FINAL REPORT

"High Frequency Monitoring of Wastewater Nutrient Discharges and Their Ecological Effects in the Florida Keys National Marine Sanctuary"

Submitted To:
Water Quality Protection Program
U.S. Environmental Protection Agency
Marathon, FL

Submitted By:
Brian E. Lapointe and William R. Matzie
Division of Marine Science
Harbor Branch Oceanographic Institution, Inc.
3754 Pine Street
Big Pine Key, FL 33043

Date: August 28, 1997

Acknowledgements

We thank our volunteer staff, including Colleen Murphey, Margaret Vogelsang, Nicole Logan, Andy Cannon, and Damon Marunyak for assistance with field sampling, processing of samples, data analysis, and graphics. Dr. Clinton Dawes (University of South Florida) kindly assisted with identification of macroalgae. Dr. Larry Brand and Arrieta Venizelos (University of Miami) are thanked for the analysis of chlorophyll a . Myron Gunsalus, Rebecca Elliot, Chandler Griffin, and other staff of the HBOI Environmental Laboratory provided low level nutrient analysis. Dr. B. B. MacIntyre (Isotope Analytical Services) performed the $\delta^{15}N$ analyses and Carl Zimmerman (Chesapeake Biological Laboratory, University of Maryland) analyzed the tissue C:N:P samples. Finally, we are grateful to G. P. Schmahl (Looe Key National Marine Sanctuary) and Bill Kruczynski (USEPA) for their assistance in various aspects of the study. Sampling in the Florida Keys National Marine Sanctuary was conducted under Permit FKNMS (LR) - 08-95.

Abstract

The Florida Keys National Marine Sanctuary (FKNMS) has identified nutrient enrichment as a critical management issue. This study assessed how physical forcing mechanisms (rainfall, wind, tides) linked land-based wastewater discharges with the initiation of algal blooms in shallow (< 10 m) inner-shelf waters between Big Pine Key and Looe Key National Marine Sanctuary (LKNMS) from January to October 1996. Three stations were selected along the offshore gradient that included: 1) an inshore station (Avenue J, "AJ") downgradient of ~ 2,000 septic tanks/cesspits in Spanish Harbor Channel, 2) a nearshore station ("PR") near a patch Reef ~ 0.5 km south of Munson Island, and 3) an offshore station ("LK") in the back Reef at LKNMS. Monthly sampling included water column concentrations of dissolved inorganic nitrogen (DIN = $NH4^+$+ $NO3^-$ + $NO2^-$; SRP = soluble reactive phosphorus); phytoplankton biomass (chlorophyll a, chl a); biomass, species composition, alkaline phosphatase activity, tissue carbon:nitrogen:phosphorus (C:N:P) contents, and natural abundance of $\delta^{15}N$ in macroalgae; and C:N:P content, blade epiphyte loads, and natural abundance of $\delta^{15}N$ of the seagrass *Thalassia testudinum.* In addition, DIN, SRP, and chl a were measured at higher frequencies (daily) prior to, during, and following selected physical events to resolve on temporal relationships between the events, nutrient enrichment of the water column, and initiation of algal blooms.

Elevated concentrations of DIN, SRP, and chl a following episodic events (i.e. high winds, low tides, and rain) demonstrated the importance of physical forcing to wastewater nutrient discharges and eutrophication. During and following a wind event (~ 20 knots, NE) in mid-February, elevated DIN, SRP, and chl a concentrations were observed at PR and LK but not at the more protected inshore station AJ. The highest DIN (mostly $NH4^+$) and SRP concentrations of the entire study were measured at AJ during low tide on March 19. With the onset of the wet season in late May and early June, DIN concentrations (mostly $NH4^+$) reached maximum values at PR and LK, which was followed by maximal chl a concentrations at all three stations during the mid-summer period. Chl a concentrations were high at all stations throughout the study and averaged 1.86 ug/l at AJ (n = 87), 0.55 ug/l at PR (n = 86), and 0.60 ug/l at LK (n = 83).

Fleshy macroalgal biomass averaged < 100 g dry wt · m^{-2} at all three stations in winter and early spring but increased to levels of 100 - 300 g dry wt · m^{-2} at AJ and PR following the onset of the rainy season in May. The red macroalga *Laurencia poiteaui* was abundant at all three stations and $\delta^{15}N$ values of this plant were highest at AJ (+ 5.0 o/oo), intermediate at PR (+ 3.5 o/oo), and lowest at LK (+ 3.0 o/oo) -- indicating increasing wastewater N contributions to this alga with increasing proximity to shore. Large-scale blooms of *Cladophora fuliginosa* were first observed at LK in February and this alga became the dominant benthic cover by late spring. Transient, short-term increases in $\delta^{15}N$ of *C. fuliginosa* from ~1 to 5 o/oo were associated with increased DIN concentrations and tissue N

following the first major rain event in late May. Increased APA of *C. fuliginosa* at LK was also associated with increased DIN in late May and resulted in significant increases in tissue P of this alga. The highest algal epiphyte loads on *Thlassia testudinum* occurred at LK rather than the inshore stations, a result of high DIN concentrations combined with high submarine irradiance at this station; these high epiphyte loads culminated in a bloom of the phaeophyte *Cladosiphon occidentalis* during May when epiphyte:blade ratios approached 3:1, among the highest values reported in the scientific literature.

In summary, these results corroborate previous findings that episodic stormwater discharges of land-based wastewater nutrients initiate harmful algal blooms in nearshore waters of the FKNMS, including the offshore bank reef at LKNMS. Our results also underscore the need for high frequency water quality sampling to obtain statistically robust annual estimates of contaminant concentrations needed for long-term "status and trends" monitoring and also to resolve on the ecologically important short-term effects of wastewater nutrient discharges.

Introduction

The Water Quality Protection Program (WQPP) of the Florida Keys National Marine Sanctuary (FKNMS) has identified water quality as a priority management issue. A major concern is the effect of increased nutrient inputs from land-based wastewater discharges on the oligotrophic coral reef and seagrass communities. Eutrophication caused by land-based human activities is a major mechanism altering coastal ecosystems worldwide (GESAMP 1990, NAS 1994) and can cause dramatic ecosystem change in tropical and subtropical ecosystems. Bell (1992) critically reviewed case studies of eutrophication in coral reef regions and noted that enrichment above concentrations of ~ 1.0 μM dissolved inorganic nitrogen (DIN) and 0.1 μM soluble reactive phosphorus (SRP) caused the demise of coral reefs in Barbados, Kaneohe Bay, Hawaii, and the Great Barrier Reef Lagoon. Nutrient enrichment associated with eutrophication typically causes phase-shifts from slowly growing corals to faster growing macroalgae and phytoplankton, as first documented in Kaneohe Bay where sewage-driven eutrophication caused blooms of phytoplankton (Caperon et al. 1971) and the green "bubble alga" *Dictyosphaeria cavernosa* (Banner 1974). Macroalgal blooms have correlated tightly with nutrient enrichment on reefs in Jamaica (Lapointe 1997, Lapointe et al. 1997), southeast Florida (Lapointe 1997), Belize (Lapointe et al. 1993), and the inner Great Barrier Reef lagoon (Bell and Elmetri 1996). Water column nutrient enrichment of seagrass meadows in the FKNMS also increases the biomass of fast-gowing blade epiphytes, macroalgae, and phytoplankton, resulting in light limitation, seagrass die-off, and habitat loss (Tomasko and Lapointe 1991; Lapointe et al. 1994). Based on empirical evidence from the many case studies around the world, eutrophication is now considered a leading cause of both seagrass (Lapointe et al. 1994, Duarte 1995) and coral reef (Bell 1992, Ginsberg 1993) decline worldwide.

Because restoration of ecosystems altered by eutrophication is difficult, early diagnosis and management of the problem is desirable. Taxonomic or biotic shifts have been used as indicators of eutrophication (Schmitt and Osenberg 1995) but because the appearance of "indicator species" signals an advanced stage of the problem, considerable environmental damage is likely to have already occurred. More desirable is an "early warning" tracer of nutrient enrichment, such as that indicating enrichment from wastewater nutrient discharges. McClelland et al. (1997) used $^{15}N/^{14}N$ ratios (= $\delta^{15}N$) as a tracer and found that septic tank DIN loads contributed significantly to blooms of the macroalgae *Cladophora vagabunda*, *Enteromorpha* sp., and *Gracilaria tikvahiae* in the Waquoit Bay estuary, Cape Cod, USA.

Lapointe (1997), also using $\delta^{15}N$ techniques, provided evidence that rainfall during summer months increased groundwater discharges of wastewater N from septic tanks or injection wells that contributed to blooms of *Codium isthmocladum* on deep (30 m) reefs offshore northern Palm Beach County, Florida.

There exists considerable potential for wastewater enrichment of coastal waters in the FKNMS. There are currently some 30,000 on-site sewage disposal systems (OSDS, conventional septic tanks and cesspits) in the Florida Keys. Because of high groundwater tables and a transmissive geological substrate, these wastewater discharges cause nutrient (Lapointe et al. 1990) and bacterial (Paul et al. 1995 a) contamination of shallow groundwaters. Continuous down-gradient flow of nutrient-enriched groundwaters has already altered water quality in nearshore waters, evidenced by increased DIN and SRP concentrations, phytoplankton biomass, macroalgal blooms, reduced dissolved oxygen, and seagrass epiphytization and die-off (Tomasko and Lapointe 1990; Lapointe and Clark 1992; Lapointe et al. 1994). Shinn et al. (1994) assessed the fate and pathways of Class V injection well effluent using core drilled monitoring wells and found evidence of fecal contamination of groundwaters in three offshore wells in the lower Florida Keys (Shinn et al. 1994). Paul et al. (1995 b), using viral tracers, found that wastewater transport from septic tanks through Key Largo limestone into surface waters occurred in as little as 11 h. Lapointe and Matzie (1996) showed that land-based stormwater-driven wastewater nutrient discharges in the summer wet season initiate nearshore phytoplankton blooms (along with anoxia and hypoxia) and that this phenomenon was observable at the offshore reefs at Looe Key National Marine Sanctuary (LKNMS).

This study used measurement of $\delta^{15}N$ to specifically assess the degree to which land-based wastewater DIN contributes to macroalgal blooms in nearshore and offshore waters of the FKNMS. Because tidal pumping and rainfall events are two important physical mechanisms that result in episodic "pulses" of groundwater-borne nutrients into surface waters (Lapointe et al. 1990; Lapointe and Matzie 1996), we assessed how episodic physical forcing (rainfall, wind, tides) link nutrient disharges from land with the initiation of blooms of phytoplankton, macroalgae, and seagrass epiphytes in coastal waters of the FKNMS. Our approach involved high-frequency sampling at three stations along an offshore eutrophication gradient before and after episodic events for DIN and SRP concentrations; biomass, tissue C:N:P ratios and alkaline phosphatase activity (APA) of macroalgae; C:N:P ratios and blade epiphyte loads of *Thalassia testudinum*; and phytoplankton biomass (chlorophyll a). We

also measured $\delta^{15}N$ in both *T. testudinum* and macroalgal tissue as a tracer of wastewater DIN enrichment.

Materials and Methods

The study was conducted between January and October, 1996, at three shallow (1-2 m) seagrass communities along an offshore nutrient gradient from Big Pine Key to Looe Key National Marine Sanctuary (LKNMS, Fig. 1). The stations included: 1) an inshore station (Avenue J, "AJ", 24° 40' 351 N, 81° 20' 320 W) downgradient of ~ 1,000 septic tanks/cesspits ~ 0.25 km off the east side of Big Pine Key in Spanish Harbor Channel (Fig. 2), 2) a station inside of Hawk Channel near a patch reef ("PR", 24o 36' 800 N, 81° 23' 670 W) ~ 0.5 km from Little Palm Island (Fig. 2), and 3) stations in the back reef at LKNMS ("LK 1", 24° 32' 564 N, 81° 24' 245 W, a shallow seagrass meadow used for sampling all parameters except for macroalgal biomass which was sampled at the adjacent "LK 2", 24° 33' 050 W, 81° 24' 339 W, Fig. 3) . At each station a 25 m transect was established by installing aluminum pins into the sediment; GPS posits for the stations were cross-checked with FKNMS staff. The GPS posits and subsurface buoys aided in the relocation and sampling.

