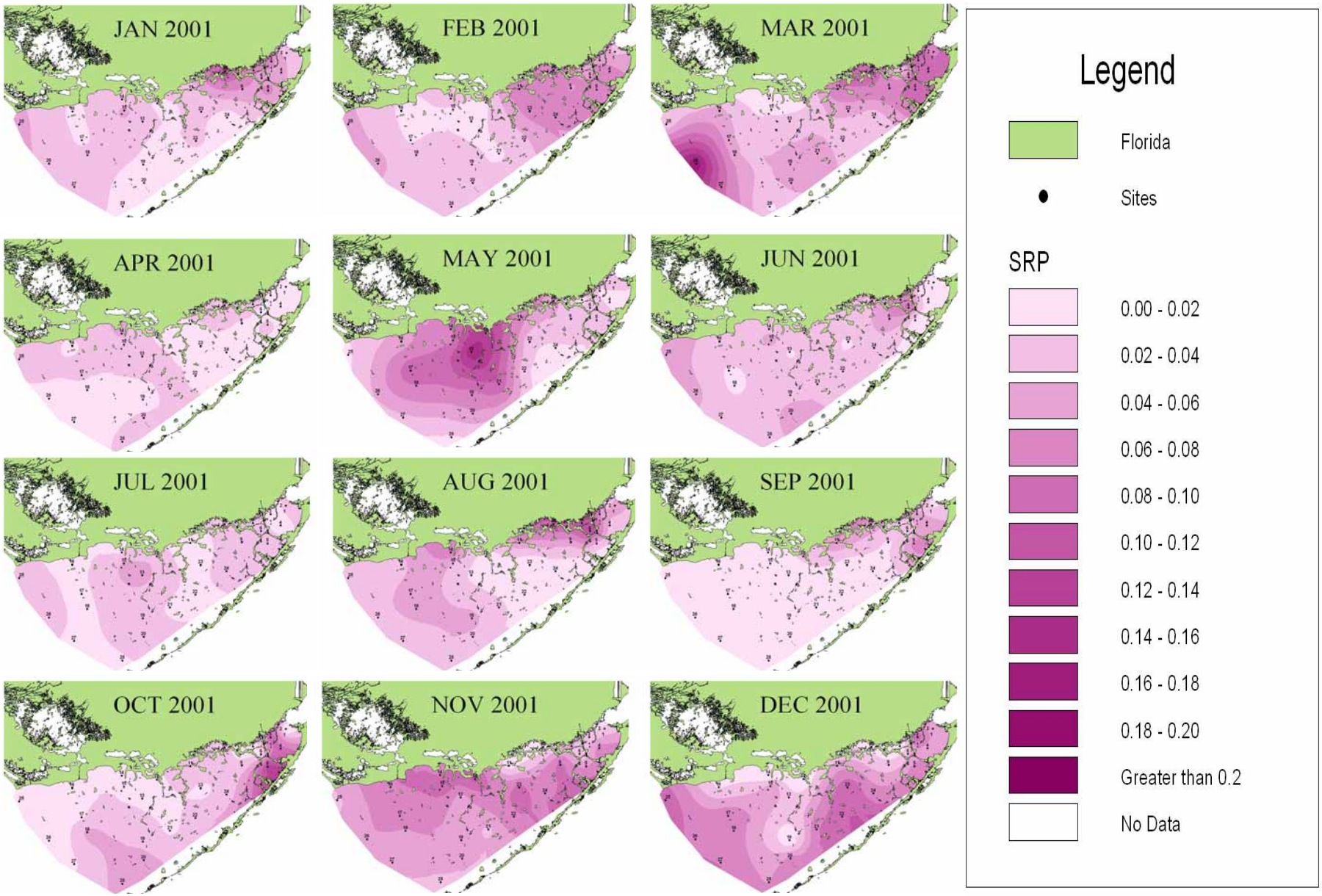

Figure 13a: Soluble reactive phosphorus concentrations (μM) across Florida Bay from January 2001 to December 2001.

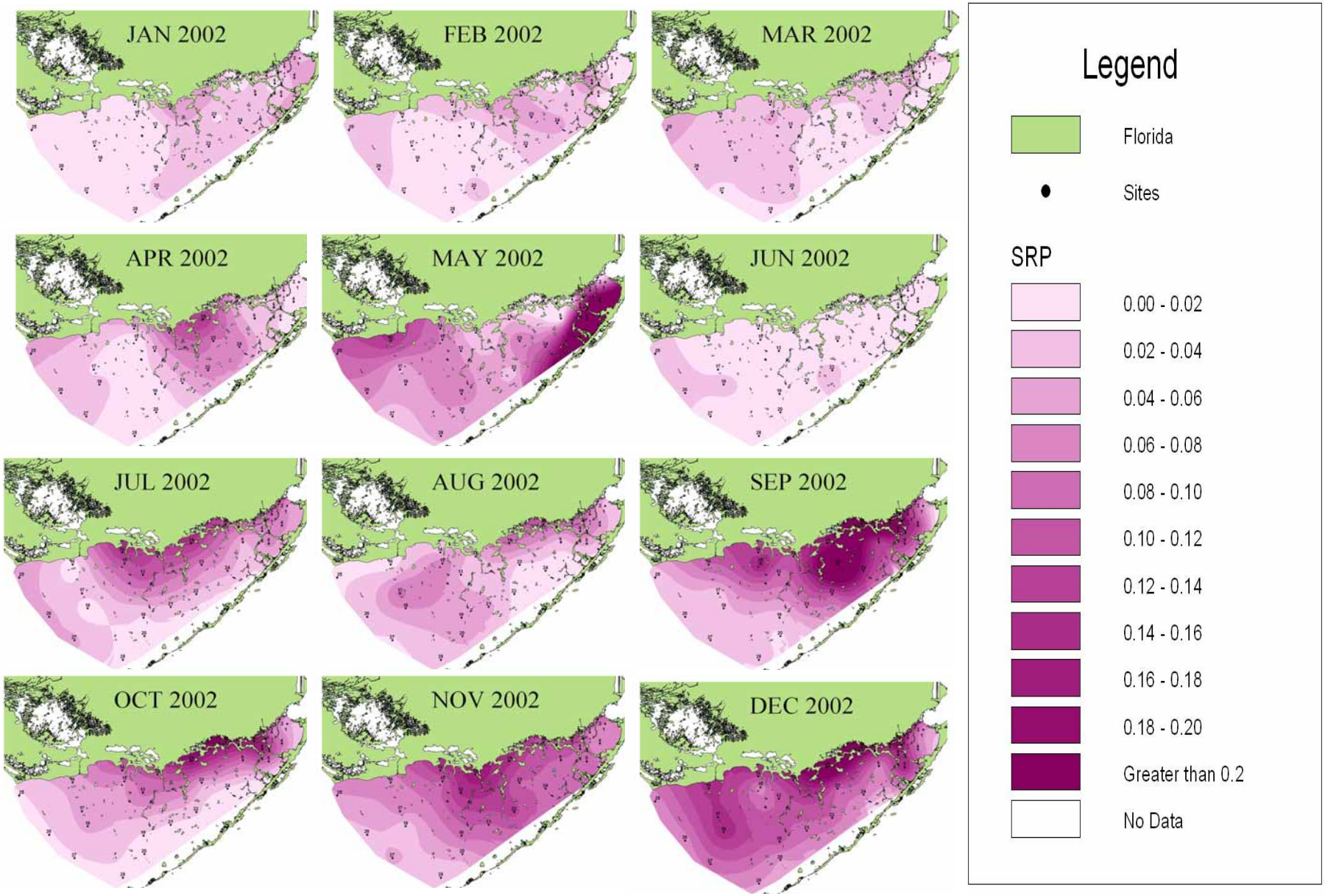

Figure 13b: Soluble reactive phosphorus concentrations (μM) across Florida Bay from January 2002 to December 2002.

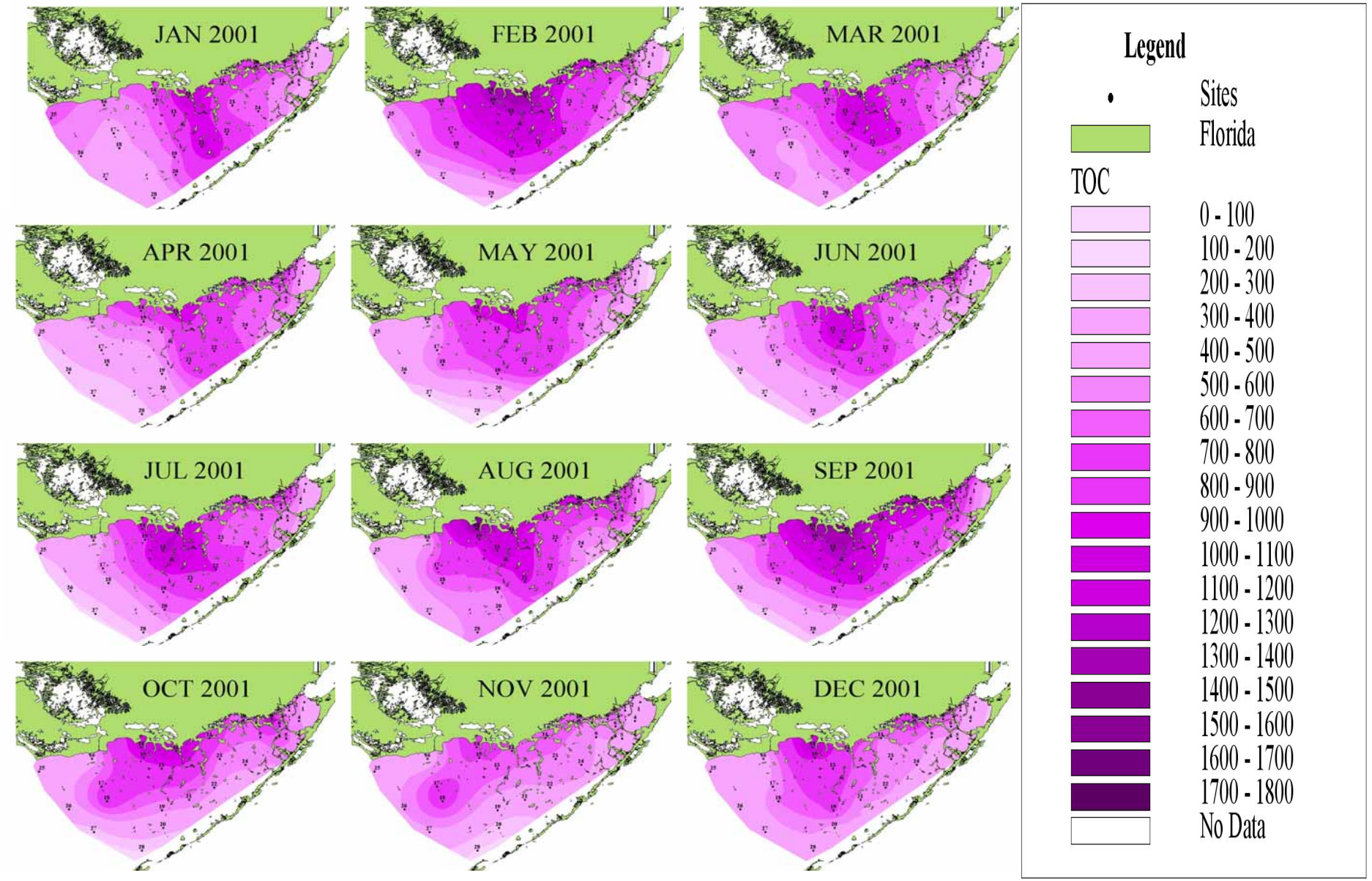

Figure 14a: Total Organic Carbon (μM) presented spatially across Florida Bay from January 2001 to December 2001.

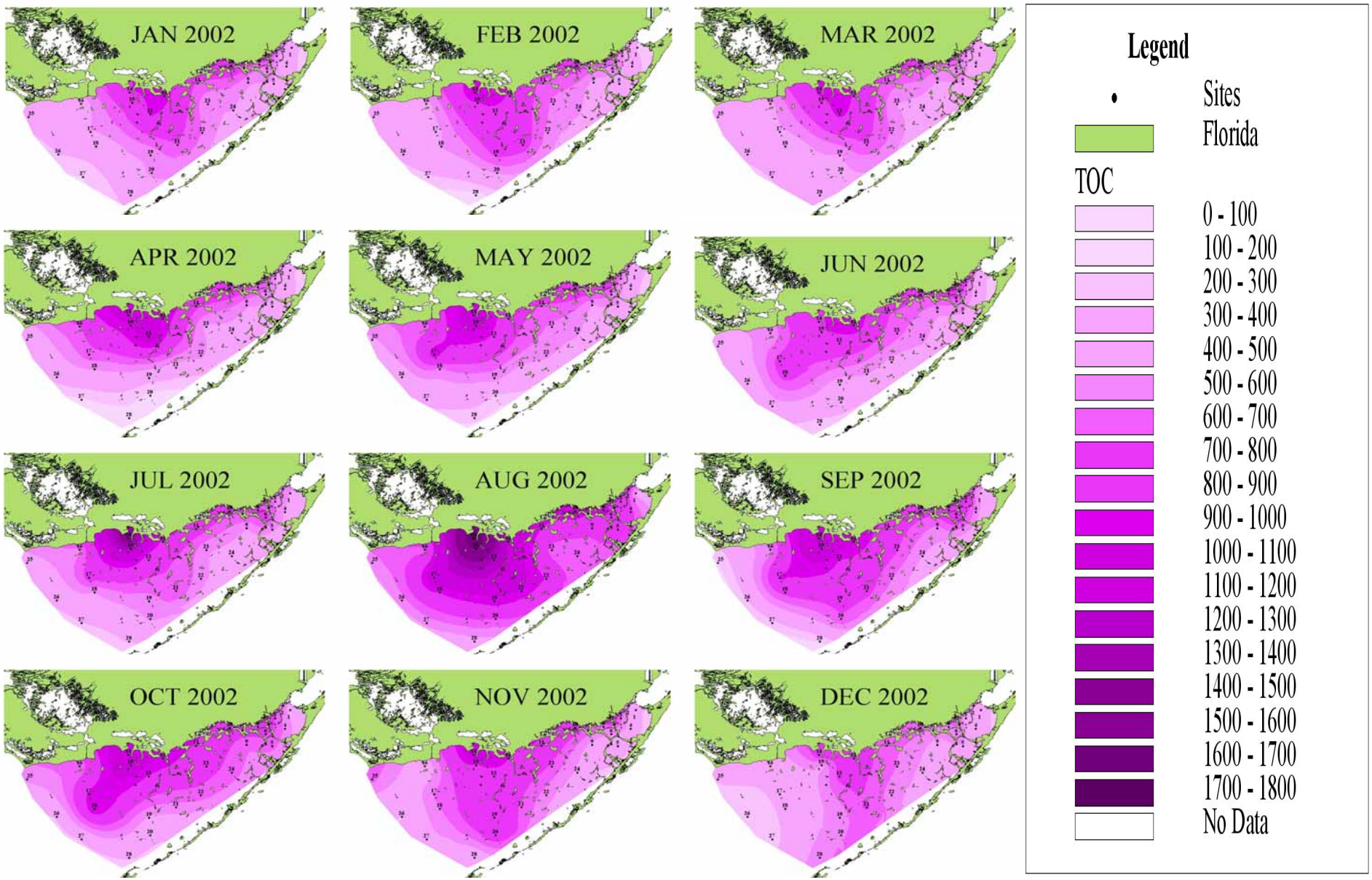

Figure 14b: Total Organic Carbon (μM) presented spatially across Florida Bay from January 2002 to December 2002.

Chlorophyll *a* - Two years of data of CHLA concentrations from standard acetone extraction techniques revealed both spatial and seasonal changes over the study period. Florida Bay CHLA concentrations were highest in the western and central Bay. The very highest concentration of CHLA we observed was in the middle of Florida Bay during the wet season of 2002. Sampling events of high relative concentrations occurred in January and June of 2001 (Figure 15a and Figure 15b). Highest concentrations occurred in July 2002 with the waters that mix mostly with the Gulf of Mexico. Throughout 2001 and with the exception of the late summer 2002, CHLA in western Florida Bay showed higher concentrations in surface water bordering with the Gulf of Mexico. These data illustrate that the algal community biomass of Florida Bay is strongly connected and coupled with both terrestrial Everglades and adjacent Gulf of Mexico waters. This results were further corroborated with the similar results we observed using PAM analyses for CHLA.

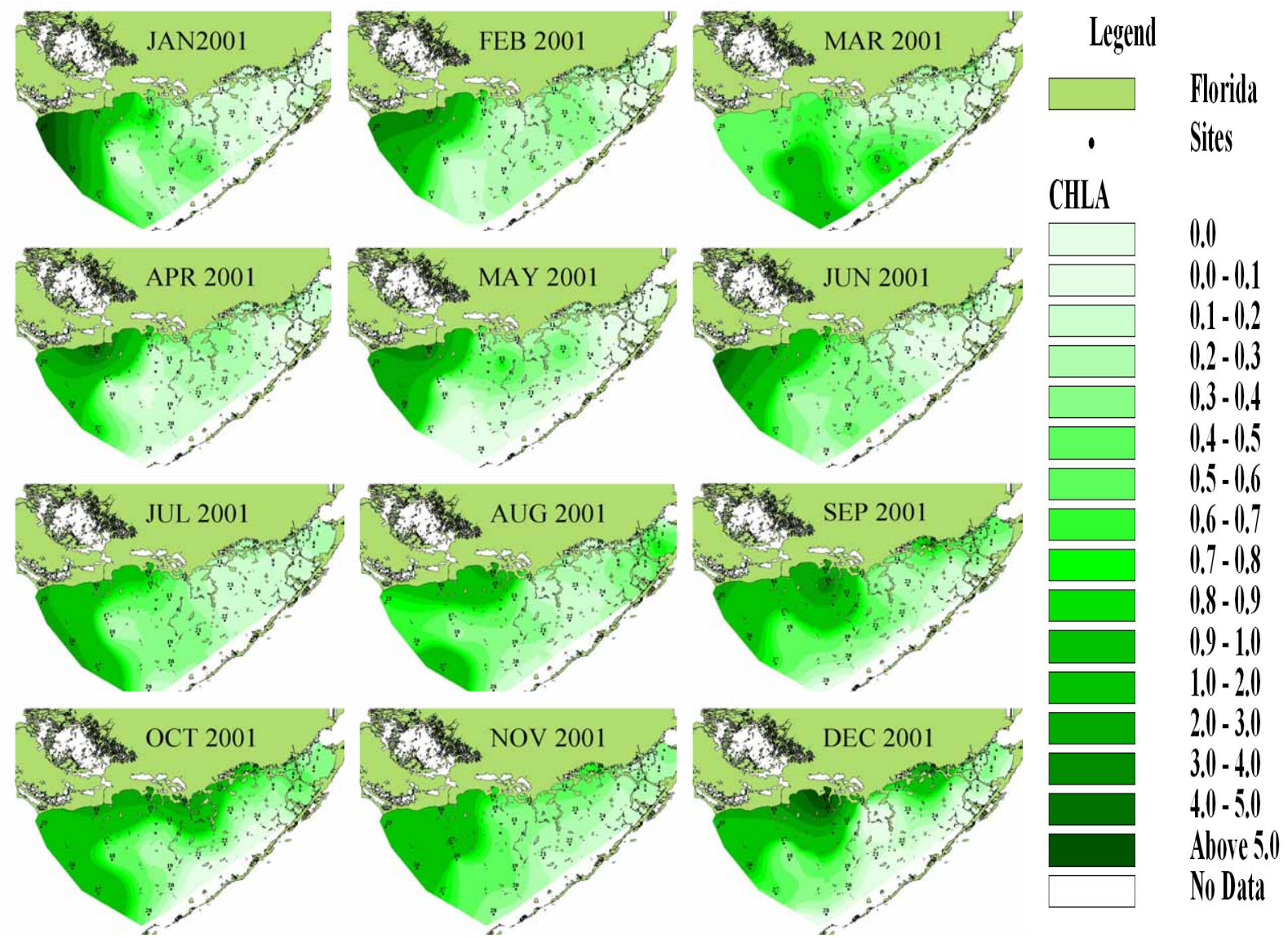

Figure 15a: CHLA concentrations (μg l^{-1}) (from acetone extractions) across Florida Bay from January 2001 to December 2001.

