

Final Report

SFP 2002: Dissolved Organic Nitrogen in the Taylor Slough, Taylor River and Florida Bay: Molecular Biogeochemistry, Bioavailability, and Potential Contribution to the Microbial Loop

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EXECUTIVE SUMMARY:

Dissolved organic nitrogen is an important component of the biogeochemical cycles in the Everglades and Florida Bay. Determining the origin and environmental dynamics of this material in this region remains crucial to reach NOAA's primary goal for the to 'protect, restore and manage the use of coastal and ocean resources through ecosystem-based management approaches'. This is particularly true considering the need to fully characterize South Florida ecosystems to be able to model the potential impact of the Everglades Restoration. While much of the DON story remains largely unknown, we have made significant progress in recent years.

Monthly surveys of DOM-related water quality parameters, including optical, chemical and microbiological properties showed that not only the DOM and DON components seems to be different between Everglades freshwater marshes, fringe mangrove swamps and Florida Bay's seagrass dominated estuaries, but some seasonal control on the composition of these materials was also observed. As such, PCA identified four composite variables indicating four separate modes of variation in the data. Our survey data for the entire study period revealed that the "Organic" factor (Salinity, TON, TOC, UVA₂₅₄, F_{max}, FI, and Peak 1) was most important in explaining overall variability in the data set and explained 37.5% of the variance in the data. The negative relationship to Salinity and some of the 'organic' parameters seems to suggest a terrestrial, Everglades-derived source for the DOM and DON. However, negative correlations with some 'organic quality' parameters with Salinity may be the result of both increased hydrological inputs to the bay during the wet season and/or the fact that more autochthonous DOM and DON is produced during the wet season in the Bay. The data strongly suggests an important autochthonous source for Florida Bay DON.

Molecular characterization studies of dissolved organic matter and nitrogen (DOM & DON) show that surface waters from Florida Bay exhibit an elevated proportion of non-humic materials compared to the freshwater Everglades, and that the DON associated with the high molecular weight DOM fraction (>1000 Dalton) was determined to mainly be in the form of protein-like materials based on ¹⁵N-NMR studies. Other research clearly shows that a significant portion of the DOM in Florida Bay is not associated with freshwater inputs from the Everglades. This seems to suggest that a much of the potentially bioavailable, protein-like materials in Florida Bay are derived from autochthonous sources. We believe the source of this autochthonous DOM most likely originates from the seagrass/benthic community. As such the $\delta^{13}\text{C}$ isotopic abundance of Florida Bay DOM was highly enriched compared to that from the Everglades and protein analyses by 1D and 2D electrophoresis showed significantly different molecular fingerprints between the Bay and anthropogenic inputs from Everglades canals. However, the exact origin and source strength of this DON in Florida Bay remains to be determined.

Finally, a detailed seasonal and regional DON bioavailability study was performed and represents the first data of its kind for the Florida coastal Everglades. We determined that the

bioavailability of DON presented a large range within the three studied ecosystems (marsh, mangroves and bay), and in the specific case of Florida Bay showed some seasonal variation probably controlled by primary productivity of seagrass communities. We found the highest bioavailability of nitrogen in the mangrove zone (24 %) with a potential BDON of 29.8 % under nutrient replete conditions. Additions of labile C and P consistently increased BDON values. This observation was true in both the wet and dry seasons. BDON in the Bay approached that of the mangroves during the wet season as a result of fresh DON produced in situ. While the bioavailability of DON in the FCE was relatively low (about 25-30%) it remains unclear why the remaining 70-75% escapes this process. With some questions regarding the DON source and dynamics in South Florida remaining, this project has made a significant contribution to the better understanding of the specific sources and biogeochemical processes that control the dynamics of DON in Florida Bay.

PROJECT GOALS AND OBJECTIVES

Florida Bay is a wedge-shaped estuary separated from the Straits of Florida by the Florida Keys which form a nearly continuous barrier (Fig. 1). Freshwater inputs to Florida Bay are dominated by direct precipitation ($\sim 98 \text{ cm yr}^{-1}$) with smaller inputs ($\sim 9 \text{ cm yr}^{-1}$) from flow through the Taylor Slough/C111 Canal basin (Nuttle et al. 2000). Freshwater flows through the Taylor Slough and C-111 canal are controlled by local water management activities and can directly influence salinities in the eastern portion of the Bay (Boyer and Jones 1999). Groundwater is not considered to be a significant freshwater source but there is continuing debate as to its true contribution (Corbett et al. 1999; Price and Swart 2001; Top and Brand 2001)

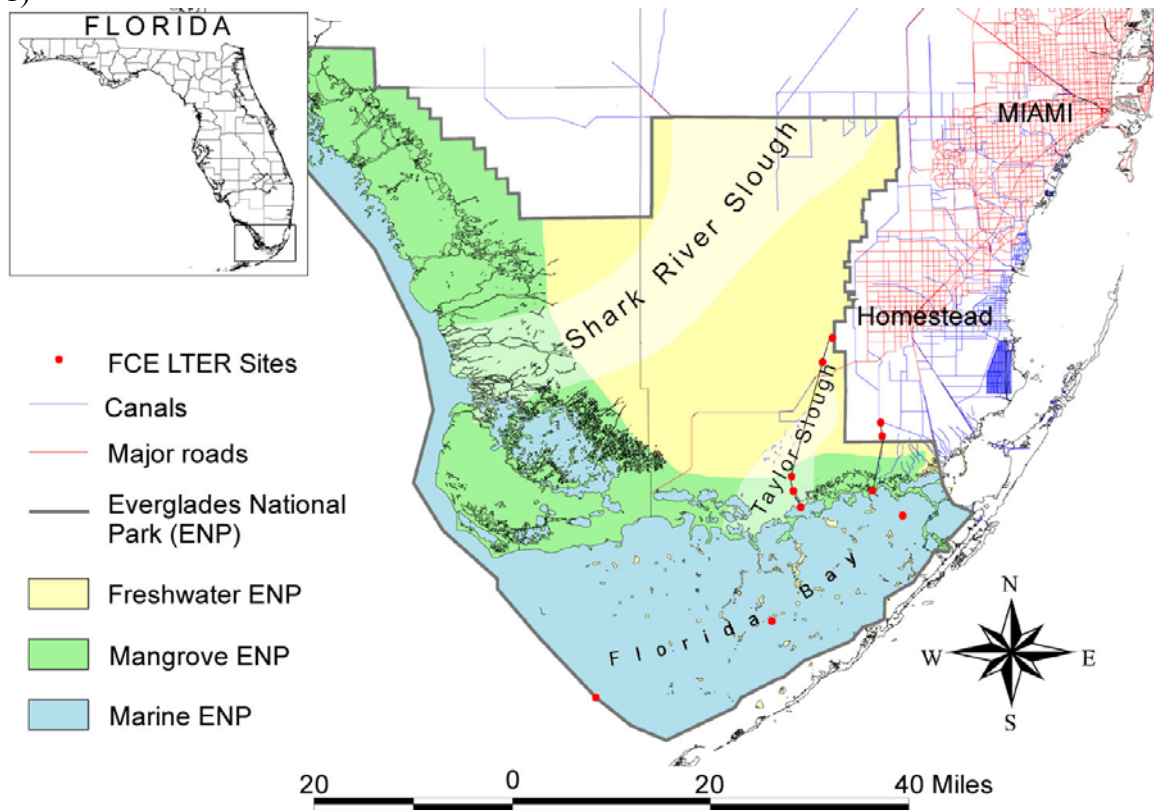


Figure 1. Map of Florida Coastal Everglades including proposed sampling sites in Taylor Slough, C-111 canal basin, and Florida Bay.

The first steps in the hydrologic restoration of South Florida have already begun (<http://www.evergladesplan.org>). The redistribution of water within the terrestrial ecosystem will necessarily affect the water entering the marine ecosystem. It is expected that sheetflow to Florida Bay will increase, in fact it already has with the removal of the southern berm on the C-111 canal in 1997 (Fig. 2, Parker 2000). Along with greater flow there is some concern that increased nutrient loading might occur (Brand 2001).

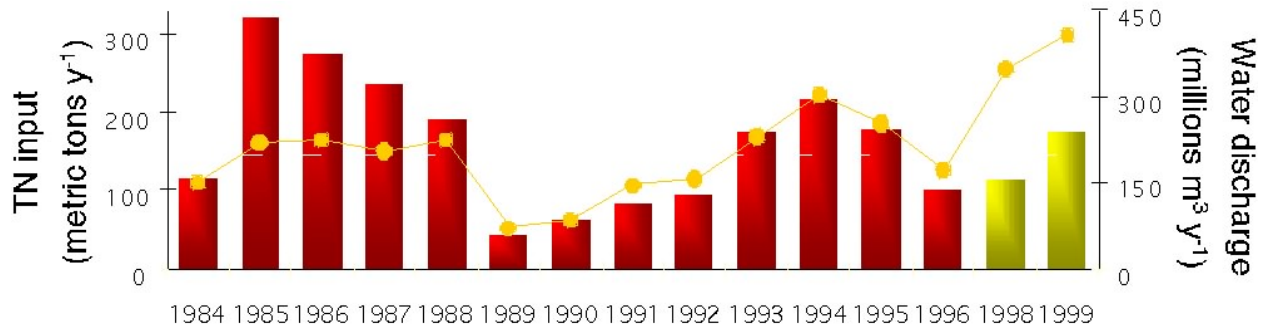


Figure 2. Water discharge (points) and total nitrogen mass load (bars) from the C-111 canal before (red) and after (yellow) the berm had been removed (Rudnick et al. 1999, Parker 2000).

Phytoplankton biomass in eastern Florida Bay is strongly P-limited (Fourqurean et al., 1993; Phipps 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). In addition to dissolved inorganic nutrients (DIN and DIP) there is a significant loading of DON from the eastern marshes (Walker 1998, Boyer and Jones 1999). DON concentrations in eastern Florida Bay are typically 5-10 times the DIN levels (Figure 3) and therefore represent a possible N source to phytoplankton and bacteria (Carlsson and Graneli 1993). The question that we propose to answer is: what are the sources of this material, what is its molecular composition and how much of the DON pool is available to the biota for growth and development?

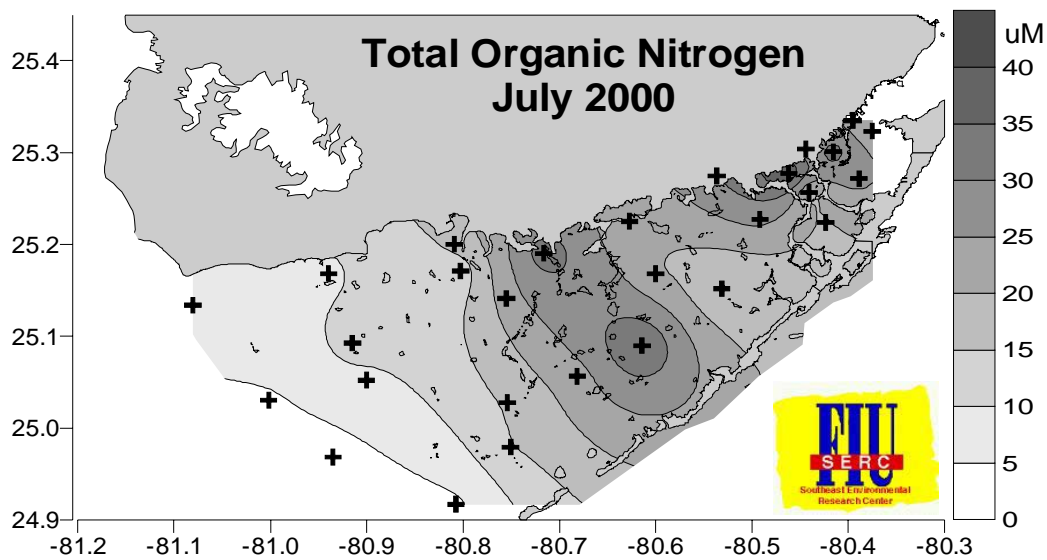


Figure 3. Concentrations of DON in surface waters of Florida Bay during July 2000. Note the higher levels in the northeast boundary.

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 (Tomas 1998, Brand 1999). However, a significant portion of the DOM 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 DOM, 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 DOM 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 few measurements of bacterial productivity have ever been published. Cotner et al. (2000) reported bacterial production estimates of 8-31 $\mu\text{g-C l}^{-1} \text{d}^{-1}$. Our results from prior NOAA support (Boyer) are summarized as follows:

1. Synchronous fluorescence measurements of DOM in incubations show that labile DOM increases with time in the dark but declines rapidly in the light.
2. Bacterial production is enhanced by addition of DOM to a greater degree than by the addition of nutrients.
3. CHLA increased with nutrient addition relative to DOM addition.
4. The change in DOC concentration over time is greatest in the DOM amended treatments; addition of N & P had a smaller effect.

These results imply that the microbial loop may be uncoupled to phytoplankton production in Florida Bay as a result of DOM input from other sources.

The quality/labability of DOM is a function of its chemical characteristics, molecular weight, elemental ratio (C:N:P:other), its age and/or degree of diagenetic transformation. Both DOC and DON concentrations are being determined across the southern Everglades landscape as part of the Florida Coastal Everglades LTER program (FCE, <http://fcelter.fiu.edu>) in an attempt to better understand key biogeochemical cycles. However, detailed information on the molecular characterization of the DOM in this system is still lacking. While bulk DOM measurements are without a doubt a key to the understanding of nutrient dynamics in wetland, estuarine and coastal systems, they do not provide critical information as to the source, chemical composition, potential bioavailability and diagenetic transformations of these materials. During the past two years our research group has generated some preliminary, but rather useful molecular information on DOM in several environments of the FCE. This information was based on a series of specific chemical analysis methods, which are described in more detail in the experimental section of this proposal. The power of these techniques in the assessment of DOM sources, and particularly in the study of DON is described below.

Spectroscopic techniques such as fluorescence have been widely used in the characterization and assessment of DOM sources in aquatic environments (Coble, 1996; Battin, 1998; McKnight et al., 2001; de Sousa Sierra et al., 1997; Lu and Jaffé, 2001). We have studied the optical properties of DOM in water samples from the estuarine and coastal areas of the western Everglades (Ten Thousand Islands and Whitewater Bay) as well as from Florida Bay. These studies were focused on applying spectroscopic techniques such as UV-visible and fluorescence spectroscopy to the assessment of terrestrially vs. marine derived DOM in the region. While absolute DOM determinations seem to indicate a conservative mixing of fresh and marine waters with a dilution of the terrestrially-derived DOM, our fluorescence studies have shown that while this is true, there is also a very clear signal of marine derived DOM that is being mixed with a ever lower terrestrial signal in the estuaries of the western Everglades. While this marine-

derived DOM is less abundant in the near-shore zone, it is composed of primarily non-humic material, most likely of carbohydrates and proteins, and may therefore be more bioavailable than the presumably more degraded terrestrially derived DOM being transported from the Everglades freshwater marshes and fringe mangrove forests. This marine derived DOM becomes dominant at salinities above 30 as depicted in Figure 4. Surface water samples from Florida Bay show an elevated amount of this non-humic, protein-like material (up to 25%) compared to the freshwater sites and most of the Ten Thousand Island and Whitewater Bay sites. However, no correlation with salinity was observed for Florida Bay waters. While this research is presently on-going, these preliminary results seem to suggest that a significant amount of the proteins in Florida Bay may be derived from autochthonous sources (e.g. seagrass beds and marine plankton) and are not solely derived from the freshwater Everglades and mangrove forests through Taylor Slough.

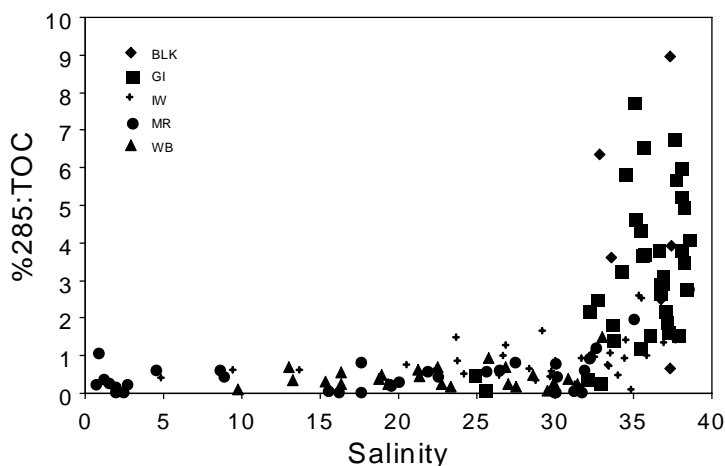


Figure 4: Relationship between surface water salinity and relative abundance (%285) of protein-like material normalized to TOC concentration for sites from the Florida coastal Everglades (Ten Thousand Islands and Whitewater Bay; Lu et al., 2001).

Water quality data from the FCE LTER Program show that the freshwater wetlands of the Southern Everglades are a consistent source of total N, (unpubl.LTER data) while the mangrove wetlands between these marshes and Florida Bay take up TN much of the year (Davis et al., 2000a,b). Fluorescence studies of this TN, which is mostly DON, suggest that the relative concentration of the synchronous fluorescence based protein like materials (relative abundance of the peak at 285 nm) are much lower than humic materials such as fulvic and humic acids (peaks at higher wavelengths; see Fig. 5). While this is an expected result for wetland waters, we have found evidence, based on fluorescence (Fig. 5), FTIR, ¹³C-NMR and pyrolysis-Gas Chromatography/Mass Spectrometry (py-GC/MS; Fig. 6) results, that the amount of proteins and carbohydrates in surface waters increases with distance from the canal, suggesting generation of biogenically derived DON as the water flows from the canal through the freshwater marsh (C-111 Basin example). This information suggests that a significant fraction of the DON in this system is not derived from allochthonous and possibly anthropogenic sources (i.e. water input from the canals) but is of an autochthonous source. Furthermore, this DON probably makes up most of the TN being supplied by the freshwater marshes. As to possible sources for this DON, both DOM leached from mangroves, sawgrass and periphyton showed a significant amount of protein-like material (based on synchronous fluorescence measurements; Lu and Jaffé, 2001).

This freshly leached DOM is quite labile, as it is sensitive to biodegradation and to a lower degree, photochemical degradation as shown in Figure 7. Its presence in surface water samples therefore, seems indicative of relatively recently produced DOM, and points at the Everglades freshwater marshes and mangrove forests as a potential source of DON to Florida Bay.

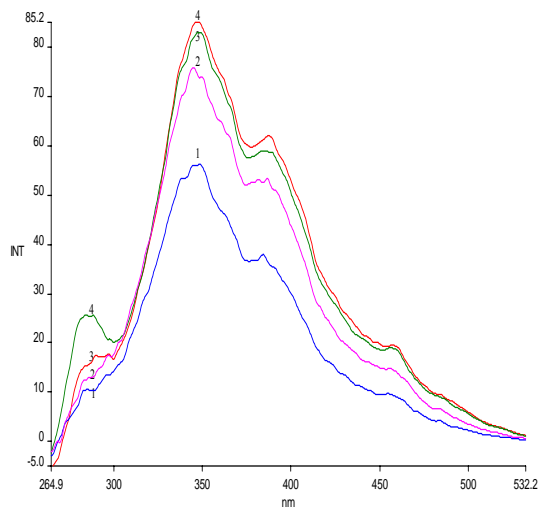


Figure 5: DOM synchronous fluorescence spectra of surface water samples from a transect from the C-111 Canal basin depicting a transect from the canal (1) through the adjacent freshwater marsh (2 to 4) with increasing distance from the canal. Relative abundance of protein-like materials are provided by peak at 285 nm. Bands at higher wavelengths are due to humic substances.

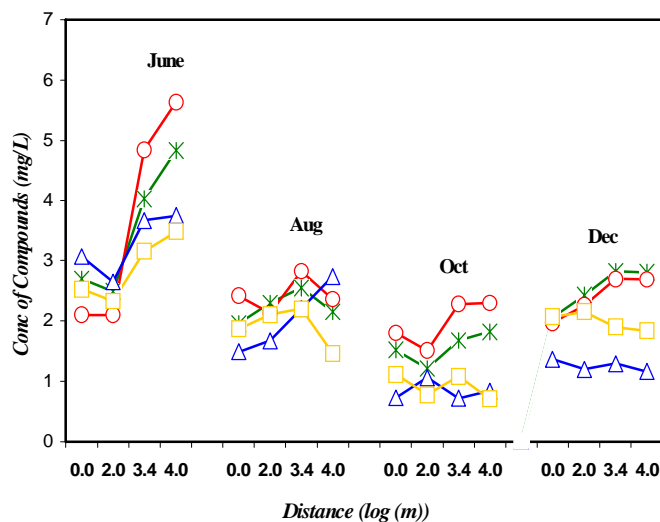


Figure 6: Pyrolysis-GC/MS results from transect of C-111 basin indicating distance from canal at different times: *) proteins; O) carbohydrates; Δ) lignins; and □) poly-aromatics (seasonal data for year 1999; see (Lu et al., 2003).

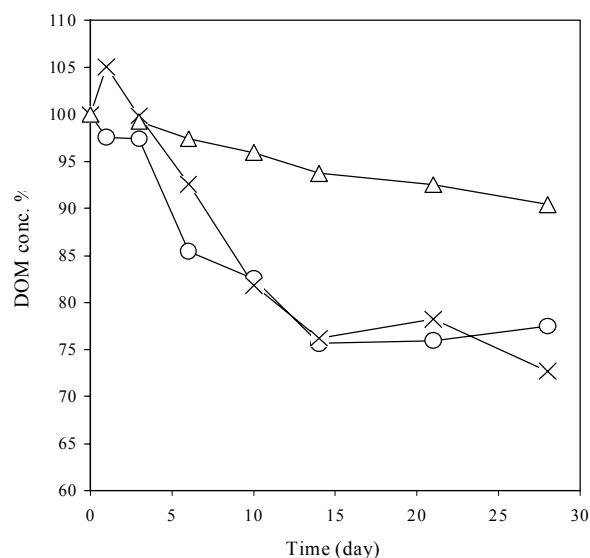


Figure 7: Relative concentrations (%) of the DOM samples (fresh leachate from *Rhizophora mangle*) incubated: x) under natural sunlight conditions; o) in the dark; and Δ) in the dark with NaN_3 added as a poisoned control.

In order to further establish a link between the DON and the presence of proteins in the DOM pool of the Florida coastal Everglades, we have analyzed proteins in several surface water samples of this system. This is a difficult task, since analytical methods for the analysis of dissolved proteins in natural water samples have not been previously developed. However, a combination of ultra-filtration with electrophoresis has allowed us to gain some insight into the protein levels and molecular distributions in the Everglades ecosystem. Water samples from C-111 canal, Taylor Slough, Florida Bay, Coot Bay, Blackwater River and the Shark River as well as leaf extracts from mangroves and sawgrass have been analyzed. Examples of the developed electrophoretic gels are shown in Figure 8. Total protein concentrations were quite variable in these samples and showed highest concentrations in the Taylor Slough and one of the Florida Bay samples, while they were lowest in Coot Bay and the Blackwater River. The C-111 sample showed intermediate concentrations representing about 40% of the Taylor Slough sample. These preliminary results suggest that different regions within the Everglades system have different protein concentration levels as well as compositions. As shown in Figure 8 the different samples show some similarities and some variations in the protein bands that were detected in the gels. While three bands (74, 69 and 53 KDa) were present in all samples including the vegetation samples, the presence of lower molecular weight bands was more variable.

The general presence of the three bands indicated above may suggest the presence of recalcitrant proteins. It could however also suggest the presence of freshly leached proteins considering that they were also present in the leaf extracts. Similarly, the low levels of the lower molecular weight proteins and the larger qualitative variability between sites may be suggestive of either higher rates of biodegradation of these proteins resulting in low concentrations, or on the contrary, they are recalcitrant remains of more highly degraded DOM. While the former explanation is more reasonable, more data is needed to better understand the biogeochemistry of protein materials and DON in general in this system.

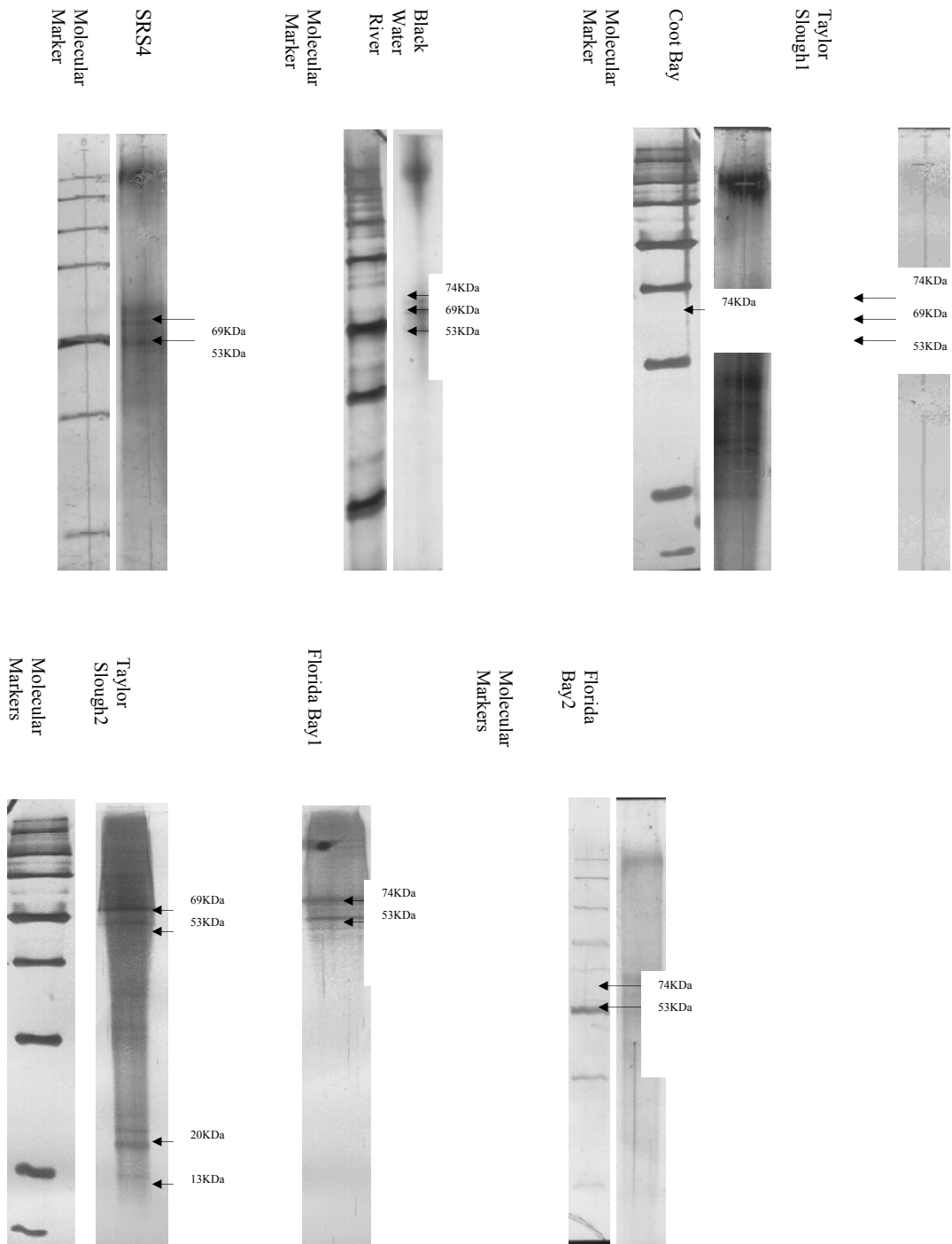


Fig.8 Electrophoretograms for all water samples. Every lane is presented with the appropriate molecular marker to its left. The molecular size the dominant bands of proteins correspond to has been noted (see (Jones et al., 2004).

In summary, our preliminary data suggest that different environments within the FCE will contribute DON to Florida Bay. While relative amount of protein-like materials in the freshwater marshes is lower than that observed for Florida Bay, the absolute concentrations of the DOM shows the opposite behavior. Therefore, both qualitative and quantitative aspects of this question need to be addressed, and the bioavailability of this material needs to be evaluated. This research project will focus on (1) a detailed molecular characterization and (2) an assessment of the bioavailability of the DOM and DON in Taylor Slough, the C-111 Basin and Florida Bay (see Fig. 1).

Research Hypothesis: Florida Everglades freshwater marshes and, to a lesser degree, mangrove forests as well as the autochthonous Florida Bay biomass are all significant sources of labile DOM and DON to Florida Bay.

CHAPTER 1: DISSOLVED ORGANIC NITROGEN (DON) AND DISSOLVED ORGANIC CARBON (DOC) REGIONAL AND SEASONAL SURVEY AND CHARACTERIZATION

Introduction:

Dissolved organic matter (DOM) is the major form of organic matter in most aquatic ecosystems (Findlay & Sinsabaugh, 2003) and this is especially true for oligotrophic ecosystems where the contribution of suspended particles is quite low (Wangersky, 1993). Estuarine and coastal waters in particular are recognized as being important in global DOM cycling. DOM influences the physicochemical characteristics of natural aquatic systems by increasing light attenuation, maintaining pH through organic acid buffering, acting as a strong ligand for many elements, affecting the heat balance, and affecting the redox chemistry of trace metals (Aitkenhead-Peterson et al., 2003). Furthermore, DOM is known to fuel the microbial loop.

As for microbial loop energetics, dissolved organic nitrogen (DON) is very important especially in oligotrophic environments where most of the nitrogen is in the dissolved organic form (Noe et al., 2001). A significant portion of DON may be remineralized by microbes depending upon ambient nutrient status, C:N:P ratio of source materials (Tezuka, 1990), and chemical stability and bioavailability of DOM (Benner et al., 1986; Amon & Benner, 1996). For example, when C:N ratios are low, bacteria may mineralize N but when C:N ratios are high, N may be mineralized only indirectly by grazers (Azam et al., 1983; Carlsson & Graneli, 1993). Alternatively, when nutrient concentrations are limiting, bacterioplankton compete with phytoplankton for inorganic nutrients (Caron, 1994). Therefore, such factors can determine whether the microbial loop is a source or sink for dissolved nutrients.

DON is the major form of nitrogen in the aquatic ecosystems of the Florida Coastal Everglades (FCE), which is an oligotrophic, subtropical wetland located in the southern part of the Florida Peninsula, USA (Wangersky, 1993; Davis et al., 2001). However, most nutrient biogeochemistry studies to date have focused on dissolved inorganic nutrients (N and P), as these fractions are directly available for phytoplankton uptake (Brand, 1999; Tomas et al., 1998). However, most of the nitrogen in the freshwater Everglades is in dissolved organic form (Noe et al., 2001; Rudnick et al., 1999) and thus represents a feasible source of nitrogen to phytoplankton and bacteria (Carlsson & Graneli, 1993).

Continuous population growth in southern Florida is ever increasing the demand for water, thus securing adequate water resources is a pressing challenge. For instance, anthropogenic activities have caused decreased freshwater inputs from the Everglades into Florida Bay (Robblee et al., 1991). This decreased flow has been blamed for a recent rapid decline in the health of Florida Bay, evident by seagrass die-off and increased algal blooms. Public awareness of the degrading health of Florida Bay has been an important impetus for one of the largest natural restoration projects designed to return a more natural flow regime to the Everglades ecosystem (<http://www.evergladesplan.org/>). However, hydrological changes might bring about fallout in the Everglades ecosystem, of which nitrogen (DON) is an important component. It is therefore important to understand the nutrient dynamics of this ecosystem. Although the Everglades ecosystem is largely phosphorous limited (Fourqurean et al., 1993; Philips & Badylak, 1996; Boyer et al., 1997; Lavrentyev et al., 1998), there are evidences that the system does respond to nitrogen inputs (Lavrentyev et al., 1998; Brand, 1999). For example, algal

blooms in Florida Bay are sometimes stimulated by N enrichment (Boyer & Keller, 2003). Still little is known about the composition, sources and bioavailability of DON in this system.