Monthly water samples were collected at each station to determine DIN, SRP, and chl a concentrations. Near-bottom (0.15 m above bottom) water samples were collected in triplicate at the three stations into clean 250 ml Nalgene bottles, filtered through 0.45 µm GF/F filters, and held on ice in the dark until return to the lab where they were frozen. Subsequently, they were analyzed for NH_4^+, NO_3^- plus NO_2^-, and SRP on a Bran and Luebbe TRACS Analytical Console at the HBOI Environmental Laboratory in Ft. Pierce, FL (see Appendix I for Quality Assurance Summary). For chl a, three 100 ml replicate water samples were filtered (after adding 1 mg of $MgCO_3$) through GF/F glass fiber filters and the filters were frozen until extraction within a few days (in the laboratory of Dr. Larry Brand, University of Miami). The filters were extracted for 30 minutes with 10 ml of dimethyl sulfoxide and then with an added 15 ml of 90% acetone at 5 °C overnight and measured fluorometrically before and after acidification for the measurement of chlorophyll and phaeopigment concentrations (Burnison 1980; Parsons et al. 1984). Fluorescence measurements were made using a Turner Designs 10-000R fluorometer equipped with a infrared-sensitive photomultiplier and calibrated using pure chlorophyll a.

In addition to the monthly samples, additional samples were collected at higher frequency (daily) prior to, during, and following physical forcing events. For example,

samples were collected between 14 and 19 February as daily average wind speed increased from 5 to 15 knots during a winter northeaster and on July 14 when winds blew from the northeast at 15 knots. Samples were collected between 15 and 20 March as a function of tidal stage ranging from a high tide on 15 March (+1.0 ft) to low tides on 18 March (-0.33 ft), 19 March (- 0.43 ft), and 20 March (-0.47 ft). Samples were collected through four different rain events on 11 March (~ 1.0 inch), 15 to 25 May (~ 4.0 inches), 1 to 3 July (7.5 inches), and 23 to 25 September (3.8 inches). Rainfall was continuously monitored (Fig. 4) with a rainfall gauge at the Big Pine Key field station and weather and tide data were obtained from the Key West National Weather Service Station as recorded in the National Climatic Database.

Monthly sampling of macroalgae included species composition, biomass, tissue C:N:P ratios and alkaline phosphatase activity (APA) of predominant species. Random samples of macroalgae (n=10) were collected along the transects with a 0.1 m^2 quadrat to estimate biomass. The samples were returned to the lab and sorted, cleaned of sediment, epiphytes, epizoa, then weighed. When possible, we sampled species common to the three stations (e.g. *Laurencia, Dictyota*) for tissue analysis to allow intraspecific comparisons among the three stations. Subsamples of two macroalgae from each station, as well as new clean blade tissue of *Thalassia testudinum,* were dried in a laboratory oven (60 ^oC) to constant weight and analyzed for C:N:P ratios at the University of Maryland's Analytical Services Laboratory in Solomons, MD. Blade epiphyte levels were also measured on *T. testudinum* at the three stations by the methods described by Lapointe et al. (1994). APA of macroalgae was measured (n = 4) by the spectrophotometric methods detailed in Lapointe et al. (1994).

We analyzed dried tissue of abundant macroalgae and *T. testudinum* sampled monthly from each station for natural abundances of stable nitrogen isotope ratios ($\delta^{15}N = {}^{15}N/{}^{14}N$). Analyses for total N and 15N atom % were performed by Isotope Analytical Services, Inc. (Los Alamos, New Mexico). The samples were processed through a Carlo-Erba N/A 1500 elemental analyzer using Dumas combustion. The purified nitrogen gas was then measured by a VG Isomass mass spectrometer.

The data were analyzed using one-way and two-way ANOVA. We assessed overall variability in water column DIN, SRP, chl a, macroalgae (biomass, C:N:P ratios, alkaline phosphatase activity, $\delta^{15}N$), and seagrass (C:N:P ratio, blade epiphytes, $\delta^{15}N$) as a function of location (AJ, PR, LK) and time (month). Additional comparisons were made using pairwise t-tests and linear regression.

Results

Dissolved Inorganic Nutrients and Chl a in theWater Column

There was a significant interaction ($F = 3.124$, $P = 0.00008$) between location and time on the mean DIN concentrations, which decreased with increasing distance from shore. Over the entire study, DIN averaged 2.10 ± 1.43 µM ($n = 102$) at AJ, 1.18 ± 0.87 µM ($n = 102$) at PR and 0.87 ± 0.68 µM ($n = 99$) at LK. Maximum DIN concentrations were 7.75 µM at AJ , 5.77 µM at PR, and 5.59 µM at LK. $NH4^+$ was the predominant DIN species at AJ, accounting for ~ 70 % of the total DIN pool. $NO3^-$ was relatively more important at the more offshore PR and LK stations, although the maximum concentrations of $NH4^+$ were ~ 3-fold higher than the maximum concentrations of $NO3^-$ at these offshore stations.

Considerable variability in DIN concentrations resulted from physical forcing. Elevated DIN concentrations at the inshore AJ station were associated with high winds, rain, and low tides. For example, DIN (primarily $NH4^+$) increased from ~ 1.0 µM to 4.0 µM as strong northeasterly winds developed between 15 and 19 February. The highest DIN concentrations measured during the entire study, 7.75 µM, occurred during the low tide sampling on March 19 (Fig. 5). Significant DIN pulses also followed rain events on 11 March, 15 to 24 May, 1 July, and 23 September. DIN also increased at both PR and LK with high northeasterly winds between 15 and 19 February and on July 14 when the maximum DIN concentrations occurred at these stations (Figs. 2 and 3). DIN spikes occurred at PR and LK following rain events on 24 May, 1 July, and 23 September. In contrast to AJ, the higher DIN concentrations at the more offshore PR and LK (Figs. 6 and 7) occurred following the onset of the rainy season in mid-May.

There was a significant interaction ($F = 1.82$, $P = 0.029$) between location and time on the mean SRP concentrations, which decreased with increasing distance from shore. Concentrations of this primary limiting nutrient were relatively lower and displayed less variability compared to DIN. Over the entire study, SRP averaged 0.06 ± 0.04 µM ($n = 102$) at AJ, 0.04 ± 0.03 µM ($n = 102$) at PR, and 0.03 ± 0.03 µM ($n = 99$) at LK. The highest SRP concentrations at all three stations were associated with the high northeast wind event between 15 and 19 February (Figs. 8, 9, 10). Annual average DIN:SRP ratios varied from 50.2 at AJ, 63.4 at PR, to 34.8 at LK.

There was a significant interaction ($F = 3.16$, $P = 0.00007$) between location and time on the mean chl a concentrations, which decreased with increasing distance from shore. Chl a was significantly ($F = 15.7$, $P < 0.00001$) higher during the summer wet season compared to the winter and spring dry season. For example, the average chl a concentration at AJ was over 4-fold higher in the wet season (2.62 ug/l) compared to the dry season (0.62 ug/l) while at PR and LK, chl a concentrations increased by ~ 50 - 70 % from the dry to the wet seasons. Over the entire study, chl a averaged 1.86 ± 2.11 ug/l ($n = 87$) at AJ, 0.55 ± 0.77 ug/l ($n = 86$) at PR, and 0.59 ± 0.46 ug/l ($n = 83$) at LK. At AJ, chl a increased to high values (> 5.0 µg/l) on 24 May following several days of intense rainfall (> 7 " rain between 15 and 25 May, Fig. 11). A similar pattern was observed at PR where the highest chl a concentrations occurred on July 1 (Fig. 12) following heavy rain (Fig. 4). The seasonal pattern in chl a at LK was similar to that at AJ, increasing in late May following the onset of the rainy season (Fig. 13).

Biomass and Species Composition of Benthic Macroalgae

Biomass of frondose, fleshy macroalgae at AJ increased from seasonal low values of ~ 25 g dry wt·m^{-2} during February to peak values of ~ 90 g dry wt·m^{-2} in late May following the onset of the rainy season (Fig. 14). During February and March, the rhodophytes *Laurencia poiteaui* and *Heterosiphonia wurdmanni* dominated the fleshy macroalgal biomass and blooms of the chlorophytes *Cladophora crispula* and *Chaetomorpha gracilis* appeared in late April and May. The rhizophytic chlorophyte *Caulerpa sertulariodes* became abundant in June following heavy rainfall and blooms of the chlorophyte *Cladophora montagneaena* and the blue green *Lyngbya majescula* developed in August and September, respectively. Biomass of the calcareous macroalgae (primarily *Halimeda opuntia*) increased from low values of ~ 150 g dry wt ·m^{-2} in the winter to maximum values of ~ 1,000 g dry wt ·m^{-2} in August (Fig. 15).

Biomass of fleshy macroalgae at PR was higher than at AJ, averaging 40 to 50 g dry wt ·m^{-2} between February and May and increasing to maximum values of ~ 300 g dry wt ·m^{-2} in September following the onset of rain in late May (Fig. 16). Between February and May, the rhodophytes *Laurencia poiteaui* and *Heterosiphonia wurdmanni* dominated the fleshy macroalgal biomass and blooms of *Ceramium nitens* appeared as an epiphyte on Gorgonians in late April. Extensive blooms of the blue-green *Lyngbya gracilis* (with *Lyngbya rivularianum* as an epiphyte) developed in June on seagrasses, corals, soft corals, and macroalgae. In contrast to the fleshy macroalgae, biomass of calcareous macroalgae at PR

showed a decreasing trend from February to October with maximum values < 140 g dry wt $\cdot m^{-2}$ (Fig. 17).

Biomass of fleshy macroalgae at LK averaged ~ 40 to 50 g dry wt $\cdot m^{-2}$ between February and April and began increasing in May, reaching maximum values of ~ 200 g dry wt $\cdot m^{-2}$ in September (Fig. 18). Between February and May, the rhodophyte *Laurencia poiteaui* and the chlorophyte *Cladophora fuliginosa* were abundant and blooms of the chlorophyte *Cladophora crispata* developed as an epiphyte on sponges, seagrasses, and soft corals between late March and July. Blooms of the blue green epiphyte *Lyngbya gracilis* covered all benthic biota in June, which was accompanied by blooms of *Lyngbya meneghinin*a and *Lyngbya semiplena* in August. Biomass of calcareous macroalgae showed a bi-modal pattern with maximum values in March and August (Fig. 19).

C:N:P, $\delta^{15}N$, and APA of Benthic Macroalgae

The rhodophyte *Laurencia poiteaui* displayed significant variation in tissue C:N:P contents among the three stations over the study period. At AJ, PR, and LK, respectively, the % C of dry weight averaged 20.48 ± 1.20, 22.84 ± 2.8, 16.49 ± 2.32; % N averaged 1.32 ± 0.30, 1.49 ± 0.22, and 0.97 ± 0.36; and % P averaged 0.028 ± 0.011, 0.024 ± 0.008, and 0.033 ± 0.011. The mean % N of dry weight in *L. poiteaui* was significantly higher at the nearshore AJ ($t = 5.9$, $P < 0.001$) and PR ($t = 3.75$, $P = 0.007$) compared to the more offshore LK. However, there was no significant ($P > 0.05$) difference in mean % P of *L. poiteaui* among the three stations.

There were also significant differences in the mean C:P and N:P ratio, but not the C:N ratio, of *Laurencia poiteaui* among the three stations. At AJ, PR, and LK, respectively, the C:N ratio averaged 19.1 ± 4.9, 18.1 ± 2.9, and 21.1 ± 4.4; the C:P ratio averaged 2181 ± 920, 2917 ± 1573, and 1408 ± 407; and the N:P ratio averaged 110 ± 21, 156 ± 59, and 69 ± 26. The C:P ratios of *L. poiteaui* were significantly higher at the nearshore AJ ($t = 2.79$, $P = 0.031$) and PR ($t = 2.53$, $P = 0.039$) compared to the more offshore LK. Similarly, the N:P ratios of *L. poiteaui* were significantly higher at the nearshore AJ ($t = 7.62$, $P = 0.0002$) and PR ($t = 4.05$, $P = 0.0048$) compared to the more offshore LK. However, there was no significant ($P > 0.05$) difference in the C:N ratio of *L. poiteaui* among the three stations.