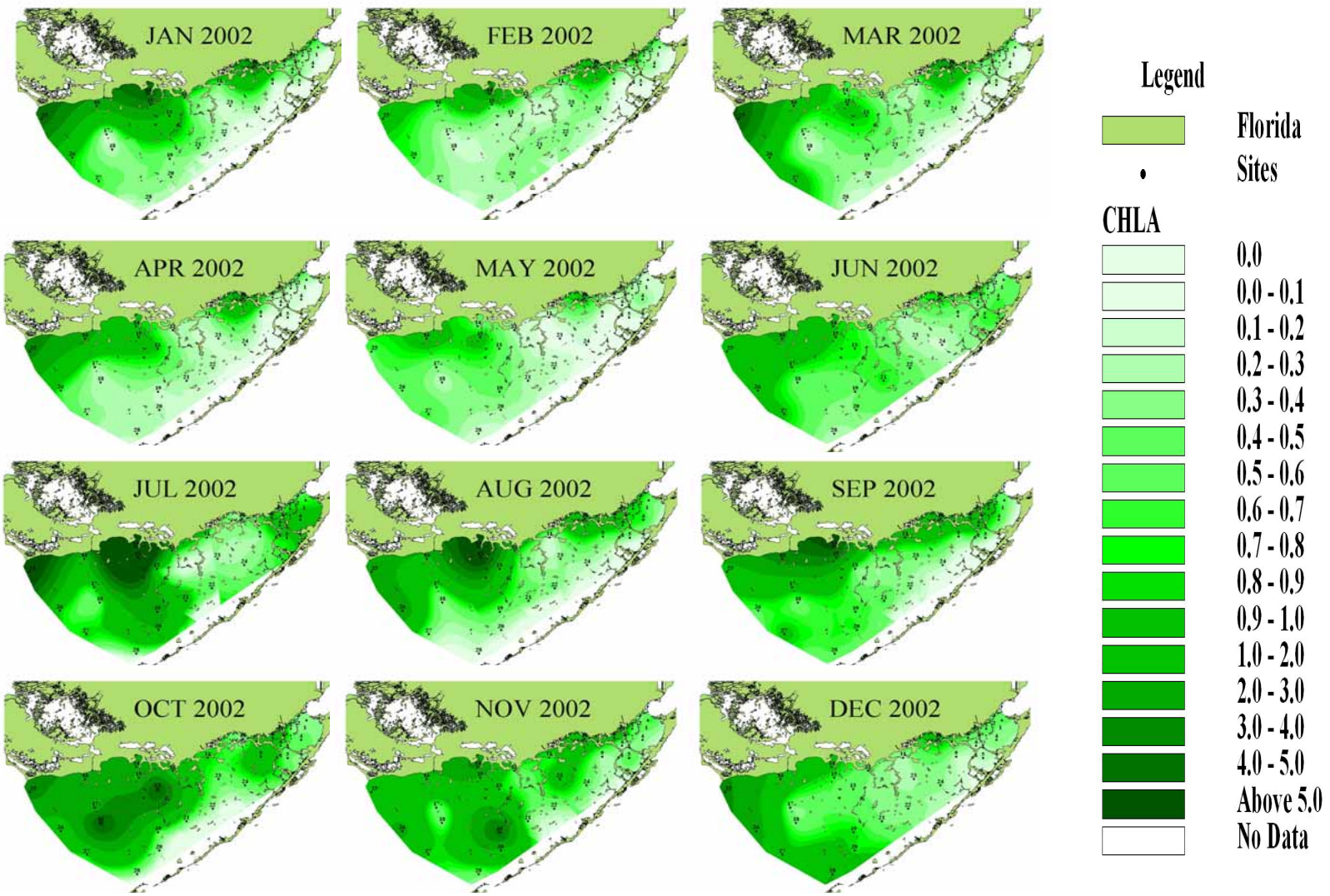

Figure 15b: CHLA concentrations (μg l^{-1}) (from acetone extractions) across Florida Bay from January 2002 to December 2002.

Bacteria Production - BP showed a general increase over the study period until December 2002 (Figure 16a and Figure 16b). The highest concentrations of bacteria production were in the central to western Bay. These productive regions are associated with terrestrial margins and primarily the south central to southwestern Everglades of Shark River Slough.

Bacteria Numbers - The overall abundance of BACT showed a higher concentration of bacteria in the north central and western area of Florida Bay (Figure 17a and Figure 17b). This result is likened to the pattern of higher abundances seen in algae, TOC and the TP during the dry season in 2001. Further, the results of the principle components analysis revealed the strong coupling of TP, algae and BACT in explaining a great portion of the variability in the entire data set over the study period.

CHLA Contribution from Cyanobacteria - Our spatial analyses of cyanobacteria in Florida Bay revealed that over the study period the highest concentrations of CHLA from this algal guild were in central Florida Bay (Figure 18a and Figure 18b). The one clear exception to this pattern was in October of 2002 when we observed higher concentrations of cyanobacteria in western Florida Bay. The period from January to June of 2001 exhibited very little contribution of cyanobacteria to the sum CHLA that we measured with the PAM analyses. Our spatial analyses of cyanobacteria revealed similar findings to the work of Lavrentyev et al. (1998) who also reported highest concentrations of cyanobacteria in western Florida Bay.

CHLA contribution from Green Algae - Green algae concentrations across Florida Bay over the study period were the lowest of the three algal guilds that we quantified using PAM analyses. In the majority of the spatial analyses there was no difference in the spatial distribution of green algae across the study area (Figure 19a and Figure 19b). These results suggest that the green algal component of CHLA has very little contribution to the microbial structure across the Bay during most of the year.

CHLA contribution from Brown Algae - Brown algal comprised the greatest portion of the total sum CHLA measured with PAM over the study period (Figure 20a and Figure 20b). Our spatial analyses of brown algae revealed highest concentration in the western portion of Florida Bay. These results are remarkably different from the spatial distribution of the other two guilds of CHLA (cyanobacteria and green algae) we measured with PAM.

Quantum Yield - Across the two-year study period a pattern of higher algal energetics, characterized by mean QY from PAM analysis, was found during what is typically known as the dry season. January and February of 2001 (Figure 21a) and November of 2002 (Figure 21b) are typified as dry season sampling points and the highest mean QY numbers were observed in these months. The spatial pattern of mean QY across Florida Bay revealed a fairly uniform magnitude across the Bay with the exception of the extreme northeast part of the Bay. These observations are further corroborated with the results of each of the individual spatial analyses of the guilds of algal data from PAM analyses.

CHLA from PAM Analyses - Concentration of CHLA measured by PAM analyses across Florida Bay for the study period revealed a spatial trend of greatest magnitude in the central and western regions of Florida Bay (Figure 22a and Figure 22b). This pattern was also concurrently observed with the standard acetone extraction method and there was a good regression (r^2= .88) over the study

period. The trends of spatial distribution did not always show the highest concentrations associated with the mangrove-freshwater land margins and freshwater Everglades derived material zone. Spatial and temporal patterns of both CHLA measurements were highly variable among site and season. In general the highest concentrations of CHLA were observed during the dry season in 2001 with March being the strongest signal with the widest coverage of higher concentrations. The pattern was just the opposite for the 2002 study period when highest concentrations of CHLA were observed in the mid and late wet season, July and October. These data suggest that the factors controlling the algal populations in Florida Bay are not best explained by season, or the derivation of freshwater from the mainland Everglades.

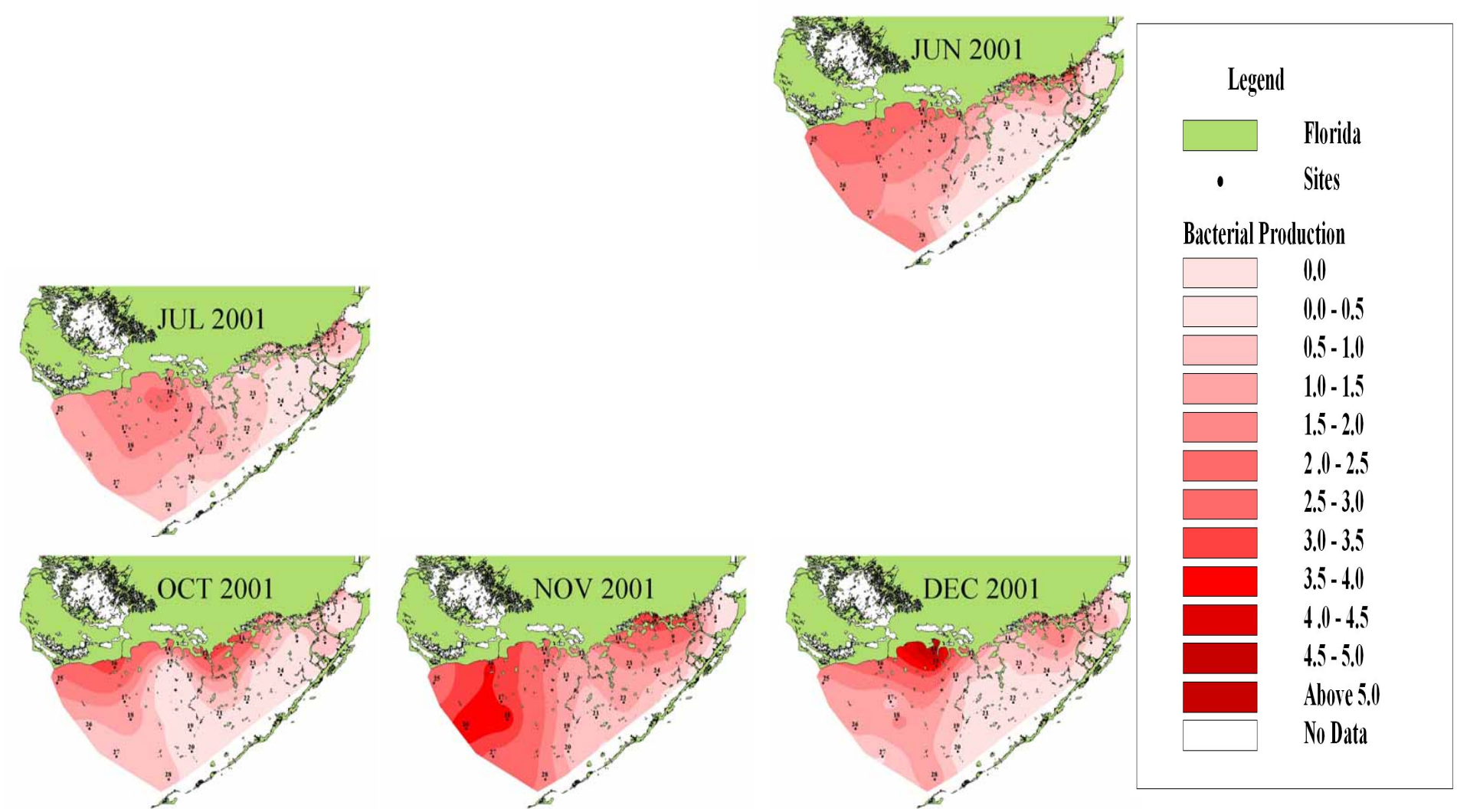

Figure 16a: Heterotrophic bacteria production (μg C l^{-1} d^{-1}) presented across Florida Bay from June 2001 to December 2001.

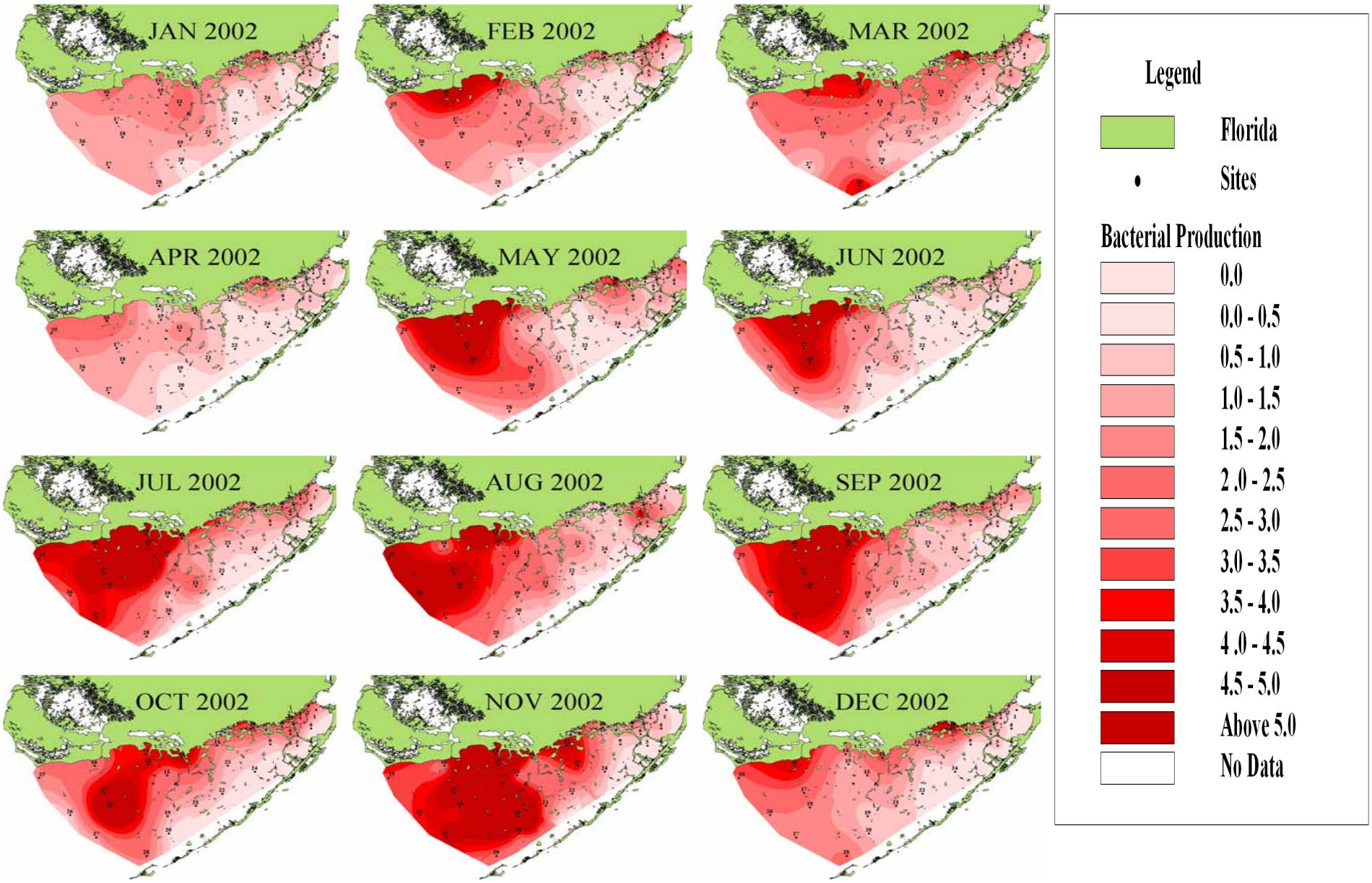

Figure 16b: Heterotrophic bacterial production (μg C l^{-1} d^{-1}) presented spatially across Florida Bay January 2002 to December 2002.

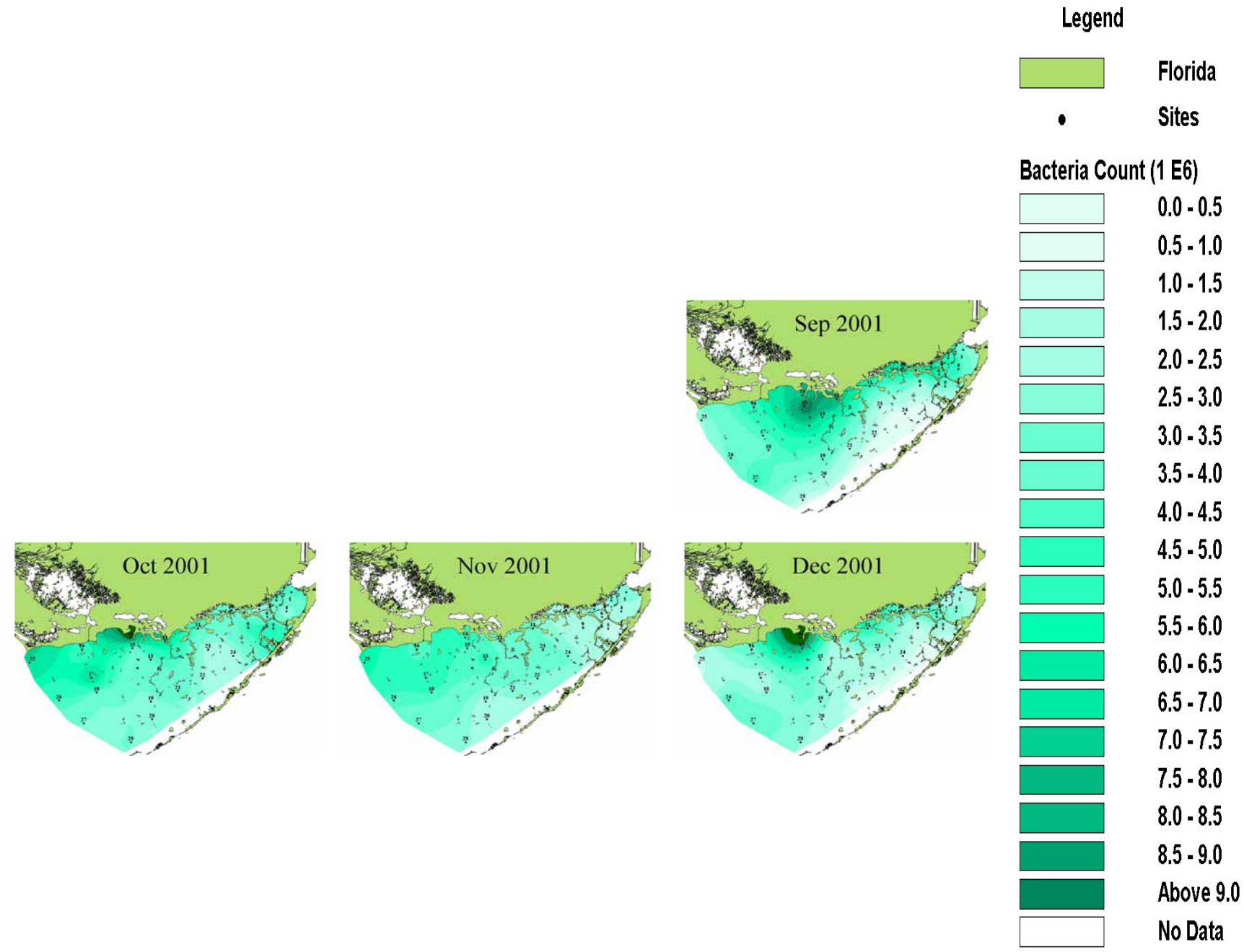

Figure 17a: Bacteria counts (10^6 cells ml^{-1}) using DAPI across Florida Bay from September 2001 to December 2001.

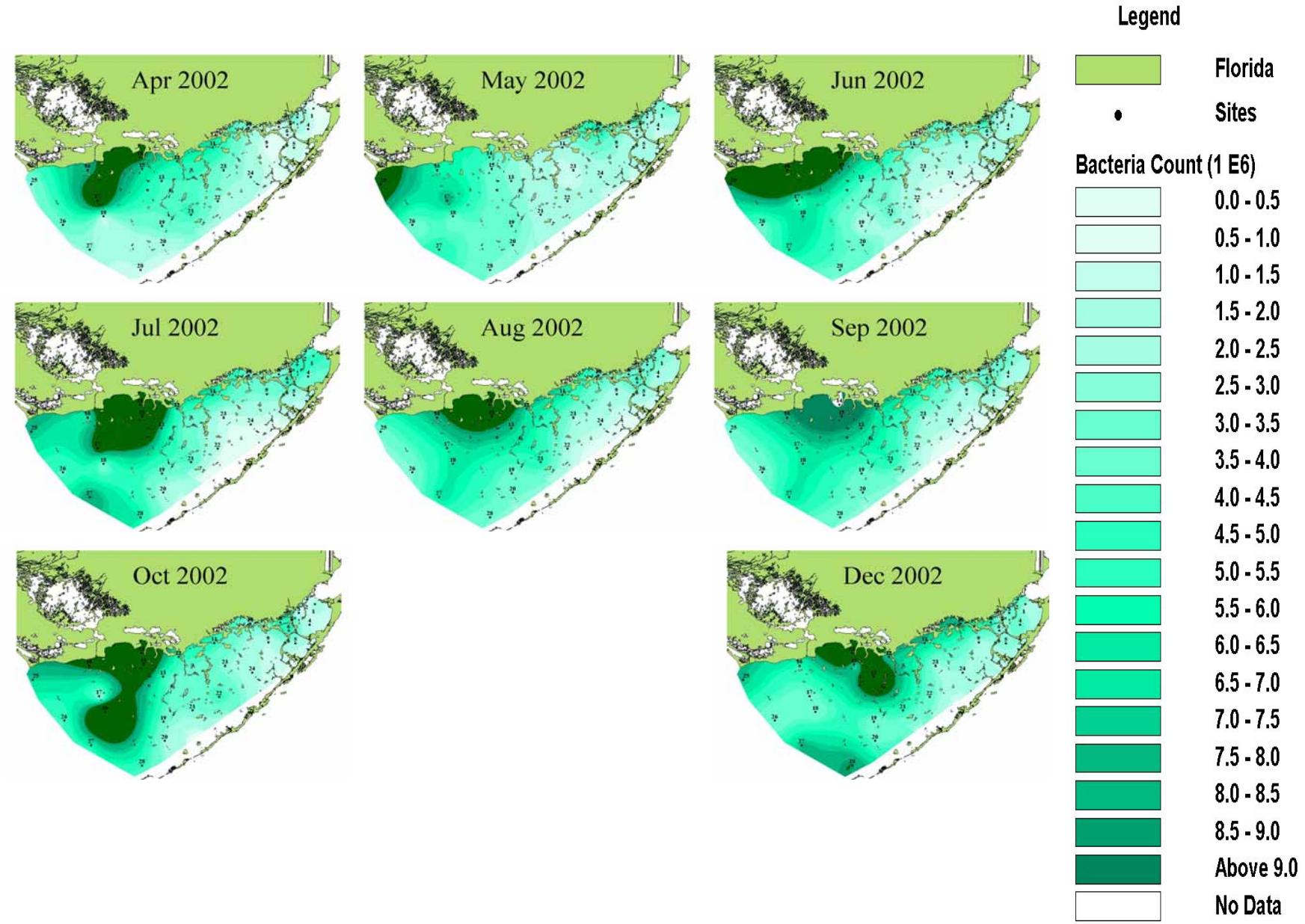

Figure 17b: Bacteria counts (10^6 cells ml^{-1}) using DAPI across Florida Bay from April 2002 to December 2002.