The main objective of this study is the characterization of the bulk and ultrafiltered high molecular weight fraction (>1000 Da) of DON (DON and UDON respectively). The UDON fraction is a significant component of the DON pool (Benner et al., 1992), and therefore may play an important role in the Everglades nitrogen cycle. Many DON bioavailability studies have focused on low molecular weight DON components (Palenik & Morel, 1990; Keil & Kirchman, 1991; Wheeler & Kirchman, 1986) while most DON presumably consists of high molecular weight compounds (Thurman, 1985; Seitzinger & Sanders, 1997). Not much is known about the bioavailability of UDON (Bushaw et al., 1996), but recent data indicate that it may be an important source of nitrogen for bacteria (Amon & Benner, 1996, 1994) and picoplankton (Berg et al., 2003). Larger molecules, once thought to be mainly refractory, have also been shown to release nitrogen-rich, biologically available compounds upon exposure to sunlight (Bushaw et al., 1996). Determining the chemical speciation and assessing the potential bioavailability of UDON are crucial steps in understanding the importance of DON to microorganisms (Feuerstein et al., 1997; Bronk & Gilbert, 1993) and its potential influence on coastal eutrophication. Most coastal eutrophication studies have been performed at temperate latitudes (Cloern, 2001); however, this work will encompass a fresh water to marine transect in a subtropical, oligotrophic coastal wetland ecosystem, namely the Florida Coastal Everglades (FCE).

In order to investigate the dynamics and characteristics of bulk DON and UDOM, we measured TOC, TON, optical properties (UV and fluorescence), water quality parameters and microbial analyses monthly at 11 stations in the Florida Coastal Everglades (FCE; see below) and determined UDOM characteristics biannually at five stations using ^{13}C and ^{15}N cross-polarization magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectroscopy and pyrolysis GC/MS (py-GC/MS). During the first year we attempted to characterize the proteins in the UDON by 1D and 2D electrophoresis, while total protein and carbohydrate analyses were performed monthly during the second year of this study.

Site Description:

We sampled 11 sites along two surveys which extend from the freshwater marshes of Taylor Slough and the C-111 Canal Basin, through the mangrove fringe into and through Florida Bay. (Figure 1), known as the Florida Coastal Everglades Long Term Ecological Research sites (<http://fcelter.fiu.edu>). We sampled monthly for water column nutrients, microbial parameters and optical properties, total proteins and carbohydrates for dissolved organic matter characterization.

Methods:

Sampling design

We sampled monthly for TN and well as NH_4^+ , NO_3^- , NO_2^- , soluble reactive phosphorus (SRP), TP, and TOC. The 3 Florida Bay sites were sampled as part of the SERC Water Quality Monitoring Program and the other 8, Taylor Slough and C-111 Basin sites were sampled as part

of the FCE-LTER Program. The distribution of these study sites spans freshwater marsh (TS 1-5), mangrove (TS 6-8), and bay communities (TS 9-11).

Nutrient Analyses

All nutrient analyses were conducted using standard methodology by the Southeast Environmental Research Center. Sample water was submitted unfiltered for TOC, 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 CO₂-free air. TN was measured using an ANTEK 7000N Nitrogen Analyzer using O₂ as carrier gas (Frankovich & Jones, 1998). TP was determined using a dry ashing, acid hydrolysis technique (Solarzano and Sharp 1980). 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, NO₂, NH₄. These parameters were obtained by flow injection analysis (Alpkem model RFA 300). Three N parameters were not measured directly but rather calculated by difference. NO₃⁻ was calculated as NO_x – NO₂; DIN was calculated as NO_x + NH₄⁺; and TON was defined as TN – DIN.

Microbial Analyses

Pulse amplitude modulated (PAM) fluorometry was used to quantify the community structure and photosynthetic parameters (quantum yield, ETR max) of the phytoplankton community of the samples. 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 was filter sterilized through a 0.2um filter to determine the blank (Z off) for plankton free water. Another 3ml of whole water were run again as to determine CHLA concentrations for blue, green, and brown algal groups (µg L⁻¹), quantum yield- a unit-less value, relative electron transport rate (µM e⁻ m⁻² s⁻¹) and parameters from a productivity-irradiance curve run across 10 light intervals (alpha ratio of Pmax and irradiance, irradiance I_k in µE m⁻² s⁻¹).

Bacterial counts were determined by epifluorescence 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 25 µg ml⁻¹ DAPI (Molecular probes©) in a filtration tower for 20 minutes prior to filtration onto a 0.2um black polycarbonate filter. The filter was mounted onto a slide with low fluorescent immersion oil and examined under a 100W Hg epifluorescence 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 was determined using ³H-thymidine incorporation incubations (Bell, 1993). We ran triplicates of each sample with a 4% final concentration formalin blank for each. With each ³H-thymidine incubation experiment we ran a blank sample for specific activity of the ³H-thymidine. We converted disintegrations per minute (dpm) from the liquid scintillation counter using then following equation:

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

Where picomoles of thymidine incorporated were calculated using the actual activity of the ^3H 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 $\text{l}^{-1} \text{h}^{-1}$. 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). We also used the same reference and value of 20 fg C cell^{-1} to determine the C content of total bacterial biomass. A C:N molar ratio of 5 to calculate the quantity of bacterial nitrogen, (BN) (Lee, 1993).

Dissolved Organic Matter Characterization

At each sampling we collected 30ml of water to conduct DOM characterization. The samples were filtered through a Whatman GF/F and 0.2 μm membrane filter, successively. The UV-Vis absorption spectra were measured with a Shimadzu UV-2102PC spectrophotometer between 250 and 800 nm in a 1cm quartz cuvette using Milli-Q[®] water as the blank. Fluorescence spectra were measured with a Jobin-Yvon-Horiba (France) Spex Fluoromax-3 fluorometer equipped with a 150W continuous output Xenon arc lamp. As a quick, simple means of distinguishing organic matter source changes, two fluorescence indices were obtained by single emission scan measurements at excitation wavelengths of 313 nm and 370 nm. For each scan, fluorescence intensity was measured at 0.5 nm increments at emission wavelengths ranging from 330 to 500 nm and from 385 to 550 nm, respectively, with a 5 nm bandpass for excitation and emission wavelengths. From the 313 nm scan the maximum intensity and maximum wavelength (λ_{max}) were determined (Donard et al., 1989; De Souza Sierra et al., 1997; De Souza Sierra et al., 1994). From the 370 nm scan a fluorescence index (FI) was calculated (McKnight et al., 2001). These two indices have been used to distinguish the DOM derived from marine/microbial and terrestrial/higher plant origin. Originally, (McKnight et al., 2001) introduced the fluorescence index as a ratio of emission intensities at 450 and 500 nm at an excitation wavelength of 370 nm. However, we noticed that after fully correcting fluorescence intensity values (including instrument bias corrections) there was a shift of emission maximum to longer wavelengths. Thus we modified the fluorescence index and used the ratio of fluorescence intensities at 470 and 520 nm, instead of 450 and 500 nm. Similar modifications are being considered by McKnight (personal comm. 2004). Since comparison of FI values among published data was difficult due to inconsistent spectrum correction, in this study, interpretation was conducted based on the comparison within our sample set.

Colorimetric analysis of total hydrolysable Amino acid (THAA)

THAA analysis was conducted according to (Castell et al., 1979). Two mL aliquot of water samples, 20 μl of 11 mM ascorbic acid, and 2ml of concentrated HCl were pipetted into hydrolysis tube. After the gas in the tube was exchanged with argon, the cap was closed tightly, and the solution was heated at 110°C for 24h in an oven. After cooling, the hydrolysate was neutralized with 10 M NaOH solution. One hundred μl aliquot of hydrolysate, 100 μl borate buffer and 100 μl fluorescamine were pipetted into a fluorescence cuvette, and was allowed to react for 1min. Then 2.5ml of Milli-Q water was added to terminate the reaction, and the

fluorescence emission at 475nm at an excitation of 390 nm was measured. Different concentration of glycine solutions (0.1, 0.2, 0.4, 0.6, 0.8 mg L⁻¹) was used as a standard.

Colorimetric analysis of total hydrolysable carbohydrates (TCHO) by TPTZ method

TCHO analysis by the TPTZ method was performed according to (Myklestad et al., 1997). A 2.25 mL aliquot of sample (surface water) and 0.25 mL of concentrated H₂SO₄ were pipetted into a hydrolysis tube, and heated at 100°C for 3 h in an oven. The hydrolysate was neutralized with 2N NaOH. A one mL aliquot of this hydrolysate and 1mL of Reagent-A were pipetted into a test tube and mixed. The solution was heated at 100°C in a water bath for 10 min. Then 1mL of Reagent-B and 2 mL of Reagent-C were add immediately and mixed. After allowing the samples to stand for 30min the absorbance at 595nm was determined by UV-visible spectrophotometry.

Reagent-A: 0.7mM potassium ferricyanide solution (Add 1 g Na₂CO₃, 11.5mg K₃[Fe(CN)₆], and 250 µL of 2N NaOH and fix the volume to 50-mL with Milli-Q water).

Reagent-B: 2mM ferric chloride solution (Add 8.2g sodium acetate, 2.1g citric acid, 15g acetic acid, and 27mg FeCl₃ H₂O and fix the volume to 50-mL with Milli-Q water).

Reagent-C: 2.5mM TPTZ (Add 78mg TPTZ and fix the volume to 100 ml with 3M acetic acid).

Statistical Analyses and graphs

All data from each of the 11 survey sites were combined for a principal components analysis (PCA) with 4 factors. All statistical analyses were conducted using Statview 4.0. Box plots of the survey data were created and grouped by wet or dry season and community type. Wet season data from the monthly surveys included observations from June to November and dry season survey were from December to May. Box plots show the 10th, 25th, 75th and 90th percentiles of data distribution.

We plotted the data for both the wet and dry seasons and averaged the data by the three community types of bay, mangrove and freshwater marsh sites. Bay sites were the end of the Taylor Slough survey and included Duck Key or TS 9, Bob Allen Key or TS 10, and Springer Bank or TS 11. Mangrove sites included TS 6, TS 7, TS 8. Marsh sites included TS 1, TS 2, TS 3, TS 4 and TS 5 (see Fig. 1).

Results and Discussion:

Results for TOC (Fig. 9) revealed the highest values for the mangrove zone. Bay values had the lowest TOC abundance and the marsh signal indicated a median level for TOC. Thus, the contribution to the total pool of carbon is most strongly affected by the mangrove carbon contribution. The signal for TOC was somewhat stronger for the mangrove community type in the wet season than in the dry season, while marsh and bay wet/dry season differences were insignificant.

The total organic nitrogen data, TON, (Fig. 10) revealed a similar pattern to that of the TOC, however there was more overlap between the three different ecosystem types for the DON compared to the TOC, suggesting that although similar, TOC and TON do have different source and production yields or present differences in the bioavailability and/or stability among the

three ecosystem types. Mean TON concentrations (Fig. 10) across the sampling transect were of about 30 μM , being somewhat higher at the mangrove sites during both the wet and dry season. The greatest range of concentrations occurred at the freshwater marsh sampling sites. This high range in the marsh was attributed to drying and rewetting of the marsh soils and periphyton mats during the dry season and beginning of the wet season (Lu et al., 2003), and/or the higher degradation rates during the summer (see NH_4^+ and NO_3^- data below), resulting in more dynamic conditions for DON cycling and transport. The highest concentrations were observed during the lowest water flow (less dilution) of the sampling period. Lowest mean concentrations of TON were observed for the marsh sites followed by the Bay and Mangrove sites. The TOC:TON ratios (Fig. 11) showed a wider range among the three sampling environments than the TON, where the highest ratios were observed for the marsh followed by the mangroves and lowest for the bay, particularly during the dry season suggesting a less nitrogen enriched DOM for the freshwater marsh sites compared to the Bay. Such enrichment seems to also agree with the relative abundance of TON vs. total N (Fig. 12), particularly for the dry season and could be the result of higher primary productivity and exudation of DON during the wet season.

Throughout the sampling period high proportions of total nitrogen were in the form of organic nitrogen (Fig. 12). Over a mean value of 90% of all the nitrogen at all three community types was organic nitrogen during the wet season. During the dry season we saw a much greater range of the proportion of organic nitrogen in the marshes. This may be associated with the leaching of inorganic nutrients from senescing marsh plants. During the wet season the highest mean ratios of TOC:TON were observed at the mangrove sites. The highest portion of bacterial nitrogen was also observed in the mangroves followed by the Bay and lowest for the marsh sites (Fig. 13). This suggests a higher level of recycling of nutrients in the mangrove sites relative to the other community types along the survey. These results are in agreement with (Jones et al., 2005) who suggested an increase in peptidoglycan contribution to the DON pool in the FCE with increasing salinity.

Salinity (Fig. 14) across the survey revealed the expected trend showing highest mean values for the bay, followed by significantly lower values for the mangrove sites and no salinity for the marsh sites. While no significant difference was observed for the bay sites, the mangrove sites showed a significantly lower salinity during the wet season due to increased freshwater discharges from the Everglades. No significant amounts of saline waters reached the marsh sites during the sampling period and mangrove sites reached only mesohaline values (see Appendix). Florida Bay salinities are usually highest during the dry season in the portions of the bay with the least runoff from the Everglades freshwater marshes, however, frequency of storms can affect this trend in the short term as was the case in this study.

The largest contribution to the inorganic nitrogen pool was ammonium. The highest variability and highest observations of NH_4^+ (Fig. 15) were associated with the marsh and mangrove community types in the dry season probably as a result of higher DON recycling (see above) during this period. Mangroves consistently showed the highest NH_4^+ concentrations, followed by the marsh and the bay sites. Small storms that punctuate the dry season may be the best explanation for the variability revealed. In particular, the ammonium leached from the mangrove and freshwater marsh soils as well as from senescent plant materials may contribute to explaining this variability.

Nitrate variability and availability (Fig. 16) was highest in the marsh sites during the dry season in agreement with the ammonia data (Fig. 15). In the wet season, all of the community types of marsh, bay, and mangrove had less variable nitrate levels that were not as high as in the

dry season. This phenomenon is most likely due to the lower concentrations of nitrate with greater water flow, high internal recycling, and higher consumption/uptake of nutrients during the wet period season. Nitrification differences between the different community types across seasons may also control nitrate dynamics.

The highest recorded values for TP (Fig. 17) and SRP (Fig. 18) across the study period and sites were observed for the mangrove and bay sites, while, as expected, the oligotrophic marshes of the Everglades showed the lowest levels. The higher concentrations in the bay (particularly during the wet season) are attributed to the higher concentrations of TP and SRP observed in western Florida Bay when compared to each of the other study sites in the bay, mangrove or marsh (See Appendix for TP and SRP by survey site).

The microbial parameters revealed differences across both season and community types. Bacterial cell counts (Fig. 19) were highest in the mangrove zone and lowest in the marsh zone. The variability of the cell counts was greatest in the mangrove zone, and particularly during the dry season. High bacterial counts may be associated with the elevated TOC and TON values at the mangrove sites and therefore, an elevated source not only of carbon but also nutrients. The high variability may be the result of nutrient ranges during the season, and hydrology driven processes.

Heterotrophic bacterial production (Fig. 20) was quite variable between the three zones, but following the general trend of the bacterial counts. The highest values were observed during the dry season for both the mangrove and marsh community types while the bay sites revealed the lowest levels of bacterial production both during the wet and dry season. This may be associated with the lower bioavailability of carbon and phosphorus that we observed in Florida Bay compared with the marsh and mangrove zones (Boyer et al., 2004a).

Chlorophyll-a concentration (CHLA; Fig. 21) were the highest in both value and range for the marsh sites, followed by mangrove and lastly the bay sites. The greatest contribution to the CHLA pool was from the brown algal group which is comprised mostly of diatoms. Bay CHLA values were the lowest and with very little variability especially compared to that observed in the marsh values. In the marsh and bay sites most of the CHLA associated with the diatom group was found associated with substrates. In the case of the marsh sites the diatoms are mostly associated with periphyton and are contained in the consortiums of periphyton in either floating mats, benthic mats or the sweater periphyton covering emergent vegetation in the marsh. For the bay sites, most of the diatoms were found to be associated with the benthos. The CHLA values reported here are water column only and are representative of only a portion of the pool of autotrophs in each of the community types.

The Quantum Yield (Figure 22) is a proxy for the energetics of phytoplankton. In both the wet and dry seasons, mean quantum yields were highest in the freshwater marsh followed by the mangrove and bay sites, basically following a similar trend to that observed for CHLA. In general, wet season values were somewhat lower than for the dry season.

The UV absorbance at 254 nm (UVA 254; Fig. 23) is a proxy for the total amount of aromatic carbon and concentration of chromophoric DOM (CDOM). The major sources of these aromatic compounds are considered to be partial degradation products of lignins and polyphenols in higher plants, soils and sediments (McKnight et al., 2003). For our study, the mangrove sites showed the highest UVA 254 values followed by the marsh and lastly the bay sites. In agreement, (Jones et al., 2005) reported higher aromaticity values, determined by ^{13}C NMR, for UDOM from FCE marsh and mangrove sites compared to the bay sites. In addition, although seagrass is known to generate relatively large amounts of polyphenols, these have been reported

to be highly photosensitive and are most likely rapidly degraded in Bay waters (Scully et al., 2004) resulting in lower UVA 254 values for the bay sites. The greatest difference in UVA 254 observed between seasons was for the mangrove sites, and consistent with the creek flushing that occurs with precipitation events.

Total fluorescence intensity distributions for wet and dry periods are shown in Fig. 24. Since fluorescence is mainly controlled by the degree of aromaticity of the DOM, it is not surprising that this parameter varied much in accordance with the UVA 254 shown above, except for the fact that the marsh sites showed a higher than expected fluorescence based on the UVA data. Therefore, the UVA correlated better with TOC than the total fluorescence did. This observation may be the result of higher relative abundances of microbial, periphyton-derived fluorophores at the marsh sites, while more condensed, higher plant/mangrove and soil derived DOM enhances the UV-VIS absorbance at the mangrove sites. Both the fluorescence and the UVA values were lowest at the bay sites and suggest a dominance of autochthonous DOM over Everglades derived DOM, and/or the photodegradation of chromophores and fluorophores in the bay region.

The maximum fluorescence wavelength (λ_{\max}) is a useful parameter to assess sources of DOM (Jaffé et al., 2004) and shown increasing values (more terrestrial in origin) from the bay to the mangroves and the marsh sites, with the mangrove and marsh sites not being significantly different (Fig. 25). In fact, a slightly stronger terrestrial value was observed for the wet season at the mangrove sites. This trend is in general agreement with the fluorescence index data (F.I.; Fig. 26) which show highest values for the bay sites (most microbial) and lower values for the mangrove and marsh sites, with the mangroves showing consistently the most terrestrial values, particularly for the wet season. A slightly higher FI values in marsh than mangrove suggests that periphyton-derived DOM is an important DOM source. This suggests again, that although mainly of terrestrial origin, the marsh sites have a noticeable microbial contribution to the DOM pool, most likely from periphyton (see also Lu et al., 2003).

Protein (Fig. 27) and carbohydrate (Fig. 28) concentrations revealed similar trends across the sampling period. In both the wet and dry season bay sites had the lowest mean concentrations of both protein and carbohydrates while these parameters were significantly higher for the mangrove and marsh sites, particularly in the case of the proteins. Both proteins and carbohydrates were highest in the mangrove zone which also had the highest TOC values. In general, the difference in concentration between the bay and the mangrove and marsh sites was larger for the proteins than for the carbohydrates, suggesting that the bay sites are either protein depleted and/or carbohydrate enriched compared to the other sites. The latter explanation is more plausible since bay water contains high proportion of carbohydrates (see below; Maie et al., 2005). It was also shown that seagrass UDOM contains a high proportion of carbohydrates (Maie et al., 2006). While no clear seasonal differences could be determined, the carbohydrates were clearly higher in the wet season for the mangrove sites possibly as a result of higher primary productivity and exudation of carbohydrates during this period.

The synchronous fluorescent peak between 280 and 290+ nm, termed here Peak 1 has been suggested as a proxy for protein-like materials in DOM (see Jaffé et al., 2004 and references therein). The Peak 1 (QSU) for the present study shows similar trends to the total protein distributions with the difference that the mangrove and marsh sites are more clearly different from one another, with the marsh sites consistently lower than the mangrove sites and the bay sites showing the lowest overall values (Fig. 29).. Protein-like materials seem somewhat higher for the mangrove and marsh sites during the wet season, while no differences were apparent for the bay sites.

PCA identified four composite variables (hereafter called Factor 1, Factor 2, etc.) that passed the rule N for significance at $P < 0.05$ (Overland and Preisendorfer 1982) indicating four separate modes of variation in the data (Table 1). Our survey data for the entire study period revealed that the “Organic” factor (SAL, TON, TOC, UVA₂₅₄, F_{max}, FI, and Peak 1) was most important in explaining overall variability in the data set (Table 1). The negative relationship to SAL and some of the ‘organic’ parameters seems to suggest a terrestrial, Everglades-derived source for the DOM and DON. However, DOM molecular characteristics (see below and Maie et al., 2005) provide evidence that DOM in Florida Bay is significantly different in composition from that in the Everglades, suggesting mainly autochthonous OM sources for the DOM in the Bay. The positive correlation between the F.I. and the %Peak1 with SAL suggests that in fact this may be the case. Therefore, the previously mentioned negative correlation for various ‘organic’ parameters and SAL may be the result of both increased hydrological inputs to the bay during the wet season and the fact that more autochthonous DOM and DON is produced during the wet season in the Bay (when an overall lower SAL is observed). These six parameters explained 37.5% of the variance in the data. The second strongest factor was composed of NO₃⁻ and NH₄⁺, and explained 12.3% of the variability. The third “Microbial” factor showed the relationship between BACT, BP, CHLA, and TP. This association is not unique to our studies of microbial and nutrient dynamics in south Florida and was also evident in our work across 28 sites we studied in the two years prior to this study (Boyer et al. 2004b). The fourth factor showed the positive relationship among SAL, TP, and SRP. Overall, 66.5% of the variability across the sampling period and across sites was explained by the PCA.

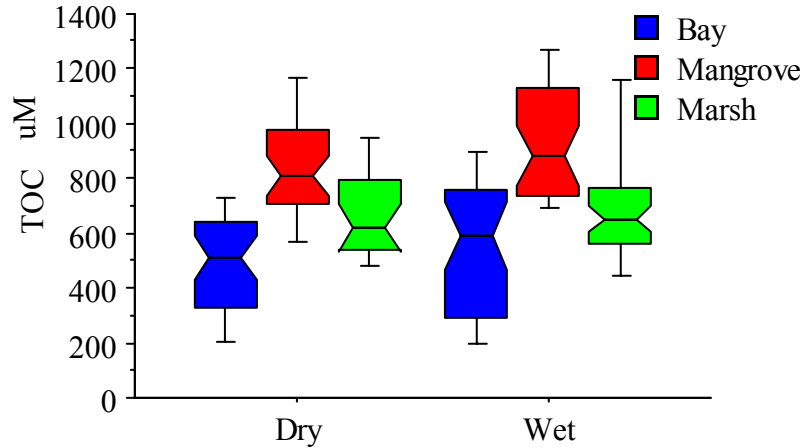


Figure 9: Total Organic Carbon (TOC μM) across the sampling survey averaged by community type for the wet and dry seasons from September 2002 to August 2004.

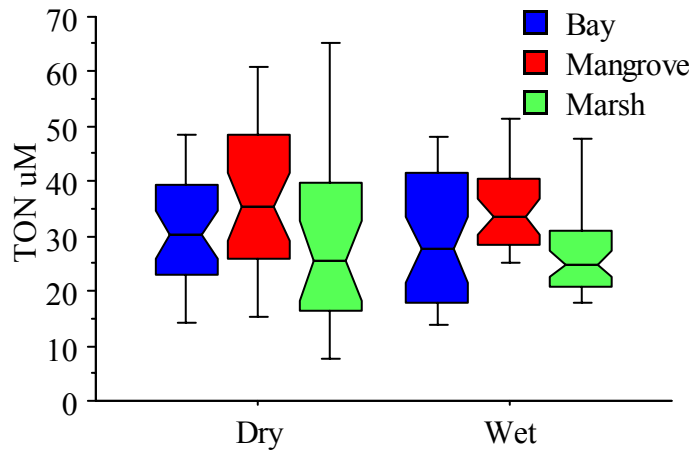


Figure 10: Total Organic Nitrogen (TON μM) across the sampling survey averaged by community type for the wet and dry season from September 2002 to August 2004.

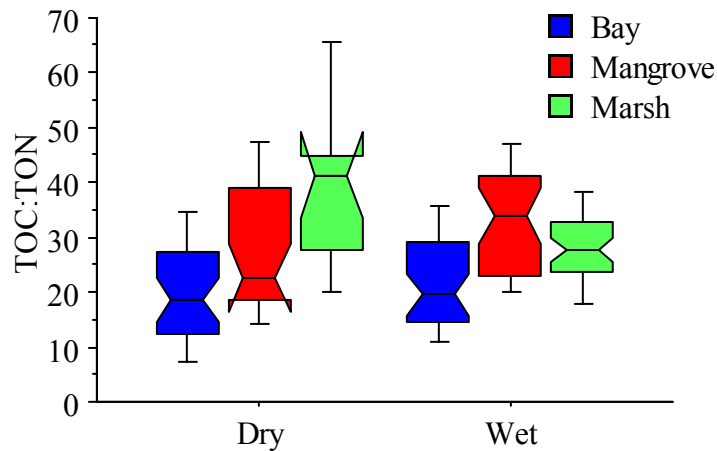


Figure 11: The ratio of TOC to TON across the sampling survey averaged by community type for the wet and dry season from September 2002 to August 2004.

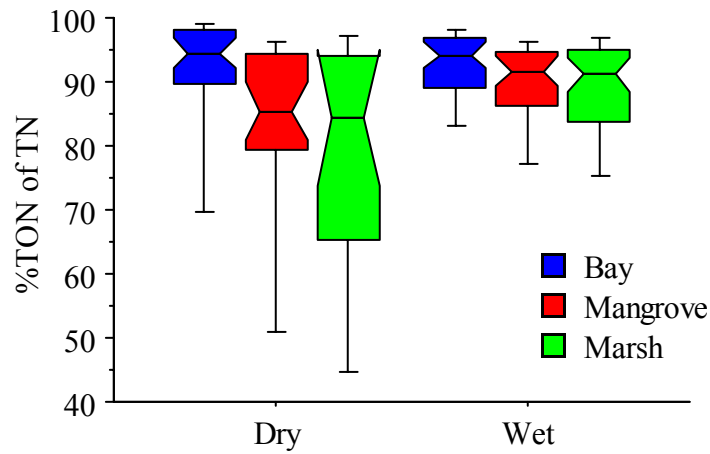


Figure 12: The percentage of TON of TN across the sampling survey averaged by community type for the wet and dry season from September 2002 to August 2004.

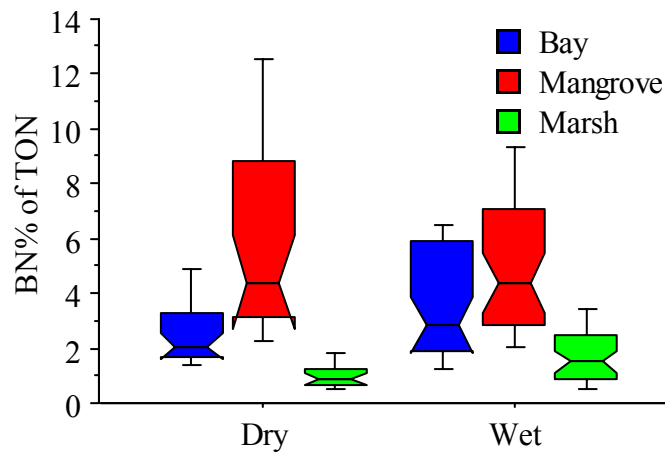


Figure 13: The percentage of Bacterial Nitrogen of TON across the sampling survey averaged by community type for the wet and dry season from September 2002 to August 2004.

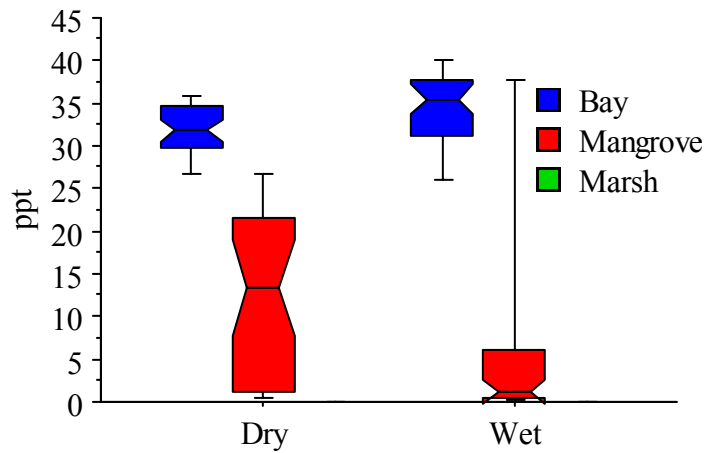


Figure 14: Salinity from each of the community types over the two year study period.

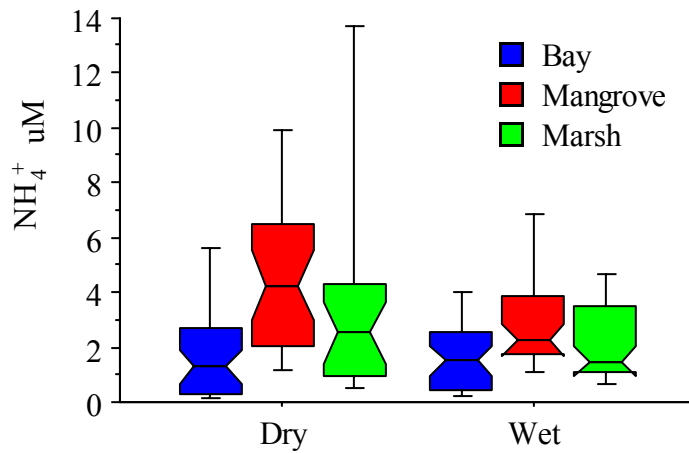


Figure 15: Ammonium (NH_4^+ μM) across the sampling survey averaged by community type for the wet and dry seasons from September 2002 to August 2004.

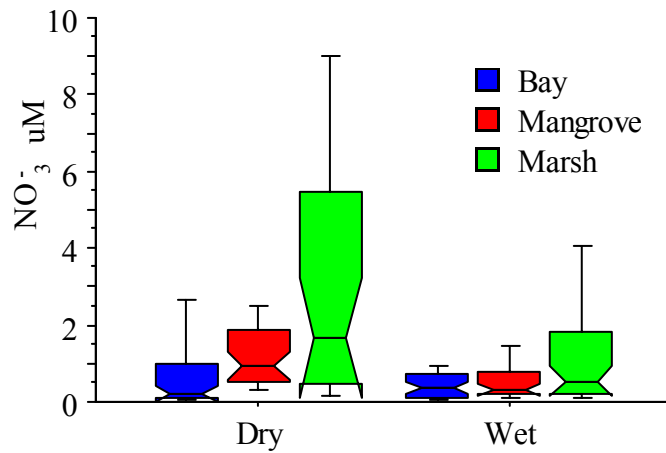


Figure 16: Nitrate (NO_3^- μM) across the sampling survey in averaged by community type for the wet and dry season from September 2002 to August 2004.

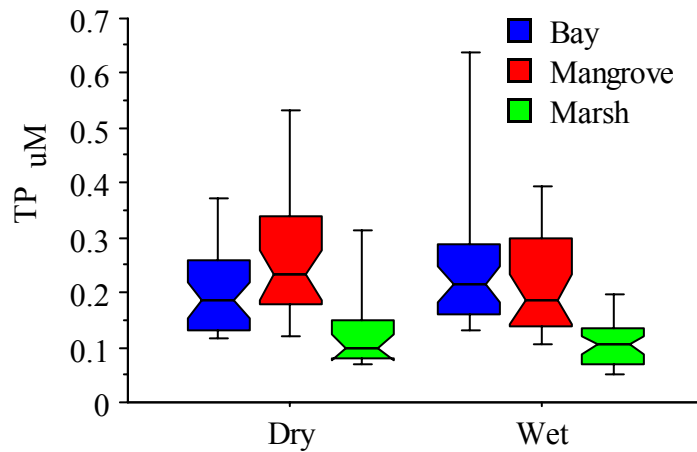


Figure 17: Total Phosphorus (TP μM) across the sampling survey averaged by community type for the wet and dry season from September 2002 to August 2004.