At the offshore LK station, there was a trend of increasing % N (Fig. 20) and % P (Fig. 21) in *Cladophora fuliginosa* tissue from winter to summer. During the entire period of study

(n = 9), % C of *C. fuliginosa* averaged 22.37 ± 2.6, % N averaged 1.07 ± 0.15, and % P averaged 0.034 ± 0.012 % dry weight. The tissue % P increased from 0.037 % to 0.062 % between April and May with the onset of rain in late May (Fig. 18). Coincidental with the increase in % P of *C. fuliginosa* tissue at LK was a significant (F = 11.54, P = 0.0004) seasonal increase in APA from values < 20 μM PO_4^{3-}·g dry wt·h^{-1} between February and April to values > 50 μM PO_4^{3-}·g dry wt·h^{-1} in May following the onset of rain (Fig. 22).

The APA of *Laurencia poiteaui* was significantly affected by location (F = 18.81, P = 0.00003) and season (F = 15.42, P = 0.0009). The mean APA of *L. poiteaui* was highest at AJ (51.6 μM SRP· g dry wt.$^{-1}$·h^{-1}) , intermediate at PR (36.8 μM SRP· g dry wt.$^{-1}$·h^{-1}) and lowest at LK (22.0 μM SRP· g dry wt.$^{-1}$·h^{-1}). APA of *L. poiteaui* increased from low values in winter to high values in summer at AJ (Fig. 23), PR (Fig. 24), and LK (Fig. 25), respectively.

The $\delta^{15}N$ values of *Laurencia poiteaui* varied among the three stations and over time during the study (Figs. 26, 27, and 28). The mean $\delta^{15}N$ of *Laurencia poiteaui* was highest at AJ (4.69 ± 1.14 o/oo), with lower values at PR (3.03 ± 0.46 o/oo) and LK (3.00 ± 1.04 o/oo). The mean $\delta^{15}N$ value of *L. poiteaui* at AJ was significantly greater (t = 3.51, P = 0.009) than those from PR and LK, and values from the latter two stations were not statistically different from each other (t = 0.057, P = 0.955).

The $\delta^{15}N$ values of the chlorophyte *Cladophora fuliginosa* at LK increased following rainfall in May and the wind event in July (Fig. 29). Between April 24 and May 23, the $\delta^{15}N$ values increased from < 3.0 o/oo to > 5.00 o/oo following the onset of heavy rain and increases in DIN at LK; the $\delta^{15}N$ values again increased on July 14 from values < 1.00 o/oo to > 5.00 o/oo following high northeast winds and DIN enrichment.

Epiphytes, C:N:P, and $\delta^{15}N$ of Thalassia testudinum

The epiphyte loads on *Thalassia testudinum* were generally high at all three stations, especially LK. The epiphyte:seagrass blade ratio averaged 0.92 ± 0.43 at AJ, 0.94 ± 0.28 at PR, and 1.85 ± 0.81 at LK. The highest epiphyte loads at AJ were observed in April (Fig. 30) while those at PR and LK occurred in June (Figs. 31 and 32). Over the entire study, the epiphyte loads at LK were significantly greater than those at PR (t = -4.309, P = 0.001) and AJ (t = -3.937, P = 0.003). The epiphytic algal community at LK between April and June included the phaeophytes *Cladosiphon occidentalis* and *Lophosiphonia saccorhiza*, the

rhodophytes *Chondria* sp., *Ceramium fastigatum*, and *Griffithsia* sp., and the blue green alga *Lyngbya gracilis*. Following a dense bloom of the chlorophyte *Cladophora montagneaena* in September and October at AJ, we observed an extensive die-off of manatee grass, *Syringodium filiforme*.

The C:N:P contents in blade tissue of *Thalassia testudinum* varied differently for N versus P. Over the entire study, the % N at AJ, PR, and LK averaged 2.40 ± 0.20, 1.82 ± 0.17, and 1.70 ± 0.11; these three average values were significantly ($t > 2.87$, $P < 0.018$) different from each other. The % P at AJ, PR, and LK averaged 0.13 ± 0.02, 0.10 ± 0.02, and 0.16 ± 0.02; these three values were also significantly ($t > -3.29$, $P < 0.009$) different from each other. At AJ, PR, and LK, respectively, the C:N ratio averaged 16.29 ± 1.05, 20.92 ± 1.12, and 22.54 ± 1.19; the C:P ratio averaged 658 ± 83, 827 ± 134, and 549 ± 90; and the N:P ratio averaged 40.6 ± 5.7, 39.5 ± 5.7, and 24.3 ± 3.3.

The average $\delta^{15}N$ value of *Thalassia testudinum* was highest at the offshore LK station, in contrast to the blade epiphytes which had the highest average value at the inshore AJ station. At AJ, PR, and LK, the $\delta^{15}N$ value of *T. testudinum* averaged 2.80, 2.77, and 3.36, or generally increasing with distance from shore. At the inshore AJ station, no clear temporal pattern in the $\delta^{15}N$ values of *T. testudinum* was evident (Fig. 33) whereas at the PR and LK stations the $\delta^{15}N$ values increased from winter to summer (Figs. 34 and 35). The $\delta^{15}N$ values of the blade epiphytes of *T. testudinum* were higher than the host plant (Figs. 36, 37, and 38) and increased during February and March when high winds and low tides resulted in elevated water column DIN.

Discussion

Episodic Water Column Enrichment

This study demonstrated significant linkages among episodic physical events, land-based wastewater nutrient discharges, and enrichment of dissolved nutrient concentrations in coastal waters of the FKNMS as far offshore as LKNMS. The observation of maximum DIN concentrations at AJ during the lowest tide of the study on March 19 illustrates the importance of tidal pumping via ebbing tides to enhanced rates of submarine groundwater discharge (SGD) of wastewater nutrients. Lapointe et al. (1990), using a heat-pulsing groundwater flowmeter, found that lateral rates of shallow groundwater flow increased by ~ three-fold during ebbing tides as compared to flooding tides. This effect would be maximum

during extreme low tides, such as that on March 19 when the highest DIN concentration (7.75 μM) was observed. In addition to increased rates of SGD, the extreme low tides also result in minimal dilution of the increased wastewater nutrient load with lower nutrient offshore water, further increasing nutrient concentrations in the coastal receiving waters.

High winds were another important physical mechanism causing nutrient enrichment. Elevated DIN and SRP concentrations were measured at all stations during the high northeasterly winds in February and July. These increased nutrient concentrations potentially resulted from several contributing factors. First, wind-driven advection associated with these events could increase cross-island hydrostatic potentials by piling up water on the Florida Bay side of the Florida Keys, thereby increasing rates of SGD and enrichment of coastal waters towards Hawk Channel and the offshore reefs (e.g. see Shinn et al. 1994). High winds cause sediment resuspension and enhanced advection and diffusion of pore water nutrients derived from SGD into the water column. High northeasterly winds also cause offshore transport of water from inshore to offshore in the lower Florida Keys; we observed this effect on March 12 and during the following days when high northeasterly winds transported highly turbid waters several miles offshore (south) of LK.

Rainfall was also an important mechanism causing episodic nutrient enrichment. Rainfall results in increased infiltration of septic tank and cesspit wastewater plumes into shallow groundwaters and also increases lateral groundwater flow rates, thereby increasing wastewater nutrient loads and DIN and SRP concentrations in adjacent surface waters of the Florida Keys (Lapointe et al. 1990). Although rainfall in the Florida Keys can have significant DIN concentrations (~ 15.0 μM, Lapointe and Matzie 1996), an extremely heavy rainfall event of 6" over several hours would enrich a shallow 2 m water column by only ~ 1 μM. Following the first rain events between May 15 and 23 when a total of ~ 4 inches of rain fell, DIN concentrations increased from < 1 μM to > 3 μM at AJ, from < 0.5 μM to > 2.5 μM at PR, and from < 0.5 μM to > 1.5 μM at LK. Obviously, the relatively low amount of rainfall over this period combined with the magnitude of this increase in DIN over several days implicates DIN sources other than rainfall alone. The increase in DIN, together with increased $\delta^{15}N$ values of *Laurecia poiteai* at AJ and *Cladophora fuliginosa* at LK following this "first flush", provide clear evidence that wastewater derived DIN was contributing significantly to the DIN pool throughout the entire study area.

These findings underscore the earlier conclusions of Lapointe and Matzie (1996) that high frequency sampling is neccessary to resolve on the episodic "pulses" of wastewater

nutrient discharges and their ecological effects in the FKNMS. This is important not only to resolve on linkages between land-based nutrient enrichment and the initiation of algal blooms, but also for longer-term monitoring programs designed to assess "status and trends" of the FKNMS. The current water quality monitoring program in the FKNMS is spatially intensive but conducted at only quarterly intervals (4 days out of the 365 days in a year) and therefore not representative of the highly variable temporal domain as demonstrated in the present study. The ongoing monitoring program is further confounded by the fact that sampling occurs over varying tidal cycles and other physical forcing events, all of which are important regulators of nutrient concentrations, algal blooms, and the status of water quality in the FKNMS. The need for medium (monthly) and high frequency (daily) sampling has been recognized the Great Barrier Reef Marine Park Authority (GBRMPA) and is currently in use in their long-term monitoring programs for eutrophication on the Great Barrier Reef (Brodie and Furnas 1992).

Land-based Wastewater Nutrients and Algal Blooms

Several lines of evidence from this study support the hypothesis that land-based wastewater nutrient discharges from the Florida Keys enhance blooms of phytoplankton, macroalgae, and seagrass epiphytes as far offshore as LKNMS. First, the concentrations of DIN, SRP, and chl a were all maximum at the inshore AJ station and the gradients consistently decreased with increasing distance from shore, pointing to a land based-source of nutrients and phytoplankton biomass in the study area. Second, the spatial pattern of decreasing $\delta^{15}N$ values of *Laurencia poiteaui* and epiphytes on *Thalassia testudinum* with increasing distance from AJ to LK, combined with episodic increases in DIN and $\delta^{15}N$ values of *Cladophora fuliginosa* at LK following rain and wind events, provides unequivicol evidence that wastewater DIN contributes to macroalgal blooms throughout the study area. Third, the increase in biomass of phytoplankton, macroalgae and seagrass epiphytes at all stations following the increase in DIN after the wet season began in May demonstrates the widespread ecological effects of even low levels of nutrient enrichment from wastewater. These results corroborate earlier studies that linked land-based wastewater nutrient discharges to algal blooms in nearshore waters of the Florida Keys (Lapointe and Clark 1992) and offshore to LKNMS (Lapointe and Matzie 1996). The wastewater enrichment throughout our study area is consistent with current meter studies that showed long-term net cumulative transport from inshore waters between Big Pine Key and Marathon in a southwesterly direction towards the offshore bank reefs (Lapointe et al. 1992, Smith 1994, Pitts, 1994) and

also previous observations of an "ammonium wake" in downstream waters of LKNMS during the wet season (Lapointe et al. 1992).

Our results also refute recent speculations that offshore upwelling of deep, cold water is the primary source of nutrients fueling the algal blooms in nearshore waters of the Florida Keys (e.g. see Szmant and Forrester 1996). There are no supporting data for that hypothesis and considerable conflicting evidence. For example, in addition to the nutrient and chl a gradients decreasing from nearshore to offshore, the predominant DIN species at AJ during this study was $NH4^+$, the DIN form in septic tank-contaminated groundwaters (Lapointe et al. 1990) and throughout surface waters of the Florida Keys (Lapointe and Clark 1992). The maximum concentrations of $NH4^+$ at PR and LK were ~ 3-fold higher than the maximum concentrations of $NO3^-$ during this study and showed distinct pulses associated with physical forcing (i.e. high winds, low tides, and rain events) that we observed to enhance offshore transport of the $NH4^+$ and chl-a rich inshore waters. Because $NO3^-$ is the predominant DIN species associated with upwelling, the episodically pulsed $NH4^+$ enrichment that we observed throughout the study area was obviously not of upwelling origin. This conclusion is further evidenced by the lack of persistent cold water intrusions in the study area and the well-known long-term transport pattern in an offshore -- not onshore -- direction in the middle and lower Florida Keys (Lapointe et al. 1992, Pitts, 1994, Smith, 1994). Thus, although upwelling events have been documented on short temporal scales at LKNMS (Lapointe and Smith 1986), these events are either anomalous (i.e., upwelling at LKNMS in July 1986 was associated with 2-3 days of 20-25 knot westerly winds) or of relatively short-term duration (i.e., time scale of hours to days) and do not reflect the dominant, long-term offshore nutrient transport process.