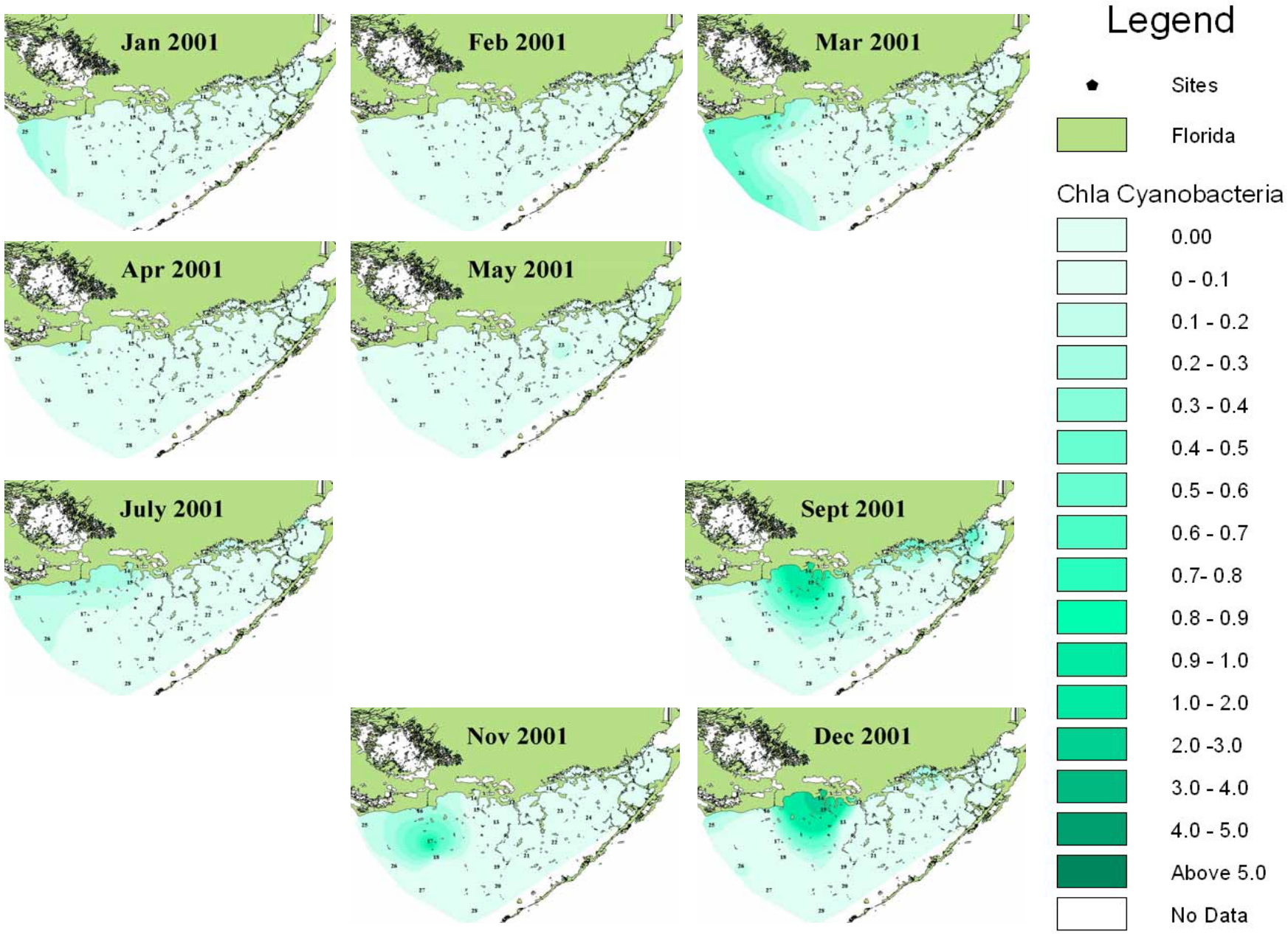

Figure 18a: CHLA cyanobacteria from PAM ($\mu g\ l^{-1}$) across Florida Bay from January 2001 to December 2001.

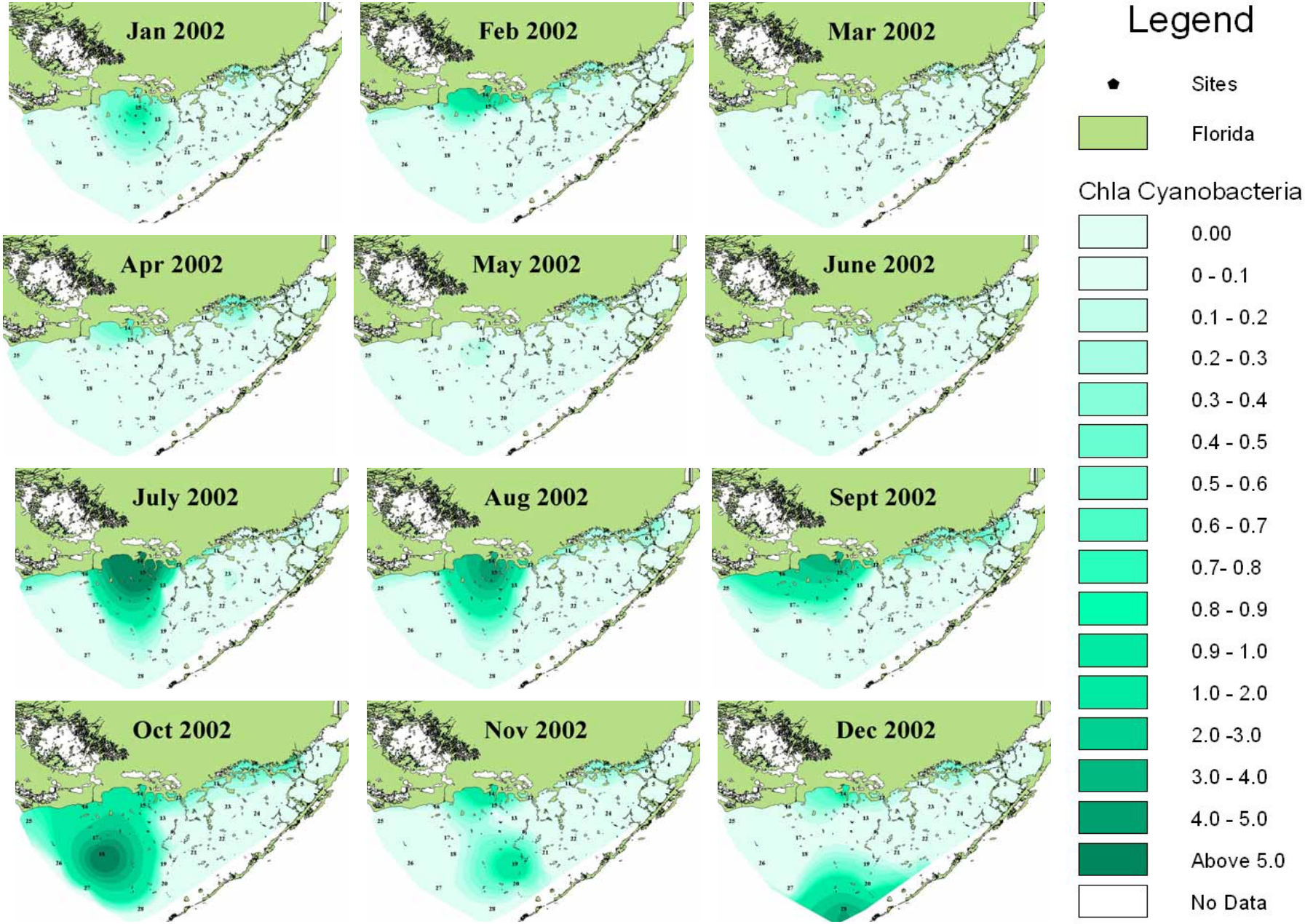

Figure 18b: CHLA cyanobacteria ($\mu g\ l^{-1}$) from PAM across Florida Bay from January 2002 to December 2002.

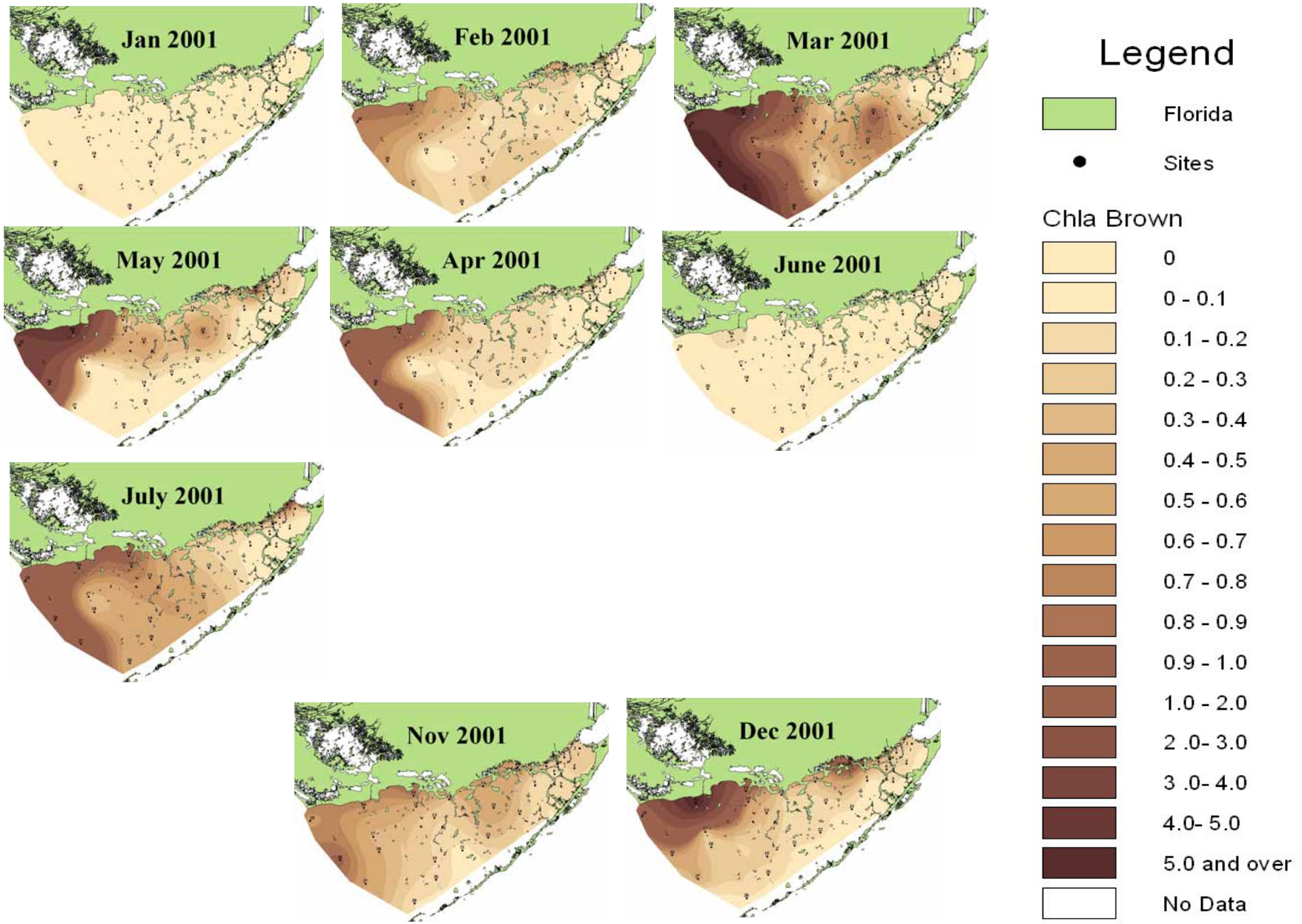

Figure 19a: CHLA brown from PAM (μg l^{-1}) across Florida Bay from January 2001 to December 2001.

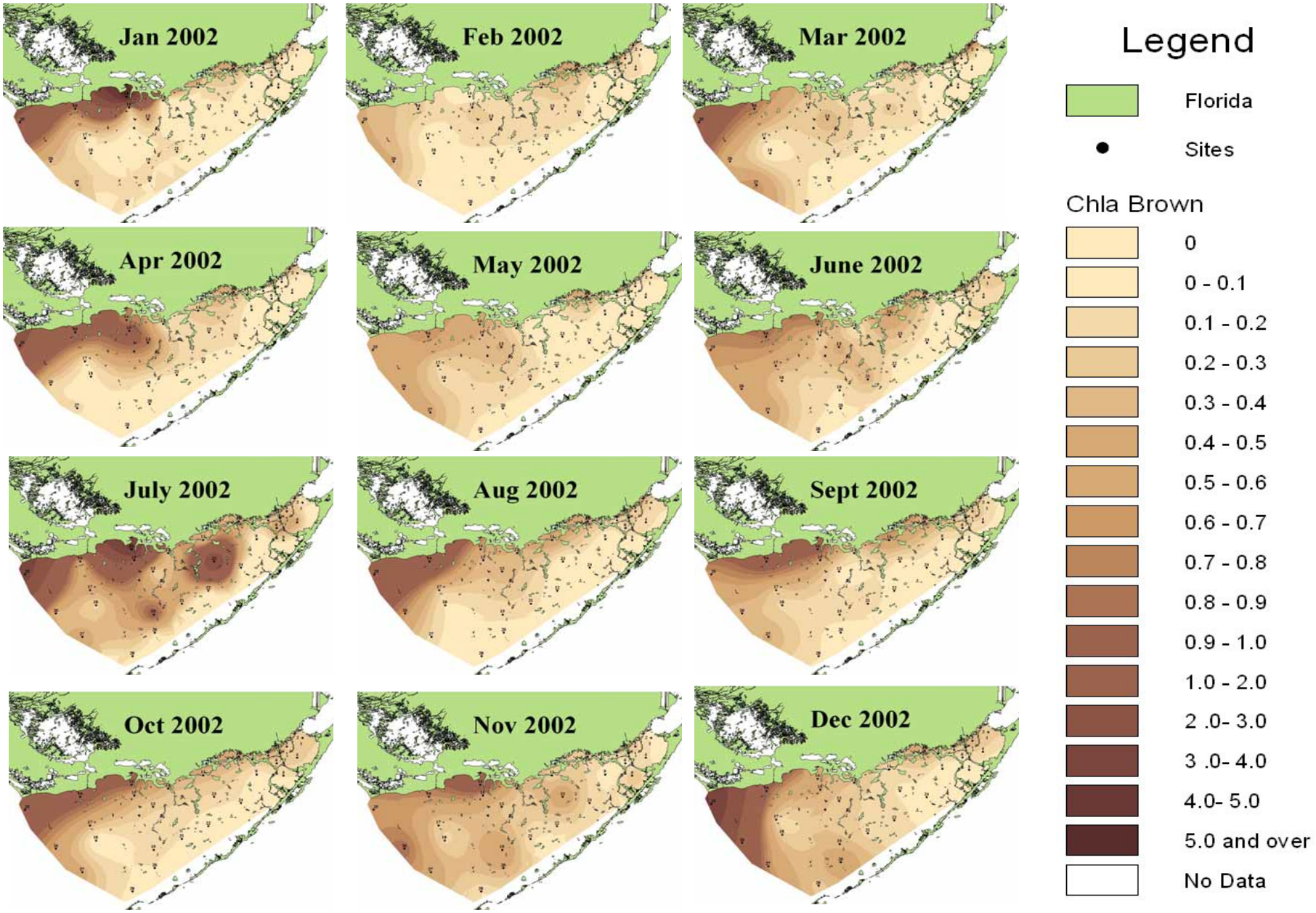

Figure 19b: CHLA brown from PAM (μg l^{-1}) across Florida Bay from January 2002 to December 2002.

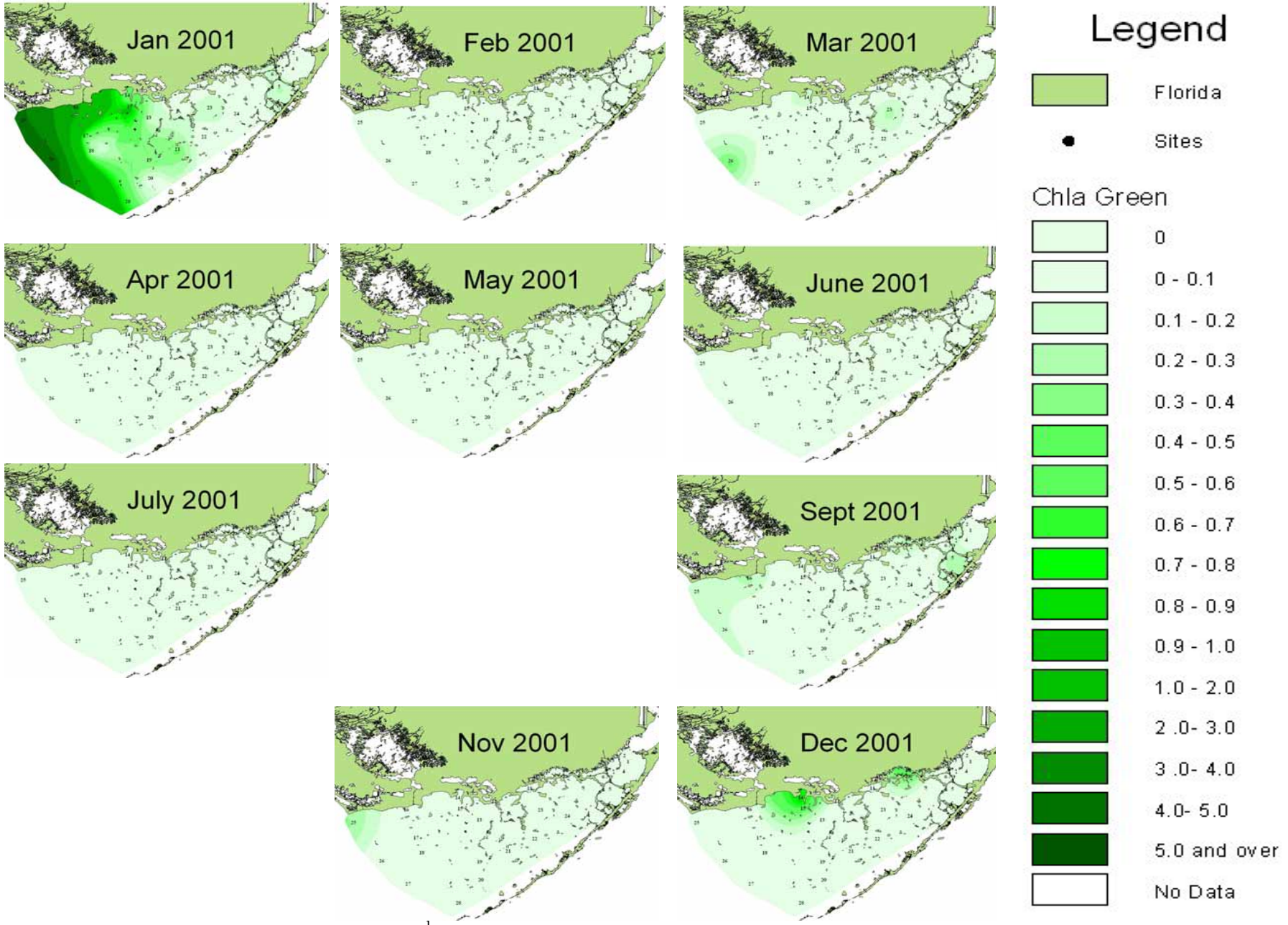

Figure 20a: CHLA Green from PAM ($\mu g\ l^{-1}$) across Florida Bay from January 2001 to December 2001.