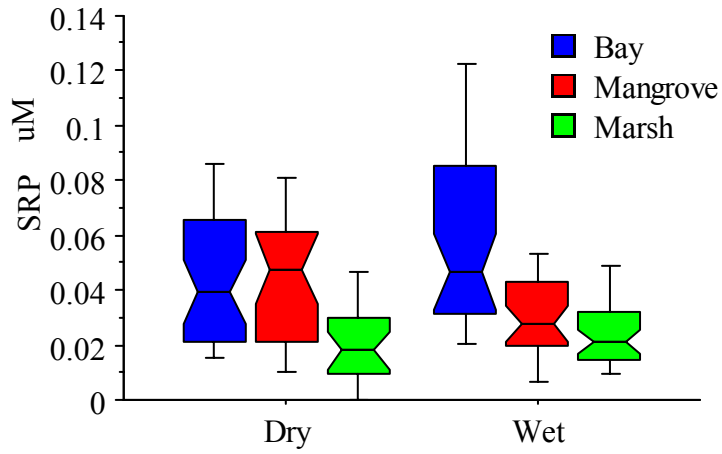


Figure 18: Soluble Reactive Phosphorous (SRP in μM) from each of the community types over the two year study period.

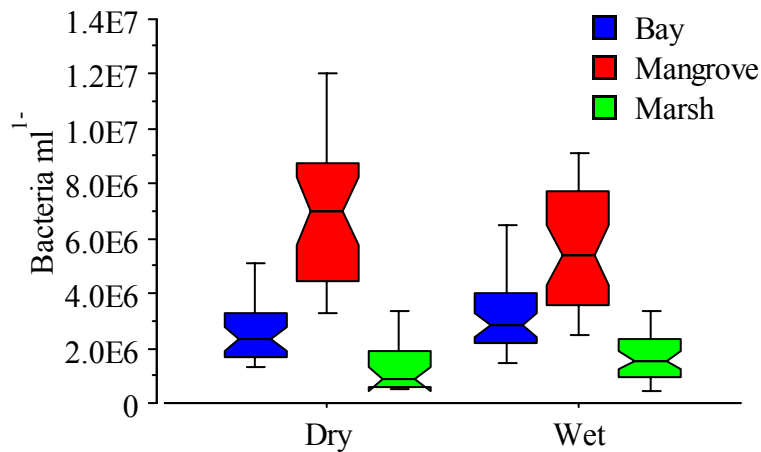


Figure 19: Bacteria counts across the sampling survey in bacterial cells ml^{-1} averaged by community type for the wet and dry seasons from September 2002 to August 2004.

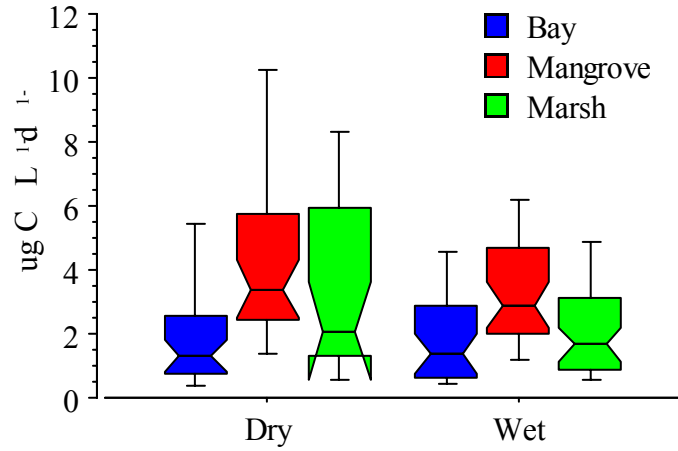


Figure 20: Bacteria production across the sampling survey in $\mu\text{g C L}^{-1} \text{d}^{-1}$ averaged by community type for the wet and dry season from September 2002 to August 2004.

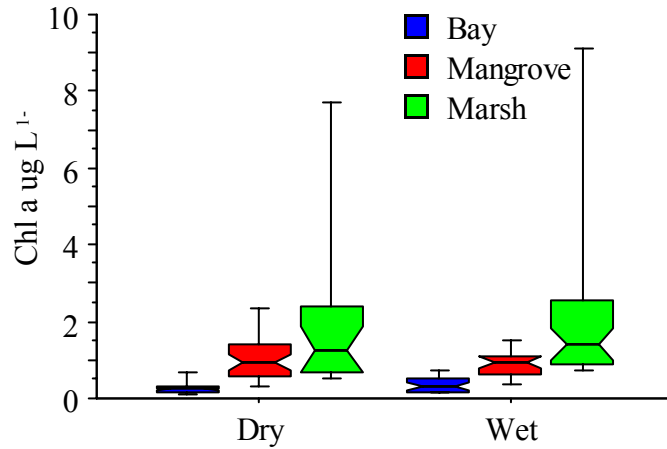


Figure 21: Chlorophyll a from the PAM (CHLA $\mu\text{g L}^{-1}$) across the sampling survey averaged by community type for the wet and dry seasons from September 2002 to August 2004.

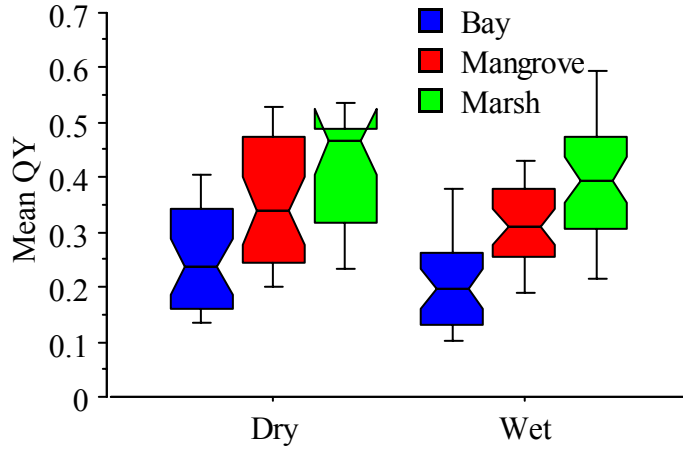


Figure 22: Mean Quantum Yield (QY) as an indicator of autotrophic production across the sampling survey in averaged by community type for the wet and dry season from September 2002 to August 2004.

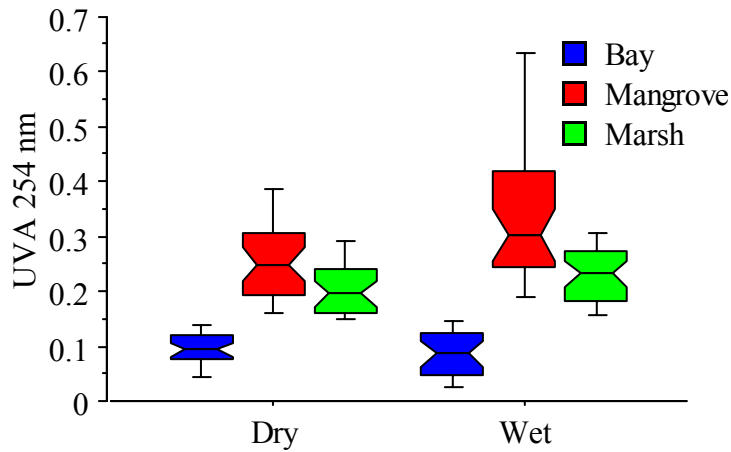


Figure 23: UVA 254 nm across the sampling survey in averaged by community type for the wet and dry season from September 2002 to August 2004.

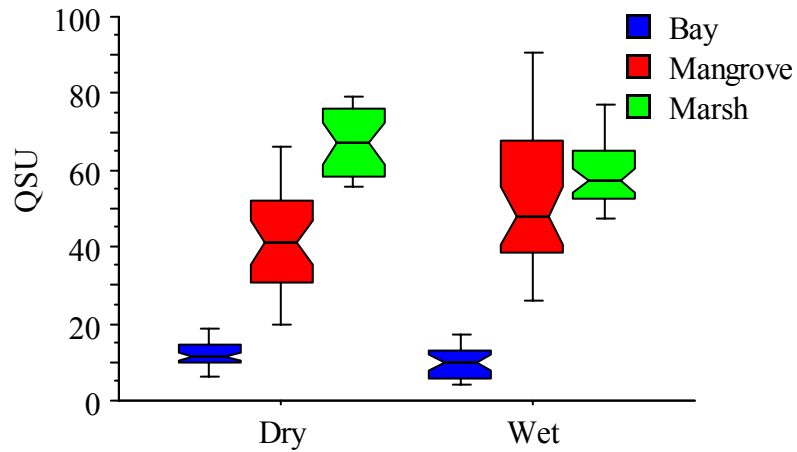


Figure 24: Total Fluorescence in Quinine Sulfate Units (QSU) from each of the community types over the two year study period.

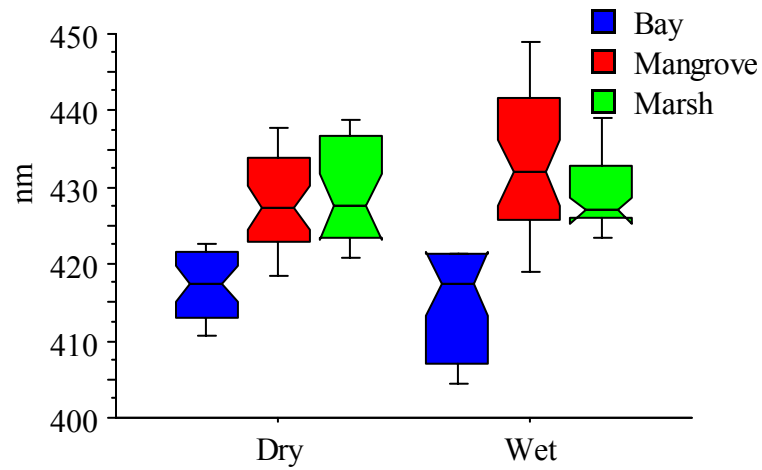


Figure 25: Maximum wavelength (nm) from each of the community types over the two year study period.

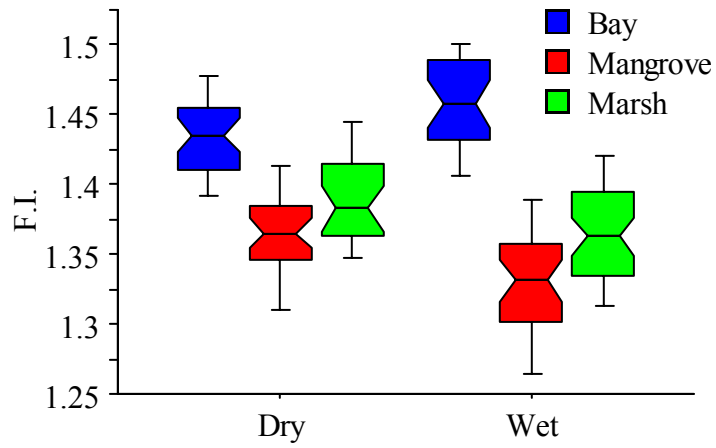


Figure 26: Fluorescence Index from each of the community types over the two year study period.

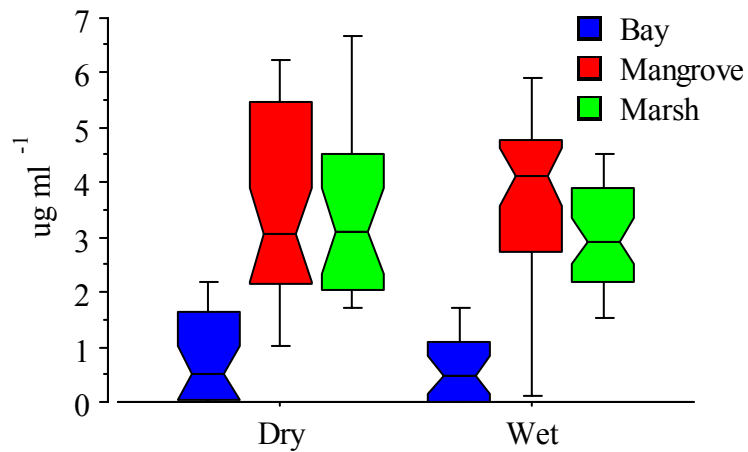


Figure 27: Protein concentration from each of the community types over the two year study period.

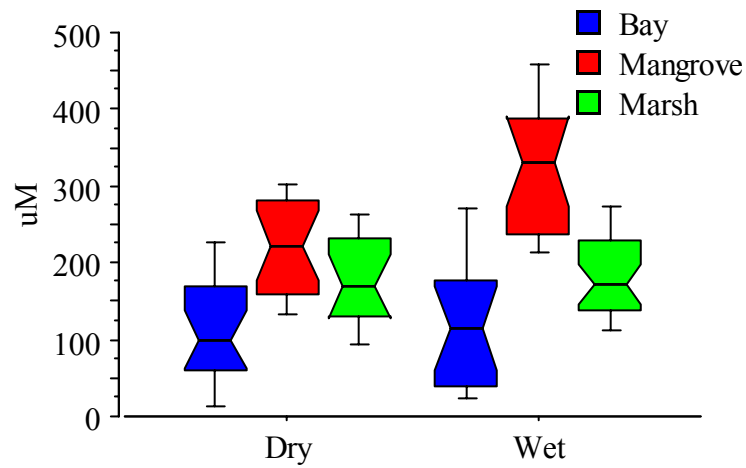


Figure 28: Carbohydrate concentration from each of the community types over the two year study period.

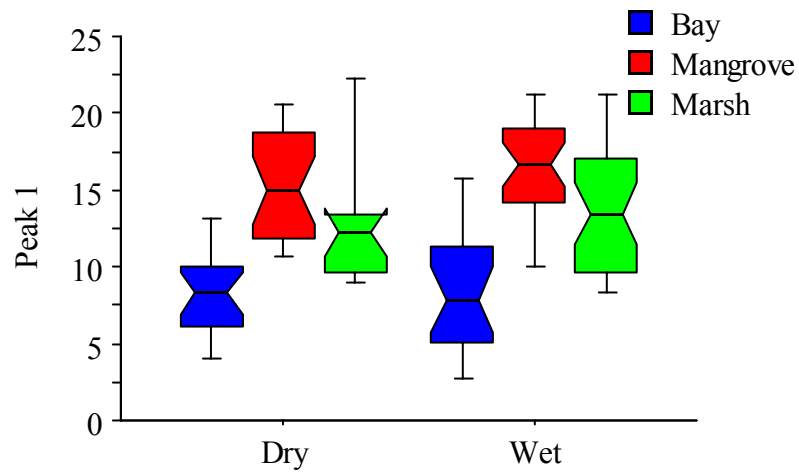


Figure 29: Peak 1, or % 285 nm from each of the community types over the two year study period.

Parameter	Factor 1	Factor 2	Factor 3	Factor 4
SAL	-0.456	-0.018	0.163	0.449
BACT	-0.115	0.280	0.592	0.117
BP	-0.279	0.372	0.655	-0.148
CHLA	-0.001	-0.002	0.621	0.219
QY	0.063	-0.001	0.386	-0.181
NO₃⁻	-0.200	0.844	0.258	0.095
NH₄⁺	0.001	0.814	0.078	-0.034
TON	0.626	-0.001	-0.402	0.030
TP	-0.307	-0.083	0.600	0.630
SRP	0.089	0.120	-0.073	0.575
TOC	0.661	0.104	-0.022	0.211
UVA₂₅₄	0.644	-0.029	0.100	0.046
F_{max}	0.658	-0.060	0.047	-0.063
FI	-0.595	0.110	-0.002	0.205
Peak 1	-0.474	0.154	-0.207	0.170
% Variance explained	35.7	12.3	12.0	6.5

Table 1: Principal component analysis for the nutrient, microbial and dissolved organic matter characterization dataset from September 2002 through August 2004.

CHAPTER 2: CHARACTERIZATION OF ULTRAFILTERED DOM (UDOM) IN THE FLORIDA COASTAL EVERGLADES.

Sampling and Methods

Sample collection

Surface water samples were collected in 25-L white low-density polyethylene Carboy bottles (Nalge Nunc International, Rochester, NY, USA) during the early part of the dry season (from Sep 2002 to Mar 2004). The bottles were cleaned by soaking in 0.5 mol L⁻¹ HCl followed by a 0.1 mol L⁻¹ NaOH solution for 24 h each. Water samples were filtered through pre-combusted (470 °C for 4 hours) 0.7 µm GF/F glass fiber filters (Whatman International Ltd., Maidstone, England), followed by concentration using a Pellicon 2 Mini tangential flow ultrafiltration (TFF) system equipped with a nominal 1000 Da molecular weight cut-off regenerated cellulose membrane (Millipore Co., Billerica, MA, USA) (Dai et al., 1998.). The water samples were concentrated to 100 ml at an inlet pressure of 10 psi, and an outlet pressure of 8 psi. For water samples collected from Florida Bay (sites 9-11), diafiltration was conducted as follows: One liter of Milli-Q[®] water (Millipore) was added to the concentrated sample and then re-concentrated to 100 ml. This process was repeated a total of three times. The concentrated samples were freeze-dried and powdered with an agate mill. While the percentage of UDOC to total DOC was not determined in this experiment, % UDOC to total DOC collected from the same sites in Sep-Oct 2002, was 14 ± 4 and 24 ± 11% for TS and FB, respectively.

Water samples for fluorescence analysis were collected separately in 30 ml brown polyethylene bottles, stored on ice, and transported to the laboratory. The water samples were filtered through pre-combusted (470°C for 4 hours) Whatman GF/F glass fiber filters prior to analysis.

Solid state ¹³C NMR spectroscopy

Solid state ¹³C NMR spectra were obtained at a ¹³C resonance frequency of 50.3 MHz on a Bruker ASX200 NMR spectrometer (Bruker, Rheinstetten, Germany) equipped with a commercial 7 mm cross polarization magic angle spinning (CPMAS) probe using a standard CPMAS pulse sequence. ¹³C chemical shifts are expressed with respect to tetramethylsilane by using the carbonyl carbon of glycine (176.48 ppm) as an external reference. Other analytical conditions were as follows: rotation frequency, 4.5 kHz; contact time, 1 ms; recycle delay, 2 s; scans accumulated, 3000-20000; spectral width, 25 kHz; filter frequency, 32 kHz; Lorentzian line-broadening, 120 Hz. NMR spectra were divided into four regions according to chemical shifts as follows: 0-45 ppm (alkyl C), 45-110 ppm (*O*-alkyl C), 110-160 ppm (aromatic C), 160-210 ppm (carbonyl C) (Kögel-Knabner, 1997). The relative abundance of these regions was reported as % of total spectral area, at reproducibility within ± 2%, determined by repacking the sample in triplicate. The first order spinning sidebands (SSBs) of aromatic and carbonyl signals (220 and 260 ppm, respectively) were corrected if necessary, according to (Knicker et al., 2000).

¹⁵N Nuclear Magnetic Resonance (NMR)

Solid state cross polarization magic angle spinning (CPMAS) ¹⁵N NMR spectra were obtained on a Bruker DMX 400 (40.56 MHz) spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) using a ramped pulse sequence (Peersen et al., 1993; Cook et al., 1996;

Abe & Watanabe, 2004), a rotation frequency of 5.5 kHz, a contact time of 1 ms, and a pulse delay of 200 ms. Between $1.1 - 4.6 \times 10^5$ single scans were accumulated and line broadenings of 50-160 Hz were applied. The chemical shift was referenced to nitromethane scale (= 0 ppm) and was adjusted with ^{15}N -enriched glycine (-347.6 ppm).

Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC/MS)

Py-GC/MS analyses were performed as previously described (Lu et al. 2003) except that in this study UDOM instead of total freeze-dried DOM samples were used. Briefly, UDOM samples (ca. 5 mg) were pyrolyzed at 650°C for 20s in a helium atmosphere using a pyroprobe 1500 pyrolyzer (Chemical Data Systems, Oxford, PA, USA). Separation of pyrolysis products was carried out on a DB5MS fused-silica column (30 m length \times 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific) at a split ratio of 1:75 under a helium atmosphere. The oven was connected to a split/splitless injection port of a Hewlett Packard 6890 GC coupled to a HP 5973 mass spectrometer. The oven temperature program was as follows: initial temperature was held at 40°C for 2 min, ramped at 7°C min^{-1} to 300°C where it was held for 15 min. The assignment of peaks was based on the comparison of mass spectra with the spectral library (NIST 98, Gaithersburg, MD, USA) and/or mass spectral interpretation. Other analytical conditions were identical with those of TMAH thermochemolysis described above.

Hierarchical cluster analysis (HCA) of pyrolysis products

Based on the relative abundance of individually identified pyrolysis products (approximately 100 compounds; peak area of individual compound to total peak area of identified compounds in pyrogram) a hierarchical cluster analysis (HCA) was performed using an agglomerative method (Ward method) with SPSS version 11.0.1 software (SPSS Inc. Chicago, IL, USA) for the interpretation of the multivariate pyrolysis data set. The objective of this study was to use this statistical approach to classify the UDOM composition of different samples using a molecular fingerprinting approach.

Results and Discussion

^{13}C CPMAS NMR analysis

O-alkyl C is the dominant C form of UDOM in the Everglades (Fig. 3 & 4 and Table 1). C111 Canal UDOM had the highest alkyl C (30 \pm 4%) and lowest *O*-alkyl C (44 \pm 7%), which suggests that the UDOM at this site is the most highly degraded compared with other studied site (see also Lu et al., 2003). This is probably due to the longer residence time of DOM in canals and/or the high microbial activity due to elevated concentrations of nutrients. Our results also suggest that a large amount of UDOM is produced within freshwater and mangrove marshes. TSPH 2 UDOM had the next lowest *O*-alkyl C and very high aromatic C. The aromatic C tended to be higher during the dry season, which is probably a result of change in hydrology. The TSPH 2 site is located in a long hydroperiod area of Taylor Slough, and is deeper during the wet season while it becomes a stagnant, shallow pond during the dry season. As such, the residence time of DOM is much longer and DOM supply from freshwater marsh plants, particularly periphyton is limited during the dry season. As a result, the DOM is most likely more degraded during the dry season. The chemical characteristics of the mangrove marsh and coastal sites were also different among seasons. We expected the input of freshwater DOM to

maximize at the end of the wet season and be at a minimum at the end of dry season. However, due to occasional rain events at the end of dry season and an earlier decrease of fresh water inflow than expected, we did not observe UDOM dynamics expected for a “typical” wet and dry season. This was also shown in the result of the pyrolysis-GC/MS analysis of UDOM (discussed below). Overall C composition of mangrove fringe is dominated by O-alkyl C through the whole season ($60\pm 7\%$). C composition of UDOM in FB was relatively stable, and O-alkyl C was the major C species representing $71\pm 2\%$ of the UDOM. Aromatic C concentrations were very small for FB ($3\pm 1\%$). The high O-alkyl C concentration in FB suggests the production of carbohydrates in Florida Bay probably by seagrass communities.

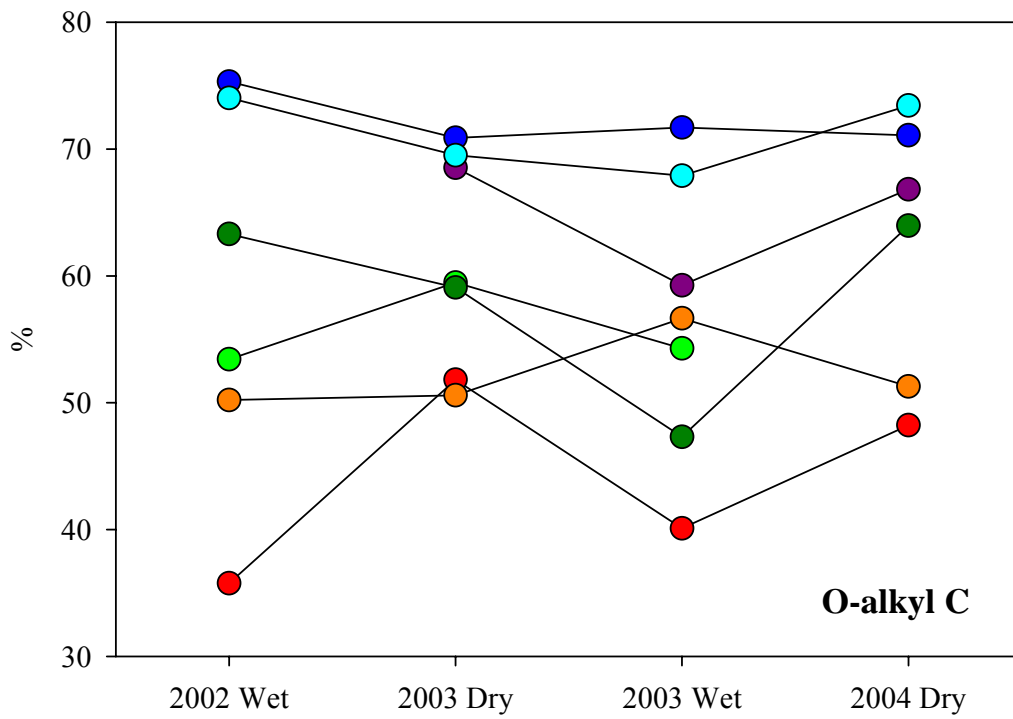
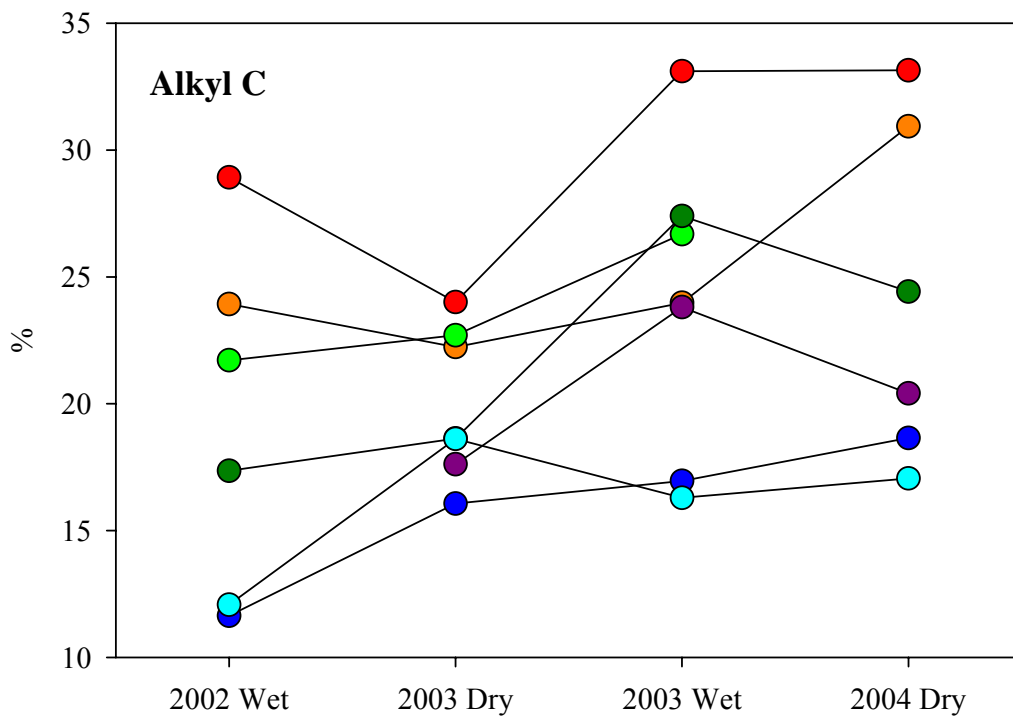


Fig.30a ^{13}C NMR based C composition of UDOM in the Florida Coastal Everglades.

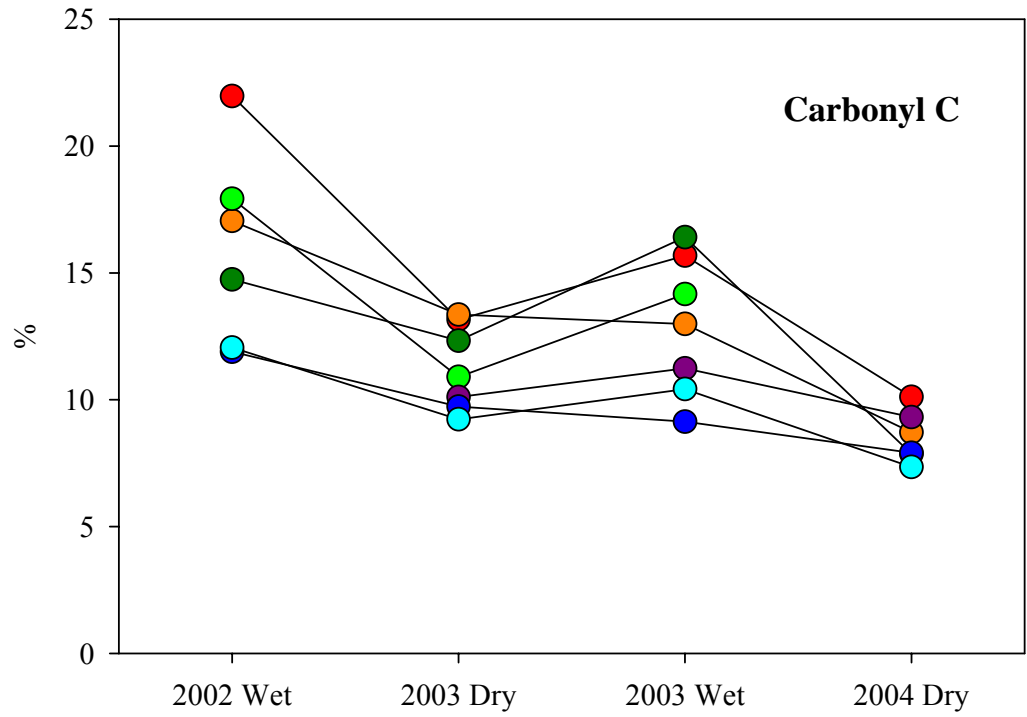
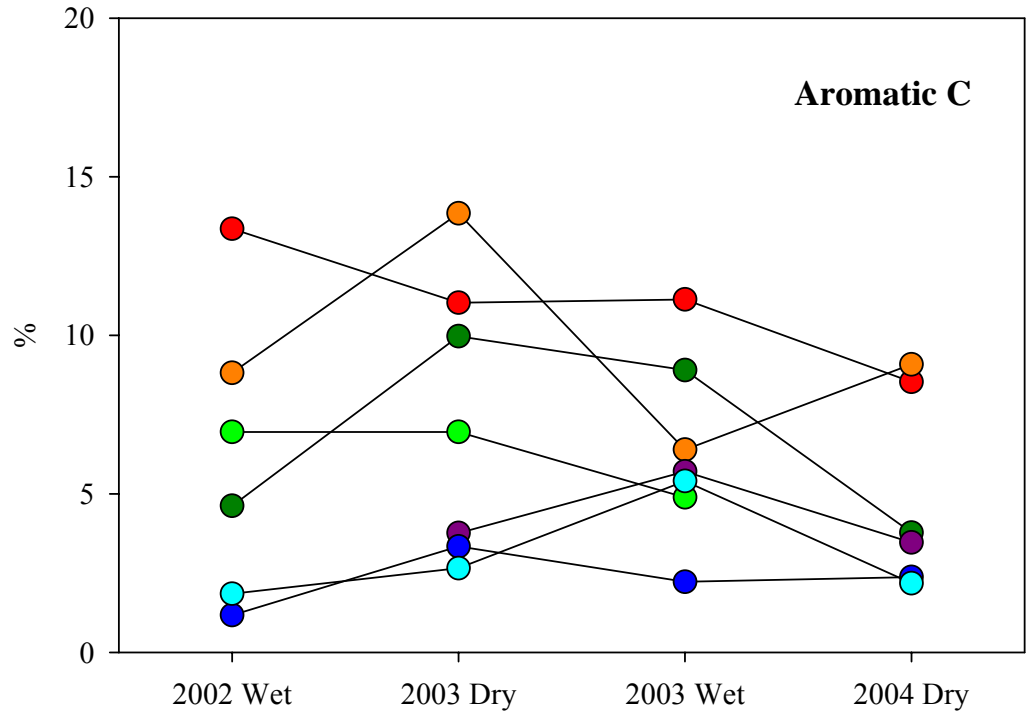


Fig.30b ¹³C NMR based C composition of UDOM in the Florida Coastal Everglades (*continued*).