The time course data on DIN, APA, and C:N:P ratios of *Cladophora fuliginosa* at LK illustrates the dynamics as to how episodic $NH4^+$-rich wastewater discharges initiate and sustain macroalgal blooms. The pulse of DIN (mostly $NH4^+$) at LK following the "first flush" rain event in May coincided with the maximum tissue % N in *C. fuliginosa*. Controlled experimental studies in Bermuda with a related alga, *Cladophora prolifera*, showed that DIN enrichment also resulted in increased tissue % N, which in turn caused increased APA due to increased N:P ratios that induced SRP-limitation (Lapointe and O'Connell 1989). An increase in APA also occurred in *C. fuliginosa* following the DIN pulse in May, which enhanced sequestering of SRP from dissolved organic phosphorus (DOP) pools. The end result was a parallel increase in tissue % P of *C. fuliginosa* even though no increased SRP concentrations were observed during this period at LK. These findings

demonstrate the importance of rapid assimilation of $NH4^+$ during episodic enrichment events and subsequent reliance on APA to sequester SRP from the dissolved organic phosphorus (DOP) pool to sustain balanced growth.

Our results also suggest that tissue C:N:P analysis alone may not be a reliable index of wastewater nutrient enrichment in macroalgae. The mean % N of the rhodophyte *Laurencia poiteaui* was higher at PR (1.49 % dry wt.) compared to AJ (1.32 % dry wt.) despite the fact that mean DIN concentrations were significantly higher at AJ, the station in closest proximity to land-based wastewater nitrogen loads. However, the wastewater impact at AJ was clearly indicated by the significantly higher $\delta^{15}N$ values of *L. poiteaui* at AJ compared to this alga at PR and LK. The lack of an enrichment signal in tissue N of *L. poiteaui* is most likely due to the confounding effects of variable light on the growth rate and tissue N of macroalgae (Lapointe and Duke 1984). For example, the *L. poiteaui* at AJ may have experienced higher light levels compared to plants at the more exposed and often turbid PR; this effect would not only increase the productivity of the *L. poiteaui* at AJ but also reduce its tissue %N. Future wastewater monitoring studies using macroalgae should emphasize the use of $\delta^{15}N$ values, rather than simple tissue C:N:P contents, due to the confounding effects of variable light (and other factors) on algal growth rates and tissue nutrient pools.

In comparison, *Thalassia testudinum* showed considerable tissue N enrichment at AJ (2.40 % dry wt.) compared to both PR (1.82% dry wt.) and LK (1.71 % dry wt.). However, the $\delta^{15}N$ values of *T. testudinum*, unlike the macroalgae and blade epiphytes, showed no decreasing trend in the $\delta^{15}N$ values offshore and actually had the highest values at LK. We interpret this as evidence that *T. testudinum* relies largely on nitrogen fixation for its nitrogenous supply (Capone and Taylor 1977) and as such may be more influenced by pore water P enrichment associated with wastewater discharges. In oligotrophic carbonate-rich waters of San Salvador, Bahamas, porewater P enrichment (but not N) increased growth of the seagrass *Syringodium filiforme* (Short et al. 1990). The die-off of both *T. testudinum* and *Syringodium filiforme* at AJ in September may have been related to light limitation and carbon imbalance from a recurrent bloom of *Cladophora montagneaena* that formed dense mats over the seagrasses. Alternatively, tissue N was high in *T. testudinum* at AJ and may reflect nitrogen toxicity, particularly during this period of seasonally maximum temperatures (Burkholder et al. 1991).

The relative importance of different types of primary producers in coastal ecosystems (i.e. phytoplankton, macroalgae, seagrasses, corals) is a key characteristic of ecosystem structure and has important consequences for ecosystem function. Numerous studies have shown large-scale phase-shifts in primary producers result from changes in limiting resources, most notably nutrient availability. Coastal eutrophication in tropical and subtropical regions during the past decades has resulted in trends away from seagrasses and corals and towards epiphytes, macroalgae, and phytoplankton. The high biomass of phytoplankton (all stations were above the eutrophic threshold for coral reefs of 0.5 ug/l, Bell 1992), fleshy macroalgae (100 - 300 g dry wt m^{-2}), and seagrass epiphytes (epiphyte:blade ratios up to 3:1 at Looe Key), and the proliferation of macroalgal "indicator species", i.e. *Cladophora* spp., *Lyngbya* spp) clearly show that the entire study area is now in an advanced stage of eutrophication.

Comparison of the chl a data at LK in this study with previous annual averages for this station dating back to 1987 illustrate the disturbing trend of eutrophication at LK during the past decade. The lowest annual average chl a measured , < 0.1 ug/l, was in the drought year of 1990 when the FKNMS was established; since then, annual chl a concentrations increased to 0.25 ug/l in 1992 and 0.68 ug/l in the present study (1996), a value well above threshold level considered eutrophic for coral reefs (see Bell 1992; Fig. 39). Although we found significant wastewater DIN impacts throughout our study area, including LK, the dramatic escalation of chl a at LK are also likely related to Florida Bay outflows that have been influenced by increasing phytoplankton blooms and turbidity in central and western Florida Bay since 1991 (Lapointe et al. 1996). The chl a and turbidity in Florida Bay is advected by prevailing currents southward through tidal channels of the middle and lower Keys and offshore to the outer bank reefs (Lapointe et al. 1992, Smith 1994, Pitts 1994). These blooms have expanded with the increased freshwater runoff and associated nitrogen loads from the Everglades since 1991 (Lapointe et al. 1996) that enhance DIN-limited phytoplankton populations in the central and western bay (Tomas 1996). This trends towards increased phytoplankton blooms and turbidity in Florida Bay has resulted from policies aimed at increasing freshwater flows to lower the salinity in Florida Bay (USEPA 1996). Thus, it is unlikely that corrective actions for controlling wastewater nutrient loads in the Florida Keys will, by itself, be adequate to fully restore water quality over the large regional scale of the FKNMS. Larger-scale control of nutrient loads from the south Florida watershed will be needed if the trend of eutrophication in Florida Bay and the FKNMS is to be reversed.

Literature Cited

Banner, A. H. 1974. Kaneohe Bay , Hawaii: urban pollution and a coral reef ecosystem. Proc. 2nd Inter. Coral Reef Symp., Brisbane 2:685-702.

Bell, P. R. F. 1992. Eutrophication and coral reefs: Some examples in the Great Barrier Reef lagoon. Water Research 26:553-568.

Bell, P. R. F. and I. Elmetri. 1995. Ecological indicators of large-scale eutrophication in the Great Barrier Reef lagoon. Ambio 24:208-215.

Brodie, J. and M. Furnas. 1993. Long term monitoring programs for eutrophication and the design of a monitoring program for the Great Barrier Reef. Proc. 7th. Int. Coral Reef Symp. 1: 77-83.

Burkholder, J. M., K. M. Mason, and H. B. Glasgow, Jr. 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. Mar. Ecol. Prog. Ser. 81:163-178.

Burnison, B. K. 1980. Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. Can. J. Fish. Aq. Sci. 37:729-733.

Caperon, J., S. A. Cattell, and G. Krasnick. 1971. Phytoplankton kinetics in a subtropical estuary:eutrophication. Limnol. Oceanogr. 16: 599-607.

Capone, D. G. and B. F. Taylor. 1977. Nitrogen fixation (acetylene reduction) in the phyllosphere of *Thalassia testudinum.* Mar. Biol. 40:19-28.

Duarte, C. M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41:87-112.

GESAMP. 1990. The State of the Marine Environment. Joint Group of Experts on the Scientific Aspects of Marine Pollution. Rep. and Stud. 39. United Nations Environmental Programme.

Ginsberg, R. N. Compiler. 1994. Proceedings of the colloquium on global hazards to coral reefs:health, hazards, and history. Rosenstiel School of Atmospheric Science, University of Miami.

Lapointe, B. E. 1997. Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnol. Oceanogr. in press.

Lapointe, B. E., and C. S. Duke. 1984. Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. J. Phycol. 20:488-95.

Lapointe, B.E. and J. D. O'Connell. 1989. Nutrient-enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda: eutrophication of a confined, phosphorus-limited marine ecosystem. Est. and Coast. Shelf Sci. 28:347-360.

Lapointe, B. E., J. D. O'Connell, and G. S. Garrett. 1990. Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. Biogeochemistry 10: 289-308.

Lapointe, B. E. and M. Clark. 1992. Nutrient inputs from the watershed and coastal eutrophication in the Florida Keys. Estuaries 15:465-476.

Lapointe, B. E., N. P. Smith, P. A. Pitts, and M. Clark. 1992. Baseline characterization of chemical and hydrographic processes in the water column of Looe Key National Marine Sanctuary.. Final Report to U. S. Dept. of Commerce, NOAA Office of Ocean and Coastal Resource Management, contract # NA86AA-H-CZ071, Washington, D. C. 103 pp.

Lapointe, B. E., M. M. Littler, and D. S. Littler. 1993. Modification of benthic community structure by natural eutrophication: the Belize Barrier Reef. Proc. 7th Inter. Coral Reef Symp., Guam 1:323-334.

Lapointe, B. E., D. A. Tomasko, and W. R. Matzie. 1994. Eutrophication and trophic state classification of seagrass communities in the Florida Keys. Bull. Mar. Sci. 54: 696-717.

Lapointe, B. E. and W. R. Matzie. 1996. Effects of stormwater nutrient discharges on eutrophication processes in nearshore waters of the Florida Keys. Estuaries 19:422-435.

Lapointe, B. E., W. R. Matzie, and D. Tomasko. 1996. Modeling relationships between external nutrient loading, water quality, and biotic phase-shifts: examples from Tampa Bay, Sarasota Bay, and Florida Bay. Program and Abstracts. Florida Bay Science Conference, December 10 - 12, 1996. Florida Sea Grant, University of Florida, 113 pp.

Lapointe, B. E., M. M. Littler, and D. S. Littler. 1997. Macroalgal overgrowth of fringing coral reefs at Discovery Bay, Jamaica: bottom-up versus top-down control. Proc. 8th Inter. Coral Reef Symp., Panama, in press.

Lindau, C. W., R. D. Delaune, W. H. Patrick, Jr. and E. N. Lambremont. 1989. Assessment of stable isotopes in fingerprinting surface water inorganic nitrogen sources. Water, Air, and Soil Pollution 48:489-496.

McClelland, J. W., I. Valiela, and R. H. Michener. 1996. Nitrogen stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. Limnol. Oceanogr., in press.

National Academy of Sciences (NAS). 1994. Priorities for Coastal Science, National Academy Press.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press. 173 pp.

Paul, J. H., J. B. Rose, S. Jiang, C. Kellogg, and E. A. Shinn. 1995 a. Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida. Applied and Environmental Microbiology 61: 2235-2241.

Paul, J. H., J. B. Rose, J. Brown, E. A. Shinn, S. Miller, and S. R. Farrah. 1995 b. Viral tracer studies indicate contamination of marine surface waters by sewage disposal practices in Key Largo, FL. Applied and Environmental Microbiology 61: 2230-2234.

Pitts, P. A. 1994. An investigation of near-bottom flow patterns along and across Hawk Channel, Florida Keys. Bull. Mar. Sci. 54:610-620.

Schmitt, R. J. and C. W. Osenberg. 1995. Detecting ecological impacts; concepts and applications in coastal habitats. Academic Press, Inc., San Diego, CA, USA.

Shinn, E. A., R. S. Reese, and C. D. Reich. 1994. Fate and pathways of injection-well effluent in the Florida Keys. U. S. Geological Survey, Open-File Report 94-276. 116 pp.

Short, F. T., W. C. Dennison, and D. G. Capone. 1990. Phosphorus-limited growth of the tropical seagrass *Syringodium filiforme* in carbonate sediments. Mar. Ecol. Prog. Ser. 62:169-174.