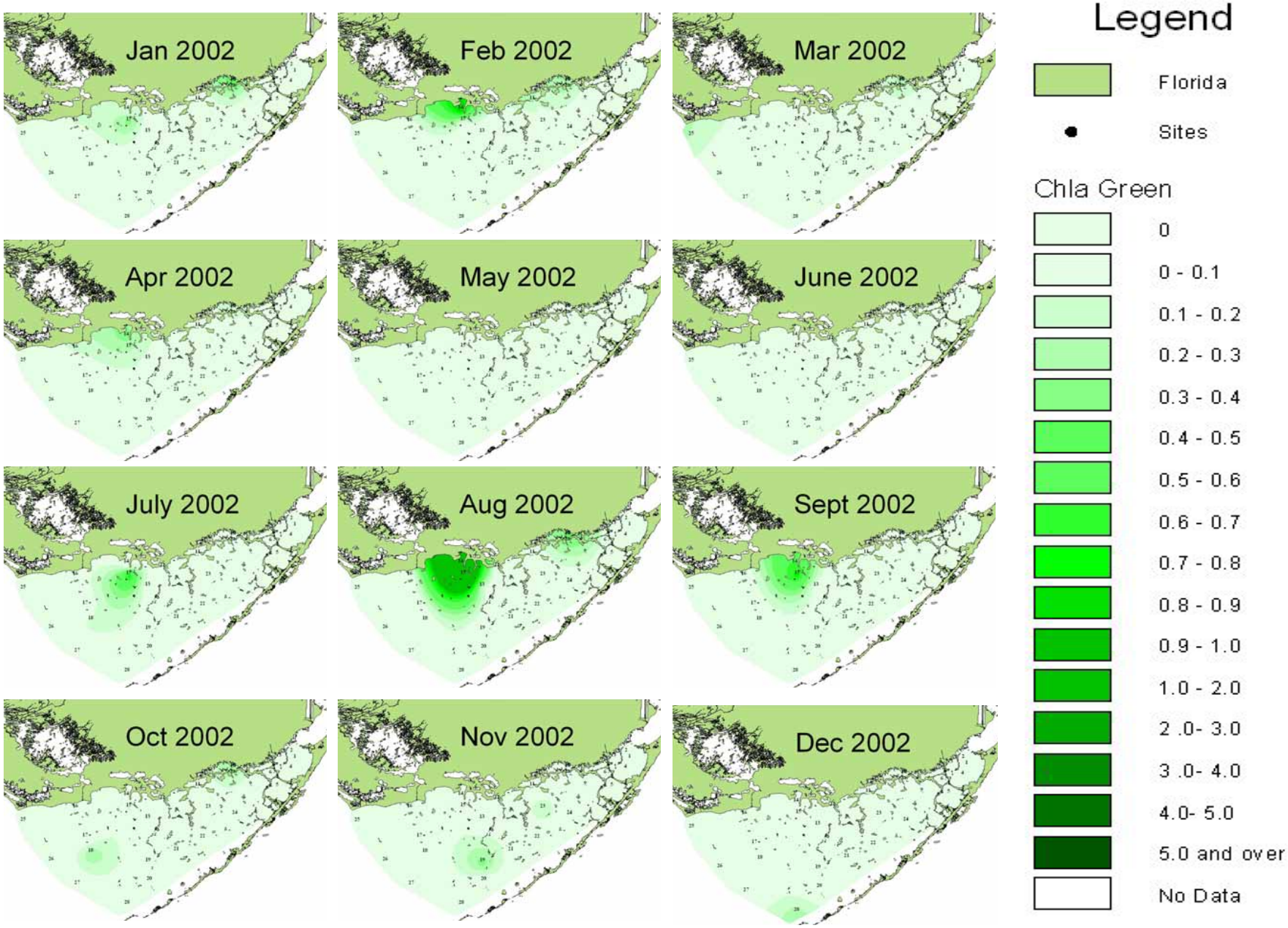

Figure 20b: CHLA Green from PAM ($\mu g\ l^{-1}$) across Florida Bay from January 2002 to December 2002.

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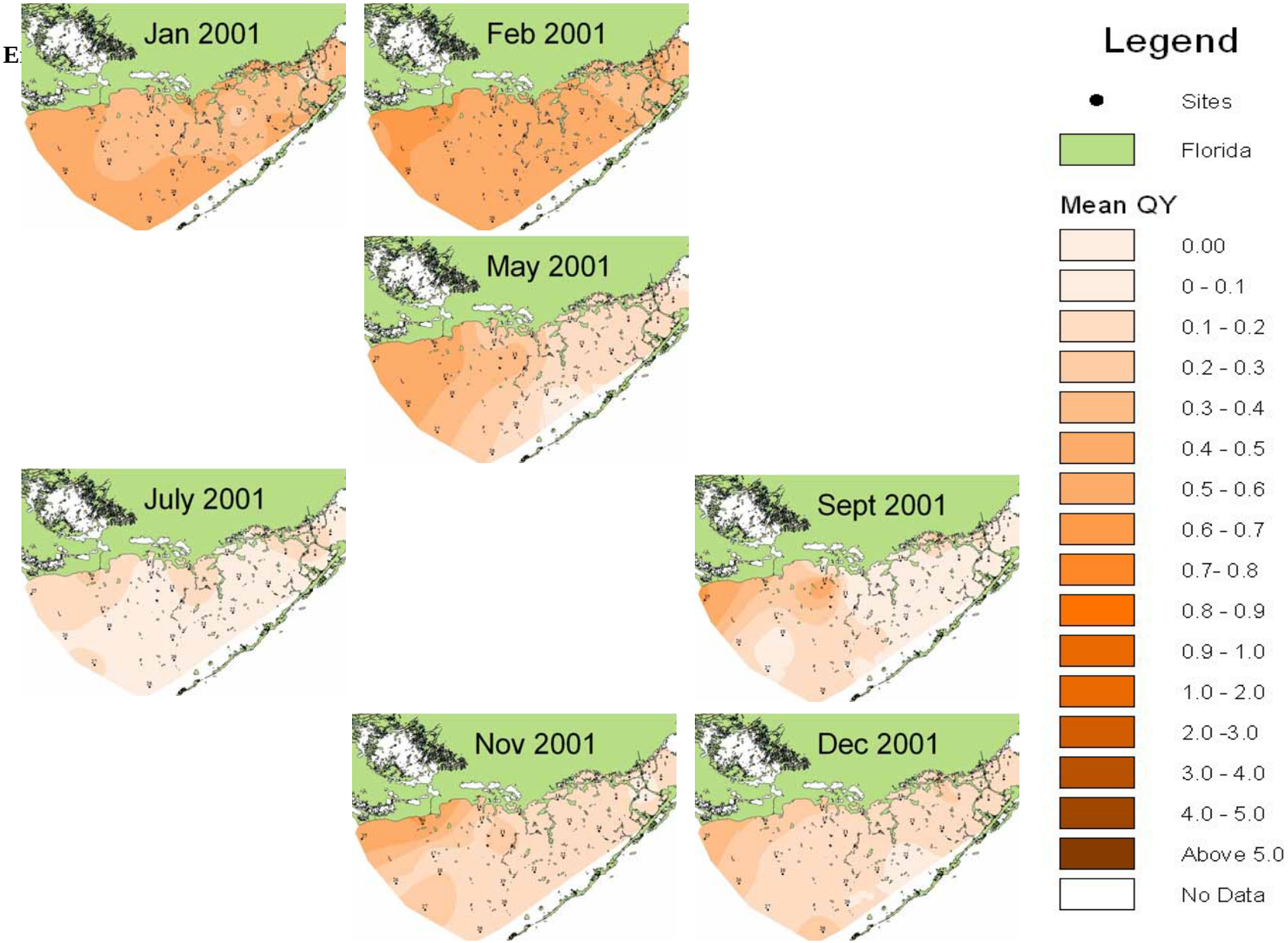

Figure 21a: Quantum Yield from PAM across Florida Bay January 2001 to December 2001.

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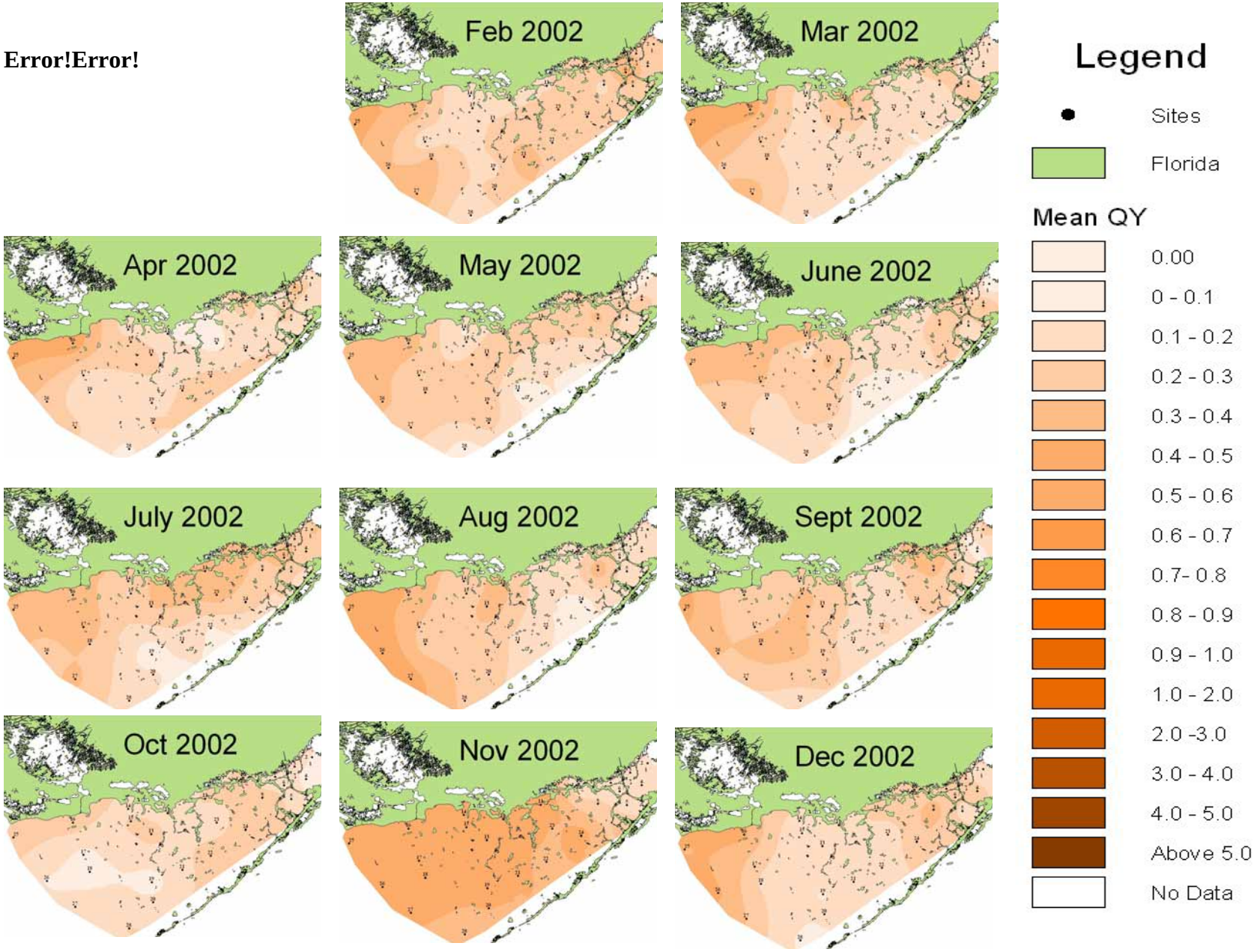

Figure 21b: Quantum Yield from PAM across Florida Bay January 2002 to December 2002.

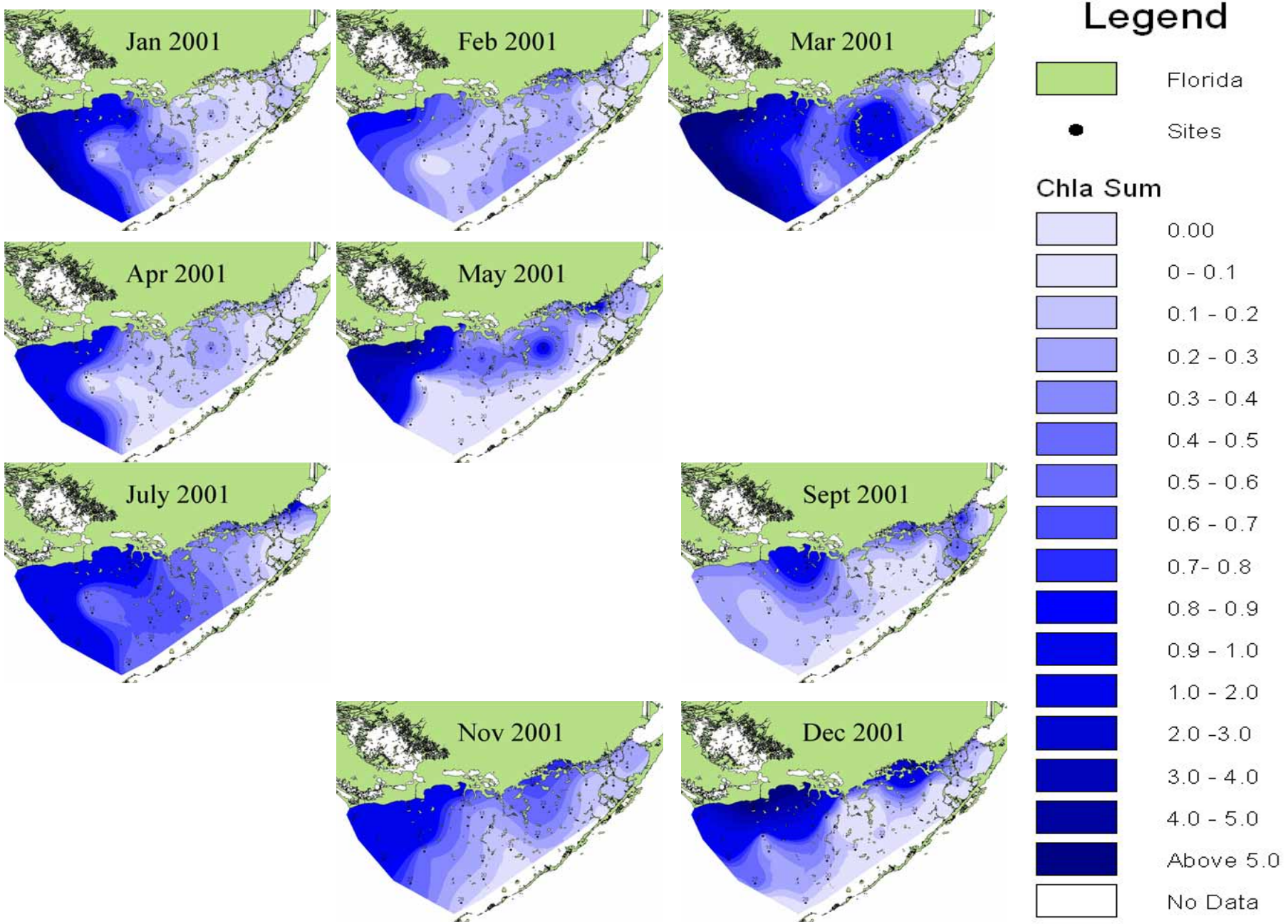

Figure 22a: CHLA ($\mu g\ l^{-1}$) from PAM analysis across Florida Bay from January 2001 to December 2001.

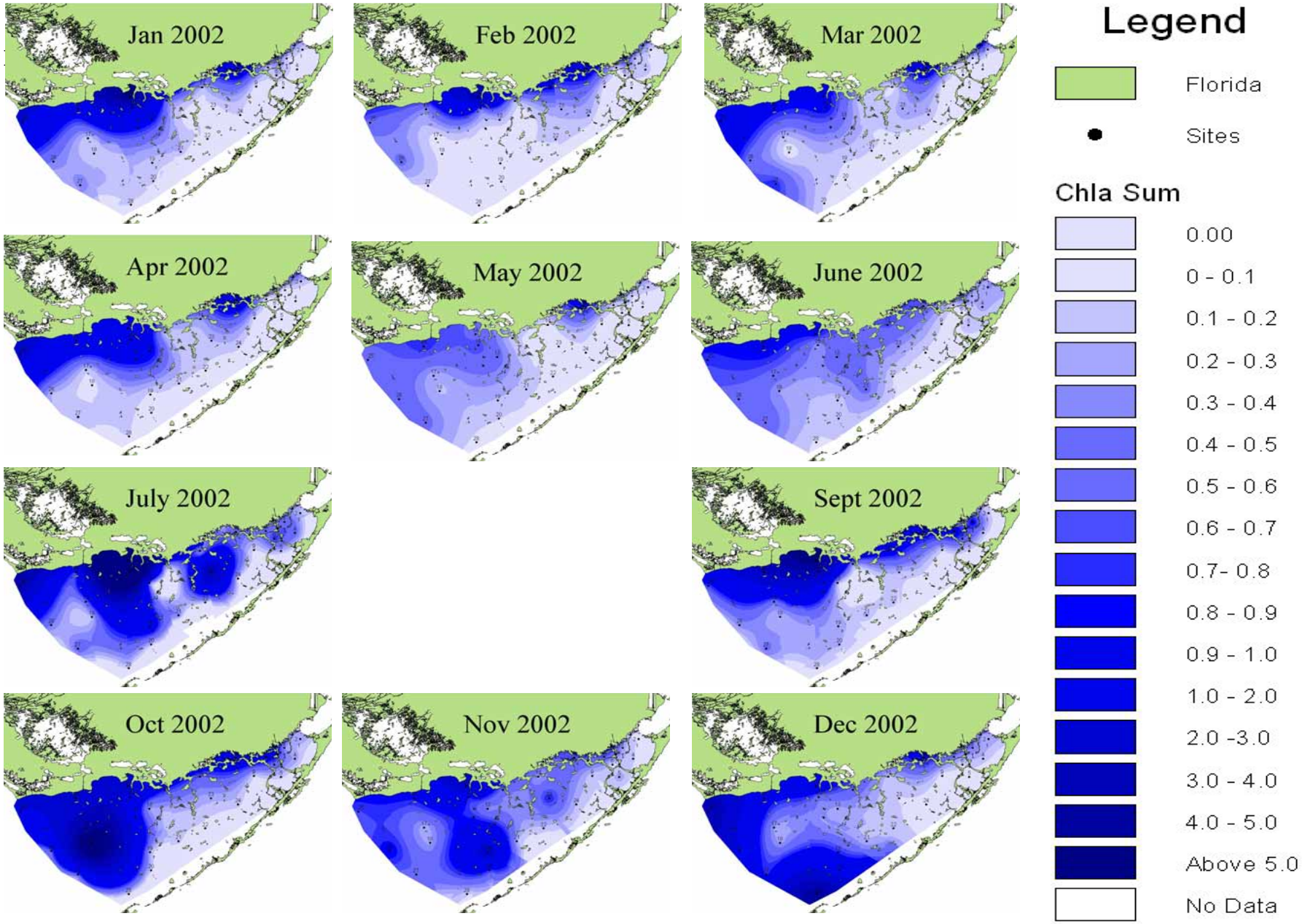

Figure 22b: CHLA (μg l^{-1}) from PAM analysis across Florida Bay from January 2002 to December 2002.

Hypothesis 1 Summary

We found that algal and bacterial dynamics and total phosphorus explained the majority of the variability in the biogeochemical data set across Florida Bay for the period from January 2001 to December 2002. The individual spatial analyses of each of the three groups of brown, green and cyanobacteria show that very low concentrations of algae are present in the northeast corner of Florida Bay. This portion of the Bay is the region where inorganic nitrogen components can be found at their highest concentration Bay-wide. These data suggest that there is little inorganic N limitation in the algal populations that we were observing over the study period. This finding was only evident through the combined analysis of all sites and all parameter across the entire study period. Data of this magnitude for Florida Bay biogeochemistry has not been available in the past for such a large-scale multivariate analysis. These data were collected at the beginning of what is the most spatially extensive wetland restoration to date for the Comprehensive Everglades Restoration Plan. One of the restoration goals of the Everglades Restoration is to deliver historical flow of freshwater to Florida Bay. Freshwater incursion to the Bay has been constrained through diversion over the past fifty years. Knowledge of water quality, biogeochemical dynamics and bioavailability changes to Florida Bay are a critical component to the restoration effects on Florida Bay.

Cyanobacteria blooms observed in Florida Bay in recent years have been a growing concern of the federal and state and municipal government, public and private organizations in South Florida. Sources of bloom formation have been postulated to result from increases in nutrient concentrations, changes in species composition and even seagrass die-off events in Florida Bay. We observed the highest concentration of cyanobacteria in the central Bay where we also observed the highest concentrations of green algae. Brown algae were in the highest concentration in the western Bay where we expected to see the cyanobacteria population blooms that had been described prior to this study. These data suggest that cyanobacterial blooms may be more associated with the organic nutrients of TON and TOC introduced into central Florida Bay from mainland Everglades.

<u>Research Hypothesis 2</u>: Under P limited conditions and high DOC concentrations, cyanobacteria are favored over eukaryotic algae by their greater cell-specific production of alkaline phosphatase.