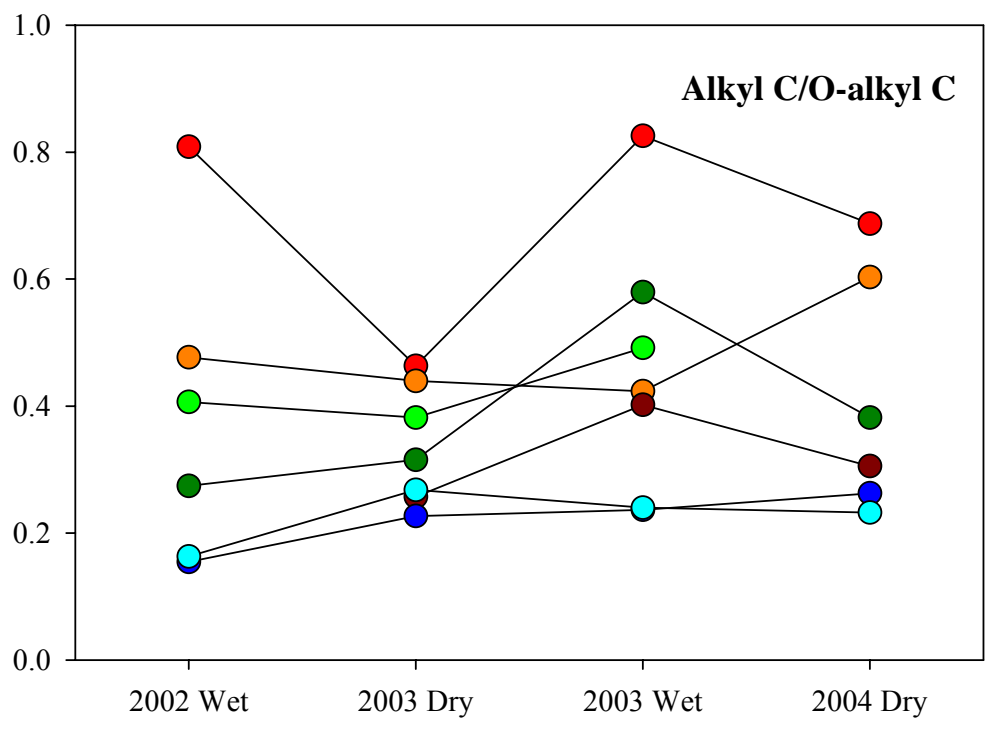
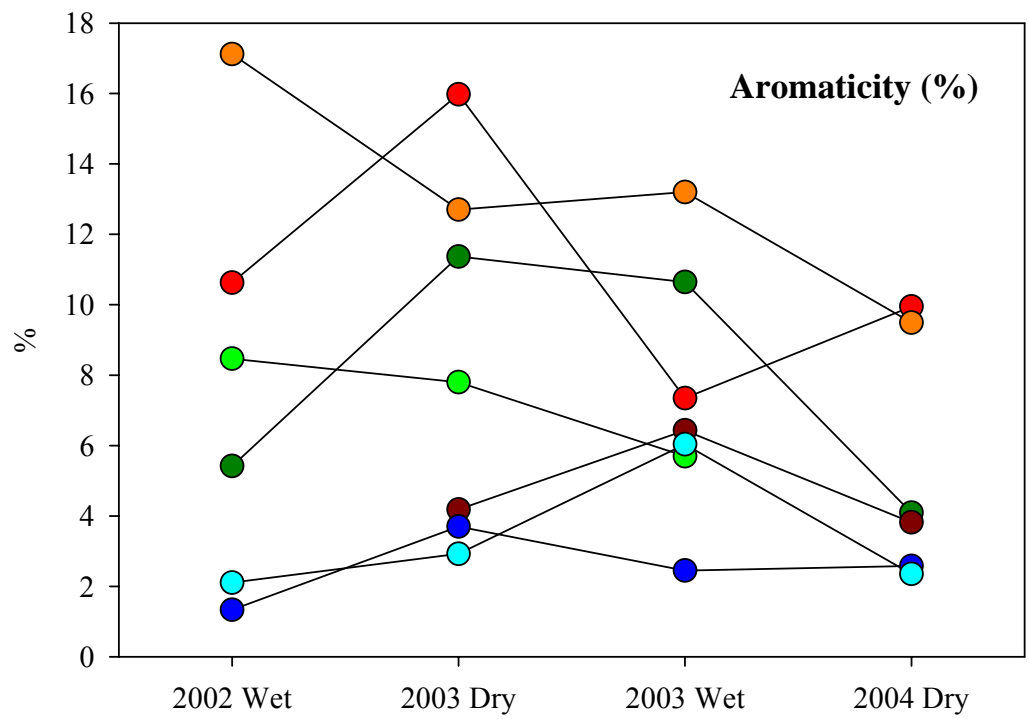


Fig. 31: Plots of the indices of diagenesis of UDOM, aromaticity and alkyl C/O-alkyl C.

Table 2: ^{13}C NMR based C composition of UDOM in the FCE

Sites	Alkyl C	O-alkyl C	Aromatic C	Carbonyl C
	0-45 ppm	45-110 ppm	110-160 ppm	160-220 ppm
C111	30 (4)	44 (7)	11 (2)	15 (5)
TSPH2	25 (4)	52 (3)	10 (3)	13 (3)
TSPH6, 7, 8	22 (4)	60 (7)	6 (2)	12 (3)
TSPH9, 10	16 (3)	71 (2)	3 (1)	10 (2)

Number in parentheses is SD.

^{15}N CPMAS NMR spectra ^{15}N

CPMAS NMR has been used to determine the speciation of organic nitrogen (Knicker, 2004). While much ^{15}N CPMAS NMR work has been applied to soil, coal, and sediment organic matter (Knicker et al., 1993; Knicker & Hatcher, 1997; Knicker et al., 2000), very little has been done specifically on DON (McCarthy et al., 1998; McCarthy et al., 1997). To the best of our knowledge this work will be the first application of ^{15}N NMR to characterize freshwater and estuarine UDOM.

Based on the ^{15}N CPMAS NMR spectra of our UDOM samples (see Fig. 32), nearly all detected N was in the form of amide-N (-220 to -280 ppm; i.e., proteinaceous N), and heterocyclic-N (-145 to -220 ppm) and amino-N (-280 to -325 ppm) were minor. The quantification of each N species was not possible due to low signal-to-noise ratio of ^{15}N NMR spectra. The dominance of amide-N coincides with other reports on the characterization of N species in natural organic matter in various environments (Knicker et al., 1993; McCarthy et al., 1997; Vairavamurthy & Wang, 2002). However, a fair criticism of ^{15}N NMR in the study of natural OM is that (1) a low signal-to-noise ratio makes the detection of aromatic-N (pyridine-N) difficult, and (2) the CPMAS technique inherently underestimates remotely protonated nitrogen (tertiary N; i.e. heterocyclic N, heteroaromatic N) due to a slow magnetization transfer rate from ^1H to ^{15}N . Even though, a high concentration of heterocyclic N has often been found in highly aromatic organic matter or charred organic residues (Knicker et al., 1993; Knicker & Hatcher, 1997; Knicker et al., 2000); this does not seem to be the case for our DOM samples.

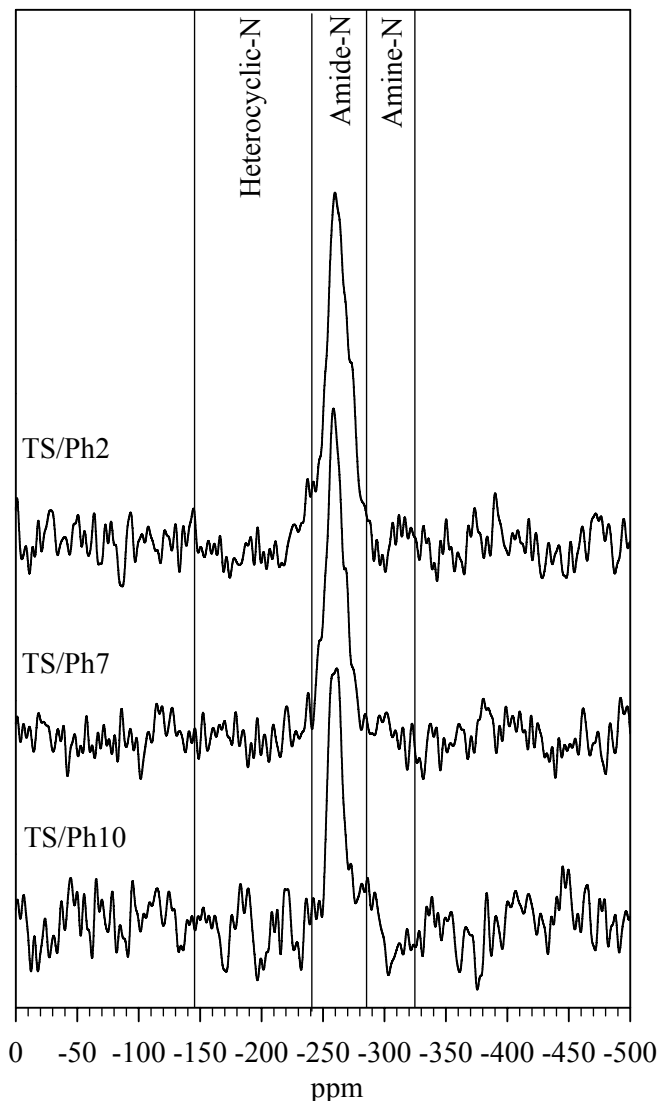


Fig. 32: ^{15}N CPMAS NMR spectra of UDOM from selected sites.

Molecular characteristics of UDOM based on pyrolysis GC/MS products

Cluster analysis of pyrolysates showed that the UDOM is largely clustered into two large groups (Fig. 33); one is typical for the terrestrial Everglades UDOM and another is typical for Florida Bay. For the terrestrial Everglades, the C111 canal and freshwater marshes, and the mangrove regions formed sub-clusters. Mangrove marsh (TSPH 6, 7, 8) UDOM distributed in both clusters depending on the sampling time. This is due to the shift of the dominant DOM source between freshwater and bay waters. TSPH2 dry season and C111 formed a large cluster, which is considered to be highly degraded. No consistent seasonal trend was observed for Florida Bay UDOM (TSPH 9, 10, 11). Freshwater marsh sites (TSPH2 and 3) are separated from mangrove marsh sites (TSPH 6, 7, 8). TSPH 2 & 3 of the year 2002 wet season was located separately from other Everglades UDOM, which was difficult to explain. At present, the reasons for this behavior remain unknown, but contributions by periphyton-derived DOM might be higher for those samples.

Overall findings suggest that the chemical characteristics of UDOM in Florida Bay are not highly variable among season and locations. The UDOM source in coastal sites changes depending on the source strength from the Everglades and therefore with hydrological

conditions. Molecular characteristics of the C111 canal water are quite different from other Everglades UDOM samples. PC1 and PC2 explained 41.7% and 38.3% of variation, respectively. Principal component analysis (PCA) of pyrolysates (Fig. 34) were clearly separated UDOM from different regions, suggesting that the UDOM composition among the ecosystem types is different at the molecular level. Therefore, it is suggested that the local vegetation is very important as a source of UDOM, including for Florida Bay.

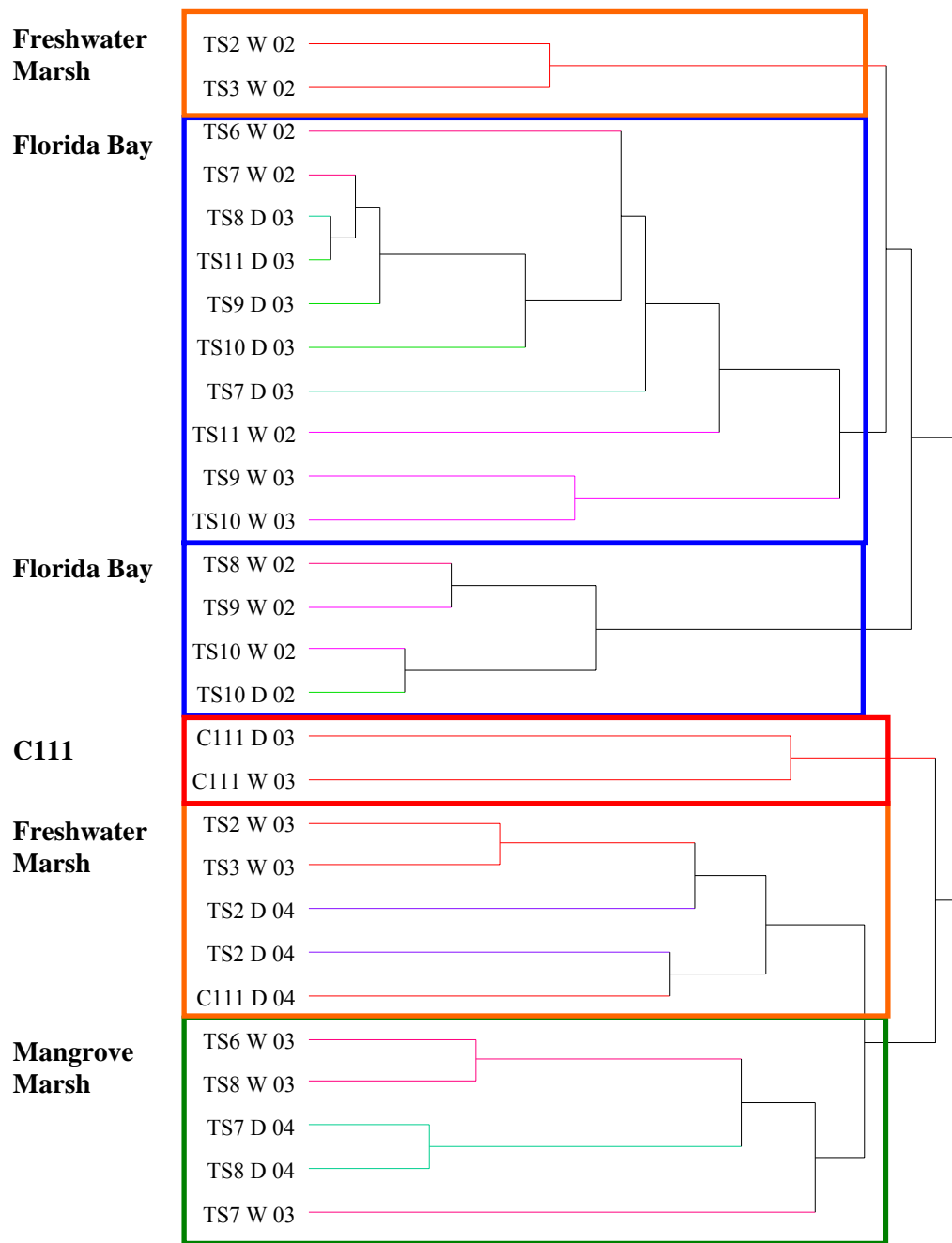


Fig. 33: Cluster analysis of UDOM in the FCE based on the pyrolysate products.

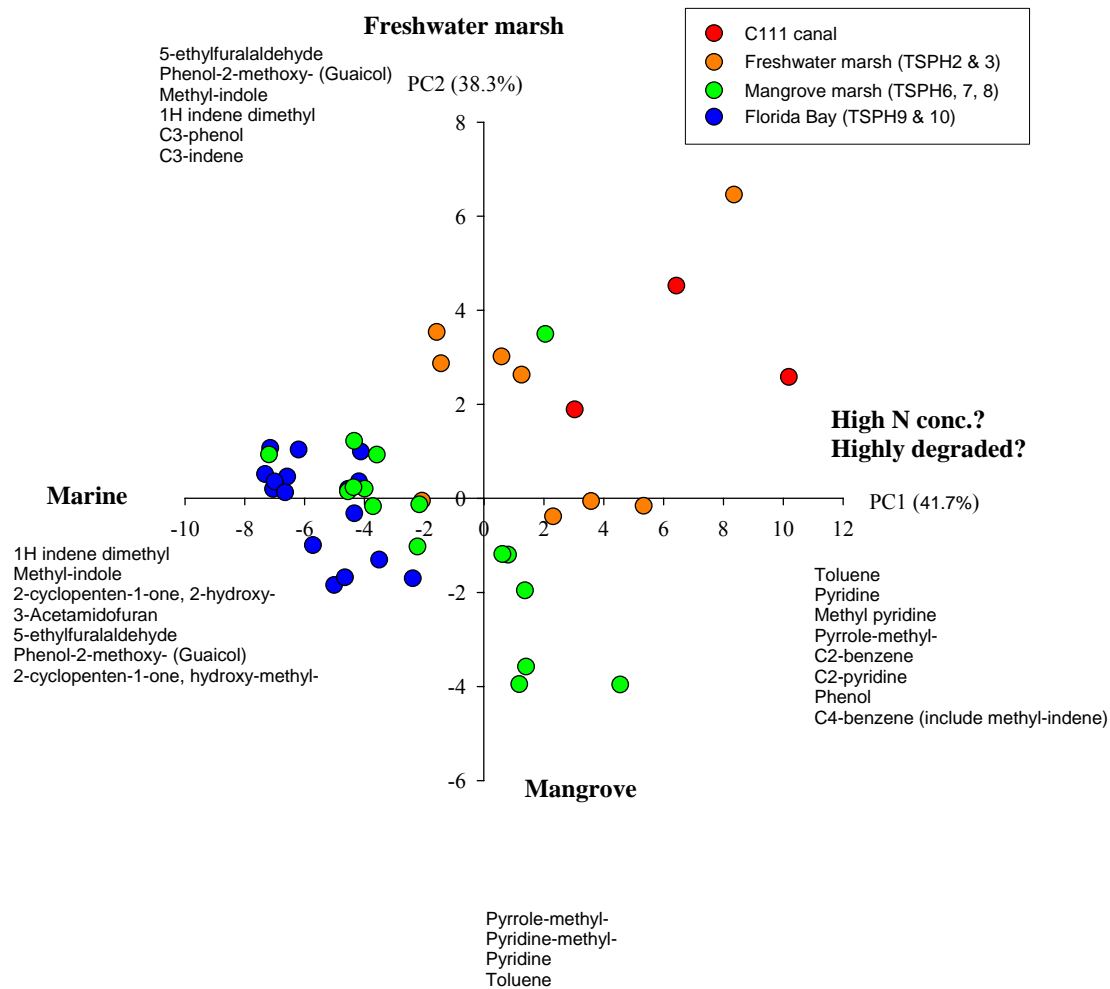


Fig. 34: Principal component analysis (PCA) of UDOM in the FCE based on the pyrolysate products.

CHAPTER 3: CHARACTERIZATION OF PROTEINACEOUS MATERIAL IN THE FLORIDA COASTAL EVERGLADES.

Methods

Proteinaceous material in dissolved organic nitrogen (DON) was collected at six geomorphologically different sites in the Florida coastal Everglades and characterized by amino acid analysis and protein gel electrophoresis. The amino acid composition of the samples suggests that the canal DON is more degraded and is subject to higher microbial inputs than the mangrove marshwater and marine end-member stations. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) results supported this observation as distinctly different protein profiles were obtained for the canal waters compared to samples collected at other stations. These preliminary results highlight the potential of combining amino acid and intact protein analysis to fingerprint the sources of DON in different aquatic environments.

Results and Discussion:

Dissolved organic nitrogen (DON) is the least known component of the nitrogen cycle. Proteinaceous material is known to typically account for 5-10% of DON in aquatic dissolved organic matter (DOM; e.g. Keil and Kirchman, 1991; Stepanauskas et al., 2000) and has been primarily studied by means of amino acid analysis (e.g. Cowie and Hedges, 1995). Although the amino acid composition of DOM appears to be relatively homogeneous across the spectrum of aquatic environments (Cowie and Hedges, 1992), the relative levels of certain amino acids have been successfully used to assess the degradation status of DON (Dauwe et al., 1999; Yamashita and Tanoue, 2003). Furthermore, amino acids enantiomeric ratios (D- versus L- form) have been used to assess the extent of the bacterial contribution to DON (e.g. McCarthy et al., 1998; Dittmar et al., 2001; Jones et al., 2005). With the advent of proteomics in the last decade, methodologies for the analysis of *intact* proteins have also evolved rapidly, hence providing an additional pathway for exploring the nature of aquatic DON. Laboratory techniques such gel electrophoresis followed by mass spectrometric sequencing (e.g. Shevchenko et al., 1996) are gradually being introduced in oceanography (Tanoue et al., 1995; Jones et al., 2004; Powell et al., 2005). Here we present data produced by a combination of amino acid, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) analysis of samples collected in the Florida Everglades.

Surface water samples (25L) were collected from six sites (see fcelter.fiu.edu) and processed through a tangential flow filtration system (1kDa) in order to isolate the high molecular weight dissolved organic matter (HMW DOM). Further processing was carried out before SDS-PAGE and 2D-PAGE as in Jones et al. (2004) in order to concentrate the proteins in HMW DOM samples. The six sampling sites were chosen to reflect geomorphologically distinct locations. C111 represents a canal site, whereas TSPH7 and TSPH8 are mangrove marsh/tidal environments. TSPH9 and TSPH10 are located in the seagrass-dominated Florida Bay and hence represent the marine/estuarine end-member (note that two samples were collected at TSPH9 with a difference of approximately a month, denoted hereafter TSPH9 and TSPH9'). Amino acid enantiomer

analysis was carried by high performance liquid chromatography as in Jones et al. (2005), based on the method of Kaufman and Manley (1998). SDS-PAGE was carried out according to Laemli (1970) and 2D-PAGE as in o'Farrell (1975).

Amino acid concentrations (Table 3) were highest at TSPH9 (64.48 μ M), followed by TSPH8 (63.15 μ M), C111 (47.34 μ M) and TSPH7 (39.69 μ M). The sample collected at station TSPH10 in Florida Bay showed the lowest amino acid concentration (17.68 μ M). Amino acids accounted for the highest fraction of DON in the canal waters (Table 1; C111; 36.9%), followed by TSPH8 (33.04%), TSPH9 (32.14%) and TSPH7 (22.27%). The lowest amino acid contribution to DON was observed in Florida Bay, at station TSPH10 (8.7%). The individual amino acid composition was similar between samples, being dominated by glycine, alanine and aspartic acid, as has been previously observed for DOM (e.g. Cowie and Hedges, 1992).

A 'degradation index' (DI) was calculated based on the individual amino acid composition of the samples (Dauwe et al., 1999; Tanoue and Hashimata 2003). The DI values (Table 3) suggested that DON is most degraded in the canal waters (C111; DI=-4.9). This is in contrast to the high amino acid contribution to DON observed for this site, but in agreement with a previous DOM characterization study (Lu et al., 2003), which indicated that the canal water DOM has a longer residence time and is more degraded than DOM at other parts of the Everglades. The mangrove marsh sites (TSPH7 and TSPH8) showed intermediate DIs (-4.5 and -3.7, respectively), while highest DI was obtained for marine waters, indicating the presence of 'fresh' DON (TSPH9 and TSPH10; -3.3 and -0.1 respectively). In order to further investigate the differences in the quality of DON between different sites, the peptidoglycan input to amino acid nitrogen (PG-AA-N%; McCarthy et al., 1998; Table 3) was employed as a measure of the bacterial input to DON. The canal site showed the highest PG-AA-N% value (14.24%), followed by TSPH7 (9.96%) and the two marine sites TSPH9 and TSPH10 (11.67% and 8.91% respectively). The lowest PG-AA-N% was observed for one of the mangrove marshwater sites considered (TS/Ph8; 6.86%).

SDS-PAGE analysis of the water samples resulted in heavily stained gels with intense background, which is characteristic of environmental applications (e.g. Tanoue et al., 1995; Jones et al., 2004; Fig. 1). However, several distinct bands were detected in most samples. A band corresponding to 25kDa was detected in sample TS/Ph7, while sample TS/Ph8 showed bands corresponding to ~36kDa, 33kDa and 25kDa. Similarly, a number of distinct bands were detected in sample TS/Ph9, corresponding to ~ 40kDa, 33kDa, 31kDa, 29kDa and 25kDa. The same four bands were detected in sample TS/Ph9', although this lane was significantly less intensely stained. Three bands at ~ 40kDa, 34kDa and 33kDa were detected in sample TS/Ph10. The protein profiles obtained for all of the Taylor Slough samples were hence very similar, with several common bands. Samples TS/Ph9 and TS/Ph9', in particular, yielded a protein profile that was almost identical. On the other hand, analysis of the canal sample resulted in a significantly different protein pattern with the dominant bands corresponding to 25kDa, 44kDa, 48kDa, 52kDa and 70kDa. The presence of a 48kDa band in the canal samples *only* is also of note. If this band does indeed represent the bacterial membrane protein identified by Tanoue et al. (1995) in a wide range of oceanic samples, it may further reflect the different nature of DON in these samples, with respect to the Taylor Slough/Florida Bay locations, in agreement with the amino acid analysis results. The C111 canal is an environment where one would expect that the most degradation-resistant molecules, such as bacterial membrane proteins (Siehnel et al., 1990; Worobec et al., 1988) would dominate the profile profile.

2D-PAGE was applied in order to obtain further resolution on the protein bands detected by SDS-PAGE. A large number of distinct spots were detected in sample C111 (Fig. 2), such as groups of spots at ~44kDa (pI 5-6), 48kDa (pI 5.2-6.2), 52kDa (pI 6.5-7) and 66kDa (pI 5-6) and the background staining remained low. These results are, to a large extent, in agreement from those obtained by SDS-PAGE of the same sample, where bands at 44kDa, 48kDa and 52kDa were observed. None of the other water samples produced satisfactory 2D-PAGE gels, possibly due to the low protein content and the presence of non-proteinaceous interfering compounds.

Our results suggest that the proteinaceous material in the canals waters is more degraded than that in Taylor Slough and Florida Bay, as well as being dominated by bacterial inputs. Distinctly different protein profiles were observed for the canal sample compared to the remaining sampling locations, potentially reflecting the difference in DON sources and/or their degree of degradation between the canal waters and other parts of the Everglades. These preliminary results highlight the problems that still exist with the application of gel electrophoresis on aquatic DOM (e.g. low sensitivity, low resolution), but suggest that combining amino acid and intact protein analysis when characterizing proteinaceous material can provide a fingerprint of DON sources in different aquatic environments, offering valuable clues on the nature and cycling of the entire DON pool.

Sampling site	C111	TSPH7	TSPH8	TSPH9	TSPH10
c. Amino acid (μM)					
L-Aspartic acid	4.2	4.5	8.4	6.6	2.1
D-Aspartic acid	0.8	0.9	1.0	1.0	0.4
L-Glutamic acid	3.5	2.9	5.3	4.8	1.5
D-Glutamic acid	0.4	0.6	0.6	1.0	0.2
L-Serine	2.4	3.7	7.6	4.6	1.5
D-Serine	0.4	0.3	0.4	0.3	0.2
L-Threonine	3.6	4.1	7.6	7.2	2.3
Glycine	8.7	5.9	7.8	9.8	1.8
L-Arginine	3.5	2.7	3.3	2.7	0.5
L-Alanine	5.0	4.6	7.8	7.7	2.1
D-Alanine	2.2	1.2	1.3	2.5	0.5
L-Valine	2.9	1.9	3.0	3.2	1.1
L-Methionine	1.4	0.3	0.5	0.8	0.2
D-Methionine	0.0	0.0	0.0	0.0	0.0
D-Valine	0.0	0.0	0.0	0.0	0.0
L-Phenylalanine	2.9	2.0	1.6	3.6	0.9
D-Phenylalanine	1.4	0.8	1.1	1.5	0.9
L-Isoleucine	0.0	0.0	0.0	0.0	0.0
L-Leucine	3.9	2.9	5.6	6.0	1.6
D-Isoleucine	0.0	0.0	0.0	0.0	0.0
D-Leucine	0.2	0.2	0.2	1.0	0.0
Σ Amino acids (μM)	47.3	39.7	63.1	64.5	17.7
aa-N%	37.0	22.3	33.0	32.1	8.8
PG-AA-N %	14.2	10.0	6.9	11.7	8.9
DI	-4.9	-4.5	-3.7	-3.3	-0.1

Table 3 Individual amino acid concentrations (μM), sum of all amino acids (Σ amino acids; μM), amino acid contribution to dissolved organic nitrogen (aa-N%), peptidoglycan contribution to amino acid nitrogen (PG-

AA-N%, calculated as in McCarthy et al. [1998] based on D-alanine concentrations) and degradation index (DI; Dauwe et al., 1999; Yamashita and Tanoue, 2003).

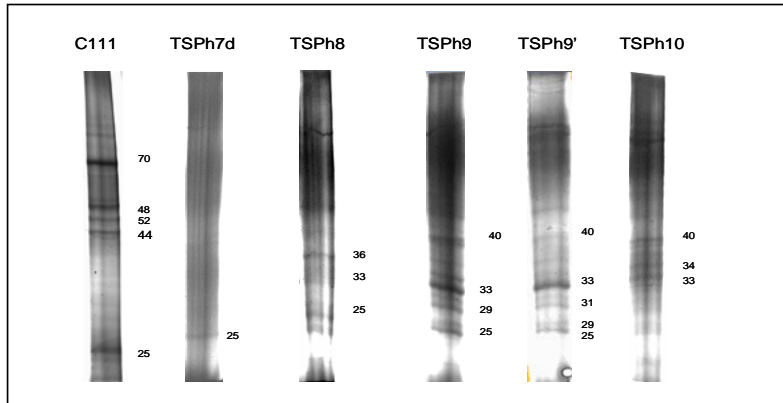


Fig.1 SDS-PAGE of water samples C111, TSPH7, TSPH8, TSPH9, TSPH9' and TSPH10. Bands of interest and their approximate molecular weights are marked in kDa.

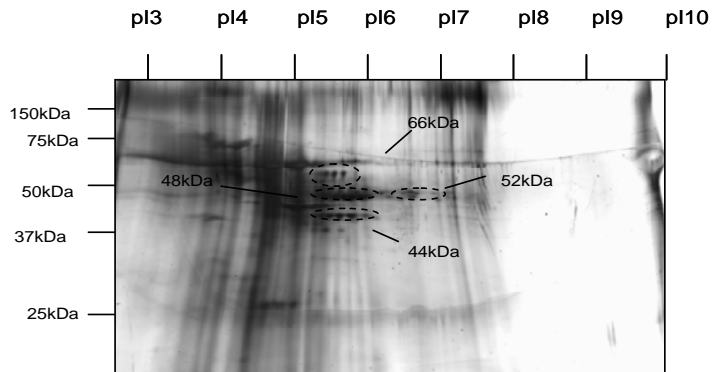


Fig. 2 2D-PAGE of water sample C111. Several group of spots were detected (e.g. groups of spots at ~44kDa, 48kDa, 52kDa and 66kDa), which are marked.

Fig. 35: Figures 35a (Fig.1) and 35b (Fig. 2) representing the 1D and 2D analyses for proteins in the Florida Coastal Everglades.

CHAPTER 4: BIOAVAILABILITY OF DISSOLVED ORGANIC NITROGEN

Bioassays Sampling Description

We sampled at 6 sites along a survey of Taylor Slough and conducted four bioassay experiments from these sites. We conducted the incubations beginning in the wet season of 2002, then the dry and wet seasons of 2003 and the dry season of 2004. Sample sites were from two Taylor Slough freshwater sites; C-111, TS 2 (Figure 36), two estuarine/mangrove sites, TS 7, Trout Creek; and two Florida Bay sites, TS 9 and TS 10.