Smith, N. P. 1994. Long-term Gulf-to-Atlantic transport through tidal channels in the Florida Keys. Bull. Mar. Sci. 54:602-609.

Smith, S. V., W. J. Kimmerer, E. A. Laws, R. E. Brock, and T. W. Walsh. 1981. Kaneohe Bay sewage diversion experiment: perspectives on ecosystem response to nutritional perturbation. Pacific Science 35: 279-397.

Szmant, A. M. and A. Forrester. 1996. Water column and sediment nitrogen and phosphorus distribution patterns in the Florida Keys, USA Coral Reefs 15:21-41.

Tomas, C. R. 1996. The role of nutrients in initiating and supporting Florida Bay microalgal blooms and primary production. Abstract. Florida Bay Science Conference, December 10 - 12, 1996. Florida Sea Grant, University of Florida, 113 pp.

Tomasko, D. A. and B. E. Lapointe. 1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels: field observations and experimental studies. Mar. Ecol. Prog. Ser. 75:9-17.

USEPA, 1996. Water quality protection program for the Florida Keys National Marine Sanctuary. First Biennial Report to Congress. December, 1996, 32 pp.

Windom, H. L. 1992. Contamination of the marine environment from land-based sources. Marine Pollution Bulletin 35:32-36.

Figure Legend

Fig. 1. Map of the Florida Keys showing locations of three monitoring stations (AJ, PR, LK) used in this study.

Fig. 2. Map of Big Pine Key showing locations of the inshore station AJ in Big Spanish Channel and the PR station just south of Munson Island.

Fig. 3. Map of Looe Key National Marine Sanctuary showing locations of LK 1 on the reef flat (shallow seagrass meadow) just behind the reef crest and LK 2 in the adjacent back reef zone that is macroalgal dominated.

Fig. 4. Rainfall during the period of study.

Fig. 5. DIN concentrations at Avenue J. Values represent means (n=3).

Fig. 6. DIN concentrations at the Patch Reef. Values represent means (n=3).

Fig. 7. DIN concentrations at Looe Key. Values represent means (n=3).

Fig. 8. SRP concentrations at Avenue J. Values represent means (n=3).

Fig. 9. SRP concentrations at the Patch Reef. Values represent means ± 1 S.D. (n=3).

Fig. 10. SRP concentrations at Looe Key. Values represent means ± 1 S.D. (n=3).

Fig. 11. Chlorophyll a concentrations at Avenue J. Values represent means ± 1 S.D. (n=3).

Fig. 12. Chlorophyll a concentrations at the Patch Reef. Values represent means ± 1 S.D. (n=3).

Fig. 13. Chlorophyll a concentrations at Looe Key. Values represent means ± 1 S.D. (n=3).

Fig. 14. Biomass of fleshy macroalgae at Avenue J. Values represent means ± 1 S. D. (n = 10).

Fig. 15. Biomass of calcareous macroalgae at Avenue J. Values represent means ± 1 S. D. (n = 10).

Fig. 16. Biomass of fleshy macroalgae at the Patch Reef. Values represent means ± 1 S. D. (n = 10).

Fig. 17. Biomass of calcareous macroalgae at the Patch Reef. Values represent means ± 1 S. D. (n = 10).

Fig. 18. Biomass of fleshy macroalgae at Looe Key. Values represent means ± 1 S. D. (n = 10).

Fig. 19. Biomass of calcareous macroalgae at Looe Key. Values represent means ± 1 S. D. (n = 10).

Fig. 20. Tissue nitrogen in the chlorophyte *Cladophora fuliginosa* at Looe Key.

Fig. 21. Tissue phosphorus in the chlorophyte *Cladophora fuliginosa* at Looe Key.

Fig. 22. Alkaline phosphatase activity of the chlorophyte *Cladophora fuliginosa* at Looe Key. Values represent means ± 1 S.D. (n = 4).

Fig. 23. Alkaline phosphatase activity of the rhodophyte *Laurencia poiteaui* at Avenue J. Values represent means ± 1 S.D. (n = 4).

Fig. 24. Alkaline phosphatase activity of the rhodophyte *Laurencia poiteaui* at the Patch Reef. Values represent means ± 1 S.D. (n = 4).

Fig. 25. Alkaline phosphatase activity of the rhodophyte *Laurencia poiteaui* at Looe Key. Values represent means ± 1 S.D. (n = 4).

Fig. 26. $\delta^{15}N$ of *Laurecia poiteaui* at Avenue J. Values represent means ± 1 S.D. (n=2).

Fig. 27. $\delta^{15}N$ of *Laurecia poiteaui* at the Patch Reef. Values represent means ± 1 S.D. (n=2).

Fig. 28. $\delta^{15}N$ of *Laurecia poiteaui* at Looe Key. Values represent means ± 1 S.D. (n=2).

Fig. 29. Correlation of dissolved inorganic nitrogen (DIN) concentrations and $\delta^{15}N$ of *Cladophora fuliginosa* at Looe Key.

Fig. 30. Epiphyte:seagrass blade ratio for *Thalassia testudinum* at Avenue J.

Fig. 31. Epiphyte:seagrass blade ratio for *Thalassia testudinum* at the Patch Reef.

Fig. 32. Epiphyte:seagrass blade ratio for *Thalassia testudinum* at Looe Key.

Fig. 33. $\delta^{15}N$ of *Thalassia testudinum* at Avenue J. Values represent means ± 1 S.D. (n=2).

Fig. 34. $\delta^{15}N$ of *Thalassia testudinum* at the Patch Reef. Values represent means ± 1 S.D. (n=2).

Fig. 35. $\delta^{15}N$ of *Thalassia testudinum* at Looe Key. Values represent means ± 1 S.D. (n=2).

Fig. 36. $\delta^{15}N$ of *Thalassia testudinum* epiphytes at Avenue J. Values represent means ± 1 S.D. (n=2).

Fig. 37. $\delta^{15}N$ of *Thalassia testudinum* epiphytes at the Patch Reef. Values represent means ± 1 S.D. (n=2).

Fig. 38. $\delta^{15}N$ of *Thalassia testudinum* epiphytes at Looe Key. Values represent means ± 1 S.D. (n=2).

Fig. 39. Annual mean values of chlorophyll-a at Looe Key (LK 1) between 1987 and 1996. Values represent means ± 1 S. D. ($16 < n < 83$).