Study Site

Four sites including Joe Bay, Duck Key, Bob Allen Key and Whipray Basin were selected from 28 sites in Florida Bay that have been continuously monitored each month for water quality trends over the last 13 years (Figure 23). These four sites have higher mean alkaline phosphatase activity (AP) and CHLA concentration relative to the other 24 sites in Florida Bay (Jones and Boyer 1999). Thus we expected to see the greatest response of microbial activity to increased substrate availability from these sites.

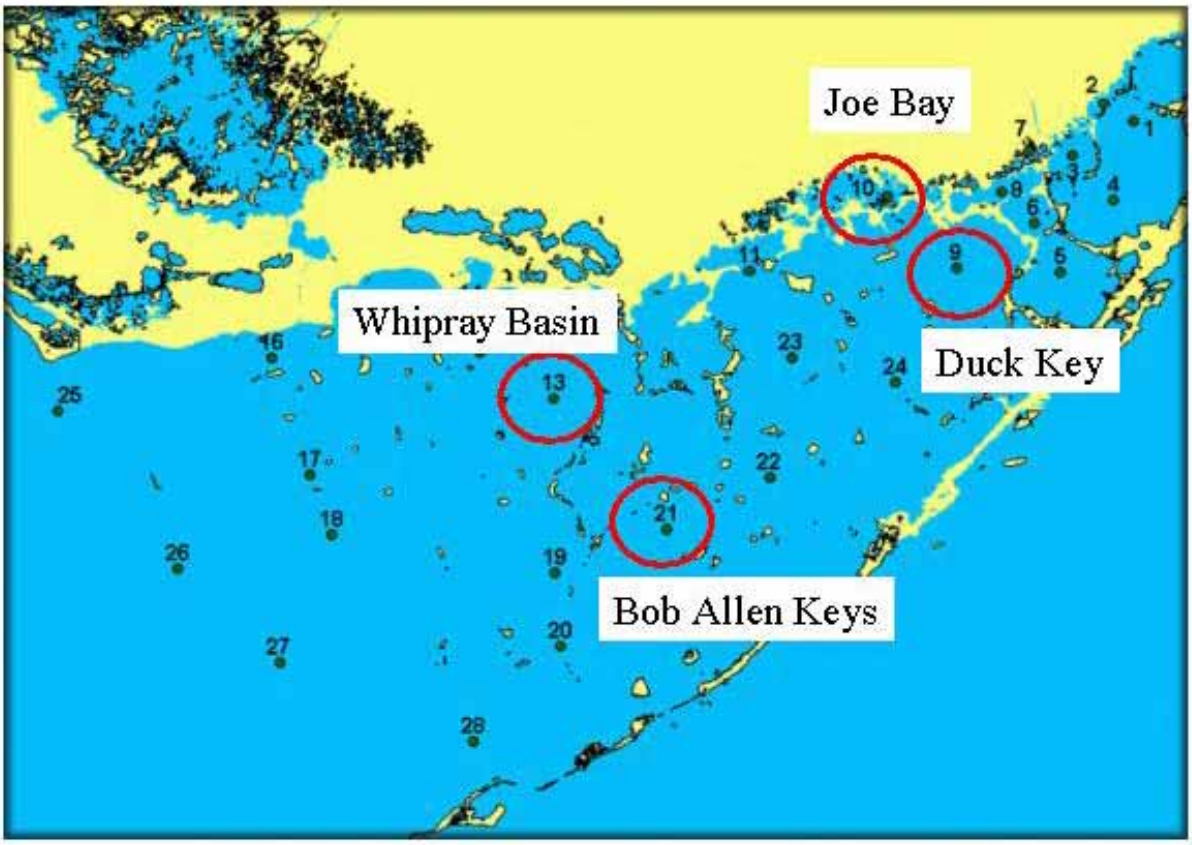

Figure 23: Map of the four Florida Bay study sites of bioavailability experiments from July 2001 to July 2002. Red circles denote the location of the four sites of Joe Bay and Duck Key in the eastern central Bay, and Whipray Basin and Bob Allen Keys of western Bay.

Methods

Bioavailability Assays (BDOC)

We sampled two sites in eastern and western Florida Bay central sites and two sites from the mangrove-Bay ecotone located in the center of the peninsula of the Everglades. We incubated water from Florida Bay to determine the effects of DOC and nutrients on the concentration and production levels of phytoplankton and bacterioplankton. For each incubation experiment water was collected from each of the four study sites. We conducted a total of five bioavailability incubation experiments from July 2001 to July 2002. Duplicate samples were collected in pre acid-washed, autoclaved distilled deionized (ADDI) water rinsed, 2.5L polycarbonate bottles. Bottles were rinsed with sample water three times immediately prior to sample collection. We sampled from a 10cm depth and kept bottles in the dark and on ice for transport. We conducted incubations with analyses to characterize the nutrients, microbial community, and DOC at each sample interval.

We expanded on our original experimental design to include further chemical and biological analyses on the water samples in latter incubation experiments. The first experiment was dark incubation with a nutrient treatment, using the same bottles and sites that we continued to sample throughout the study period. The second experiment was more comprehensive and included a DOC amendment treatment for light and dark bottles. Concurrently we combined this treatment with a nutrient treatment. In the second experiment we conducted bacteria production incubations and determination, but we had not yet switched to our DAPI bacteria count method. Experiments 3, 4 and 5 were conducted in the same manner using the following methodology for each. Treatments of DOC enrichment, nitrogen and phosphorus enrichment, and a control were applied in both the light and dark in the last three incubation experiments. Incubations were conducted in 2.5L polycarbonate bottles, clear for light treatments and wrapped with aluminum foil tape for dark treatments. Bottles were incubated by floating on a line in the FIU campus lake to expose the incubating water to ambient light, temperature, and wave action. Nutrient treatments were conducted by amending a measured amount of sample water with stock solutions of NH_4NO_3 and KH_2PO_4 with N and P enrichment concentrations of 10 μM and 1 μM, respectively. DOC treatments were conducted by concentrating water by tangential flow fractionation filtration (TFF) with a Millipore Pellican-2 system with a 1 KDalton pore diameter membrane. Using this method, particles smaller than the membrane pore size are discarded in the wastewater and only particles larger than 1 Kdalton are retained. For the second incubation experiment, DOC concentrate was obtained from Shark River (Florida Coastal Everglades (FCE) LTER site SRS 4), while the water for the DOC concentrate for the third, fourth and fifth experiments, was collected from Taylor Slough (FCE LTER site TS/Ph 6 or Argyle Henry). During the TFF process we concentrated the DOC to a final concentrate that was from 8 to 12 times the original DOC concentration. We dispensed the DOC concentrate to DOC treatment bottles to a final concentration of 2 times the original level by volume. Control bottles were measured and poured in the same technique as the enriched treatments but otherwise left unaltered.

Nutrient analyses

Samples were collected from the incubation bottles on 1, 3, 5, and 10 days after treatment with the variation of the third and fifth experiments being sampled on day 6 instead of day 5. For the last 3 experiments, bottles were also sampled at time 0, directly after treatment. The sampling

procedure consisted of collecting water from the incubation bottles in sterile, amber HDPE bottle for transport. Incubation bottles were homogenized by gentle inversion 3 times and the transport bottles were rinsed with sample water three times prior to filling. Immediately following sampling water was prepped for analyses. All nutrient analyses were conducted as described in the testing of Research Hypothesis 1 in the previous section.

Dissolved Organic Matter Characterization

At each sampling we collected 20 ml of water to conduct DOC characterization. We filtered the sample though a Whatman GF/F then conducted analyses to determine fluorescence emission and synchronous fluorescence analyses. All samples were processed within 7 days of collection. We used a Perkin Elmer LS50B spectrofluorometer with a 150 W Xenon light source to analyses 3ml aliquots of sample. Samples were first run for UV-visible data that were used to correct the data we collected for fluorescence intensity, maximum wavelength and the fluorescence indices (Jaffe et al., 2003).

Results and Discussion

Bioavailability of DOC

Bioavailability at each of the four study sites was variable across time, space and treatment. Overall TOC production in light versus dark incubations revealed their mean trophic status was net C utilization rather than C production (Figure 24). These findings suggest the algae that are associated with observed blooms in Florida Bay are codependent with the bacteria and heterotrophic microbial community. Further, results of our treatments suggest that the Florida Bay microbial community responds to both inorganic N and P enrichment and organic enrichment.

There was a significant treatment response on bioavailability of DOC in both the nutrient and DOC amendments. Our experiments revealed both algal and bacterial parameters were affected by each treatment. Our findings demonstrated the water column microbial population is overall heterotrophic but that there were specific responses by each of the algal groups. There was a response in the percent bioavailability to DOC enrichments that exceeded inorganic nutrient amendment that was exceeded in some incubation experiments by the response to the DOC amendment (Tables 2, 3, and 4). We also saw a greater response of higher bioavailability of carbon across treatments occurred in the western central Bay sites (Tables 2, 3, and 4). When we tested our bioavailability responses between sites with a t test we found there was only a significant difference between the Duck Key study site in the eastern central Bay and the western central Whipray Basin site. We also saw more evidence of the same pattern in a trend between Duck Key and the other western study site of Bob Allen Keys. Our combined seasonal incubation experiments also indicated that the Florida Bay microbial population responded more in the wet season with greater bioavailability of DOC than in the dry season.

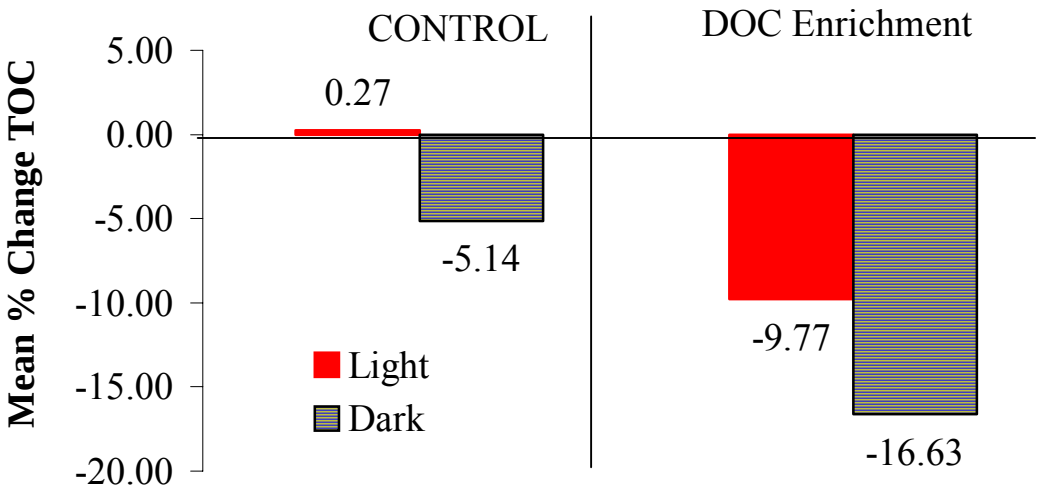

Figure 24: Bioavailability of total organic carbon shown as Mean % change from the December 2001, May 2002, and July 2002 experiments.

Data were fitted to a one phase exponential decay equation (Figure 25) where X is time, and Y is DOC concentration. Y starts out equal to Span+Plateau and decreases to Plateau with a rate constant K (d^{-1}) (Table 2a-c, Table 3a-c and Table 4a-c). Potentially bioavailable DOC (%BDOC) was calculated as Span/(Plateau+Span)*100. We were able to use the dark bottle incubation data from the third, fourth and fifth experiments for these analyses.

$$Y = Span * e^{-K*t} + Plateau$$

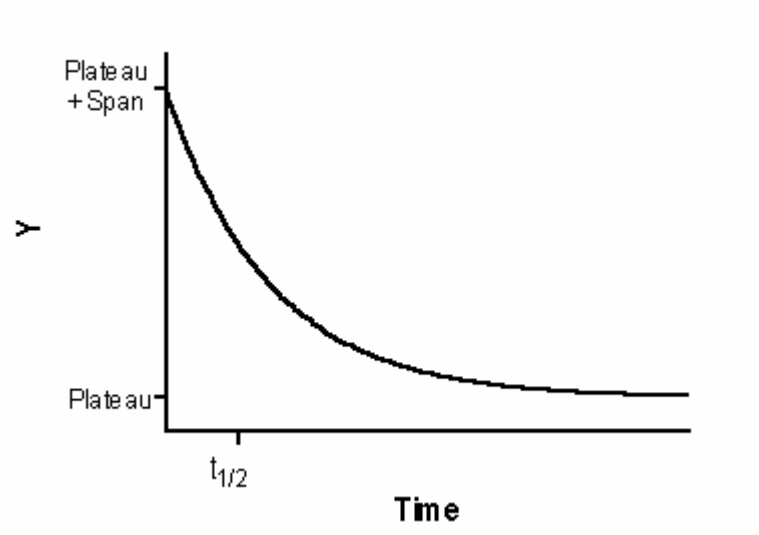

Figure 25: Example of one phase exponential decay equation.

Table 2a: One phase exponential fits for the third water column incubation experiment for the control treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	19.0	14.2	26.5	13.8
K (d^{-1})	1.07	0.82	0.37	0.29
Plateau (µM)	516.8	810.0	821.8	548.4
Half Life (d)	0.65	0.85	1.86	2.38
%BDOC	**3.8**	**1.7**	**3.1**	**2.5**
R^2	0.122	0.413	0.445	0.397

Table 2b: One phase exponential fits for the third water column incubation experiment for the N+P treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	8.2	34.2	79.5	46.3
K (d^{-1})	0.08	0.01	0.93	0.16
Plateau (µM)	502.0	369.1	782.5	497.0
Half Life (d)	8.45	54.78	0.75	4.40
%BDOC	**1.6**	**4.3**	**9.2**	**8.5**
R^2	0.096	0.456	0.296	0.673

Table 2c: One phase exponential fits for the third water column incubation experiment for the 2XDOC treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	11.2	55.4	159.2	181.2
K (d^{-1})		0.240		
Plateau (µM)	1070.0	1324.2	1217.5	1005.4
Half Life (d)		2.893		
%BDOC	**1.1**	**4.0**	**11.6**	**15.3**
R^2		0.476		

Through use of non-linear one-phase exponential decay curves, we evaluated the loss of organic carbon over the experiments. These values are comparable to other studies (Wetzel 1992; Stepanauskas 2000), where the researchers observed 2 - 16% bioavailability for their marsh sites. These are the first bioavailability numbers to be determined this way from Florida Bay. The rate constants tell only one part of the story; the plateaus for all sites were high relative to initial levels. This means that the rate of degradation was rapid in the beginning but that only a small portion of the total DOC pool was degraded. It is important that any future modeling efforts incorporate this Plateau/Span information or else DOC decomposition will be overestimated. Where changes in DOC

were not a linear decrease over the experiment, the model did not converge and K, half life, and R^2 coefficient were not derived. In these exceptional cases, BDOC was calculated as mentioned above.

We tested bioavailability across time and found that there was a significant difference between the dry and wet seasons. The dry season bioavailability was significantly different from the last wet season experiment ($p=0.0004$) with a mean BDOC of 5.56% in the dry season and 16.86% in the late wet season when the last experiment was conducted. There was no significant difference between the two wet season experiments.

Mean BDOC from both treatments of 2XDOC and N+P enrichments revealed that a significant increase occurred in the response of the microbial community in bioavailability of the organic carbon. We grouped all of the bioavailability data cross-sites t-test analysis of %BDOC. With N+P and 2XDOC, there was significant increase in the bioavailability of DOC when compared to control: 2XDOC and control t-test stat 2.402 (1 tailed t stat 1.79) ($df=11$, $p=0.03$); N+P and control t-test stat 2.373 (1 tailed t-test stat 1.79) ($df=11$, $p=0.03$). There was no significant difference between the N+P and 2XDOC treatment although the mean BDOC was higher for the 2XDOC treatment. Thus, the nutrients provided in organic form lead to bioavailable changes that were equivalent to when inorganic nutrients were provided. An increase in organic material load from central Everglades, especially during the wet season, can produce a response equal to or greater than the response from inorganic nutrient enrichment. This suggests that both inorganic and organic forms of nutrients can be limiting factor for bacteria or phytoplankton populations in the area.

The highest bioavailability that we observed was from the Whipray Basin site with our 2XDOC treatment. The bioavailability seen at this site in the first wet season experiment was twice what we observed at any other site under any treatment over time and suggests that this site responds more quickly to ‘fresh DOC’ and more intensely to organic additions.

Table 3a: One phase exponential fits for the fourth water column incubation experiment for the control treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	47.2	44.6	65.8	42.8
K (d^{-1})	1.48	0.002	0.005	0.001
Plateau (µM)	485.5	770.2	1077.1	421.3
Half Life (d)	0.47	279.4	147.2	457.6
%BDOC	**8.9**	**5.5**	**5.8**	**9.2**
R^2	0.500	0.90	0.64	0.735

Table 3b: One phase exponential fits for the fourth water column incubation experiment for the N+P treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	14.2	133.5	297.1	23.7
K (d^{-1})		0.005	1.65	
Plateau (µM)	493.8	698.6	858.3	443.8
Half Life (d)			0.42	
%BDOC	**2.8**	**16.0**	**25.7**	**5.1**
R^2		0.870	0.983	0.395

Table 3c: One phase exponential fits for the fourth water column incubation experiment for the 2XDOC treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	201.2	137.5	1224.6	108.8
K (d^{-1})	0.586	0.113	0.001	0.107
Plateau (µM)	1386.2	1757.9	999.2	1465.0
Half Life (d)	1.18	6.16	423.0	6.46
%BDOC	**12.7**	**7.2**	**55.1**	**6.9**
R^2	0.936	0.673	0.735	0.448

Table 4a: One phase exponential fits for the fifth water column incubation experiment for the control treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	24.9	111.1	91.5	58.4
K (d^{-1})		0.003	0.004	
Plateau (µM)	523.6	678.8	742.8	687.5
Half Life (d)		263.0	176.3	
%BDOC	**4.5**	**14.1**	**11.0**	**7.8**
R^2		0.410	0.605	

Table 4b: One phase exponential fits for the fifth water column incubation experiment for the N+P treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	7.5	206.1	222.1	219.2
K (d^{-1})	1.42	0.001	0.002	0.001
Plateau (µM)	504.2	627.2	625.4	541.9
Half Life (d)	0.5	474.4	304.0	449.6
%BDOC	**1.5**	**24.7**	**26.2**	**28.8**
R^2	0.270	0.885	0.922	0.796

Table 4c: One phase exponential fits for the fifth water column incubation experiment for the DOC * 2 treatment from each of the four study sites in the western and eastern Bay.

Water Column	Duck Key	Joe Bay	Whipray Basin	Bob Allen Keys
Span (µM)	165.8	289.6	320.0	463.8
K (d^{-1})	0.004	1.48	1.09	0.07
Plateau (µM)	1416.7	1512.1	1530.0	1343.8
Half Life (d)	166.7	0.5	0.6	10.4
%BDOC	**24.7**	**16.1**	**17.3**	**25.7**
R^2	0.587	0.932	0.459	0.789

Bioavailability in the control incubations (Tables 2a, 3a and 3b) across Florida Bay does not appear have a pattern that is regional in scale. The highest bioavailability and one of the very lowest control bioavailability we observed across the study period occurs at northeastern central study site of Joe Bay (1.73% and 14.06%). Joe Bay is in the ecotone between the Everglades mainland and Florida Bay and it cloistered behind miles of winding mangrove waterway. It is interesting that BDOC is highest in the western Florida Bay sites and in those sites closest to the mangrove estuary and some of the lowest BDOC were in the middle of Florida Bay further from the freshwater ecotone. This suggests that the amount of bioavailable DOC from the marsh and mangrove that is transported to Florida Bay is low. The half lives among sources are different as well, meaning that the

bioavailable fractions are probably of individual origin. This concept was born out from the chemical characterization (λ_{max}) (Boyer et al. 2004).

Our BDOC for the 2XDOC amendment is higher than any reported value in the literature, excluding BDON data (bioavailable dissolved organic nitrogen) (Table 5). This suggests a feedback loop where higher concentrations of DOC introduced to Florida Bay from the mainland Everglades, either through pulse events such as hurricanes, massive rains, or human altered water events, can lead to higher bioavailability and eventually higher DOC concentrations. An increase in organic material load from central Everglades and especially during the wet season can produce a response equal to or greater than the response from and inorganic nutrient enrichment. This suggests that both inorganic and organic forms of nutrients can be limiting factors for bacteria or phytoplankton populations in Florida Bay.

Table 5: Bioavailability results of our study compared with those of researchers in estuaries reported across the globe with + standard error when reported or available.