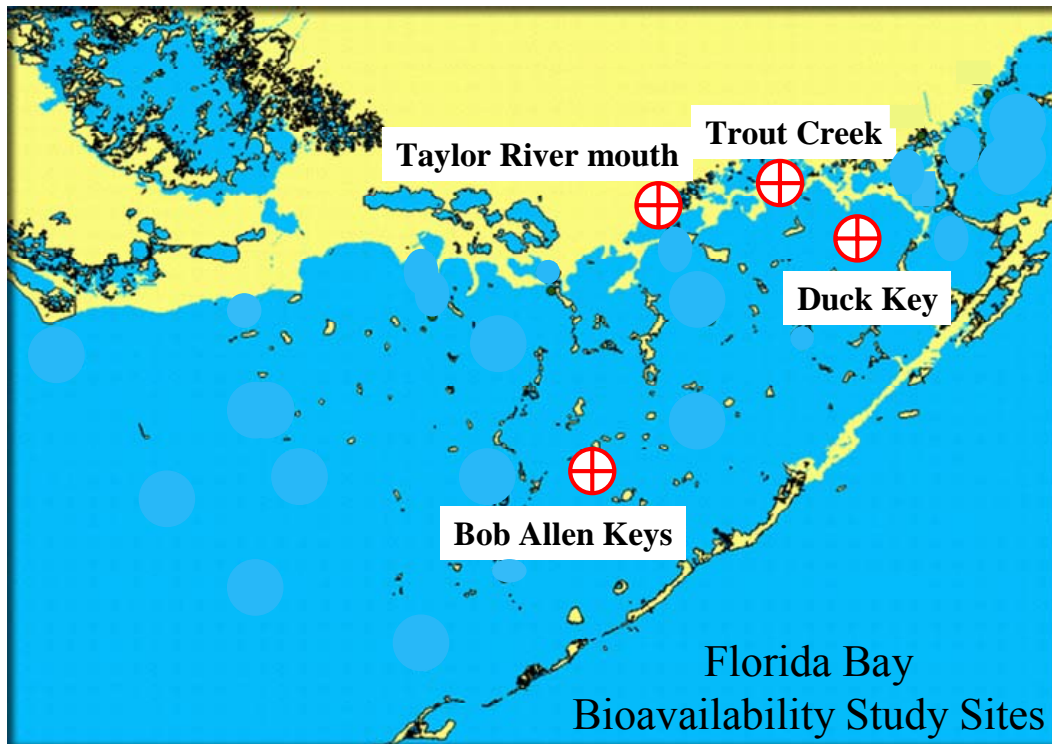


Figure 36. Map showing collection sites for the bioavailability experiments. Freshwater sites TS 2 and C-111- across the canal from TS 4 .

Methods

We conducted a series of incubation experiments under ambient nutrient levels (Control) and under N limited but C and P replete conditions (C+P). We collected 2, 8 liter bottles from each location on the survey and brought the water back to FIU campus for immediate processing.

Water was filtered through a 0.2 μM cartridge filter and stored in the dark at 4°C for 1-3 days in a sterile, brown Nalgene bottle. We prepared inoculate by filtering water from each of the sites through a 0.8 μm filter and storing these samples in brown Nalgene bottles at 4°C until the incubation experiment. We added 2 ml inoculate to each of the bottles. Bottles received their bacterial inoculates from the same site where water was collected for the incubation experiment.

We placed 2000 ml of the cartridge filtered sample water into 2.5 L polycarbonate incubation bottles. We kept 2 replicates from each site for a control and to two replicates we added Glucose (Dextrose $C_6H_{12}O_6$) to a 500 μM concentration and P ($NaH_2PO_4 \cdot H_2O + NaH_2PO_4 \cdot 2H_2O$) to a 5 μM concentration for the incubation bottles. Bottles were placed under the counter in the dark and sampled at Day 0, 1, 2, 3, 5, and 8. After the completion of the first bioassay and incubation experiment we adapted our sampling period to reflect further degradation of the dissolved organic nitrogen. Thus, for the second, third and fourth experiments, sampling times were 0, 2, 4, 8, and 16 days into the incubation. Samples were collected, heterotrophic bacteria production, bacteria counts, total nutrients of Si, TP, TN, and TOC. Dissolved constituents of NH_4^+ , NO_3^- , NO_2^- , SRP were collected at the same time and filtered with a Whatman GF/F. Total and dissolved samples were analyzed in the same manner as the water quality monitoring of the survey sites described in the previous methods section in this report.

At each sampling we collected water and used an alkaline phosphatase activity (APA) 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 of whole water 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 APA ($\mu M h^{-1}$).

At each sampling we collected water and used a Leucine MCA assay experiment to determine the production of extracellular enzymes to mineralize organic nitrogen in the form of aminopeptidase (Hoppe et al., 1988). We incubated 3ml of whole water for 24 hours after adding Leu-MCA. We determined initial and final readings using a Gilford Fluoro IV Spectrophotometer (excitation = 380nm, emission = 440nm) and the change over the period to determine the Leucine MCA activity ($\mu M h^{-1}$).

Results and Discussion:

As seen in the first chapter of this report, over 90% of the total nitrogen is in the organic form. Thus, the bioavailability of DON (BDON) is of primary importance in the N cycling of the ecosystem. Out of the three community types tested (marsh, mangrove, and bay), the mangrove sites generally had the highest BDON followed by the marsh and bay (Fig 37).

TN concentrations were higher in the mangroves than in the marsh for 3 of the 4 experiments; however the total concentration of nitrogen in the Bay was higher than in the mangrove zones at the time of the experiments (Appendix 1). Thus, there is no clear relationship between DON concentrations and BDON across the sites.

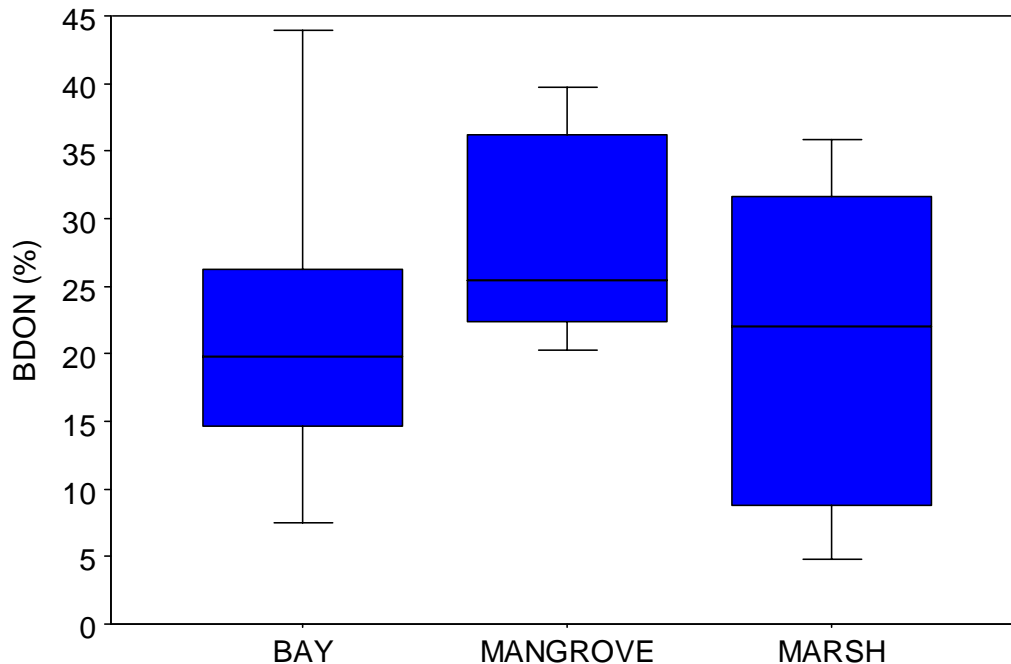


Figure 37. Bioavailability of dissolved organic nitrogen from the ambient water shown for each of the community types.

Marsh Sites

For the Control assays under ambient nutrient concentrations, there was no significant difference in BDON between seasons (17.5 %, Fig. 3), but there was a larger range during the wet season. The addition of a labile carbon source (glucose) and PO_4^- stimulated the degradation of DON only in the dry season, but it was not significant (24.0 vs 17.5 %). Since most of this DON would not be transported to the mangroves until advent of the wet season, it remains on site as a pool of reactive DON.

Mangrove Sites

There was no significant difference in BDON between seasons or the Control assays (24.3 %, Fig. 38), but there was a larger range during the dry season. The C+P treatment stimulated the degradation of DON in both seasons but was significant only in the wet season (29.8 vs 23.3 %). This implies that the mangrove DON was potentially more labile in the wet season but was also limited under the ambient conditions. We have no idea how much of this DON originated from the marsh and how much was produced in situ, but we expect that, if exported to the Bay, this labile DON fraction would eventually be mineralized.

Bay Sites

BDON in the wet season was significantly higher than in the dry season for control incubations (21.5 vs 15.0). Since much of the DON in the bay is produced in situ, we expected that it would be more bioavailable. C+P additions increased the BDON for both seasons but the differences were not statistically significant. Wet season BDON in the Bay was similar to BDON in the mangroves.

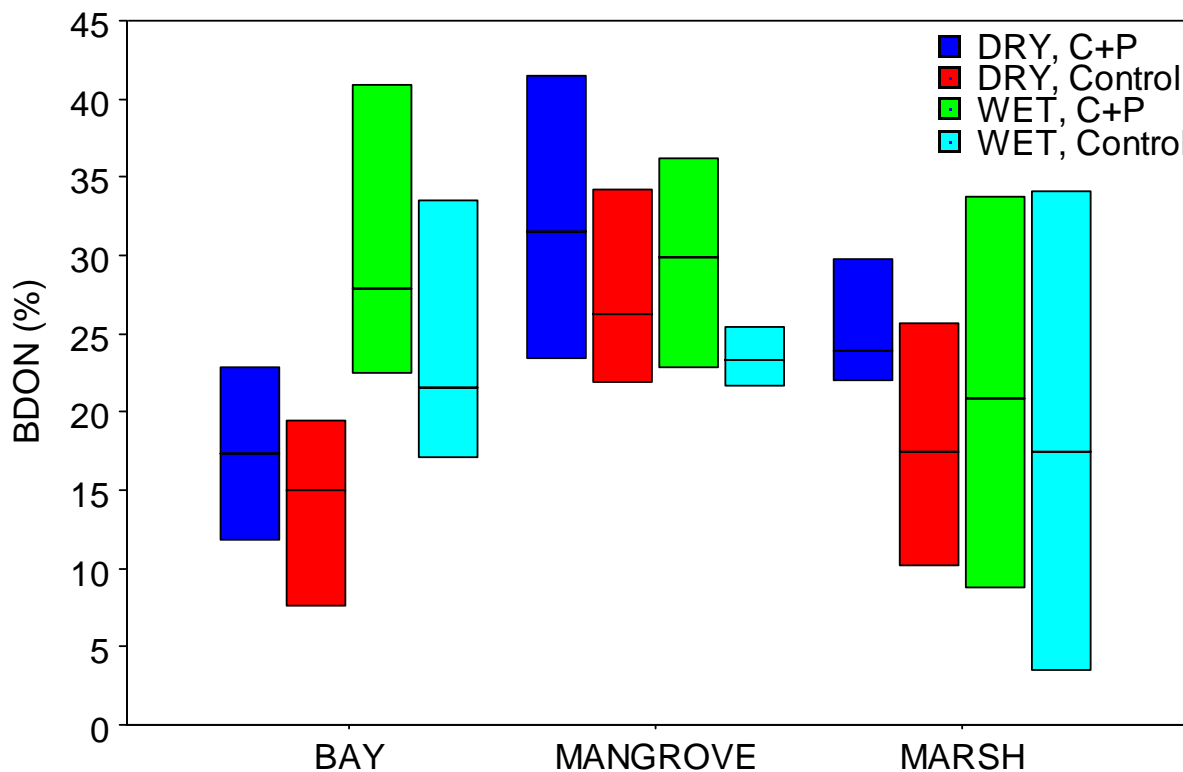


Figure 38. Bioavailability of dissolved organic nitrogen from the ambient water shown for each of the community types by season.

These bioassays provide a baseline for the bioavailability of nitrogen controlled by heterotrophic bacteria. Bioavailable nitrogen in the marsh may be more tightly recycled in the marsh in wet season when atmospheric nitrogen is more readily available through precipitation events and less senescence of bioavailable nitrogen in terrestrial plants occurs. Microbial degradation of DON in the mangrove zone may also be less subject to the pulses of nitrogen provided in precipitation events and high production periods and more dependent on nitrogen inputs from the mangrove roots and submerged ecology. In the dry season, mangrove sites also showed the greatest range of bioavailability, which may be associated with the nitrogen inputs from mangrove leaf loss during dry season storm events. In the wet season, marsh and bay sites showed a greater range of bioavailability. Strong storm events seen during the wet season may be the source of this range in bioavailability.

In summary of our findings for the bioavailability of nitrogen in the oligotrophic survey of the Florida Coastal Everglades, we found the highest bioavailability of nitrogen in the mangrove zone (24 %) with a potential BDON of 29.8 % under nutrient replete conditions. This observation was true in both the wet and dry seasons. BDON in the Bay approached that of the mangroves during the wet season as a result of fresh DON produced in situ.

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Appendix 1. Monthly Survey Data

TS 2

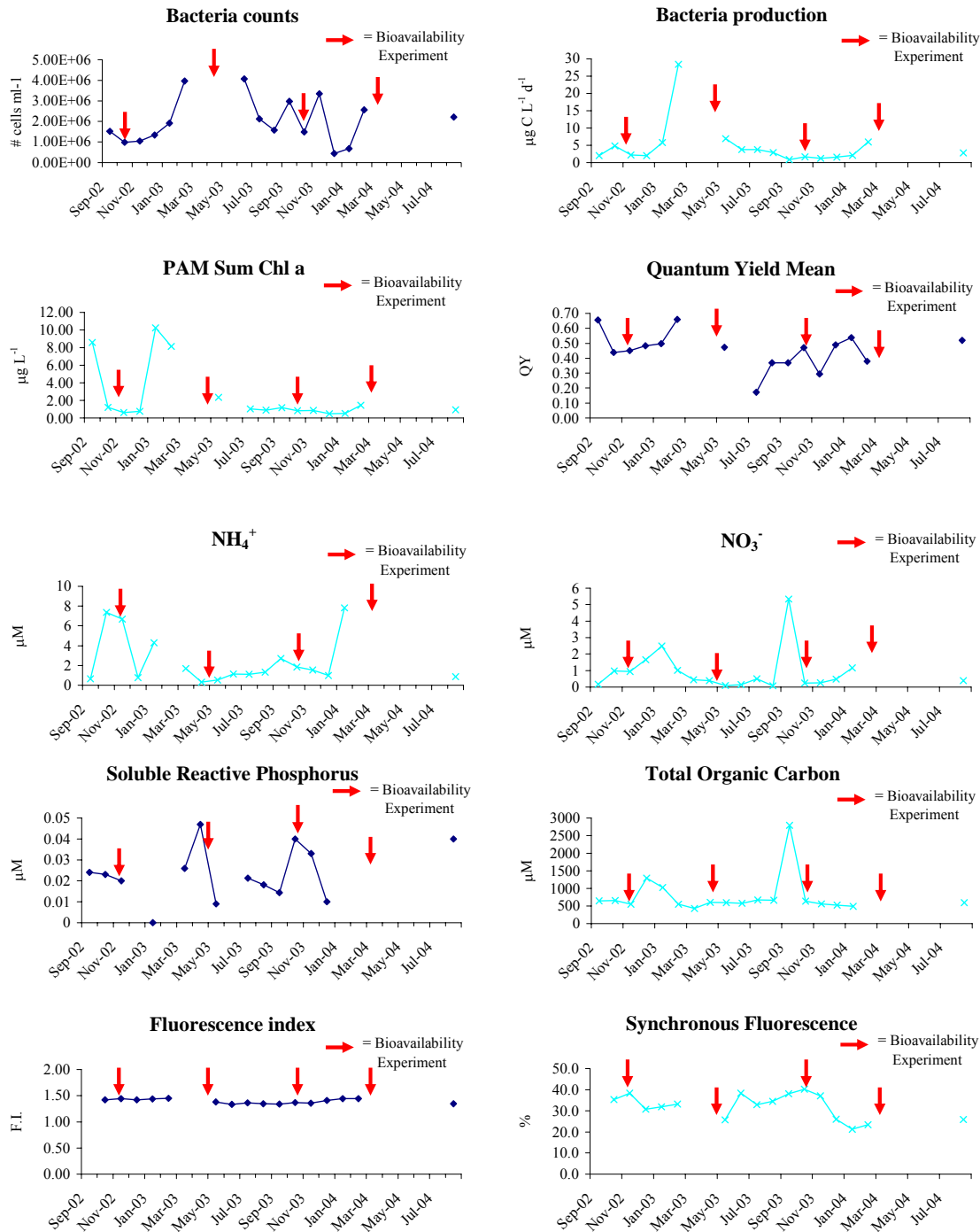


Figure 4a: Monthly survey data from TS2 from the sampling period September 2002 to August 2004. Red arrows indicate the first day of each of the bioavailability experiments during the study period.

TS 2

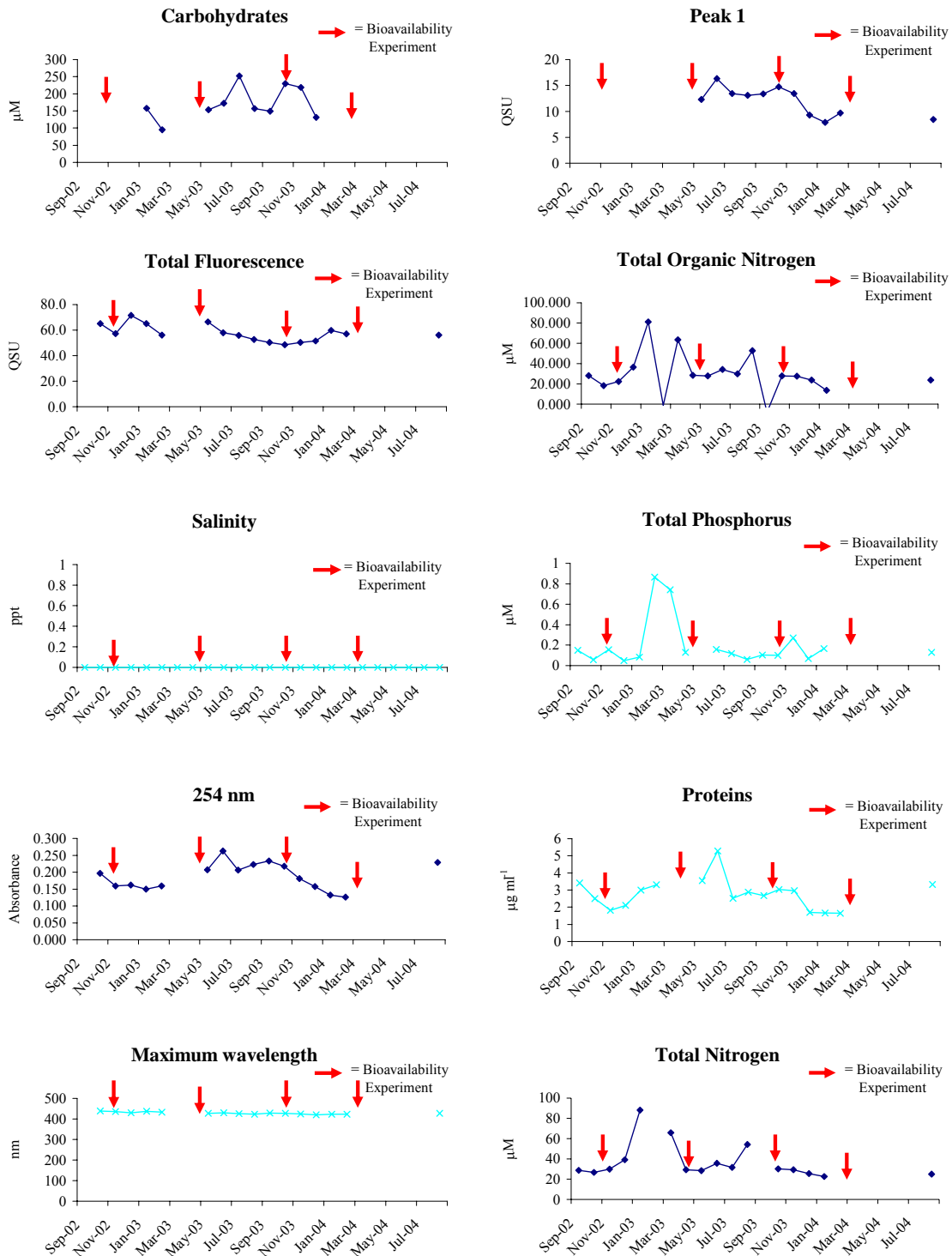


Figure 4b: Monthly survey data from TS2 from the sampling period September 2002 to August 2004. Red arrows indicate the first day of each of the bioavailability experiments during the study period.

TS 7

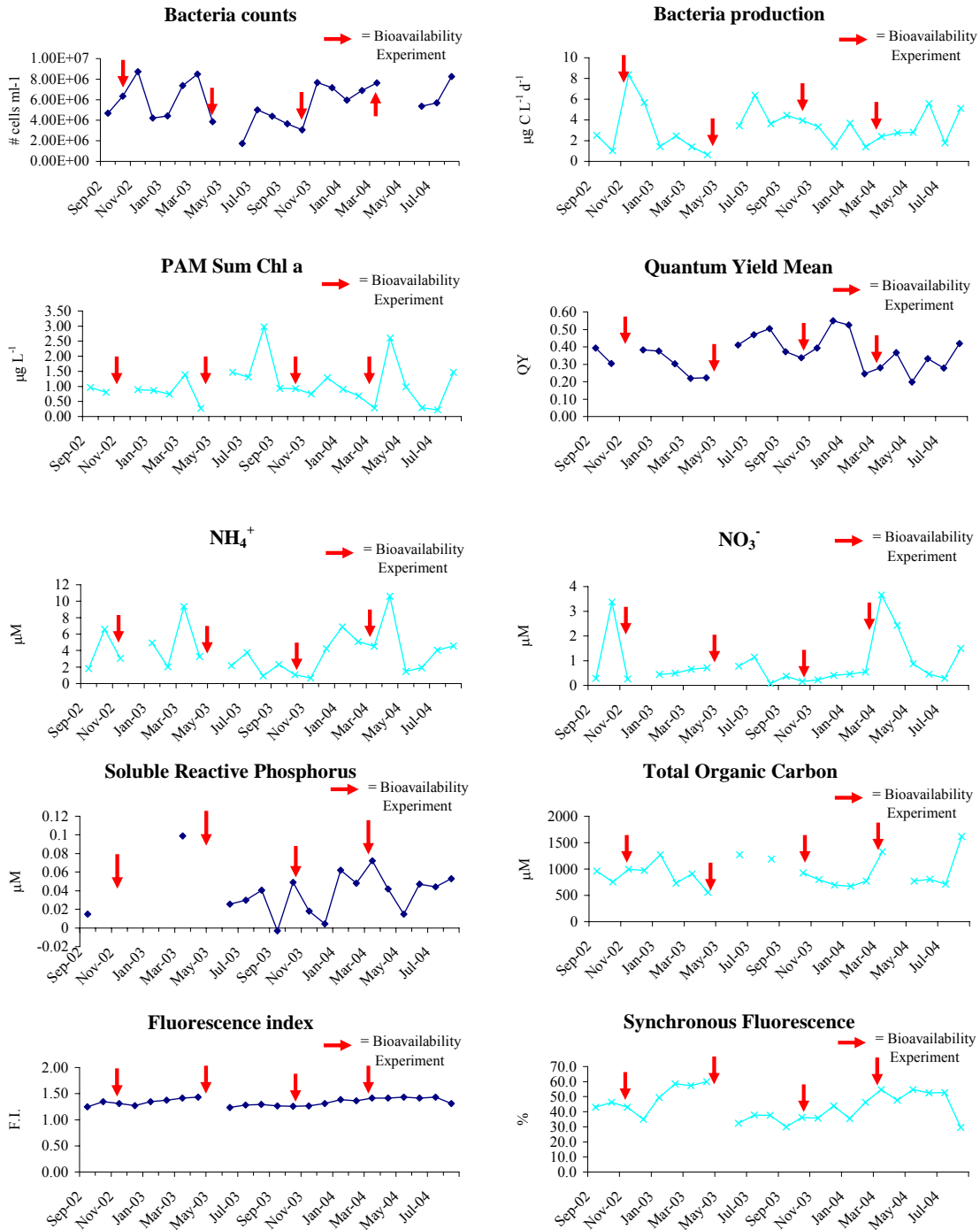


Figure 5a: Monthly survey data from TS7, or Taylor River mouth, from the sampling period September 2002 to August 2004. Red arrows indicate the first day of each of the bioavailability experiments during the study period.

TS 7

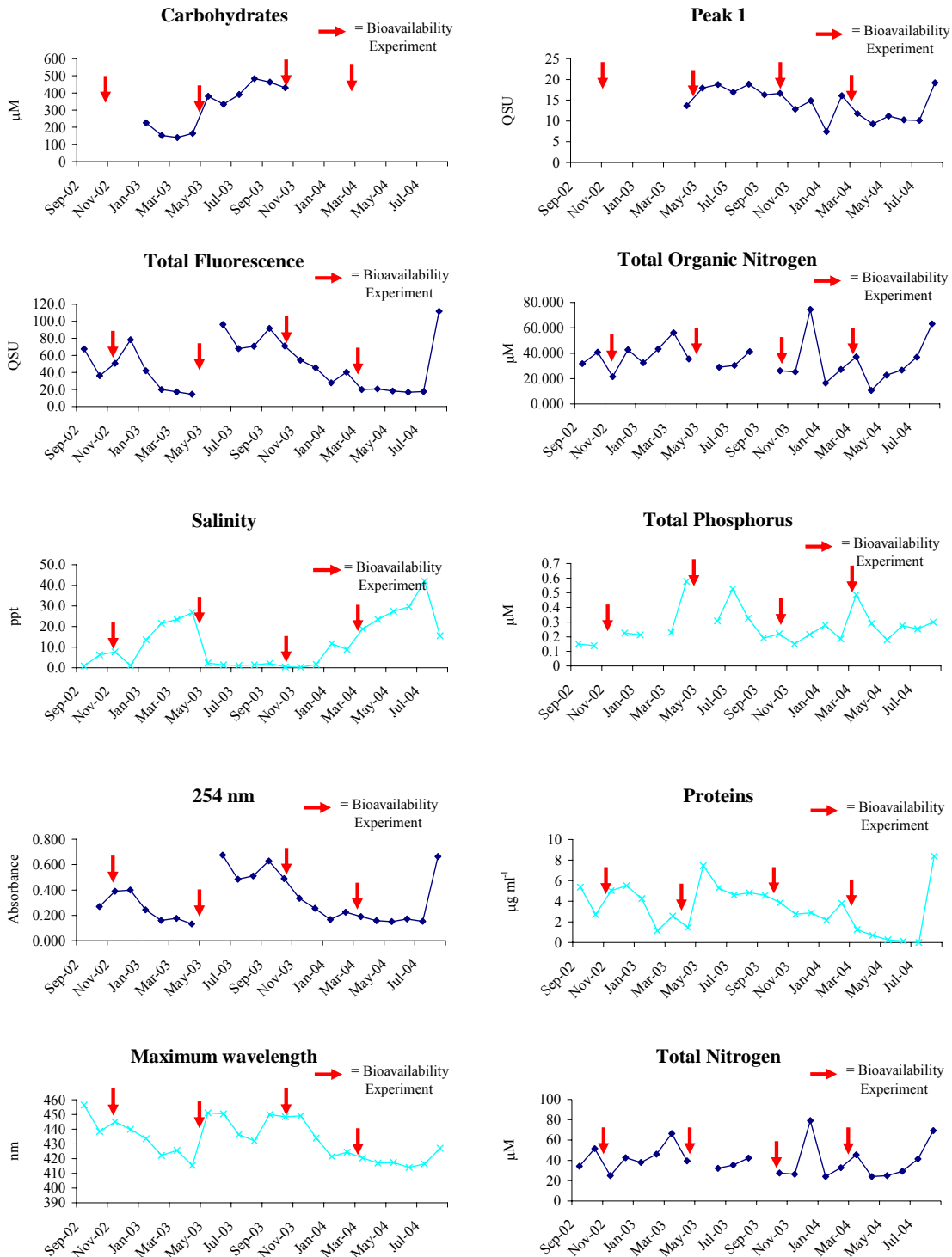


Figure 5b: Monthly survey data from TS7, or Taylor River mouth, from the sampling period September 2002 to August 2004. Red arrows indicate the first day of each of the bioavailability experiments during the study period.

TS 9

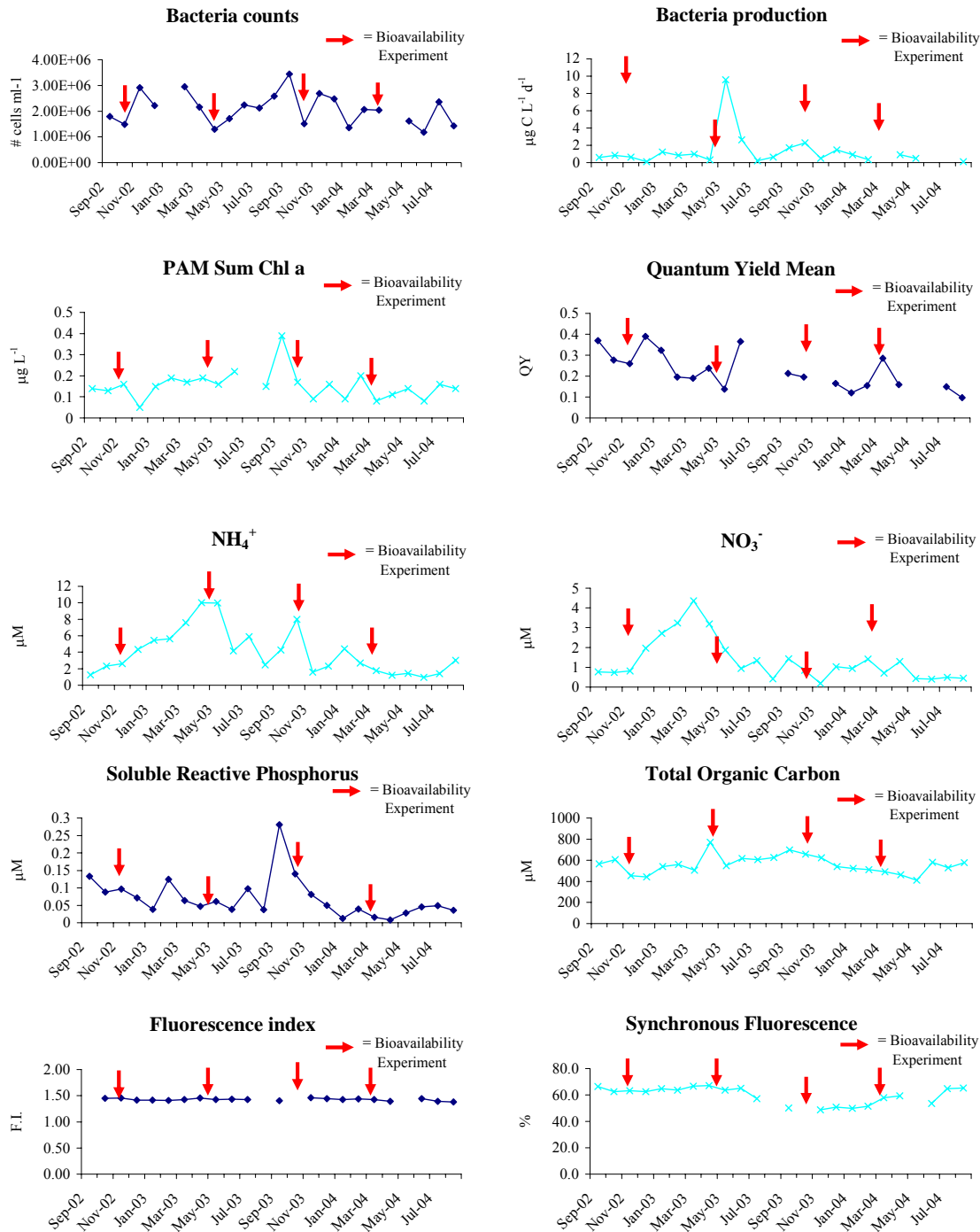


Figure 6a: Monthly survey data from TS9, or Duck Key, from the sampling period September 2002 to August 2004. Red arrows indicate the first day of each of the bioavailability experiments during the study period.