THE FLORIDA KEYS

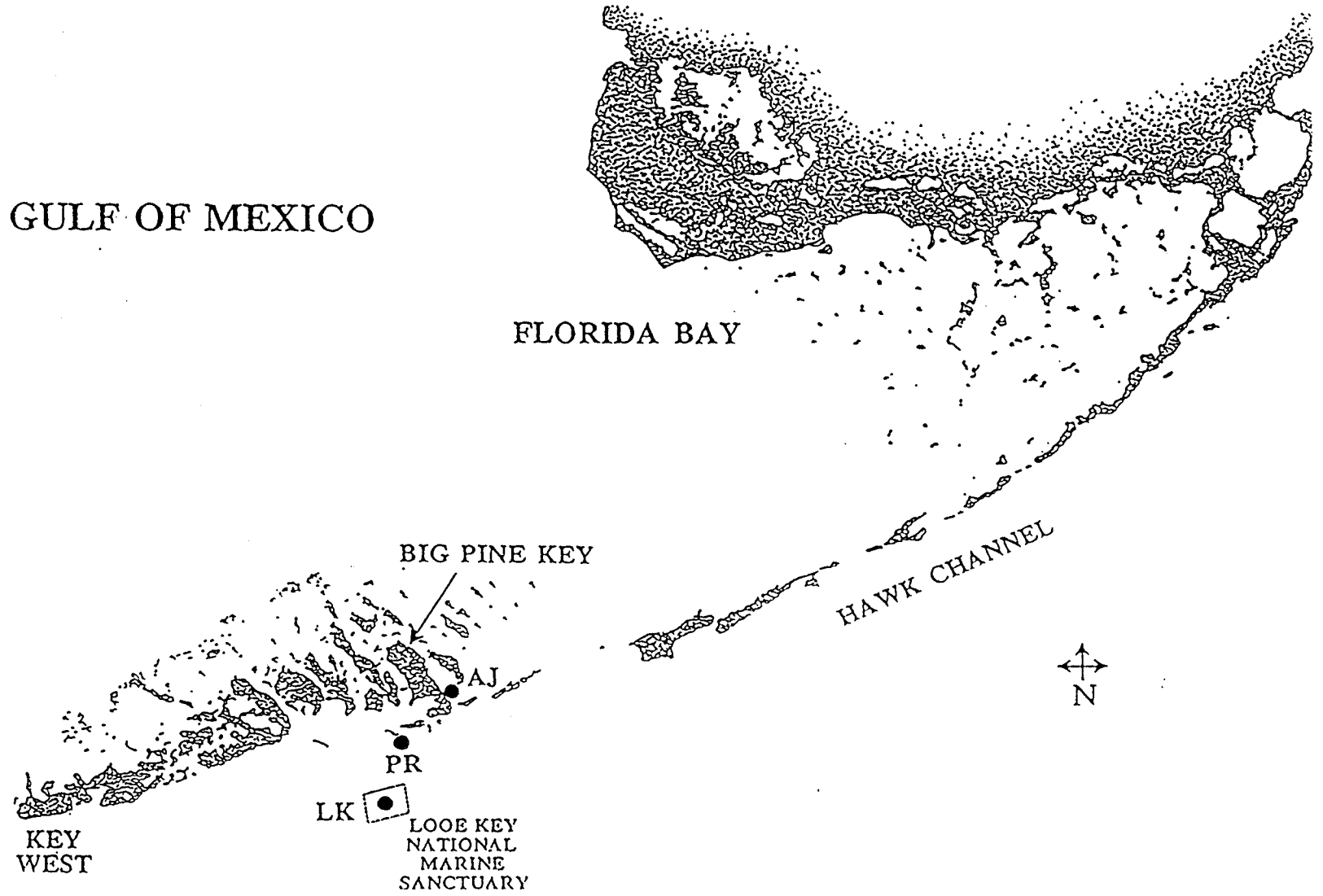


Figure 1

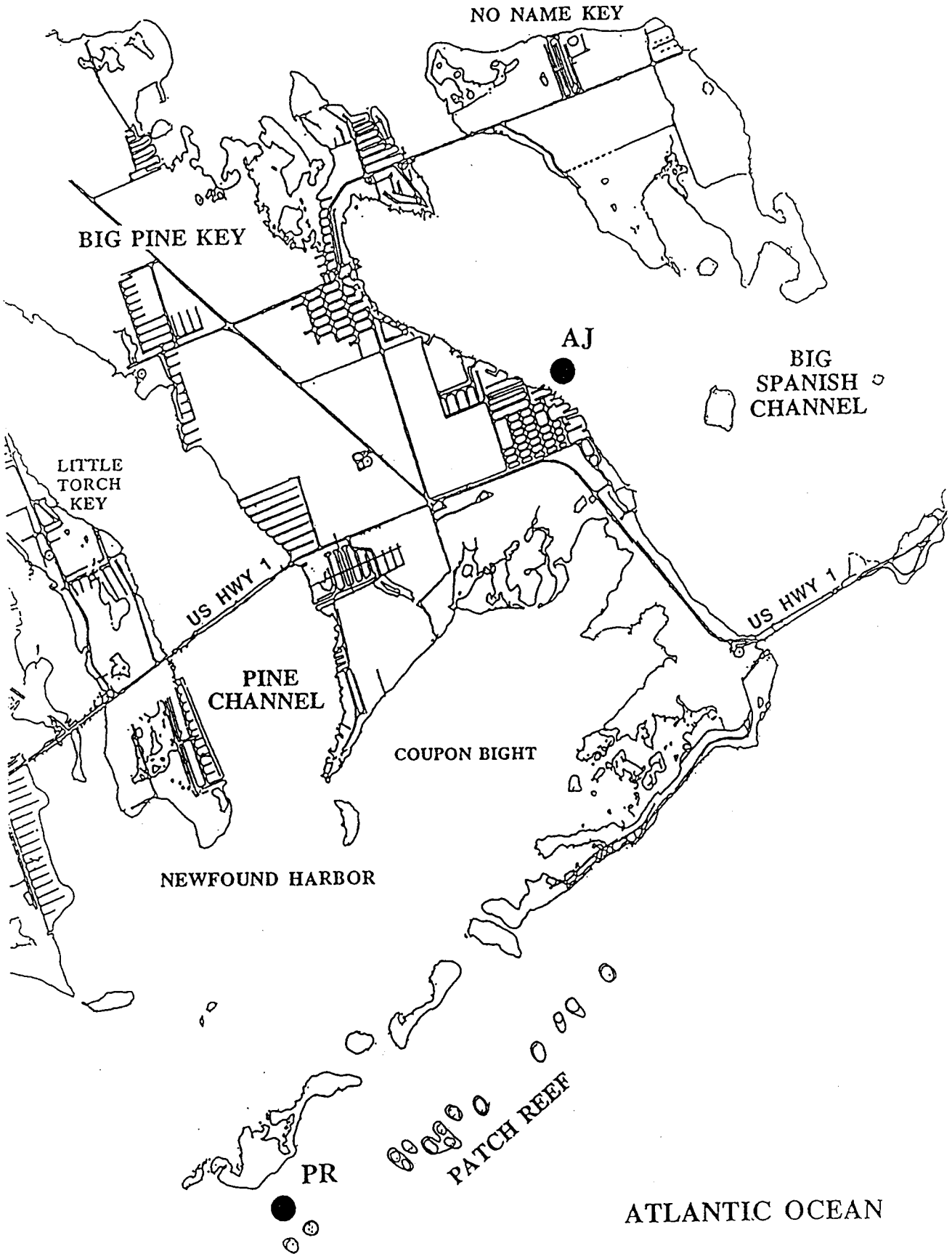
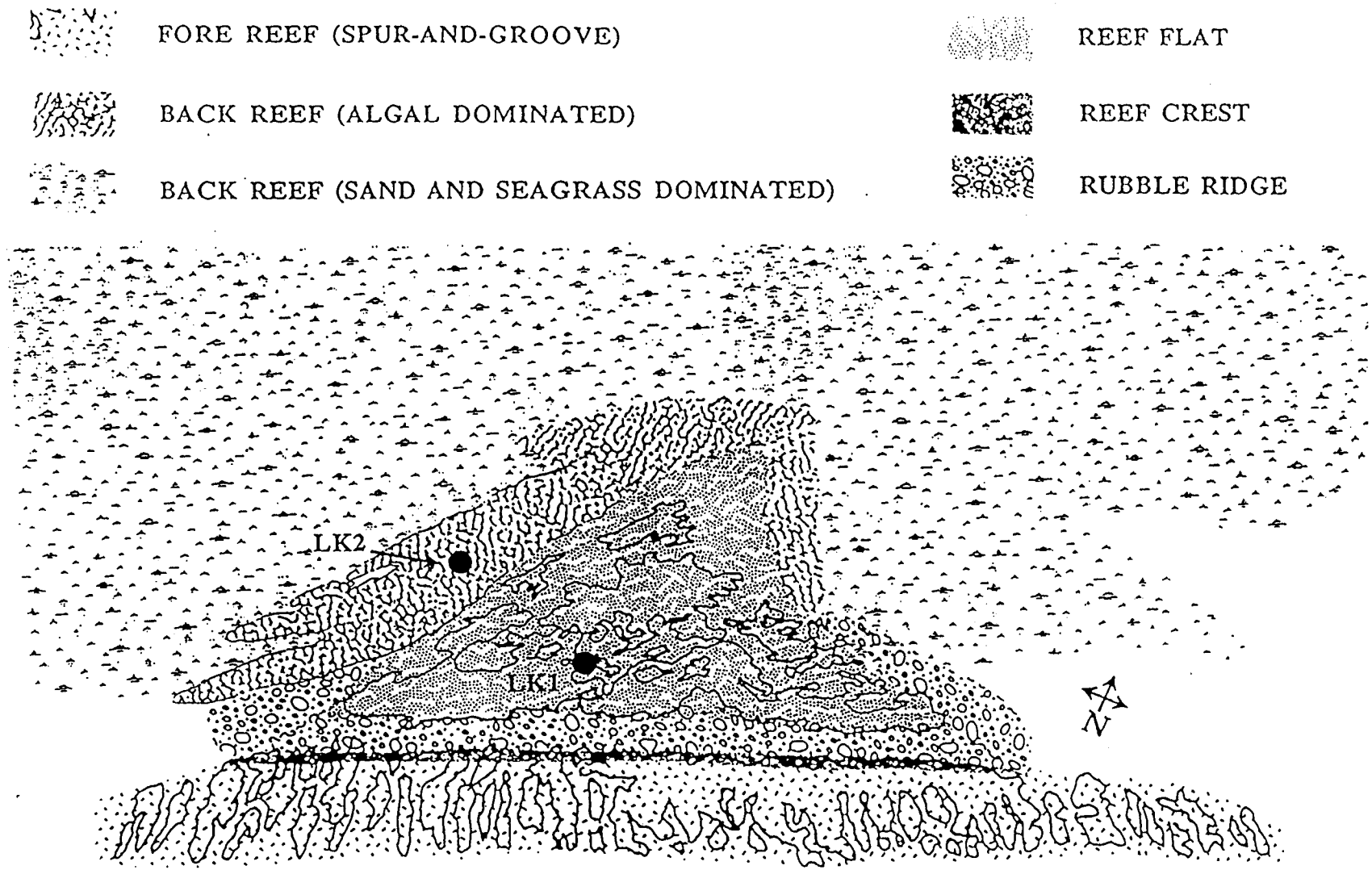

NO NAME KEY
BIG PINE KEY
AJ
BIG
SPANISH
CHANNEL
LITTLE
TORCH
KEY
US HWY 1
US HWY 1
PINE
CHANNEL
COUPON BIGHT
NEWFOUND HARBOR
PR
PATCH REEF
ATLANTIC OCEAN

Figure 2



LOOE KEY NATIONAL MARINE SANCTUARY

Figure 3

RAINFALL

BIG PINE KEY

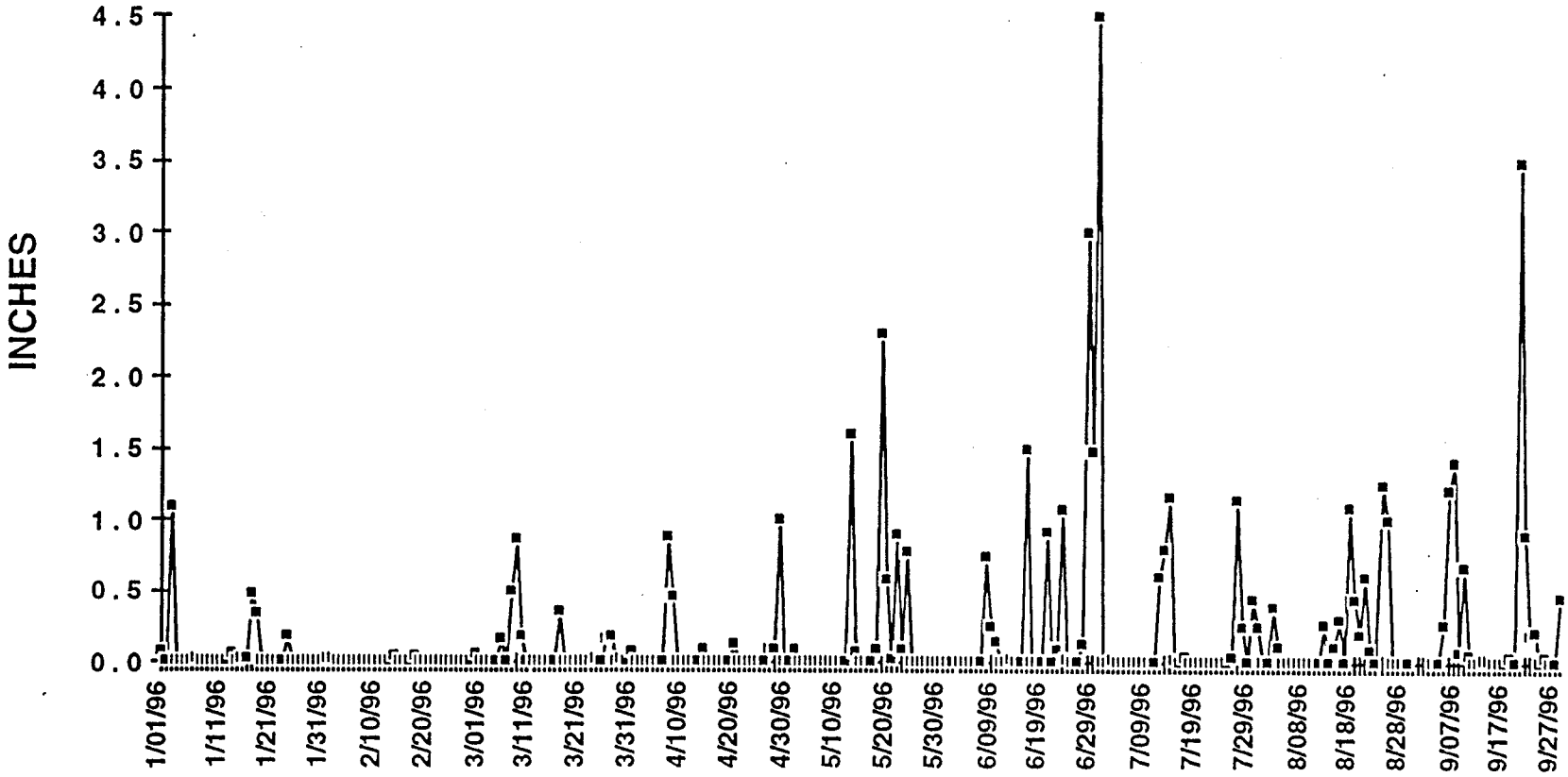


Figure 4

DISSOLVED INORGANIC NITROGEN

(DIN)

AVENUE J (AJ)

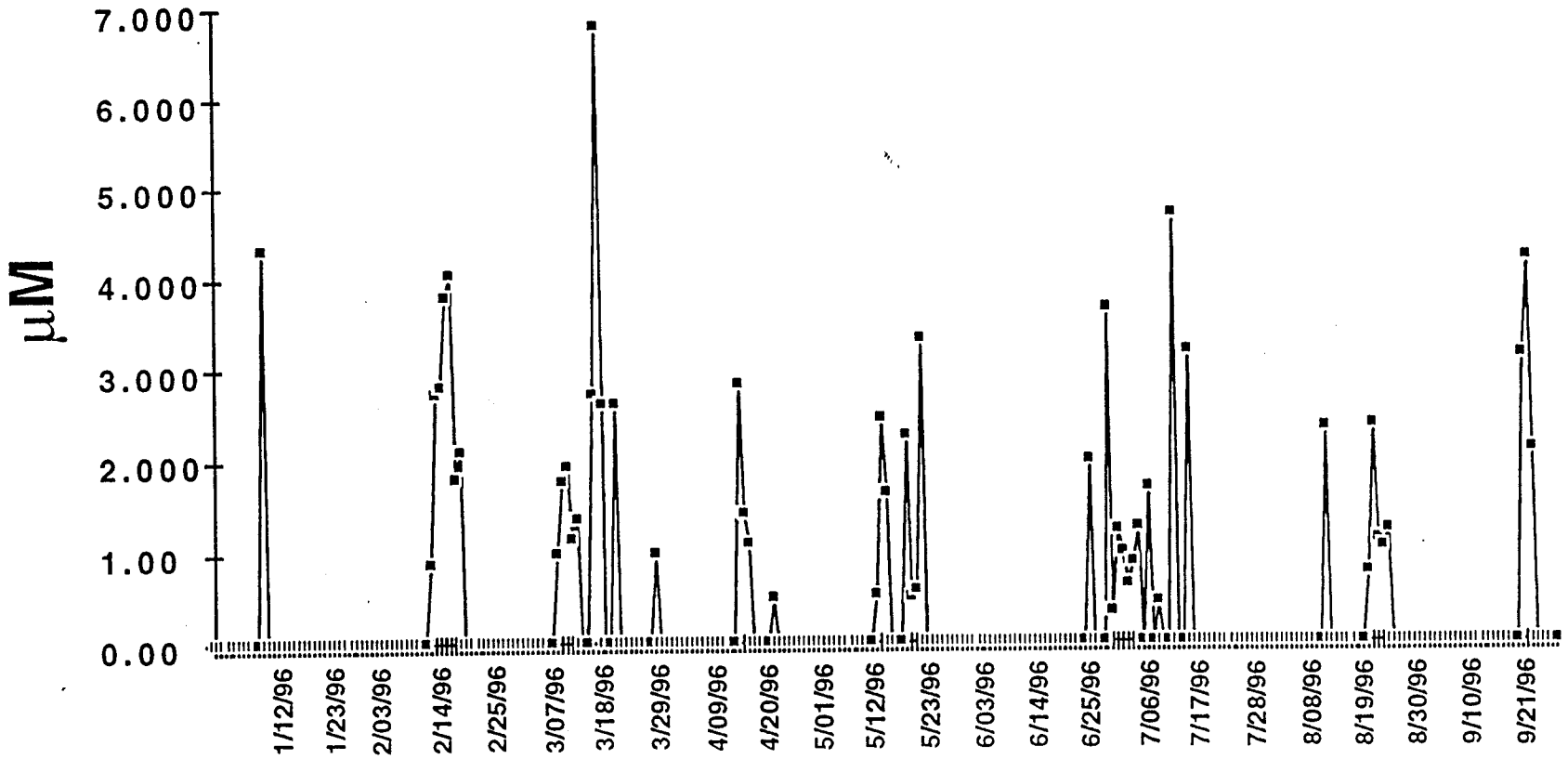


Figure 5

DISSOLVED INORGANIC NITROGEN

(DIN)