Location of study	% BDOC and BDON	Focus of study	Literature cited
Cape Fear River, USA	9.0 + 4.5 % (BDOC)	Total microbial community	Avery et al. 2003
Rio de Janeiro, Brazil	6.6 + 17.4 % (BDOC)	Bacteria only	Fajalla et al. 2002
Baltic Sea	2 – 72 %	Heterotrophic community	Stephanaukas 2000
Taylor River, ENP, USA	3.5 % (BDON)	Bacteria only Dark incubations	Boyer et al. 2004
Taylor River Slough freshwater ENP, USA	9.2 % (BDON)	Bacteria only Dark incubations	Boyer et al. 2004
Duck Key, Florida Bay USA	6.8 **+** 1.5 % (BDOC)	Total microbial community Dark incubations	This study
Joe Bay, Florida Bay, USA	7.1 + 3.6 % (BDOC)	Total microbial community Dark incubations	This study
Whipray Basin, Florida Bay, USA	6.6 + 2.3 % (BDOC)	Total microbial community Dark incubations	This study
Bob Allen Keys, Florida Bay USA	6.5 + 2.1 % (BDOC)	Total microbial community Dark incubations	This study
Florida Bay USA	6.7 + 1.1 % S.E. (BDOC)	Total microbial community Dark Incubation	This study
Sacramento-San Joaquin River Delta	11 - 15 % (BDOC)	Total microbial community	Sobzjak et al. 2002

Ectoenzymatic Activity Response

Microbial AP activity was variable among sites, incubation experiments and over the experiment interval. Our results show that there was a mid experiment maxima at Bob Allen Keys, the western central site furthest from the mainland Everglades, in the fourth incubation experiment (Figure 26). We saw this same mid experiment response from each of the four study sites in the fifth

incubation experiment (Figure 27). These mid-incubation maxima occurred only in the N+P treatment and only in the light incubations. The 2XDOC treatment revealed the highest endpoint for all experiments in the light and for 3 of the four sites in the dark incubations (Figure 28). We attribute this to the enzyme activity of the heterotrophic population and their rapid response to the increase in DOC availability. Heterotrophic population AP activity did not respond to N+P additions during the incubations as the autotrophic population had. The contribution of the heterotrophic population to AP activity was a surprise. We had expected the cyanobacteria to be the main contributor to what we quantify as AP activity and attributed this as the key component to the release of SRP to initiate the blooms of cyanobacteria. Because we saw a response, or increase in AP activity in both light and dark incubations, both heterotrophic and autotrophic microbial populations contributed. This suggests a close coupling between the heterotrophic and autotrophic populations regarding the cycling of DOC especially when DOC becomes more available in Florida Bay.

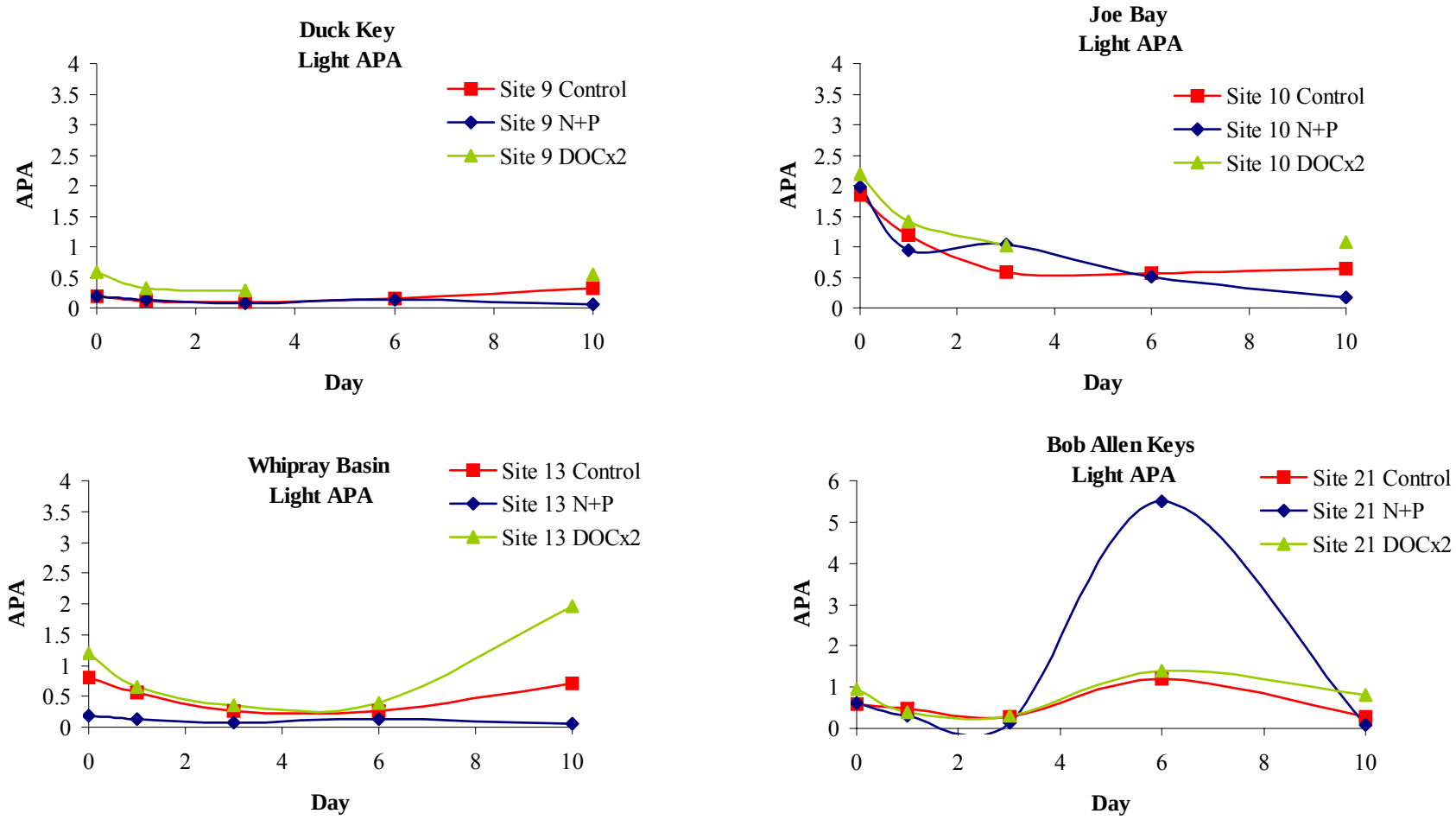

Figure 26: Light alkaline phosphatase activity (µM l^{-1} hr^{-1}) for each of the four study sites across Florida Bay for the fourth incubation experiments.

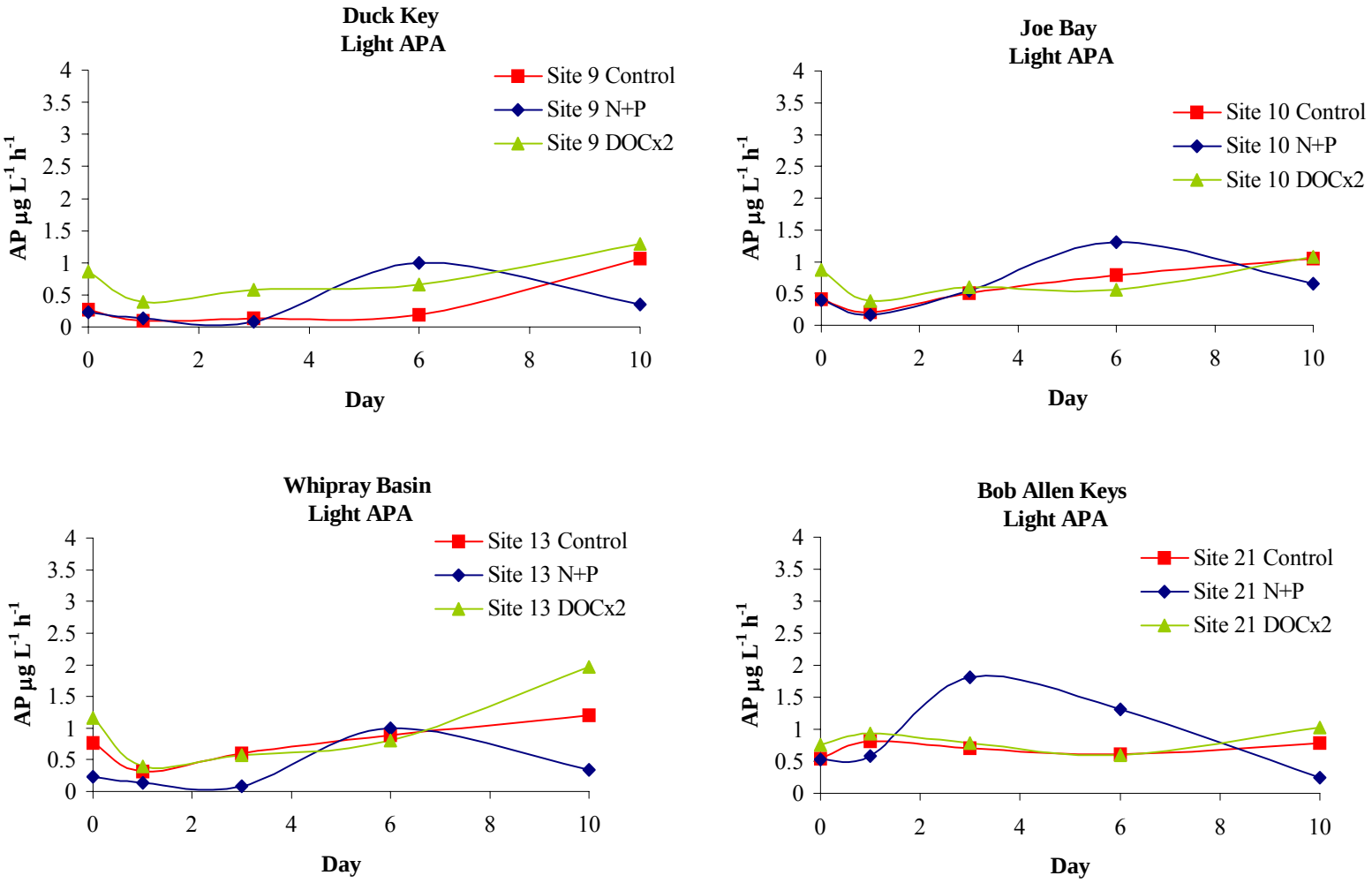

Figure 27: Changes in AP activity for each of the four study sites over the fifth incubation experiment.

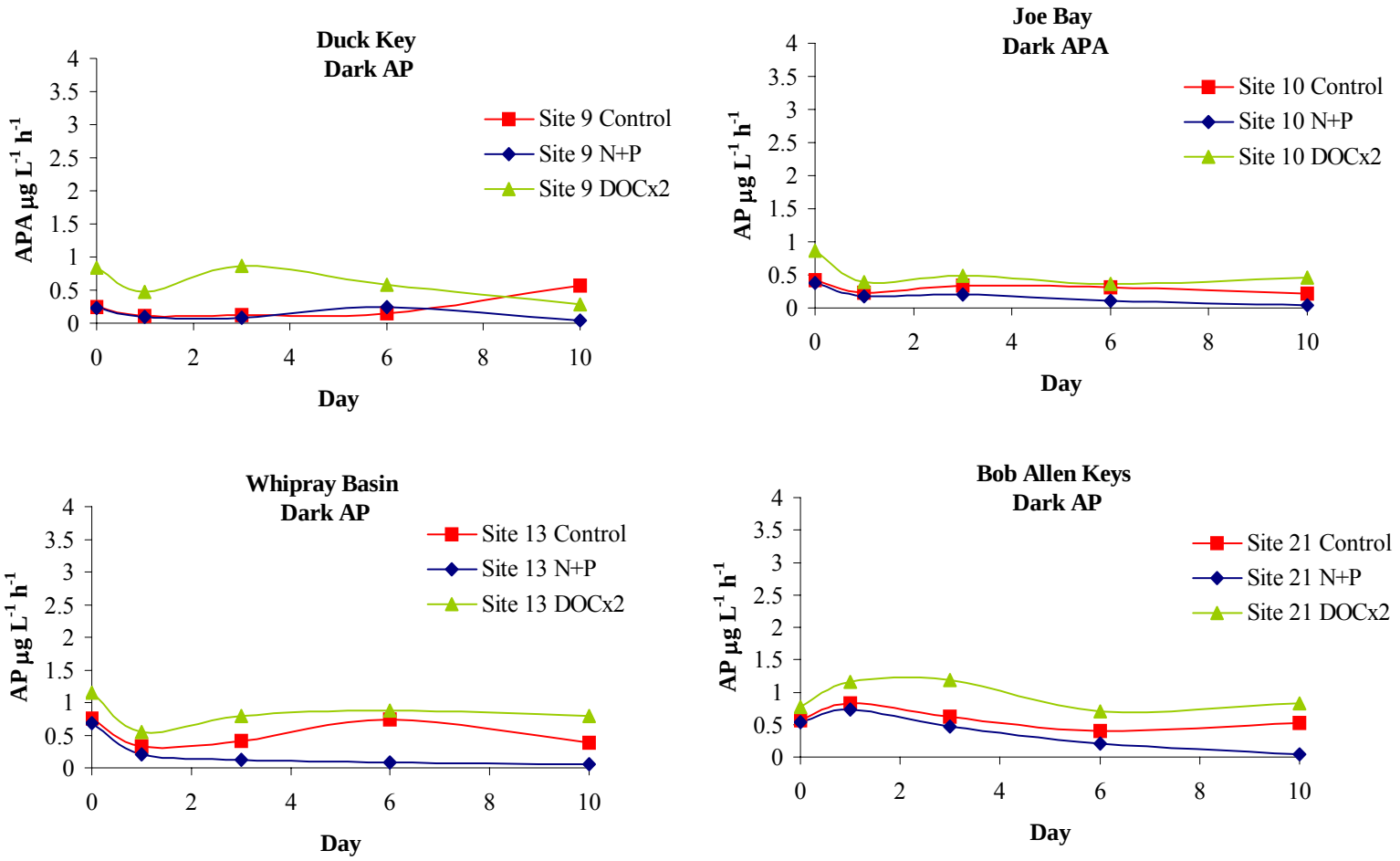

Figure 28: Changes in AP activity for each of the four study sites from the fifth incubation experiment.

Algal Response

Total CHLA concentrations were dynamic across time, treatment and location of sampling. The greatest increases always occurred in the middle of the incubation experiments and then a decrease to near intial concentrations. The highest initial concentration of CHLA and highest observation (N+P) was found at Joe Bay.

These mid level maxima were associated with N+P treatments, as seen with several of the AP activity responses (Figures 29 and 30). However, the endpoints most often showed that the greatest final treatment response was attributed to the 2XDOC treatment. In particular, these responses were associated with the brown algal group.

There was only one response of the cyanobacteria concentration in all of the incubation experiments. This response was from the N+P treatment and was only observed at Joe Bay. Joe Bay also had a higher beginning concentration of CHLA from cyanobacteria. There was no change in the overall concentration of cyanobacteria from our DOC amendment treatment of the organic material from Taylor River. Our findings revealed that cyanobacteria was a very small contribution of the overall total CHLA concentration and less than the other two guilds of brown or green algae.

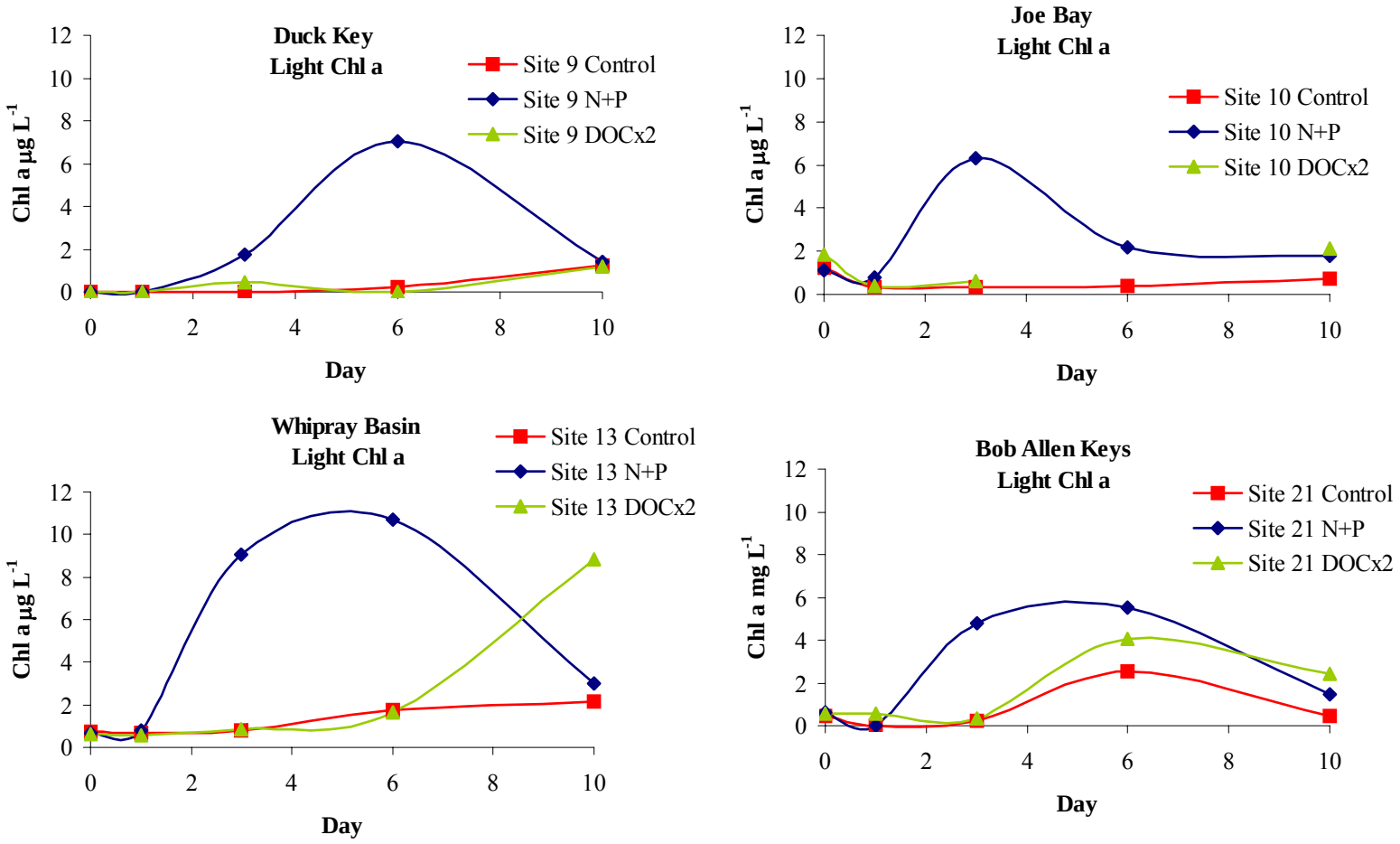

Figure 29: Changes in CHLA concentrations (µg l^{-1}) from light incubated bottles in the fourth incubation experiment.

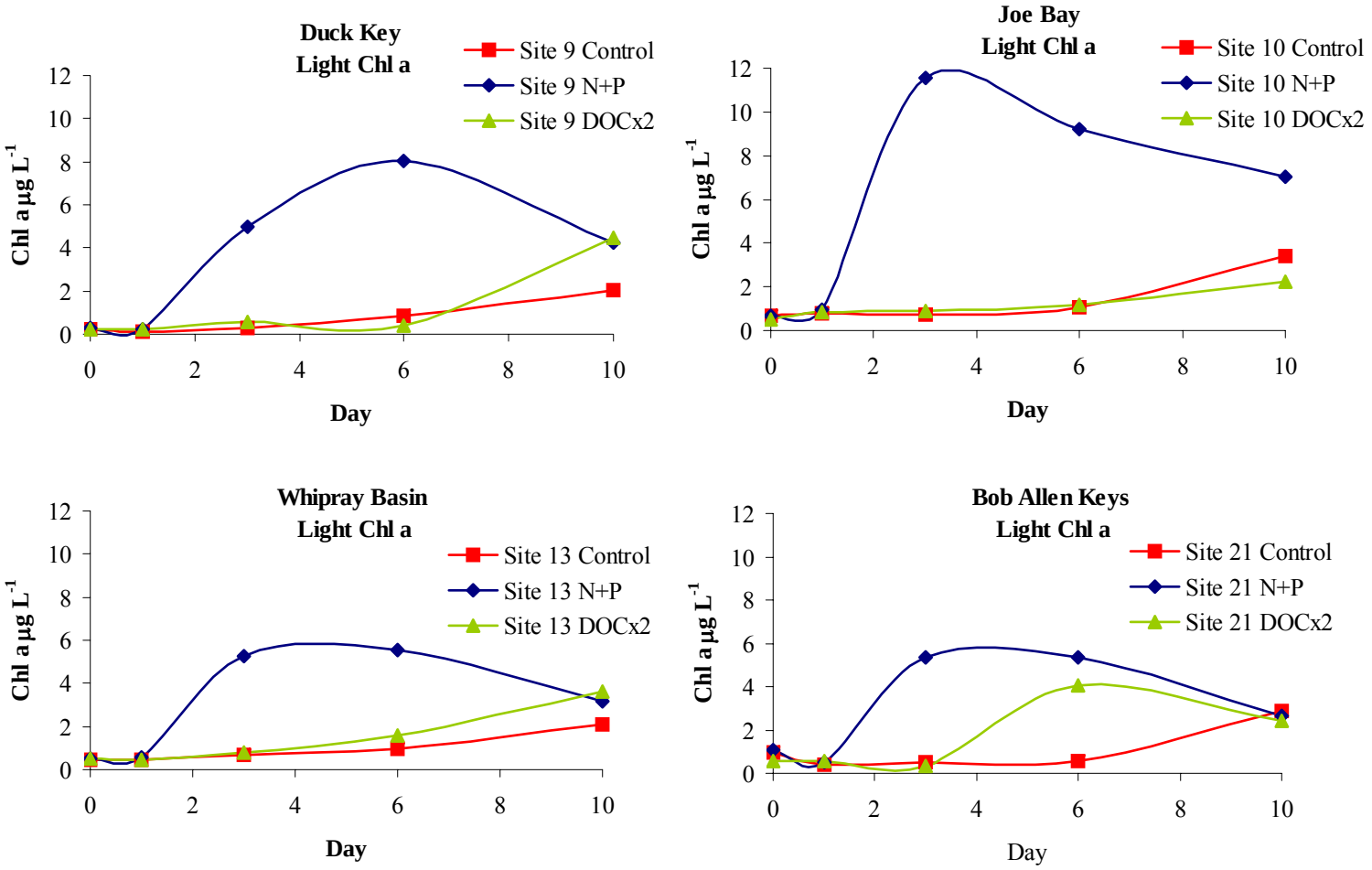

Figure 30: Changes in concentration (µg l^{-1}) of CHLA from the fifth incubation experiment.