TS 9

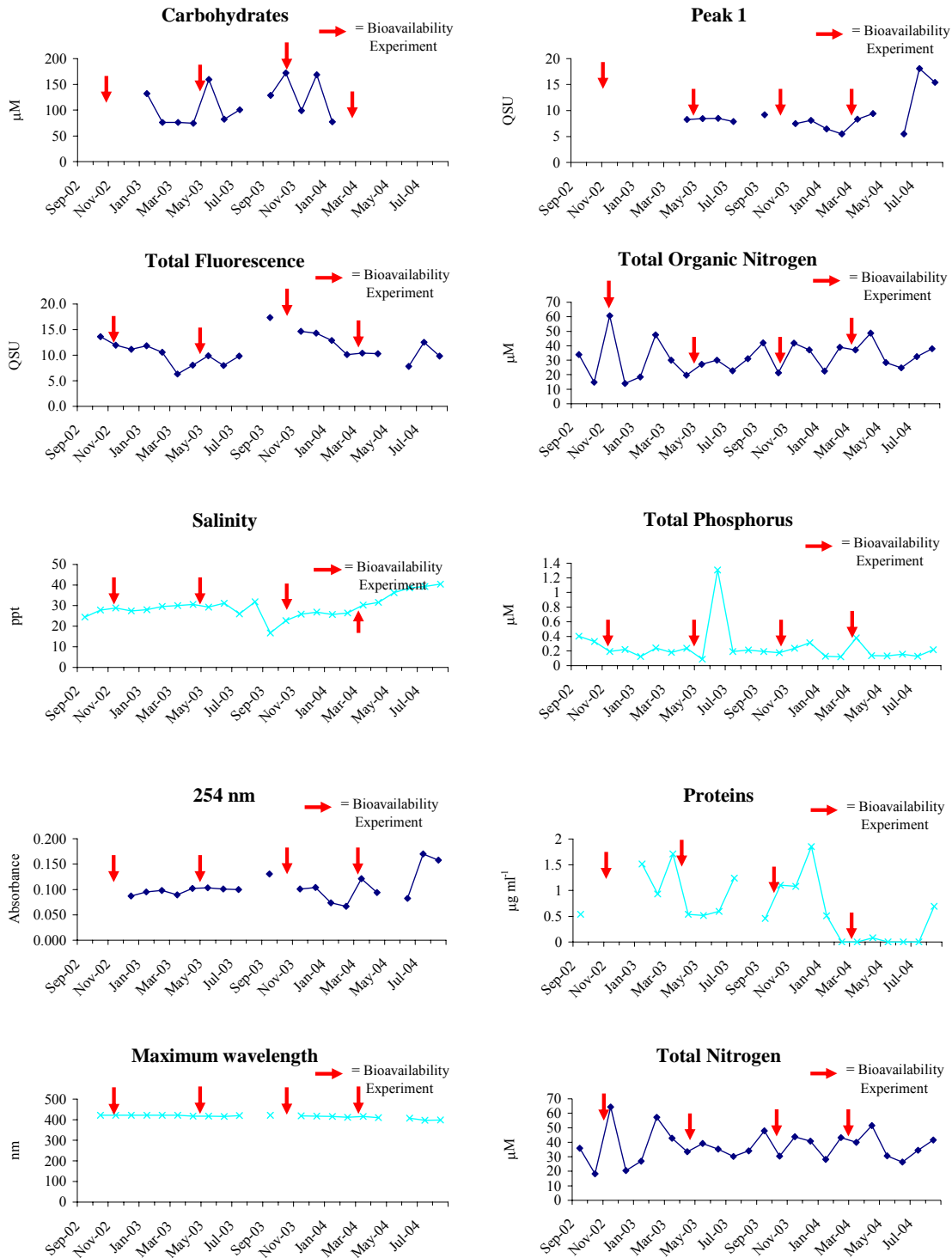


Figure 6b: Monthly survey data from TS 9, or Duck Key, from the sampling period September 2002 to August 2004. Red arrows indicate the first day of each of the bioavailability experiments during the study period.

TS 10

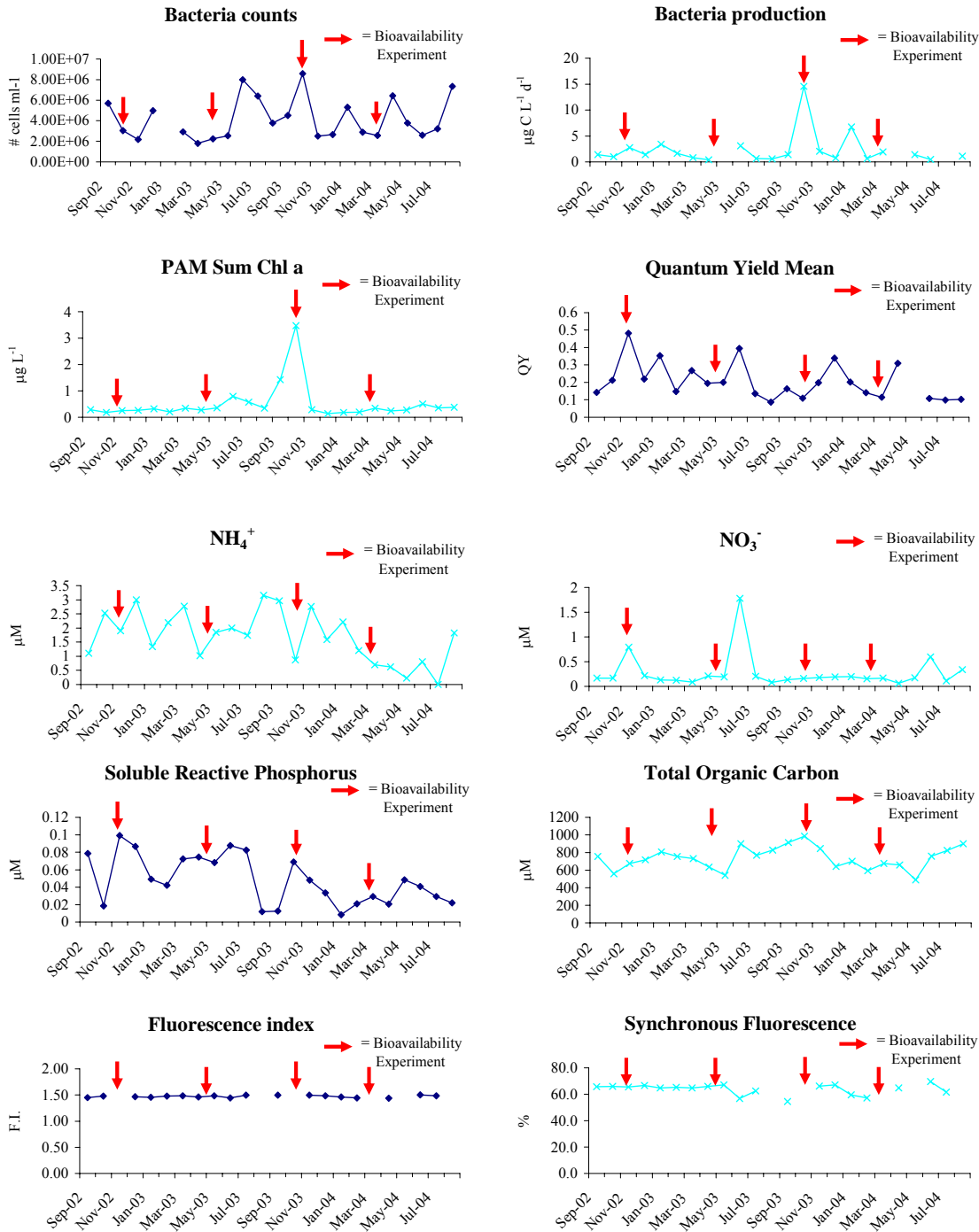


Figure 7a: Monthly survey data from TS 10, or Bob Allen Keys, from the sampling period September 2002 to August 2004. Red arrows indicate the first day of each of the bioavailability experiments during the study period.

TS 10

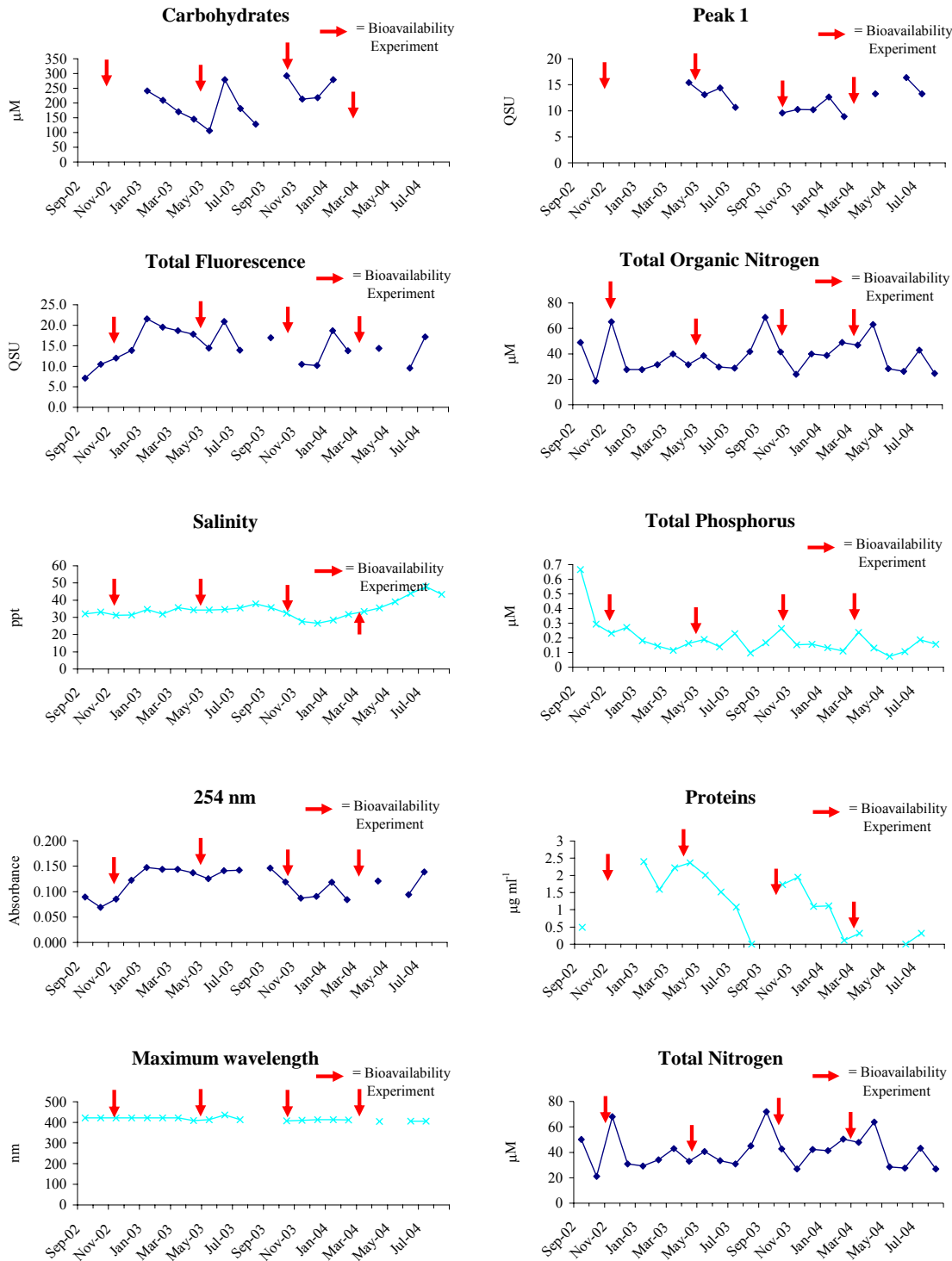


Figure 7b: Monthly survey data from TS 10, or Bob Allen Keys, from the sampling period September 2002 to August 2004. Red arrows indicate the first day of each of the bioavailability experiments during the study period.

TS 1

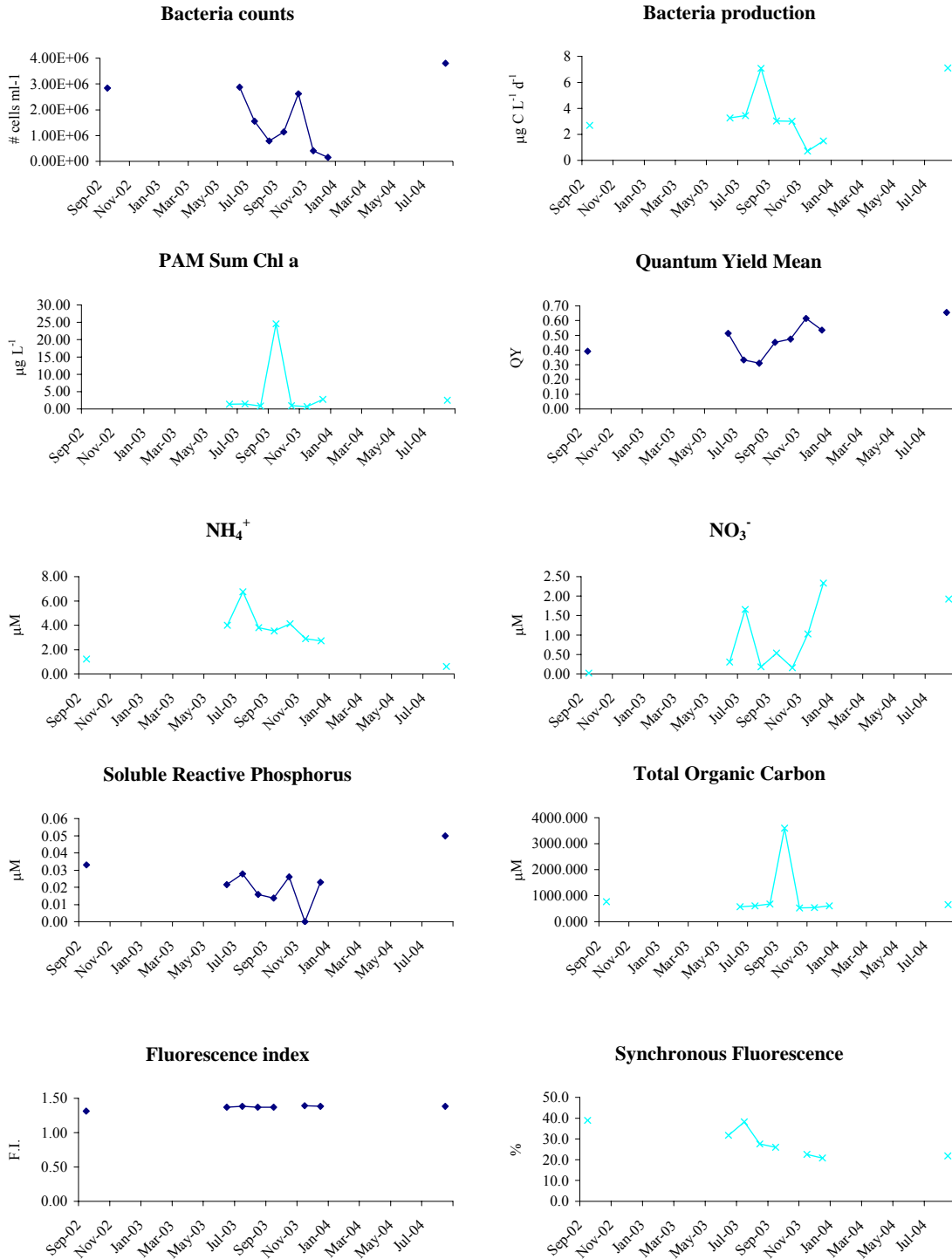


Figure 23a: Monthly survey data from TS 1 from the sampling period September 2002 to August 2004.

TS 1

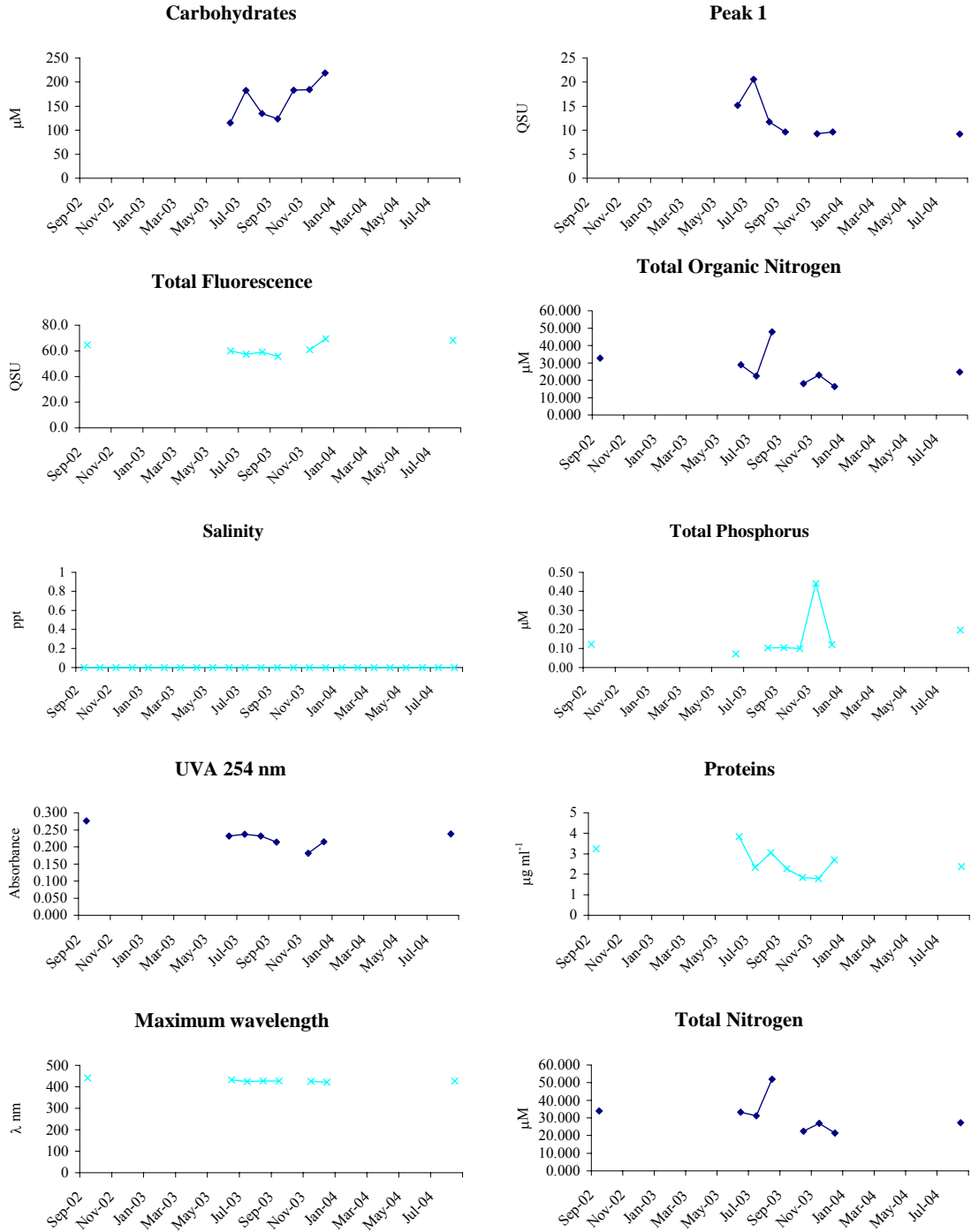


Figure 23b: Monthly survey data from TS 1 from the sampling period September 2002 to August 2004.

TS 2

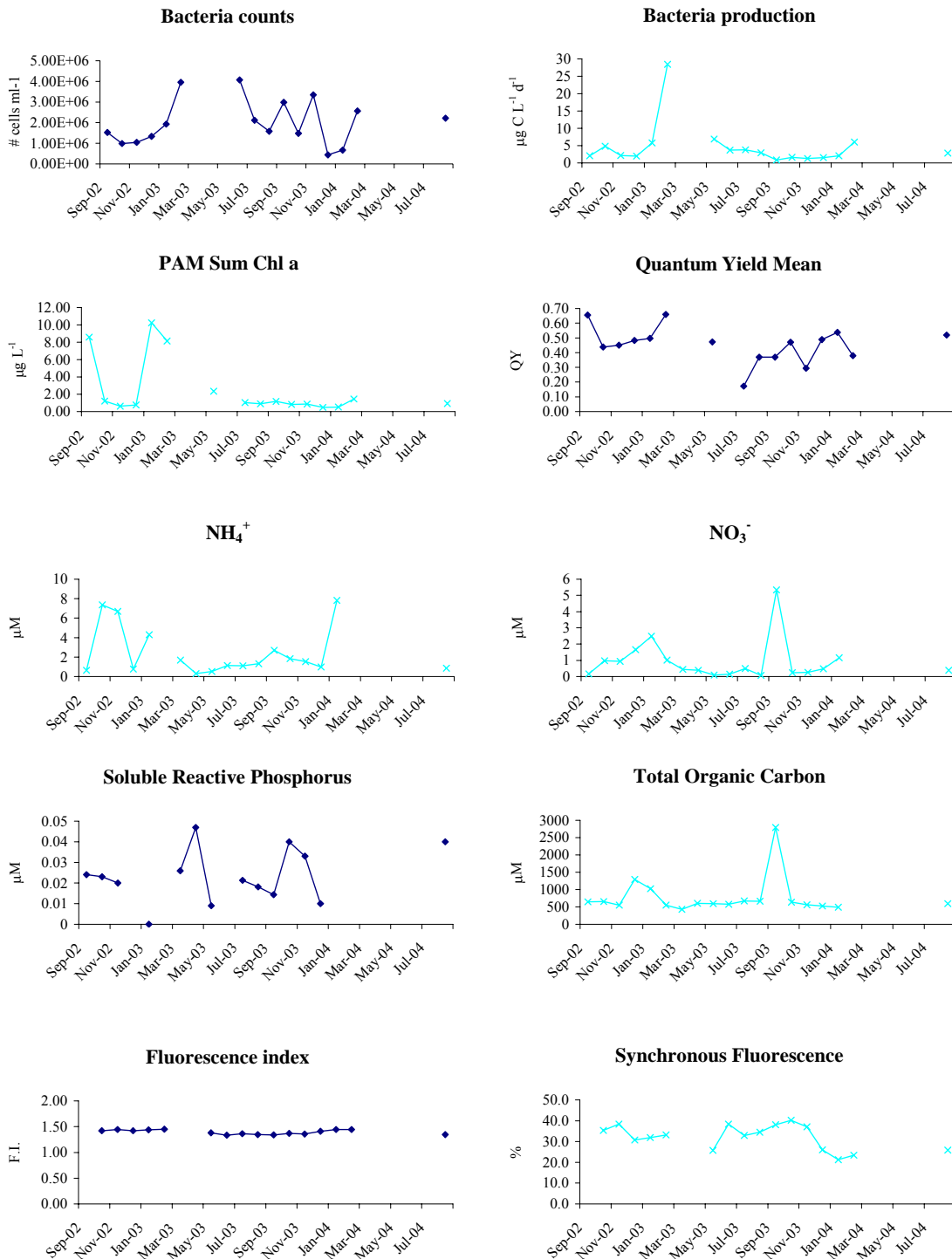


Figure 24a: Monthly survey data from TS 2 from the sampling period September 2002 to August 2004.

TS 2

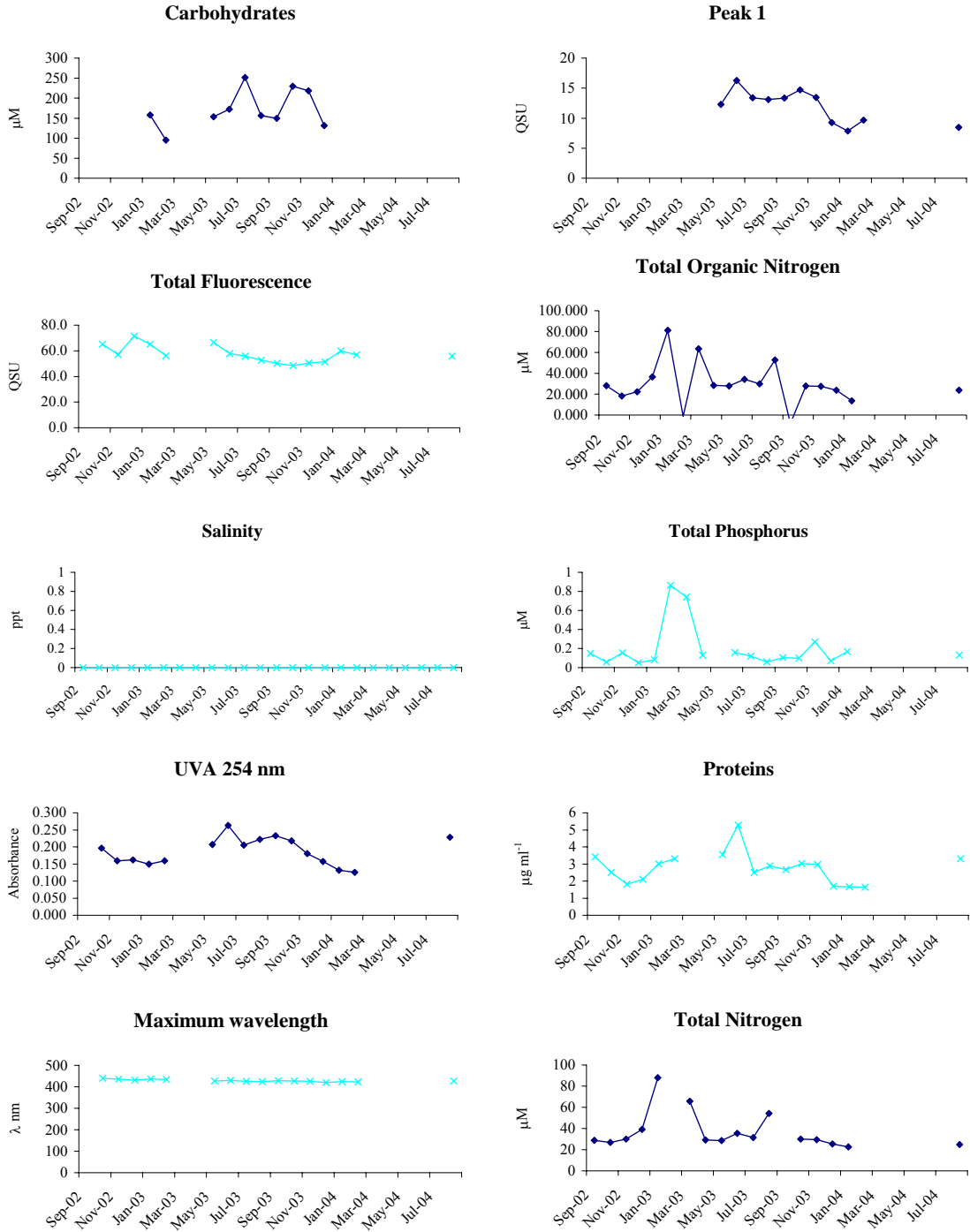


Figure 24b: Monthly survey data from TS 2 from the sampling period September 2002 to August 2004.

TS 3

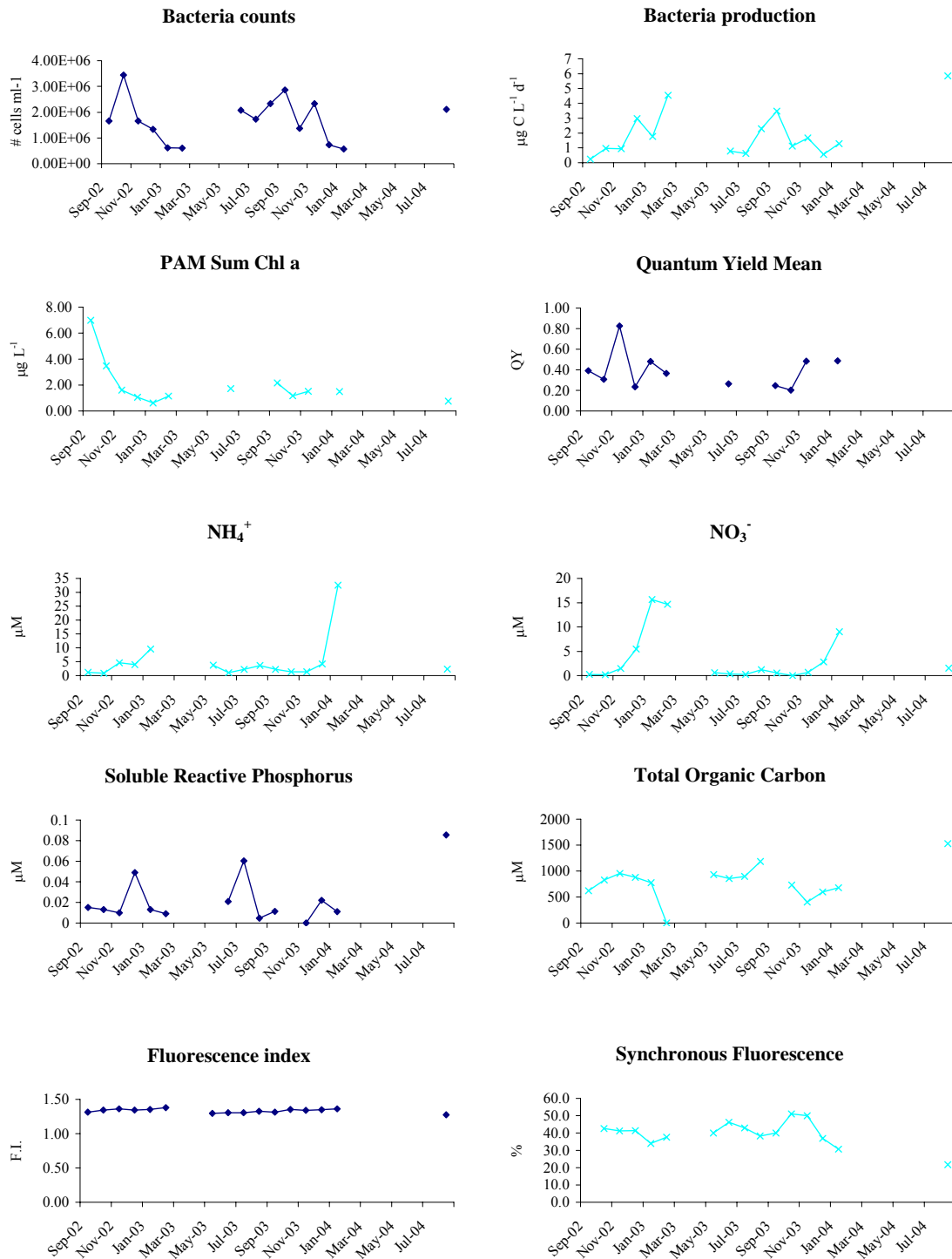


Figure 25a: Monthly survey data from TS 3 from the sampling period September 2002 to August 2004.