PATCH REEF (PR)

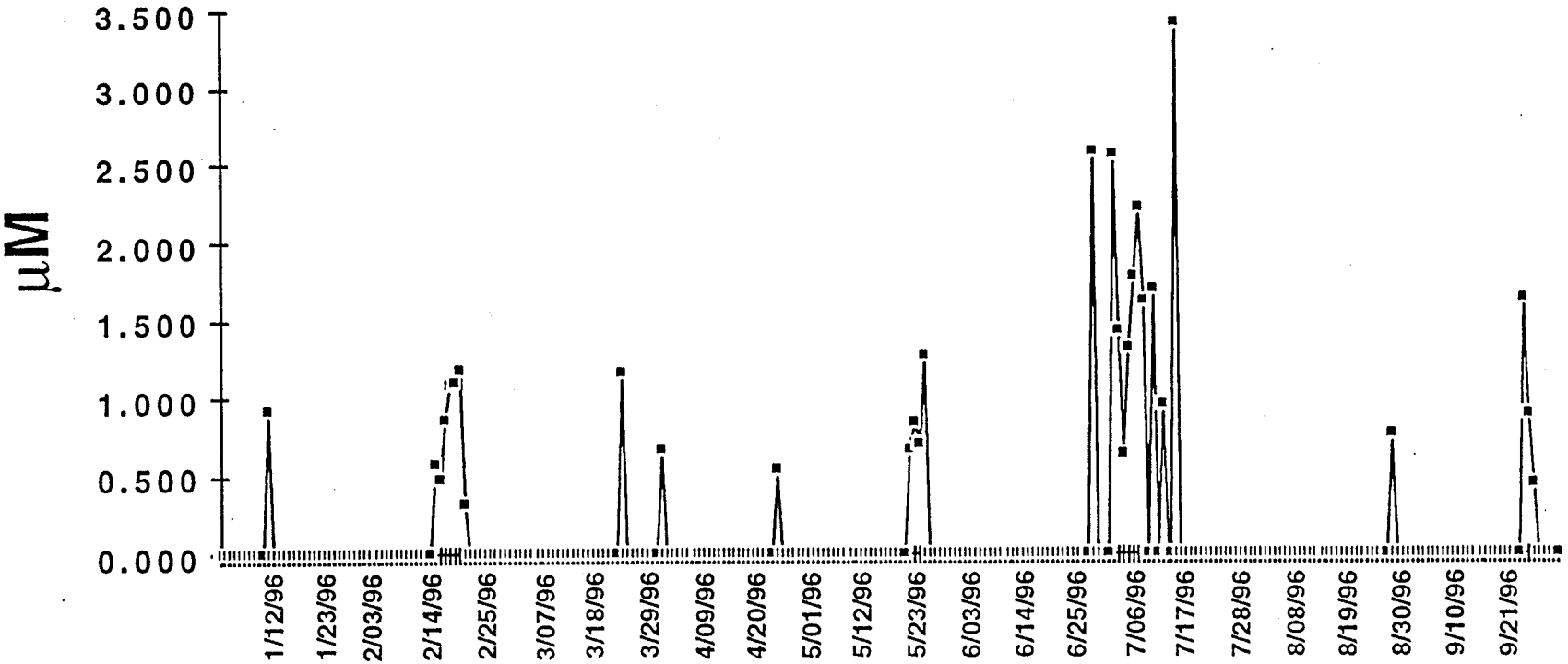


Figure 6

DISSOLVED INORGANIC NITROGEN

(DIN)

LOOE KEY (LK)

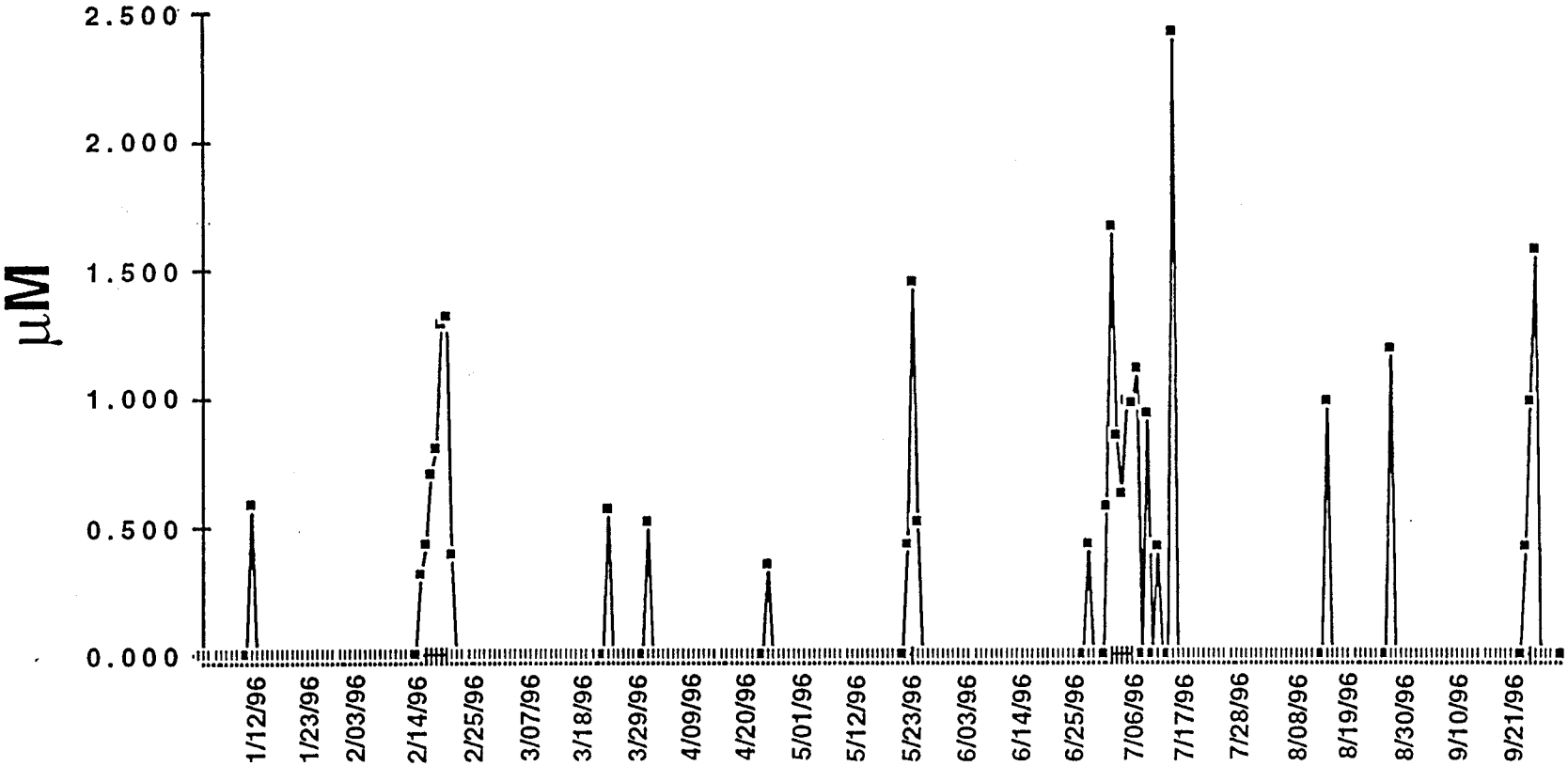


Figure 7

SOLUBLE REACTIVE PHOSPHORUS

(SRP)

AVENUE J (AJ)

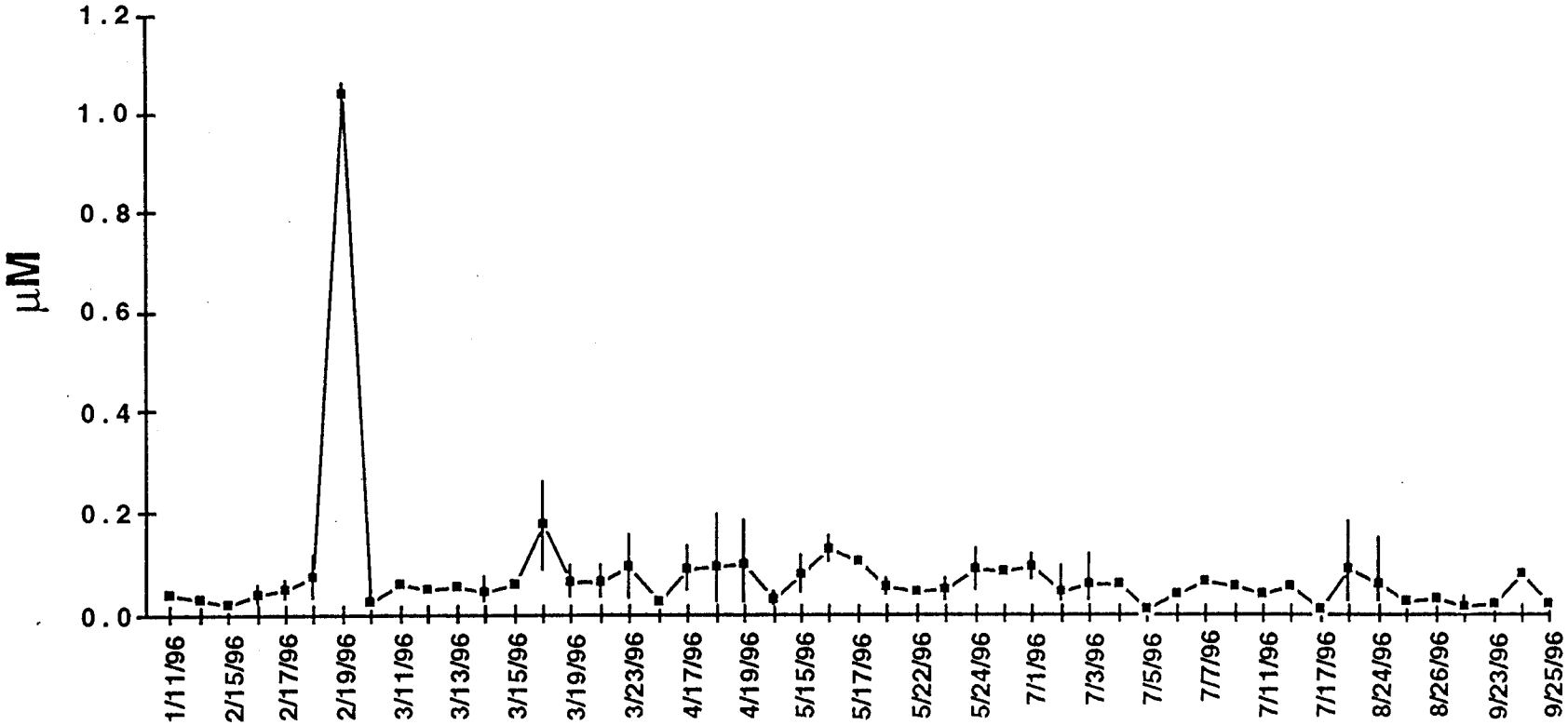


Figure 8

SOLUBLE REACTIVE PHOSPHORUS

(SRP)