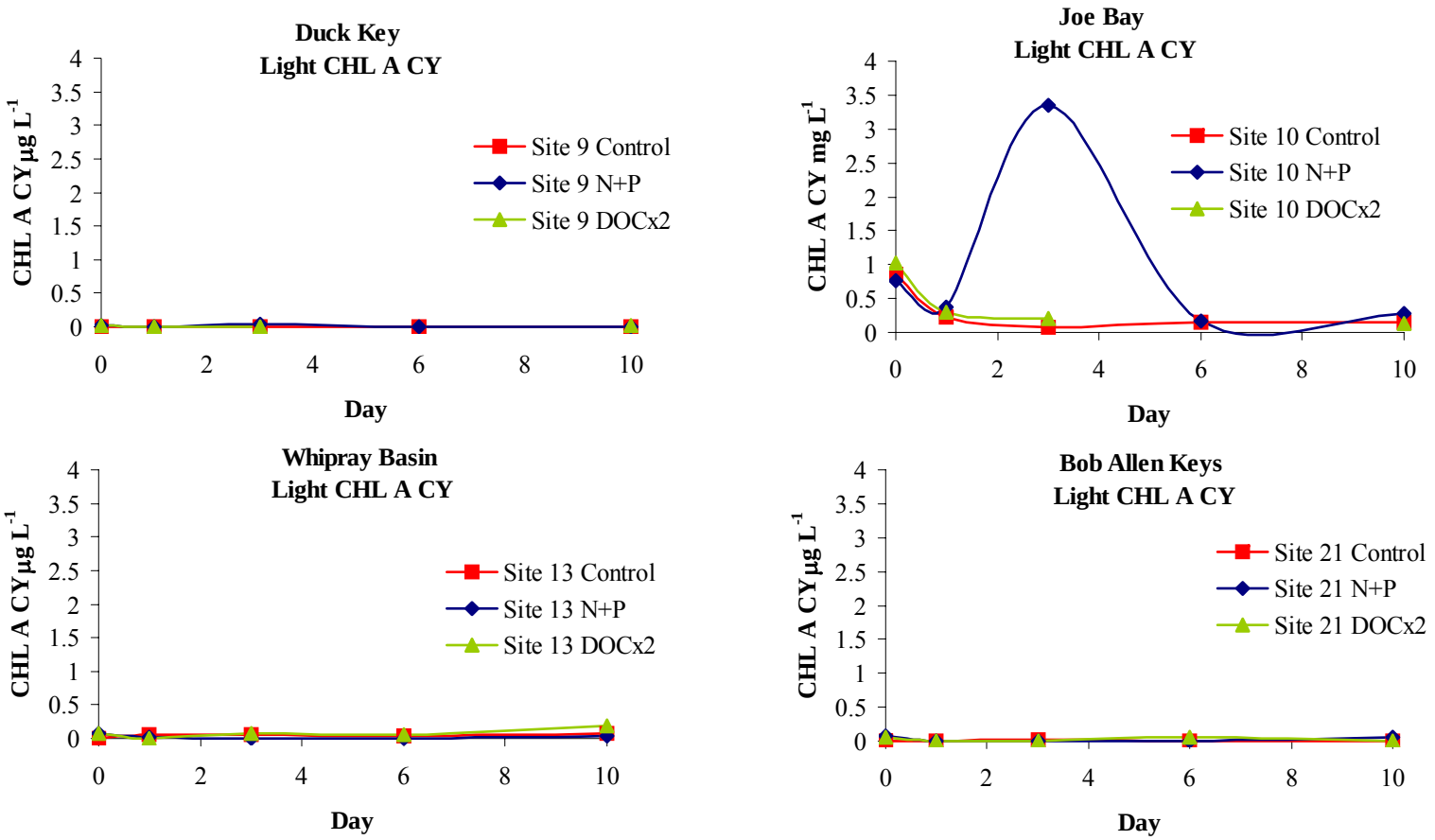

Figure 31: Changes in CHLA contribution from cyanobacteria over the fourth incubation experiment for all four central Florida Bay sites.

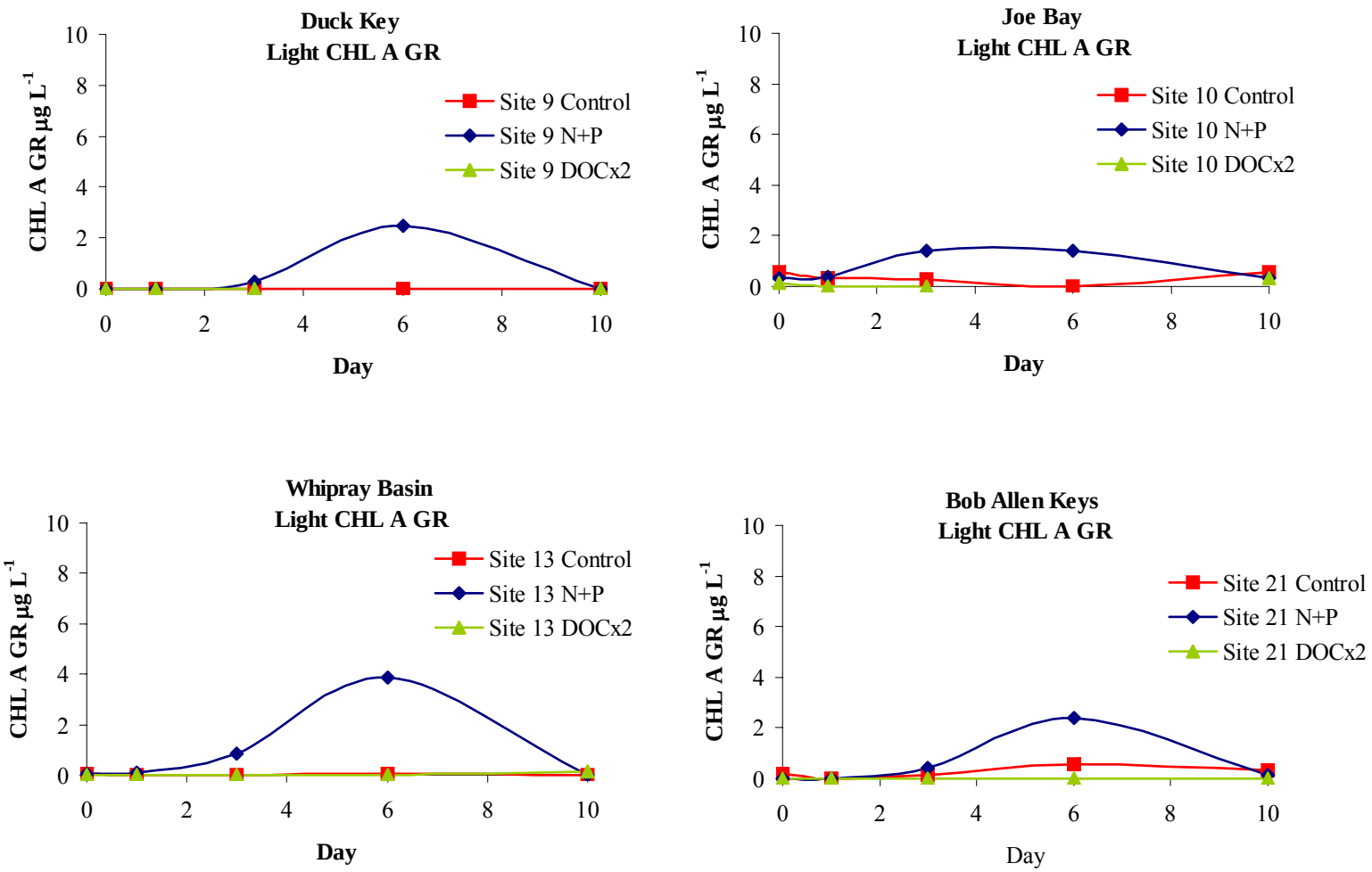

Figure 32: Changes in Chla concentration ($\mu g\ l^{-1}$) contribution from green algae over the fourth incubation experiment.

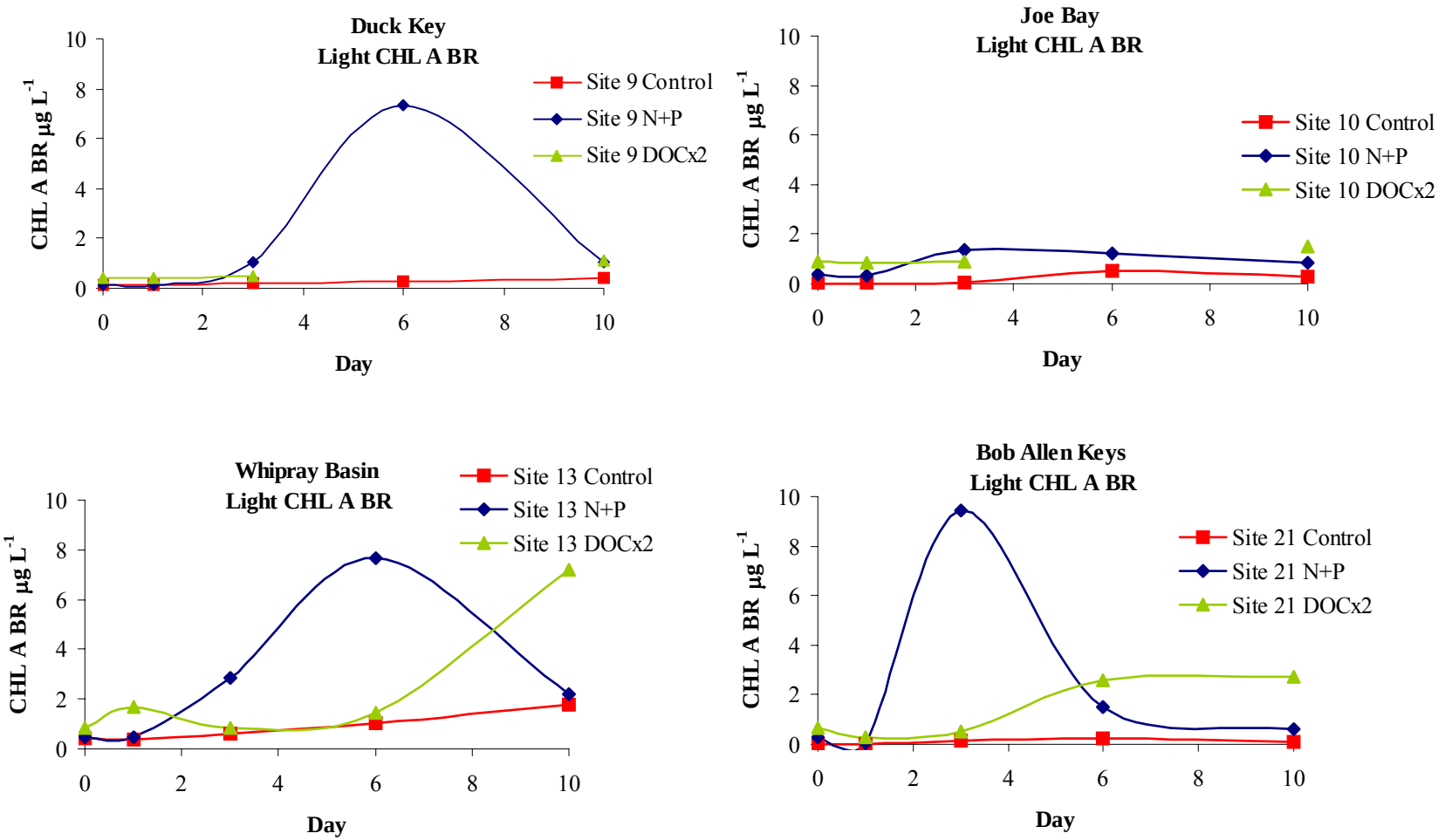

Figure 33: Changes in concentration (µg l^{-1}) of CHLA contribution from brown algae during the fourth incubation experiment.

Bacterial Response

For BACT, the strongest endpoint response to any of the treatments was water incubations from the Whipray Basin study site and was in the 2XDOC treatment (Figure 34). Whipray Basin also has the highest ambient concentrations of DOC of any of the four study sites. The implication is that with DOC there is higher bacteria biomass and that this phenomenon can exist especially from higher DOC background levels. We see this same effect with the %BDOC. There was also a response from the N+P treatment in bacteria production at Whipray basin. Production was also higher than the control in the DOC amendment treatment (Figure 35).

BACT were highly variable throughout the incubation experiments. The treatment effect was variable between sites. The daily variability in BACT (Figure 34) and heterotrophic production (Figure 35) suggests that the bacterial population is under the control of a variable that may be cyclic and perhaps may require a more frequent scale of observation.

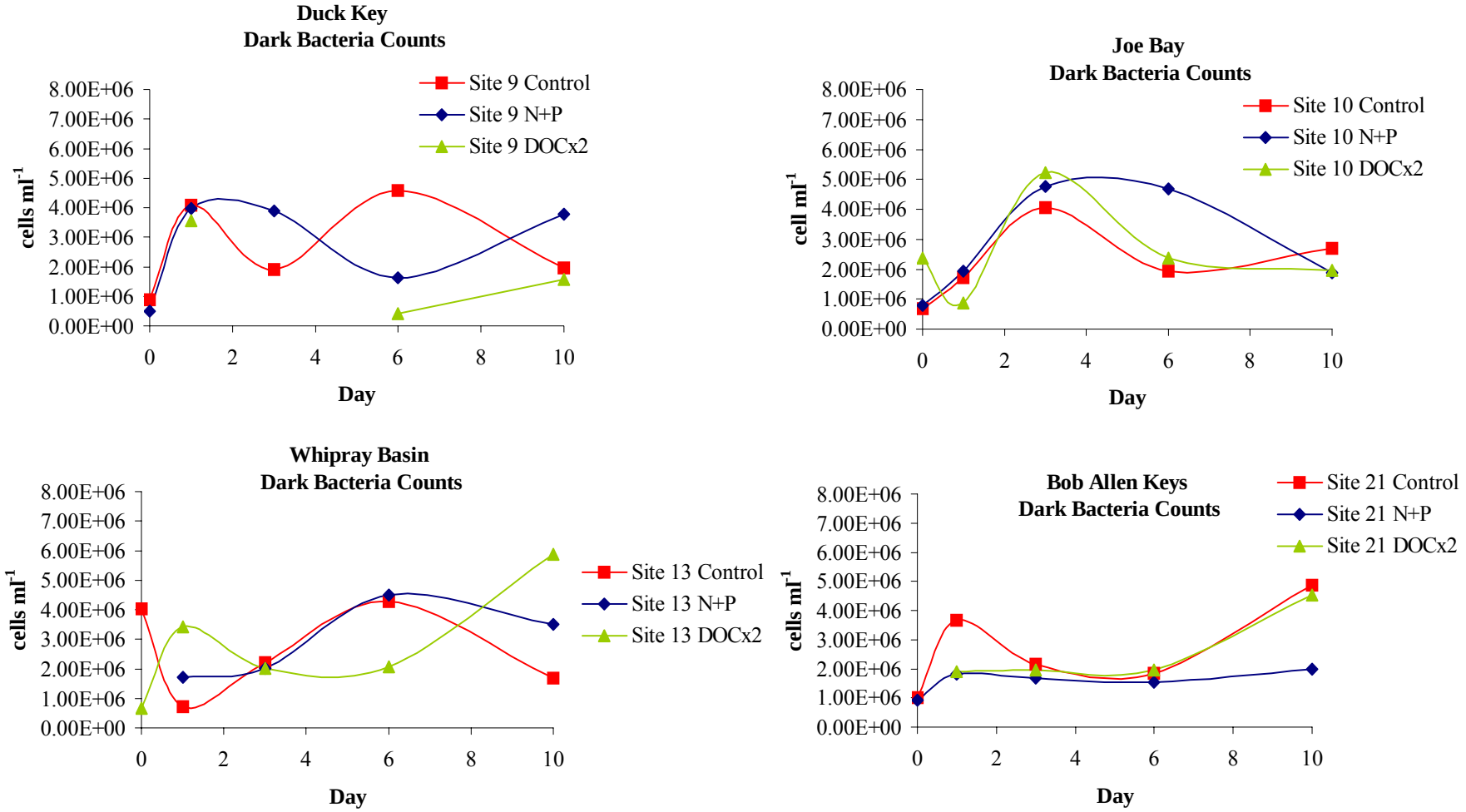

Figure 34: Dark bacteria ml^{-1} from the fourth incubation experiment in the beginning of the wet season 2002.

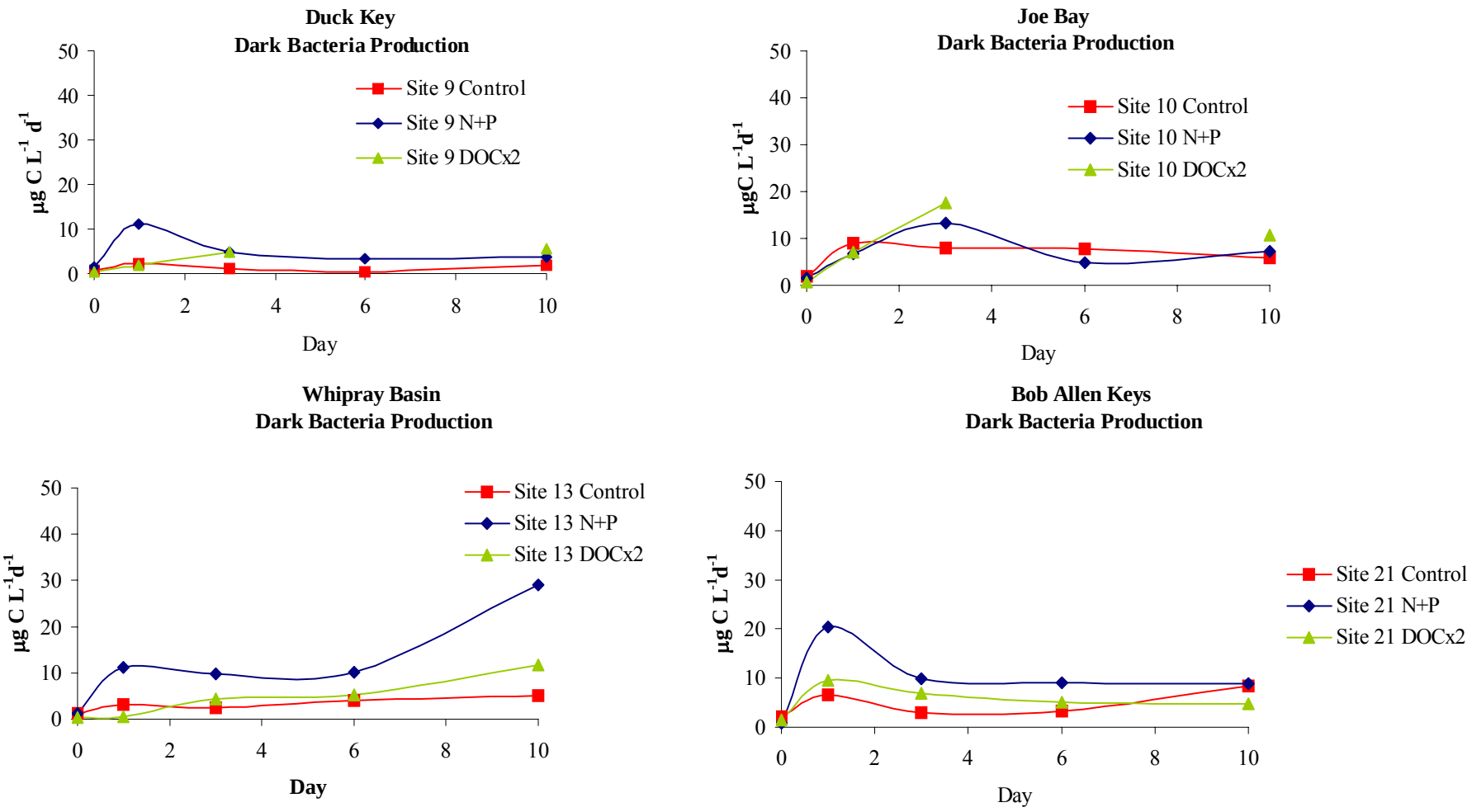

Figure 35: Changes in bacteria production (µg C l^{-1} d^{-1}) from dark bottles in the fourth incubation experiment.