TS 3

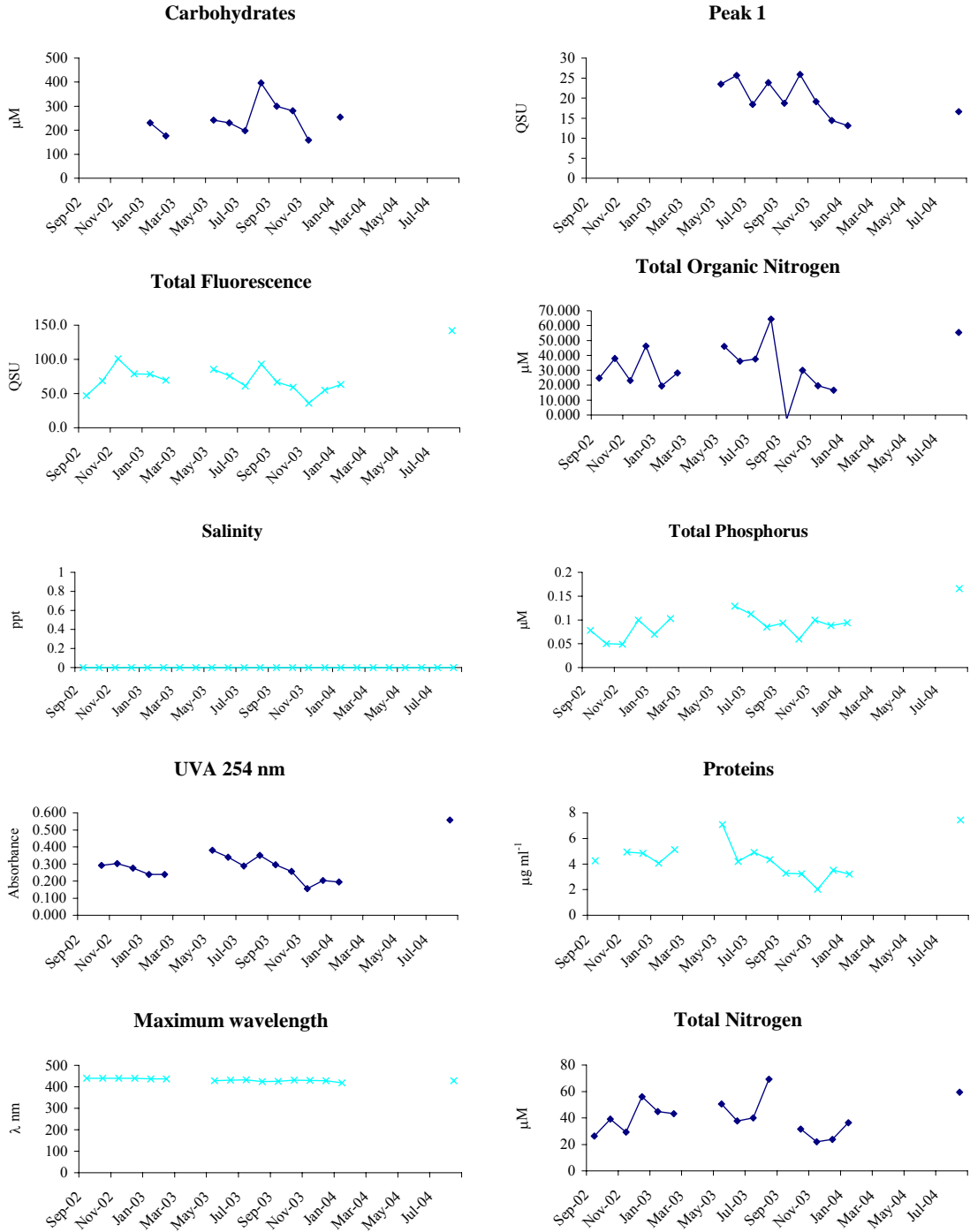


Figure 25b: Monthly survey data from TS 3 from the sampling period September 2002 to August 2004.

TS 4

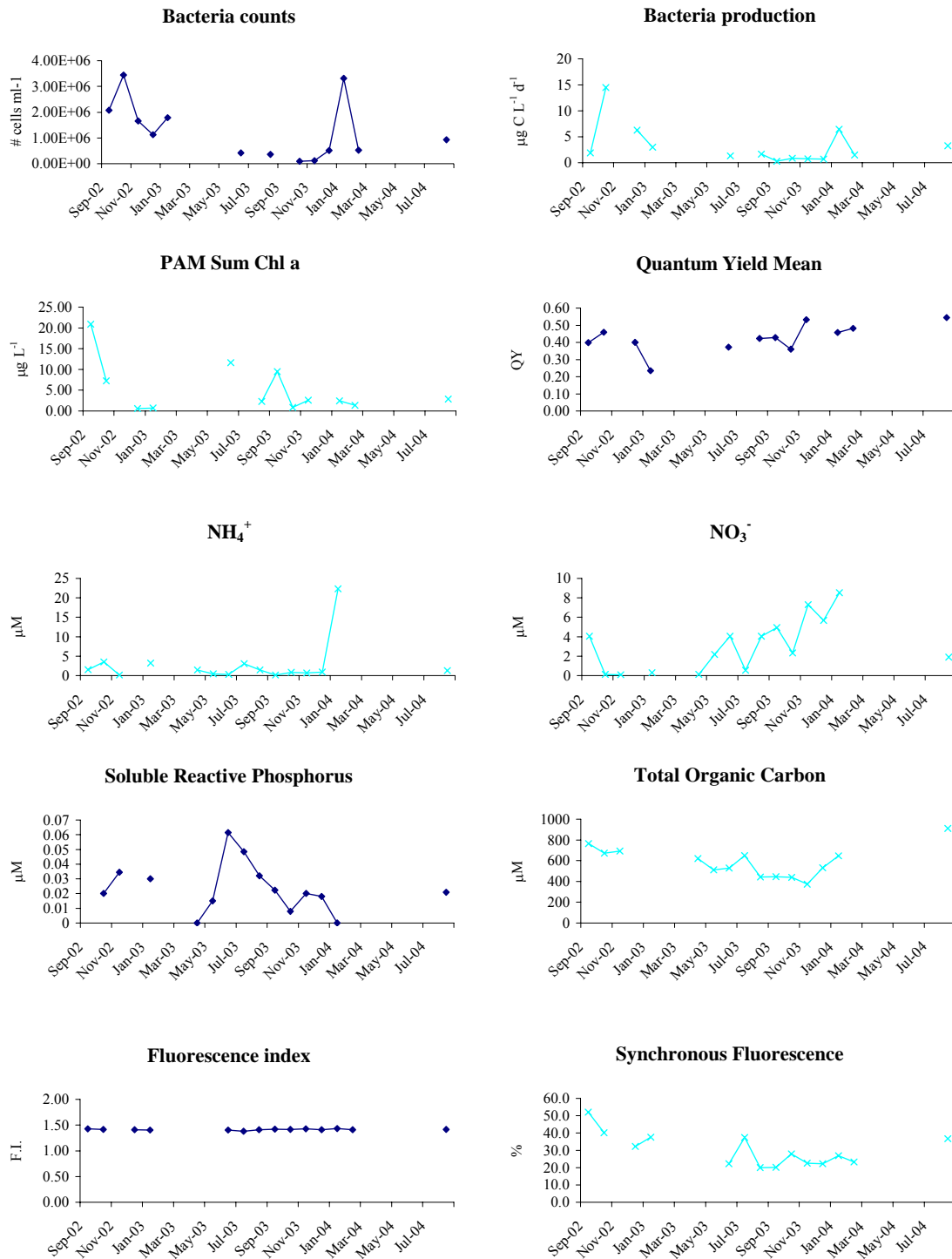


Figure 26a: Monthly survey data from TS 4 from the sampling period September 2002 to August 2004.

TS 4

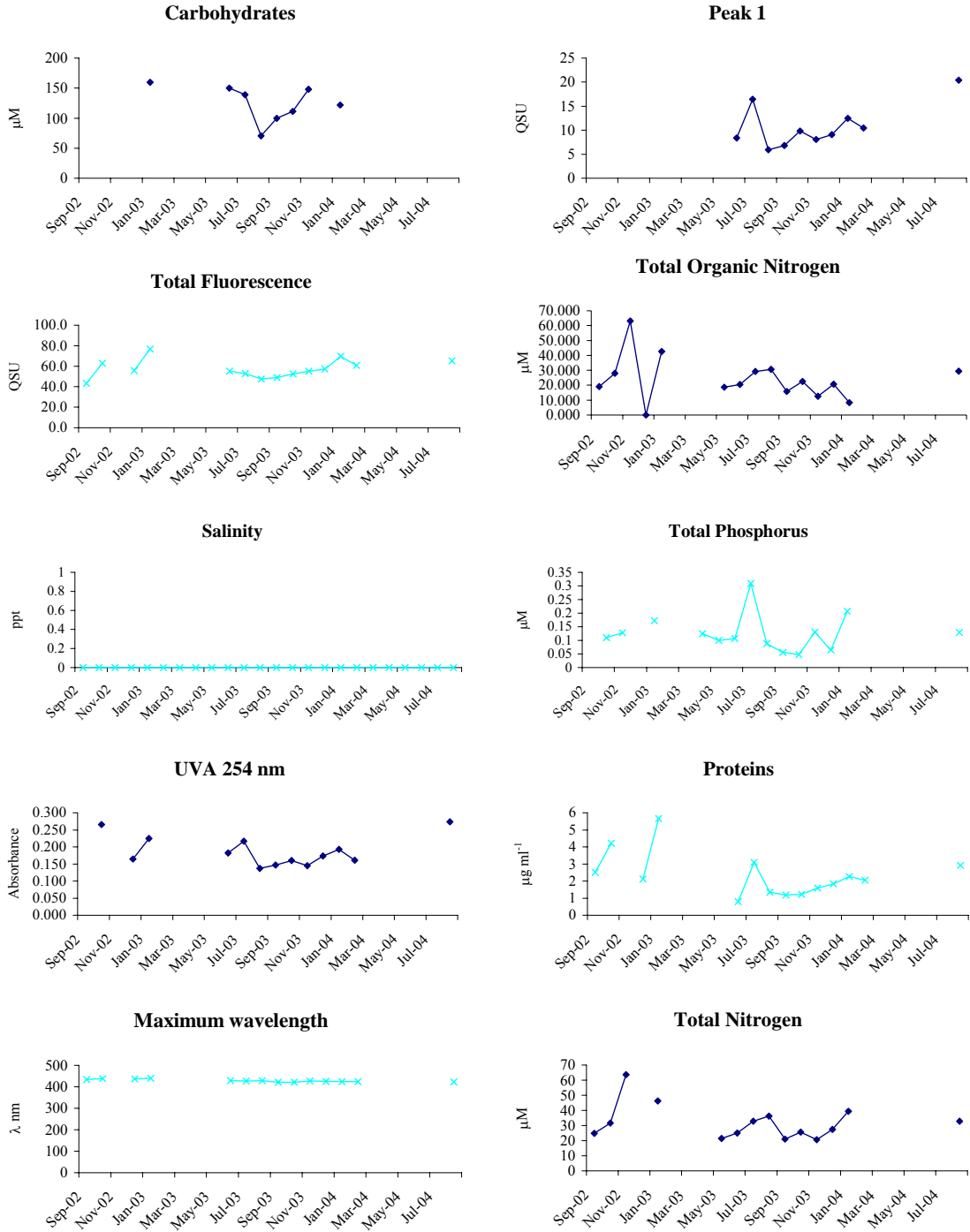


Figure 26b: Monthly survey data from TS 4 from the sampling period September 2002 to August 2004.

TS 5

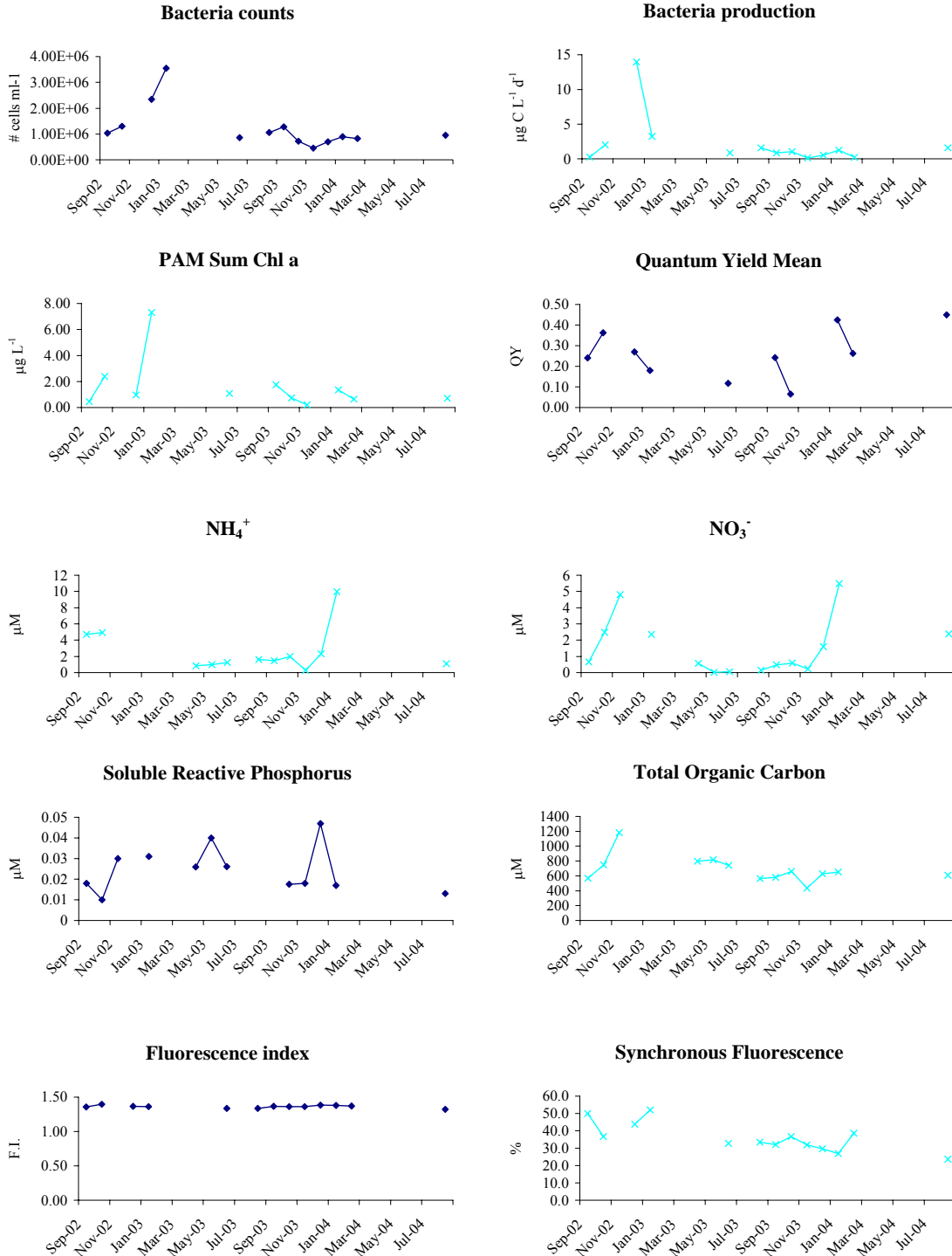


Figure 27a: Monthly survey data from TS 5 from the sampling period September 2002 to August 2004.

TS 5

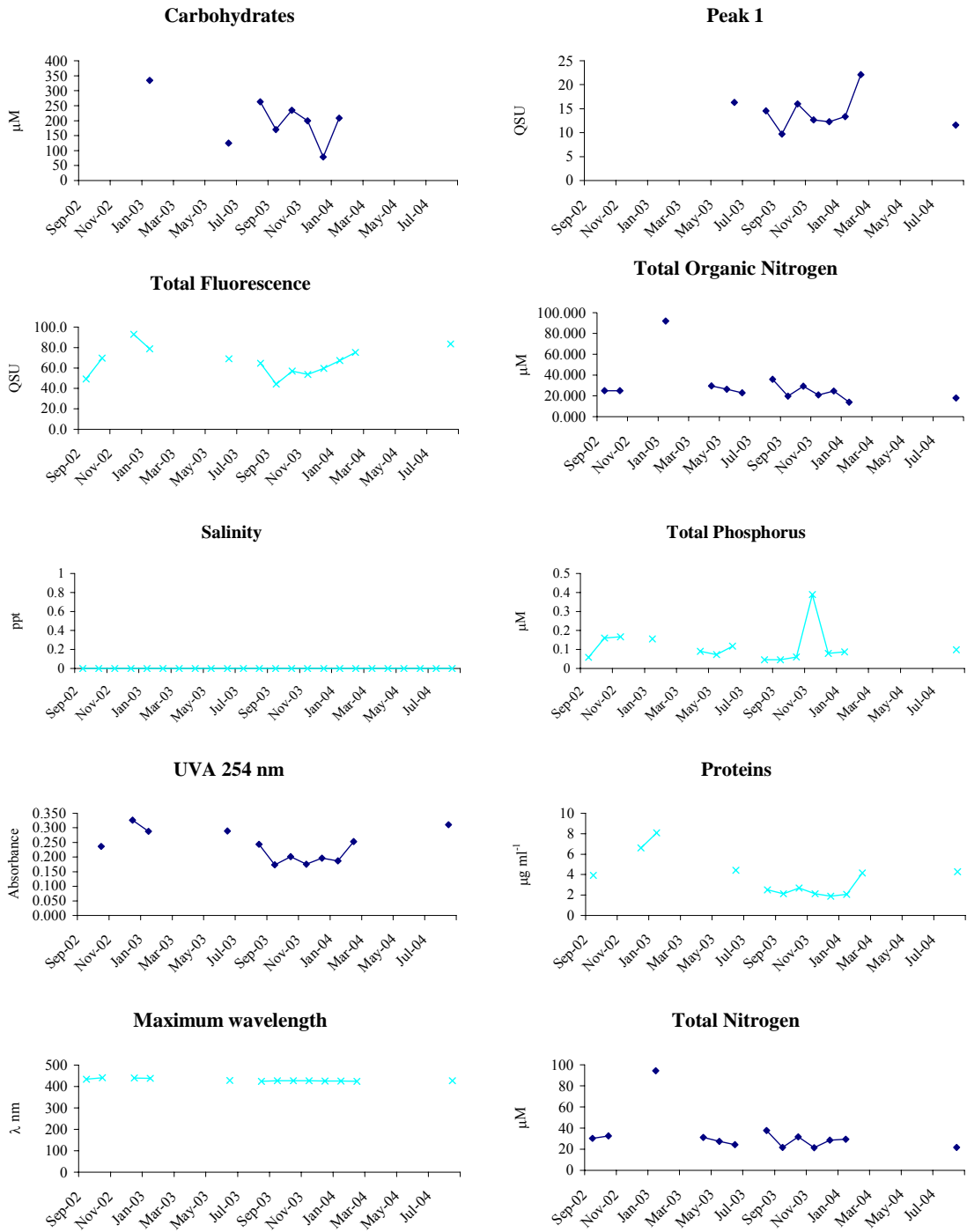


Figure 27b: Monthly survey data from TS 5 from the sampling period September 2002 to August 2004.

TS 6

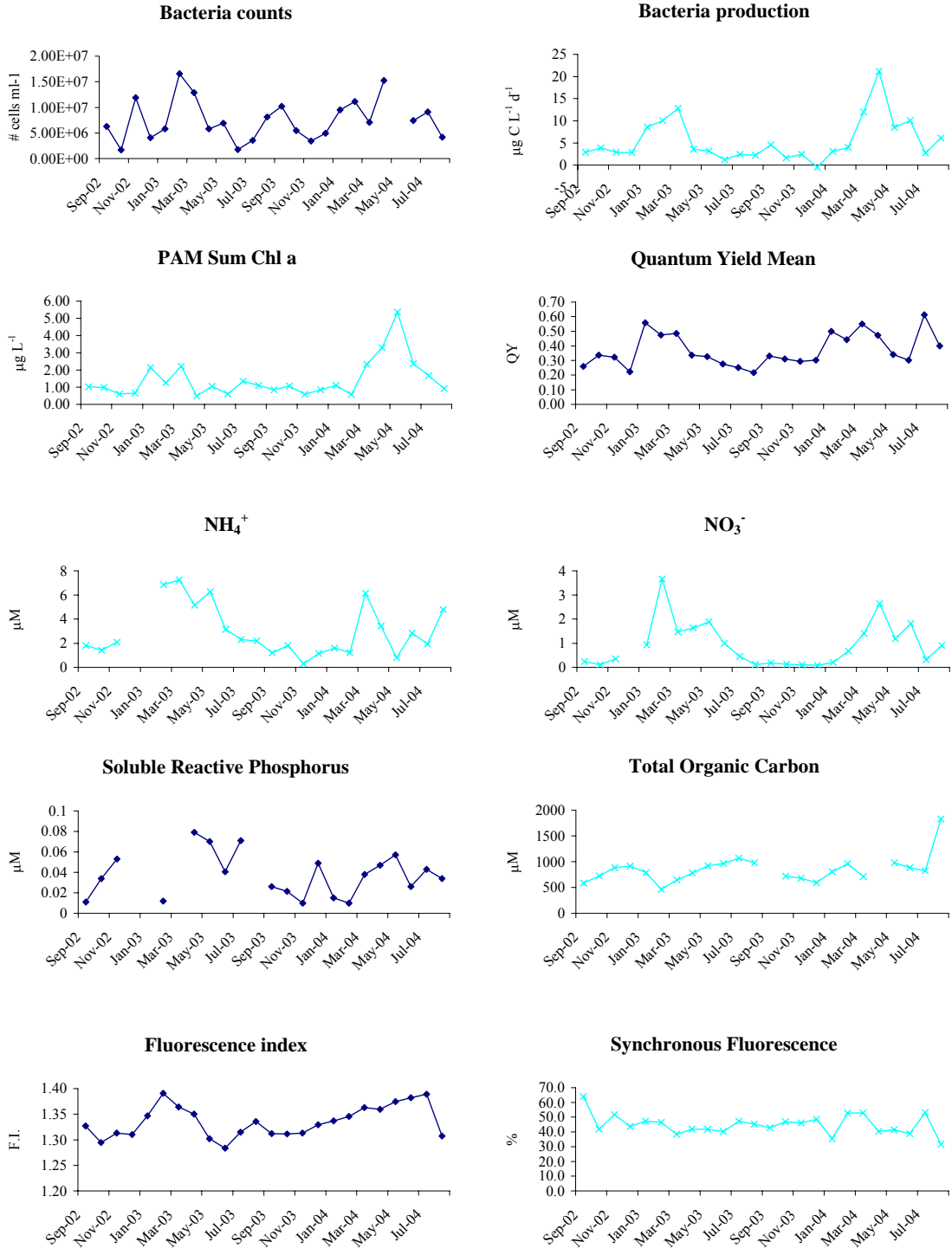


Figure 28a: Monthly survey data from TS 6 from the sampling period September 2002 to August 2004.

TS 6

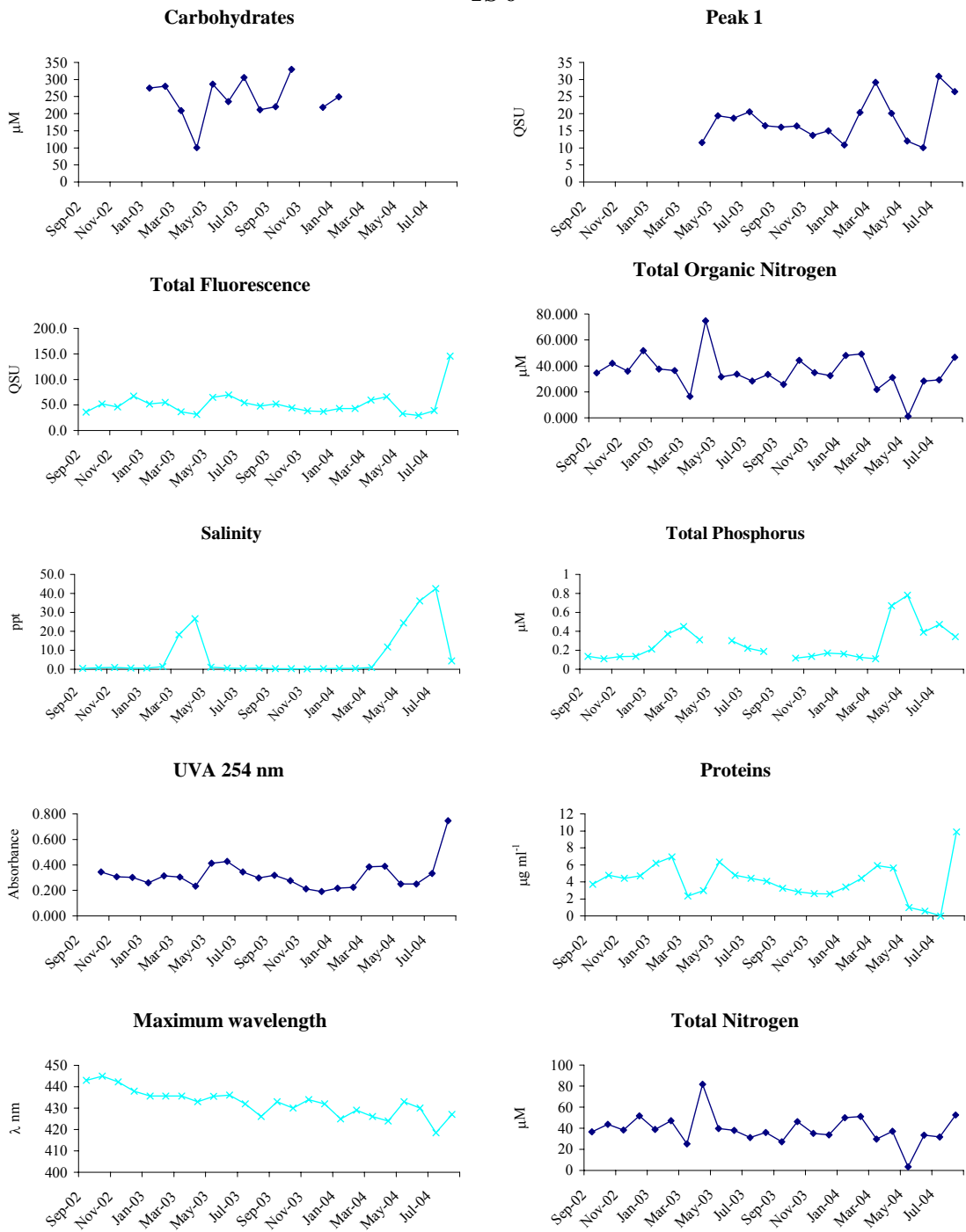


Figure 28b: Monthly survey data from TS 6 from the sampling period September 2002 to August 2004.

TS 7

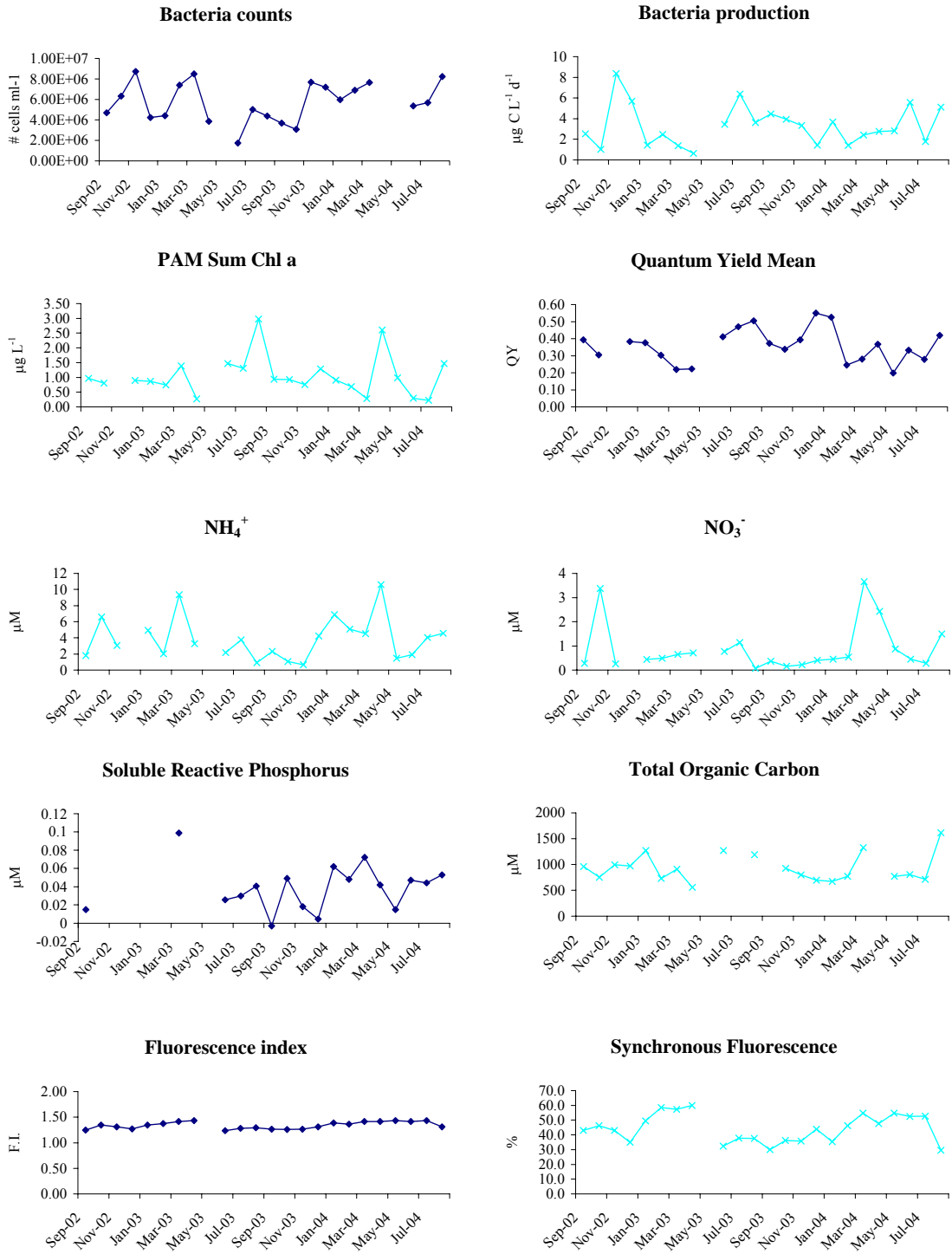


Figure 29a: Monthly survey data from TS 7, or Taylor River mouth, from the sampling period September 2002 to August 2004.

TS 7

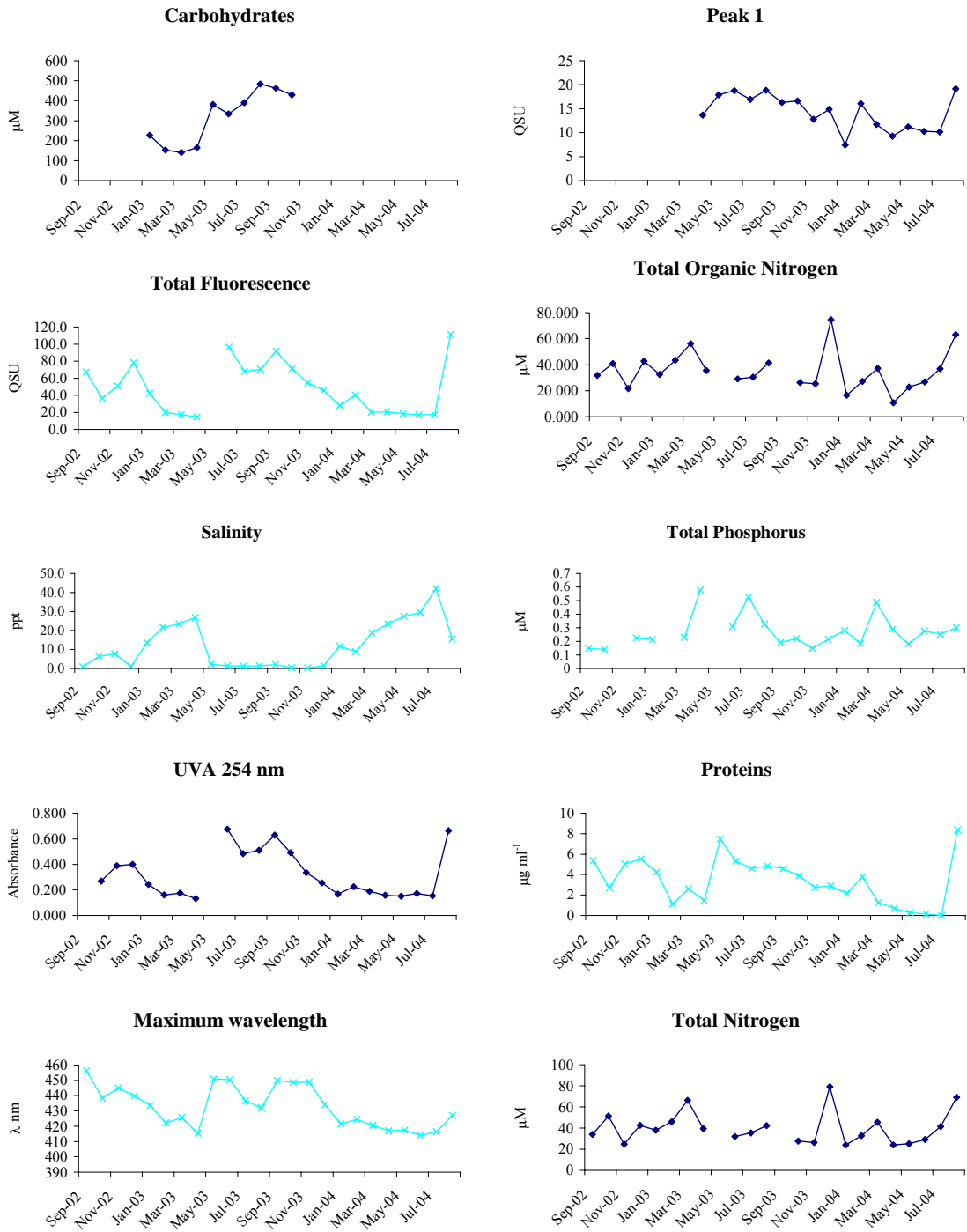


Figure 29b: Monthly survey data from TS 7, or Taylor River mouth, from the sampling period September 2002 to August 2004.

TS 8

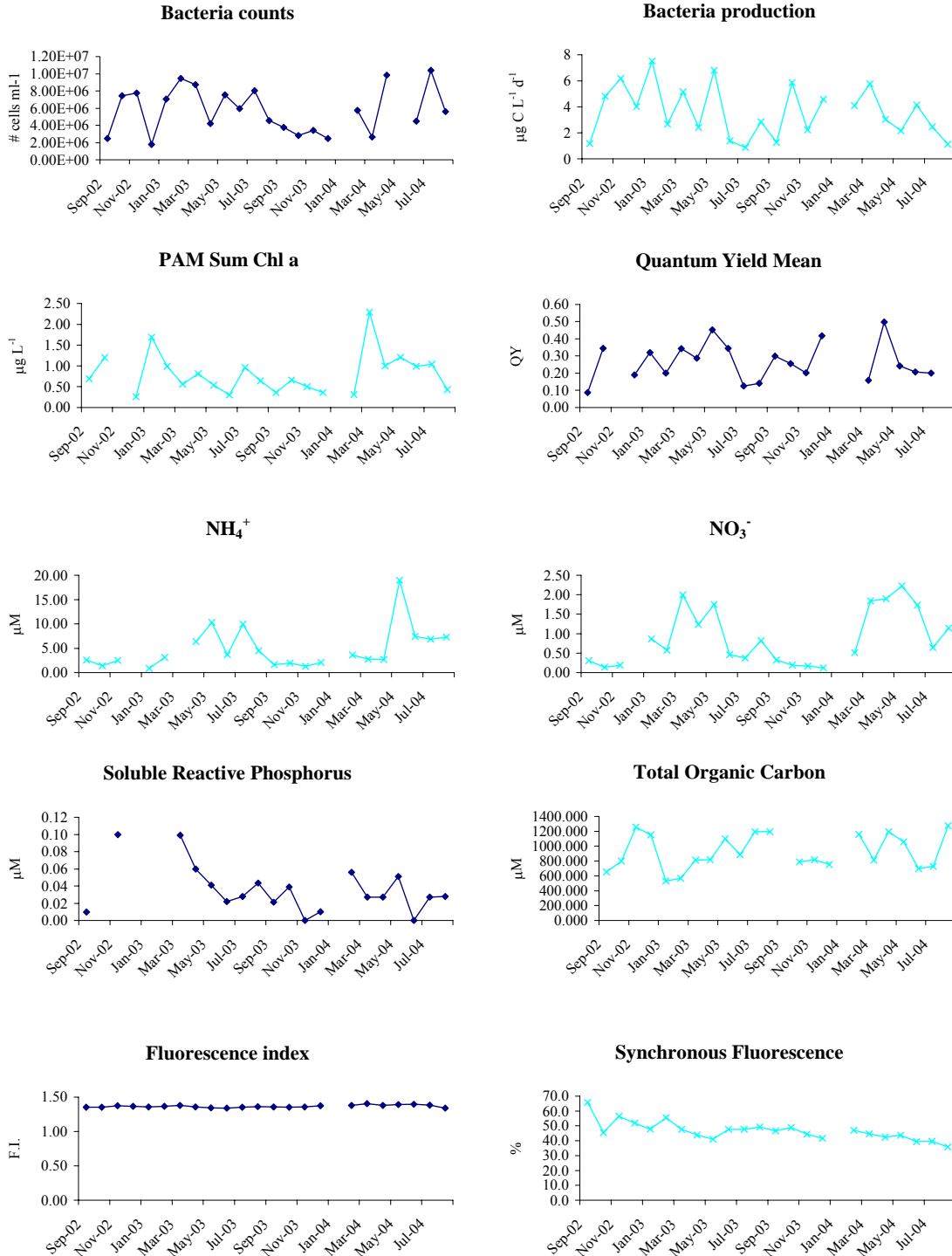


Figure 30a: Monthly survey data from TS 8, or Joe Bay from the sampling period September 2002 to August 2004.

TS 8

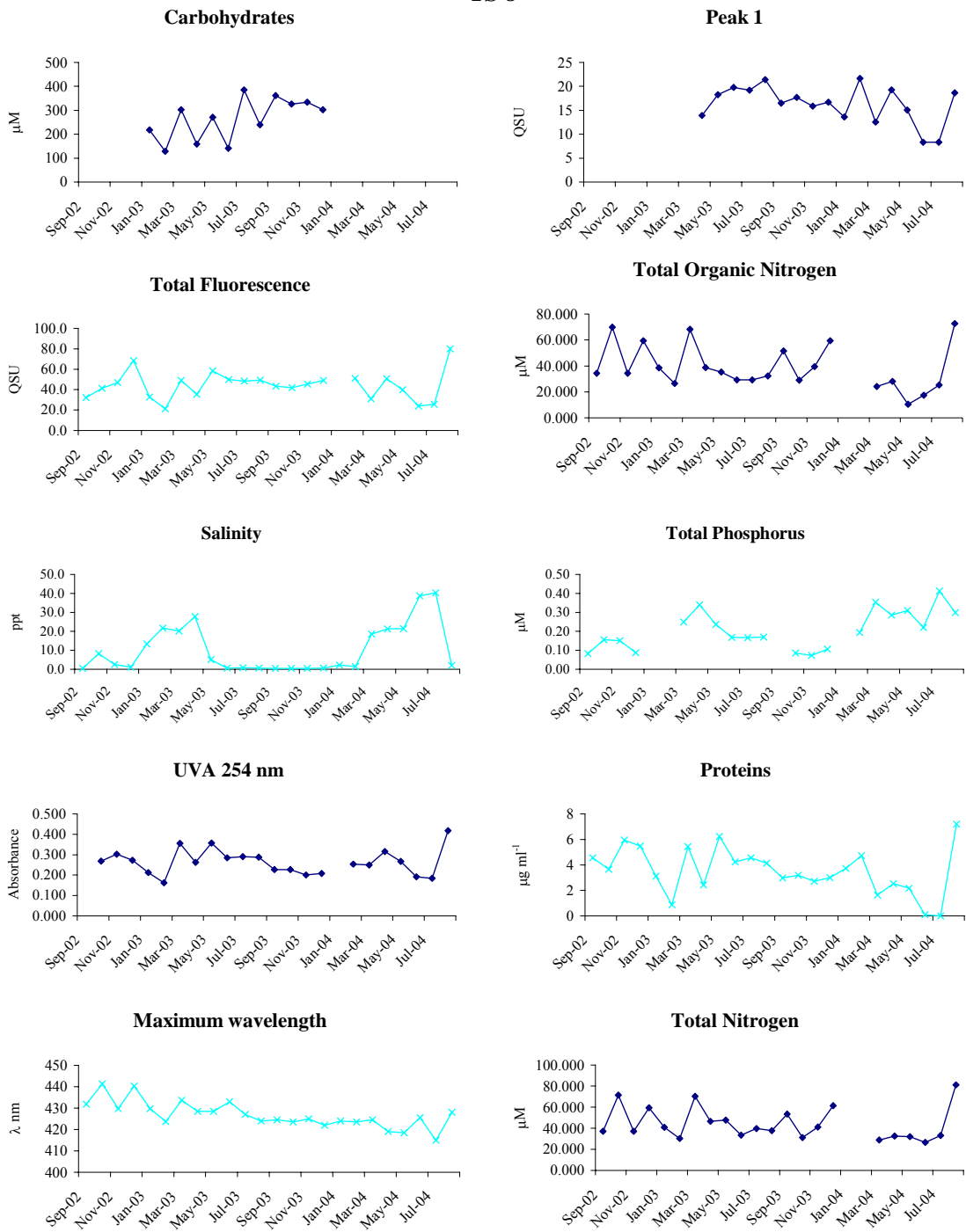


Figure 30b: Monthly survey data from TS 8, or Joe Bay from the sampling period September 2002 to August 2004.

TS 9

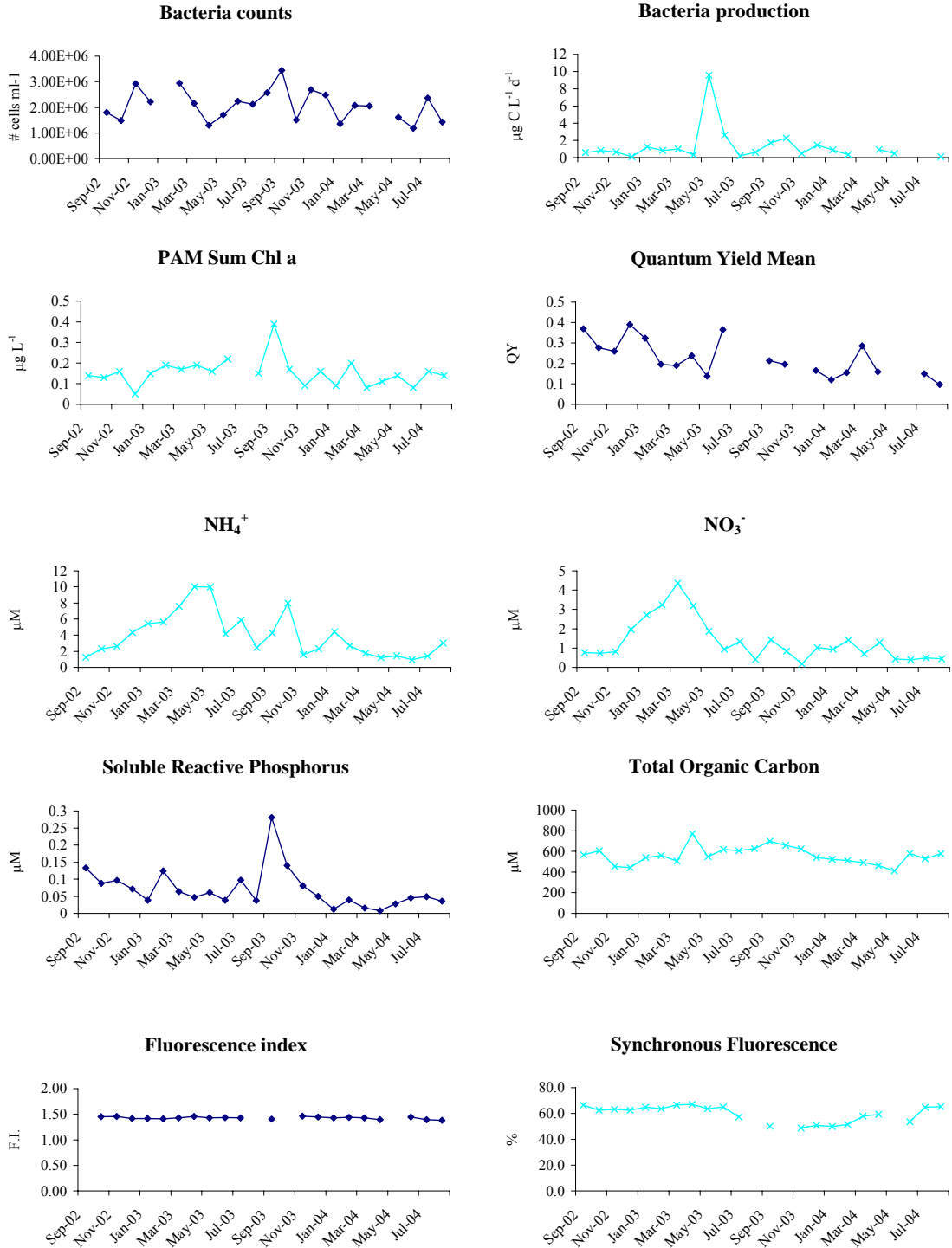


Figure 31a: Monthly survey data from TS 9, or Duck Key, from the sampling period September 2002 to August 2004.

TS 9

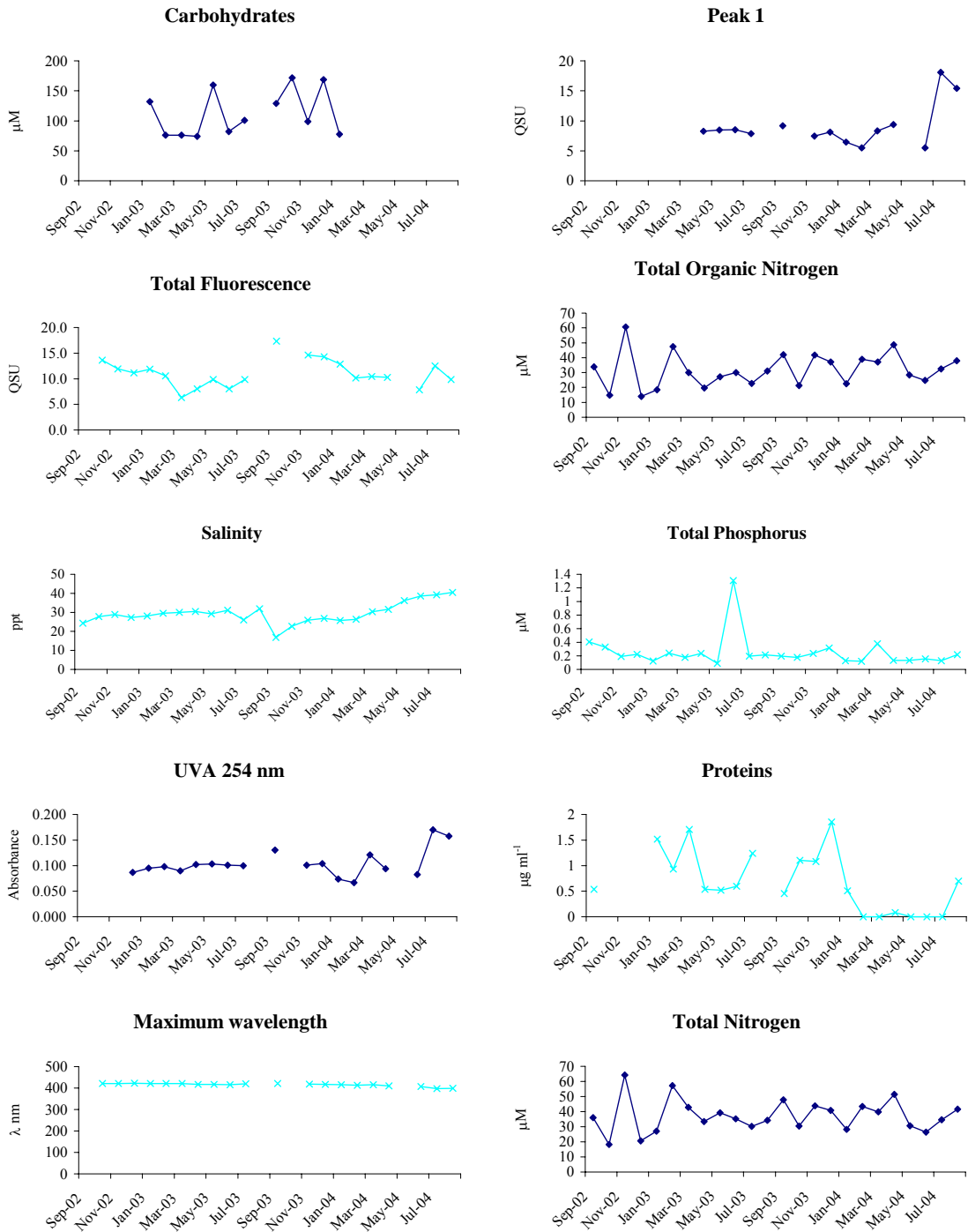


Figure 31b: Monthly survey data from TS 9, or Duck Key, from the sampling period September 2002 to August 2004.

TS 10

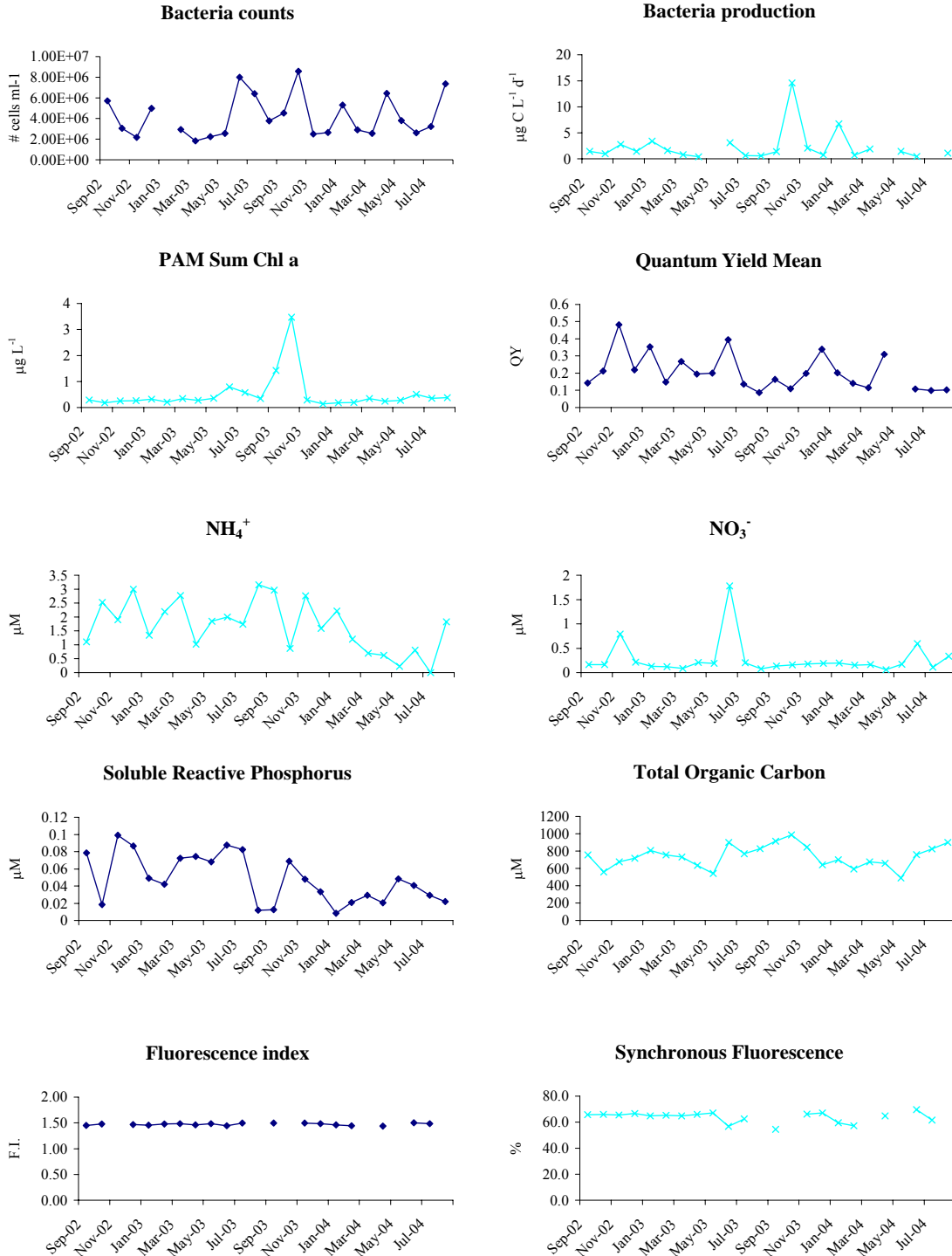


Figure 32a: Monthly survey data from TS 10, or Bob Allen Keys, from the sampling period September 2002 to August 2004.

TS 10

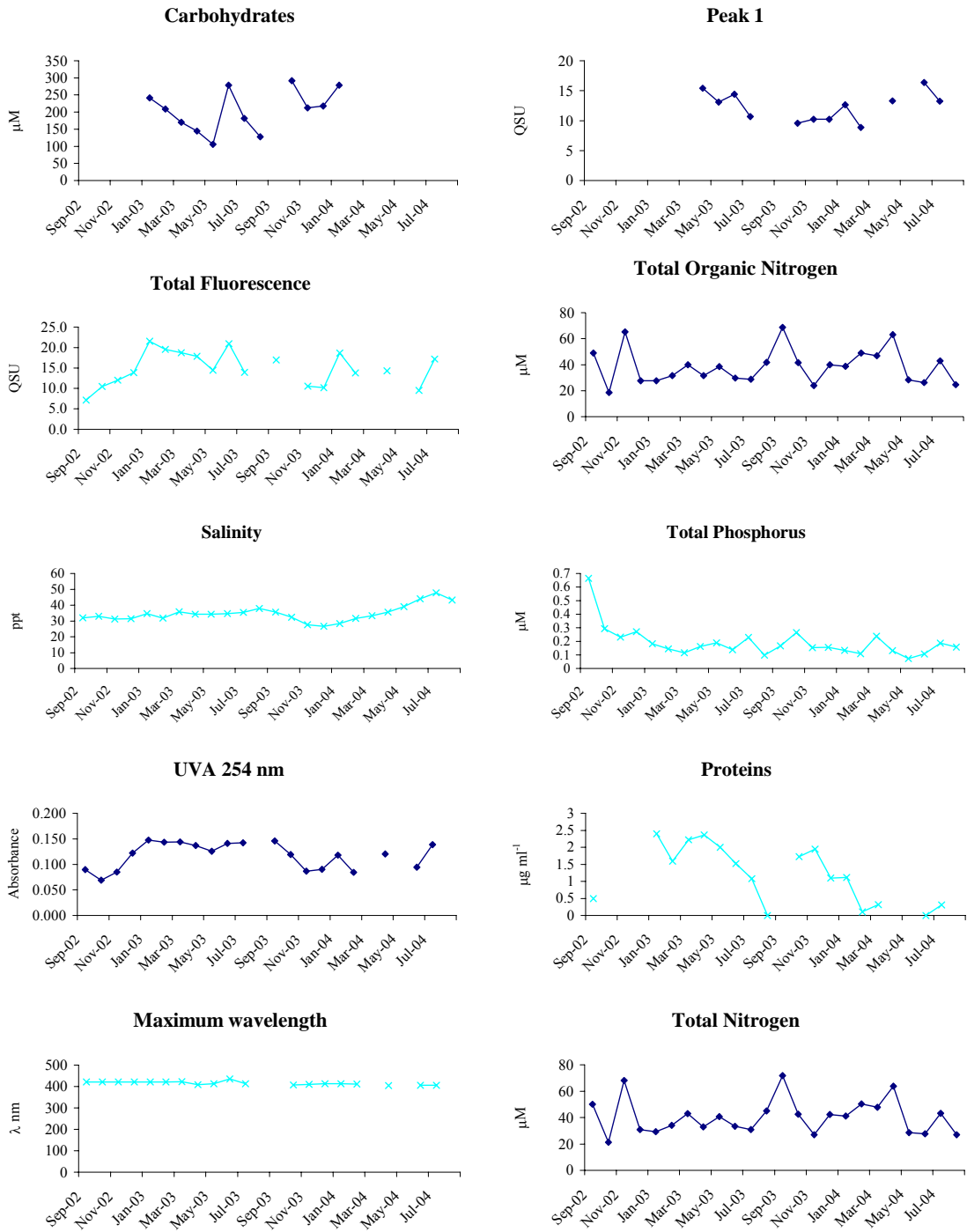


Figure 32b: Monthly survey data from TS 10, or Bob Allen Keys, from the sampling period September 2002 to August 2004.

TS 11

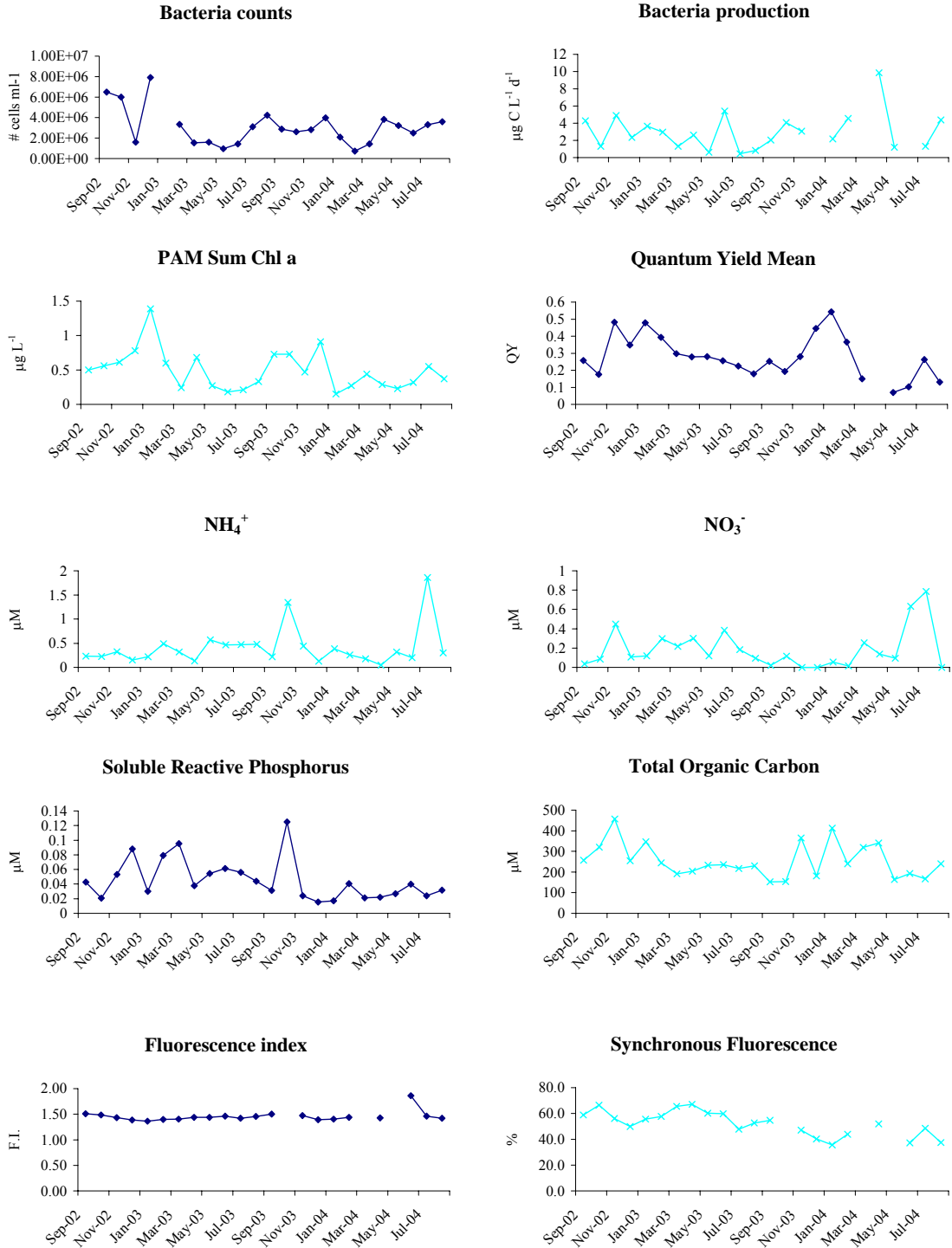


Figure 33a: Monthly survey data from TS 11, or Sprigger Bank, from the sampling period September 2002 to August 2004.

TS 11

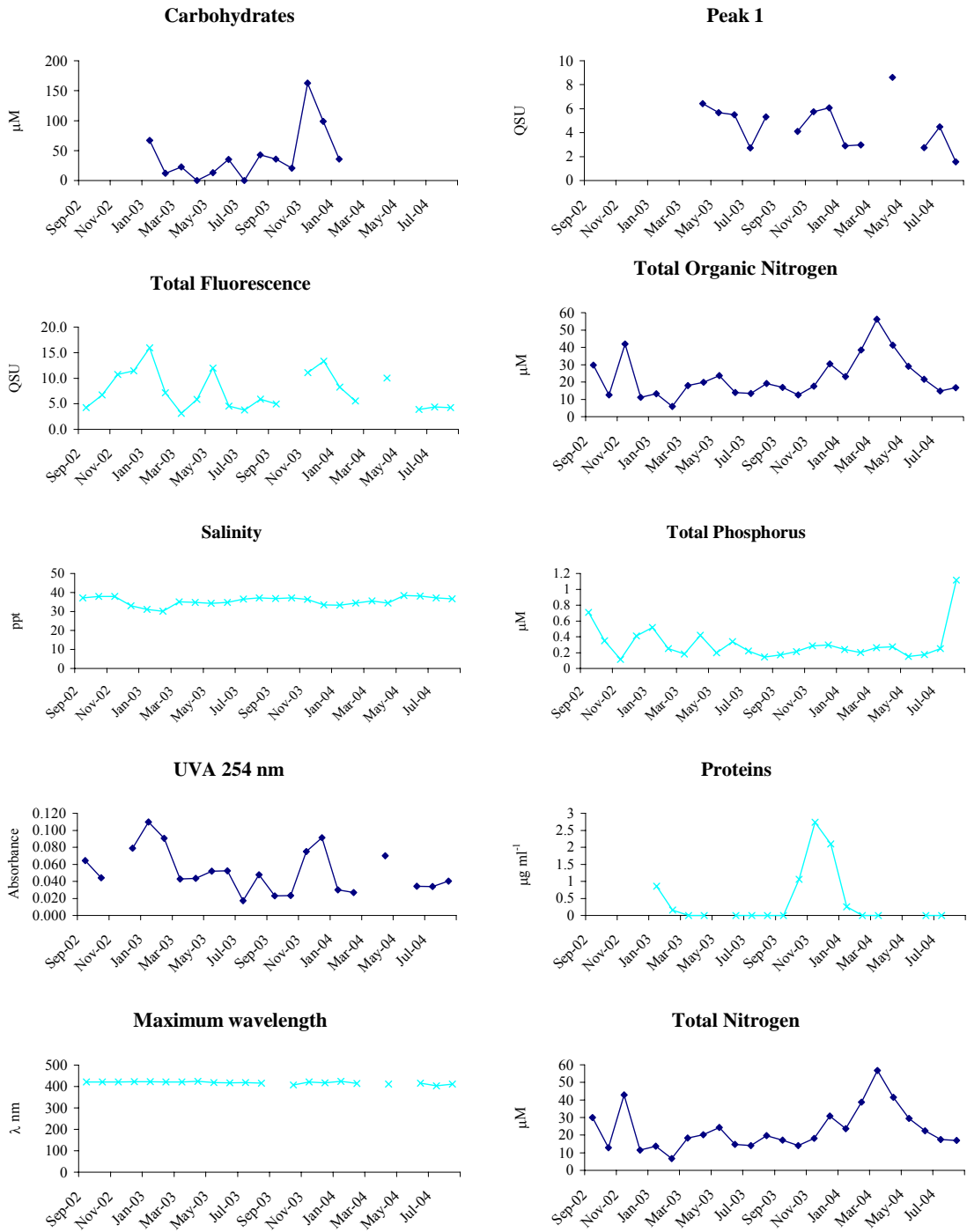


Figure 33b: Monthly survey data from TS 11, or Sprigger Bank, from the sampling period September 2002 to August 2004.