Nutrient and DOC responses

Concentrations of DOC were naturally highest at Whipray Basin(Figures 36 and 37), where we saw some of the most dynamic responses in the bacteria numbers and CHLA concentrations. These responses were most associated with the 2XDOC treatment but there was also a significant effect of the inorganic N+P enrichment. These %BDOC are reported by site (Table 5), analyzed (Tables 3a-cthrough 5a-c), and discussed earlier in the text. The positive feedback loop with higher DOC concentrations, comes higher bioavailability that is proposed in this report is driven largely by the responses seen in the wet season at Whipray Basin, with its higher ambient DOC concentrations.

TP and SRP concentrations were most affected by the N+P additions, with very little change over the incubation interval from either ambient or 2XDOC enrichment The behavior of the TP (Figure 38) and SRP (Figure 39) both are cyclic in their fluctuation over the experiment. These patterns are as variable as those seen in the bacteria and bacteria production numbers and suggests the two factors are coupled. We found these same results with the PCA of the 2 year dataset of all microbial, nutrient and physical observations. Our DOC amendment provided twice the concentration of the ambient TP. We did not see any initial amendment of SRP from our 2XDOC treatment and expected none, as the tangential flow fractionation process removes all inorganic constituents in wastewater.

Dissolved Organic Matter Characterization

The characterizations of the DOC were not as dynamic as the microbial and nutrient responses over the incubation interval. Out of the four characterizations that we performed from each experiment we did not see any temporal or spatial change in the organic matter characterization. We also saw very little difference between treatments in any of the initial conditions. We also saw that our N+P treatment had no residual effects on the molecular characterization of the ambient dissolved organic material. We also saw the DOC amendment was very similar, in molecular behavior and fluorescence properties, to the ambient DOC in the controls. This provides some validation that indeed our DOC amendments were similar in structure to the ambient DOC. Further, it supports the argument that the microbial population of Florida Bay responds to allochthonous carbon inputs to the water column, such as those conditions that may exist in pulsed energy events such flooding events.

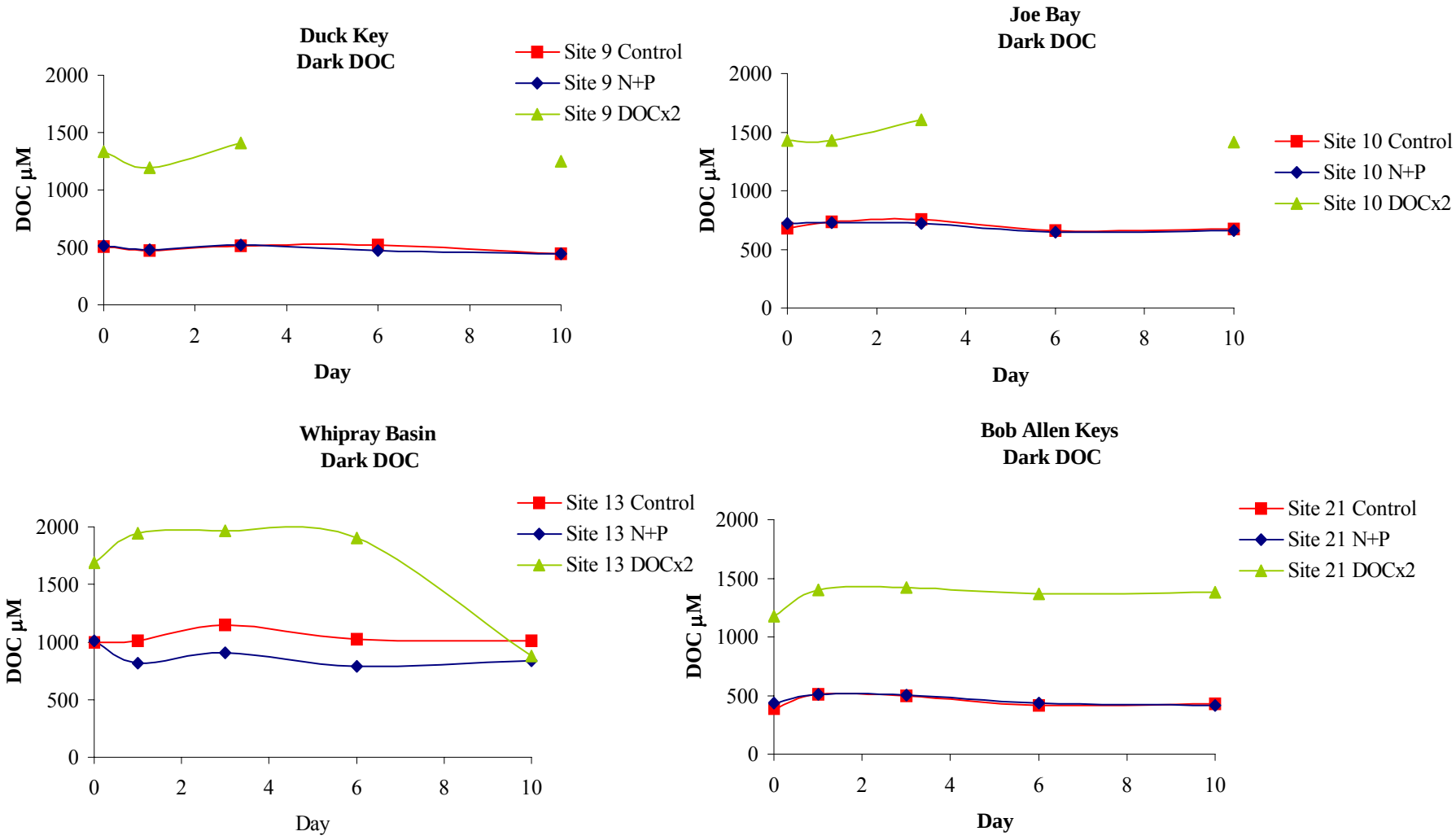

Figure 36: Changes in Dissolved Organic Carbon concentration (µM) from dark bottles in the fourth incubation experiment.

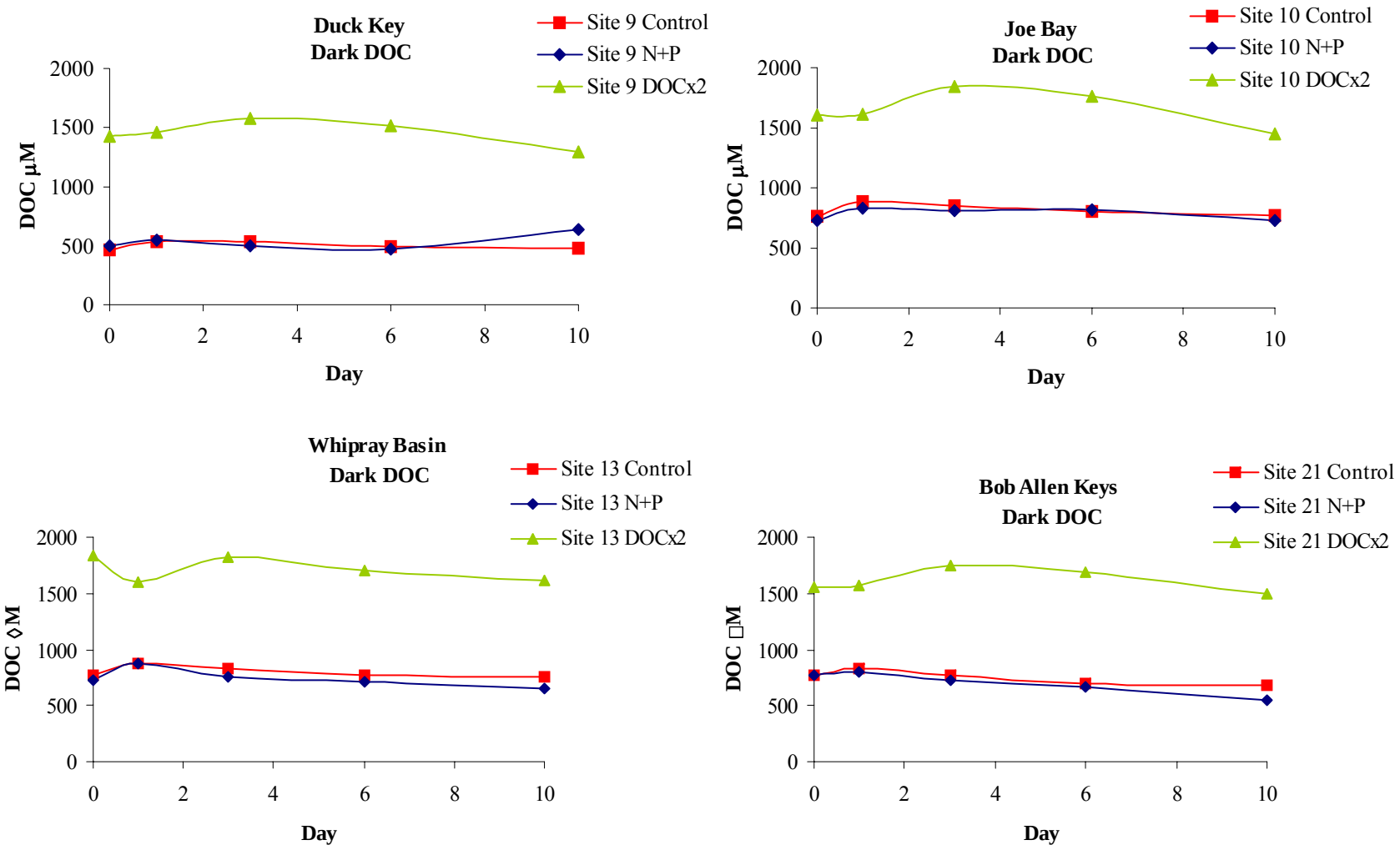

Figure 37: Concentrations of dissolved organic carbon (µM) showing the difference between treatments across sites over the fifth incubation experiment.

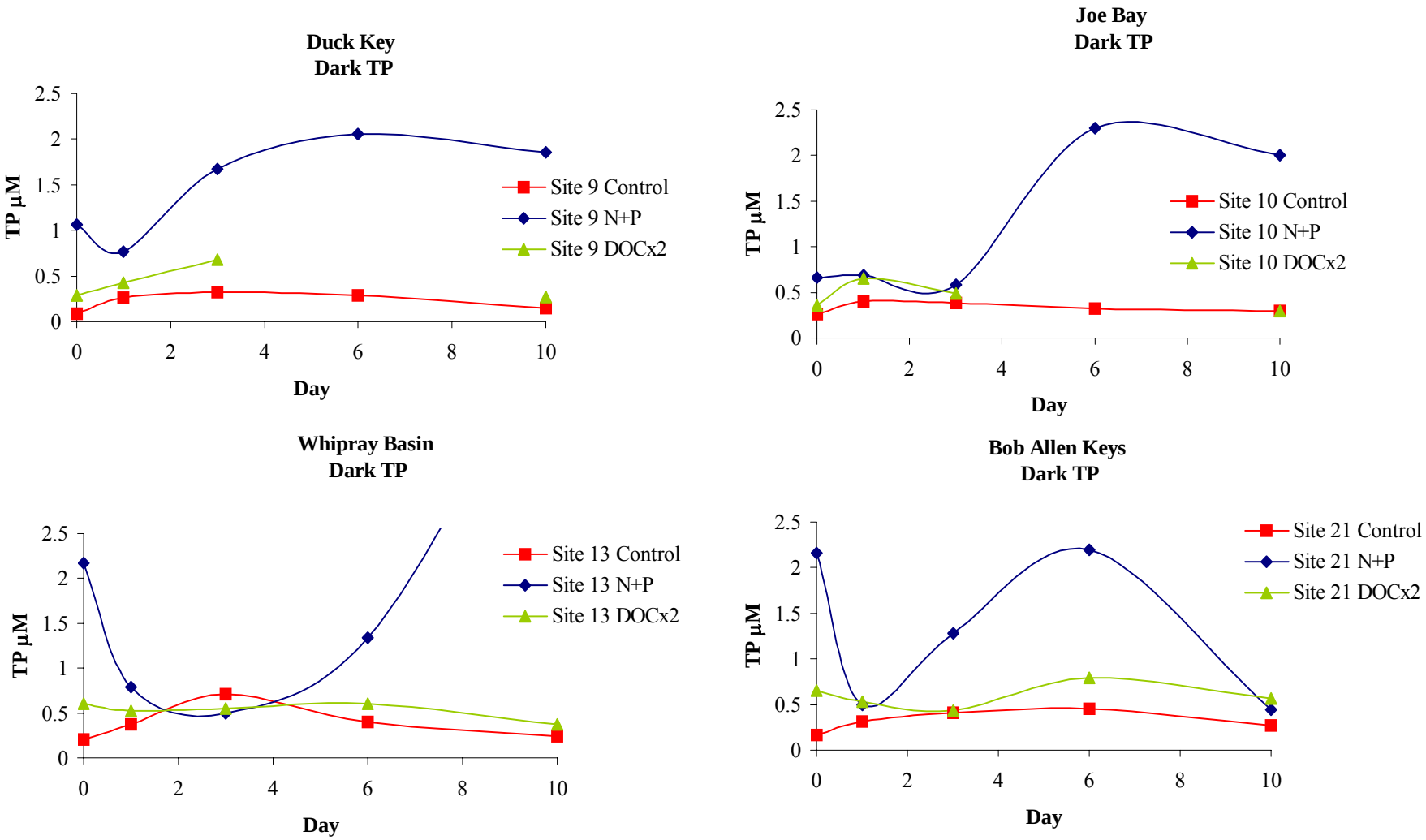

Figure 38: Change in TP (µM) concentrations in dark bottles over the fourth incubation experiment.

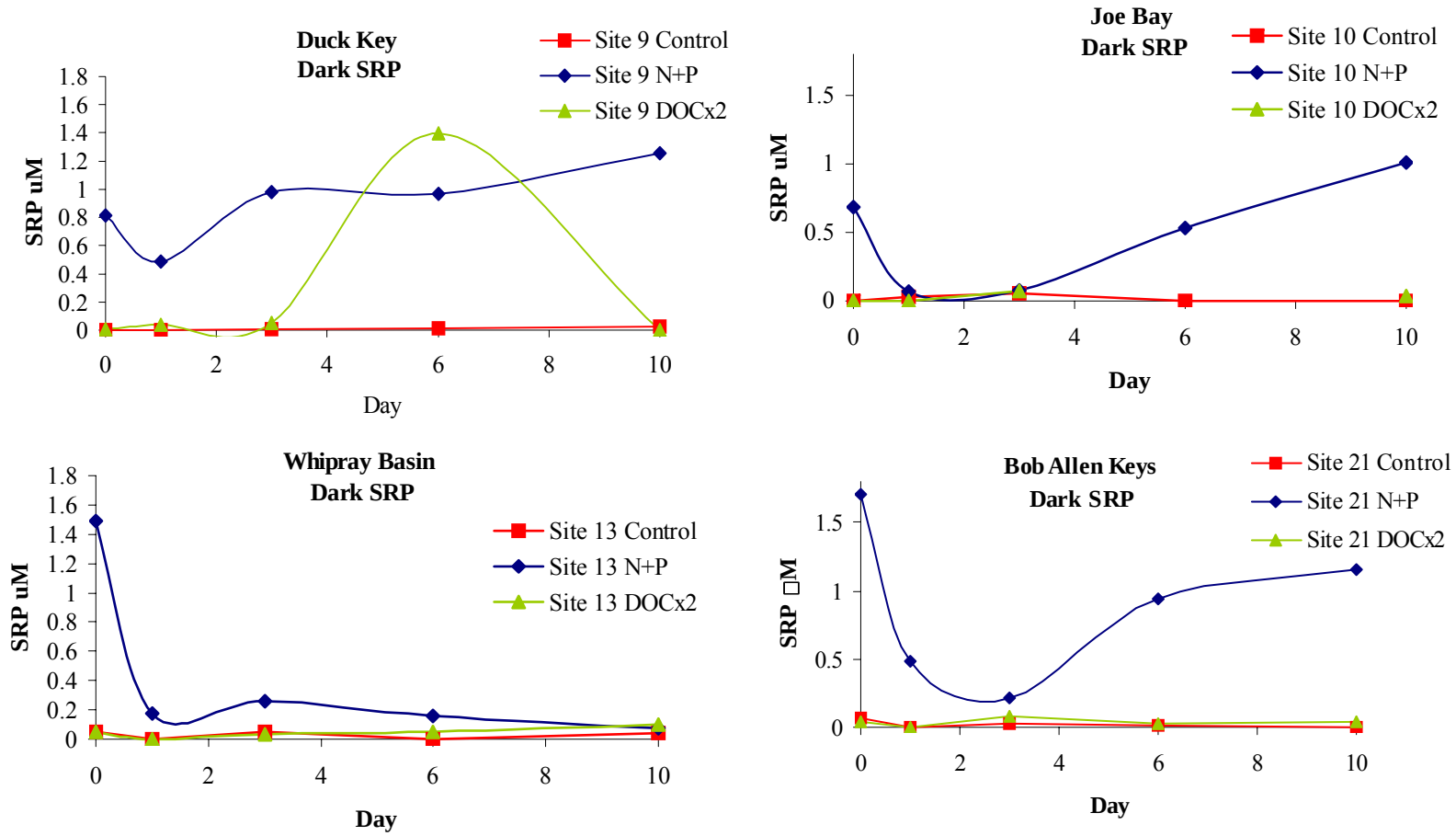

Figure 39: Changes in Soluble Reactive Phosphorus (μM) concentrations in dark incubation bottles during the fourth experiment

Hypothesis 2 Summary

These experiments indicate that both autotrophic and heterotrophic microbial populations in Florida Bay are co-limited by organic and inorganic nutrient availability. Both treatments of organic and inorganic enrichment revealed significant and positive response in the bioavailability of organic carbon. Potential bioavailability ranged from 1.1 – 55.1 %, with the most labile forms occurring in Whipray basin. Thus, bioavailability of organic materials to heterotrophic and autotrophic communities are tightly coupled. This has major implications in the fate of DOC from the Everglades and Florida Bay ecosystems and corresponding carbon budget models. The experiment implicates bioavailable nutrients as the key factor controlling the onset and persistence of microbial blooms and that %BDOC increases with increased availability of DOC. The greatest amount of change in bioavailability occurred in the wet season when DOC introductions from mainland Everglades might be mostly likely to induce algal blooms.

The combination of treatments that we tried in these experiments helped further our understanding of the mechanisms that induce changes in the algal community in Florida Bay. We saw a shift in the algal community in cyanobacteria and green algae to brown algae with the introduction of greater concentrations of organic carbon into the water column. With N+P addition, we observed a mid experiment increase in concentration of cyanobacteria and green algae that was greater than the response seen with the inorganic nutrient additions. The response was most seen in the brown algae. Thus, in none of our experiments were we able to induce a bloom affect such as those described in Florida Bay. We were able to stimulate growth of brown algae, which include diatoms, with the DOC treatment, although blooms of this sort have not been previously described in Florida Bay. These brief blooms of all algal groups and dynamic trends in bacterial numbers and TP suggest that the three are tightly coupled, as seen in the 2 year microbial, nutrient and physical factor model in this report.

Throughout these experiments several results helped elucidate the coupling between bacterial and algal dynamics in Florida Bay and their dependence on total and dissolved constituents. We saw that there was a response in both heterotrophic and autotrophic response parameters from our bioavailability assays. Both bacterial and cyanobacterial alkaline phosphatase production is likely one of the key processes facilitating large population growth of autotrophic communities. These communities will lead to increased heterotrophic microbial activity by exuding highly labile carbon and a cycling of bioavailable nutrients. This implies that once initiated a diverse microbial bloom can persist by autochthonous resource cycling. The controls have the very same P limited conditions that occur in Florida Bay where very little DOC is bioavailable to the microbial organisms in this system. The mineralization of DOC becomes increasingly important in determining the overall production of carbon in the estuary. The positive feedback of higher availability of DOC which lead to higher bioavailability was shown through two lines of data in this project. First we saw the significant positive effect of the 2XDOC treatment on %BDOC, but also we saw significantly higher %BDOC with the onset of the wet season. We expect events where massive transport of water occurs to have similarly high %BDOC.

Incubation experiments are being continued to examine the bioavailability and influence of organic nitrogen along freshwater-marine ecotones in Florida Coastal Everglades LTER sites that extend through Florida Bay. The results of the current experiment combined with continued research of alternate substrate bioavailability add to a growing base of understanding of the fate and cycling or nutrient resources in Florida Bay and coastal marine systems in general.

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