PATCH REEF (PR)

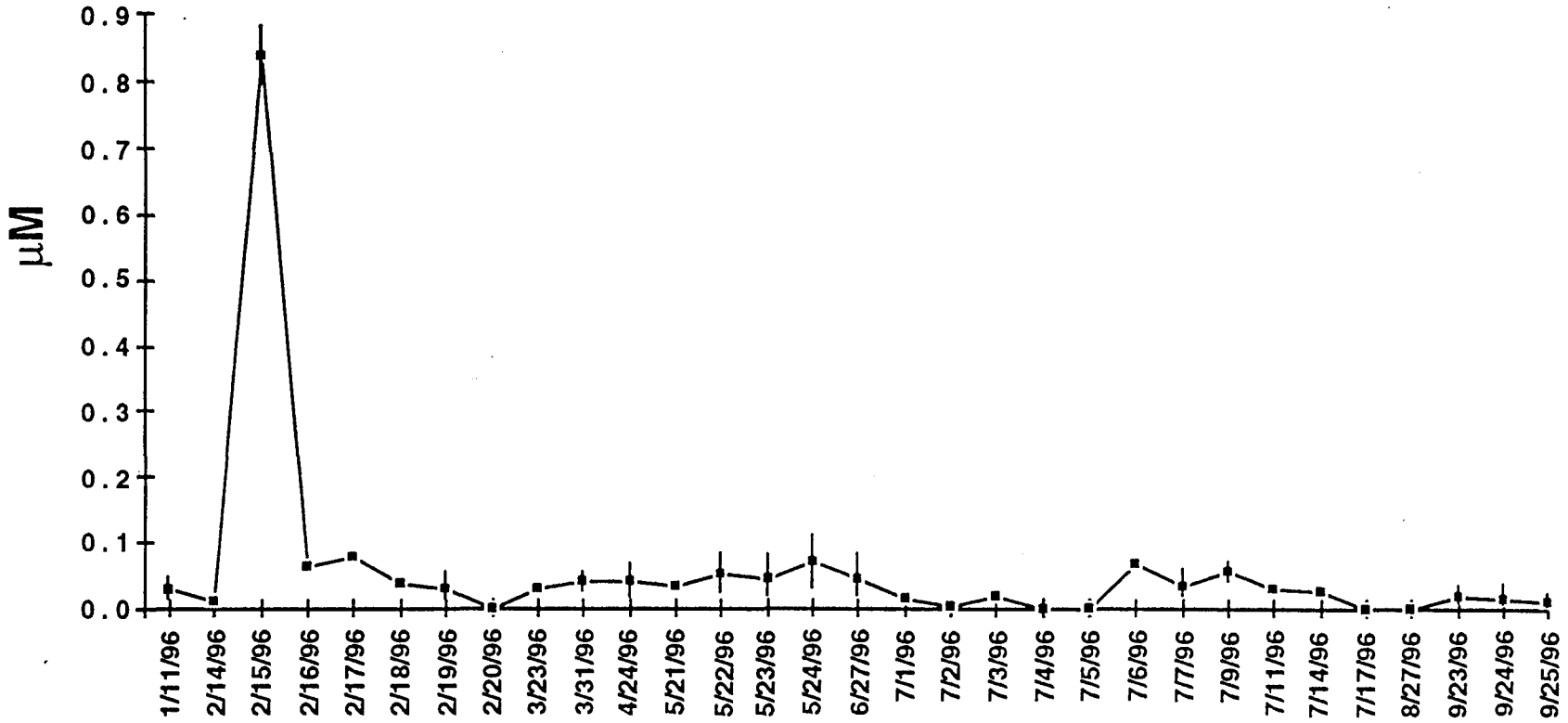


Figure 9

SOLUBLE REACTIVE PHOSPHORUS

(SRP)

LOOE KEY (LK)

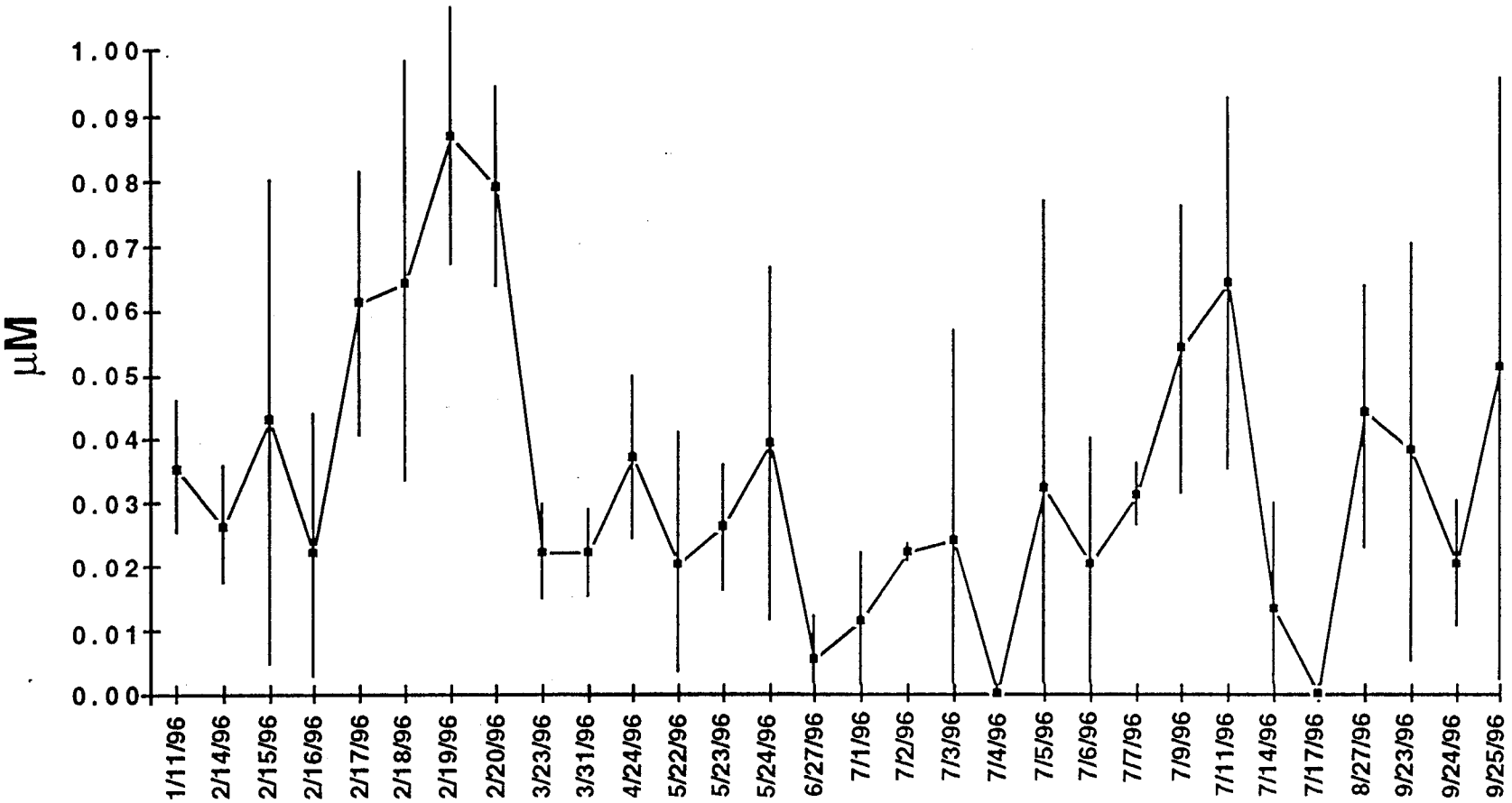


Figure 10

CHLOROPHYLL-a

AVENUE J (AJ)

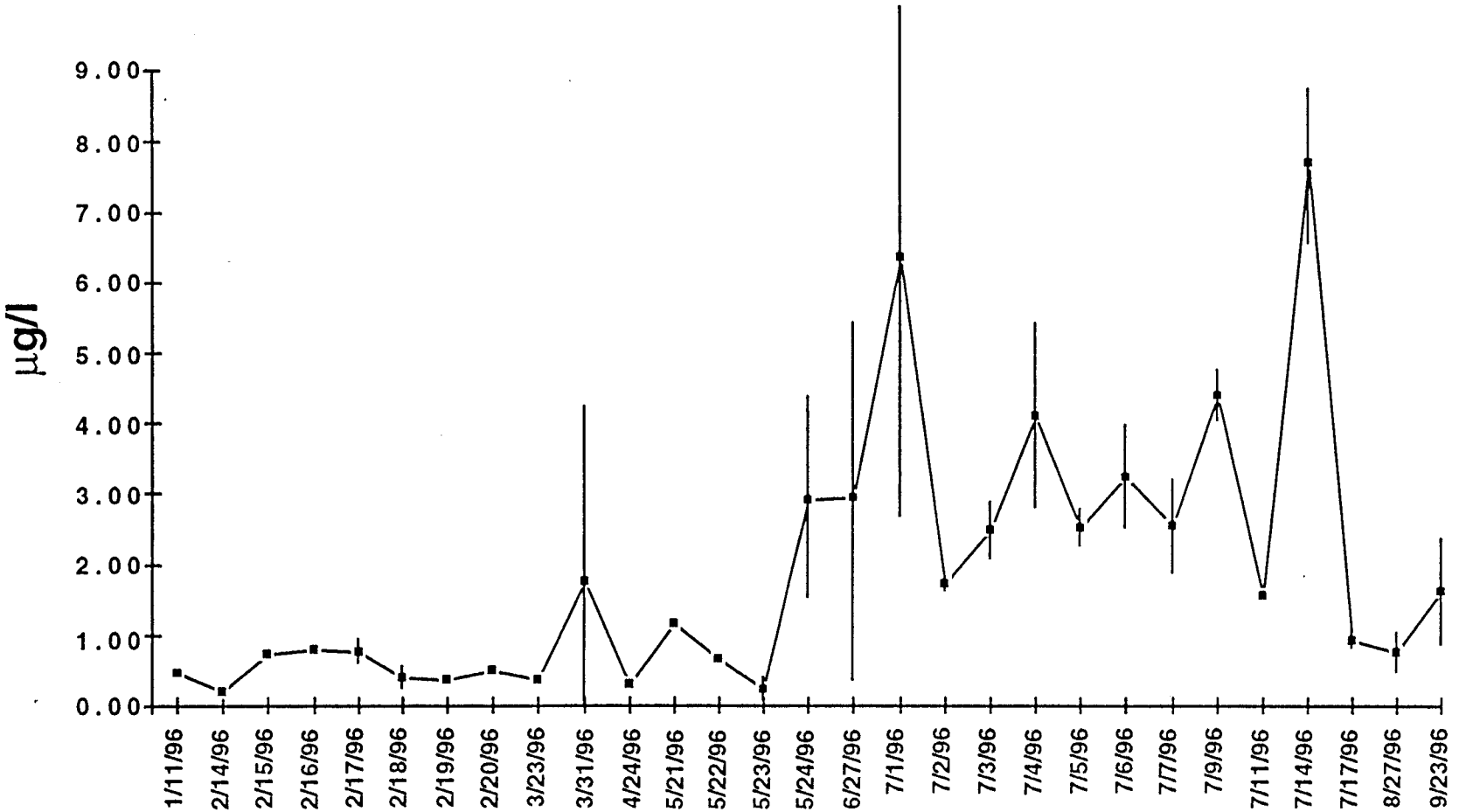


Figure 11

CHLOROPHYLL-a

PATCH REEF (PR)

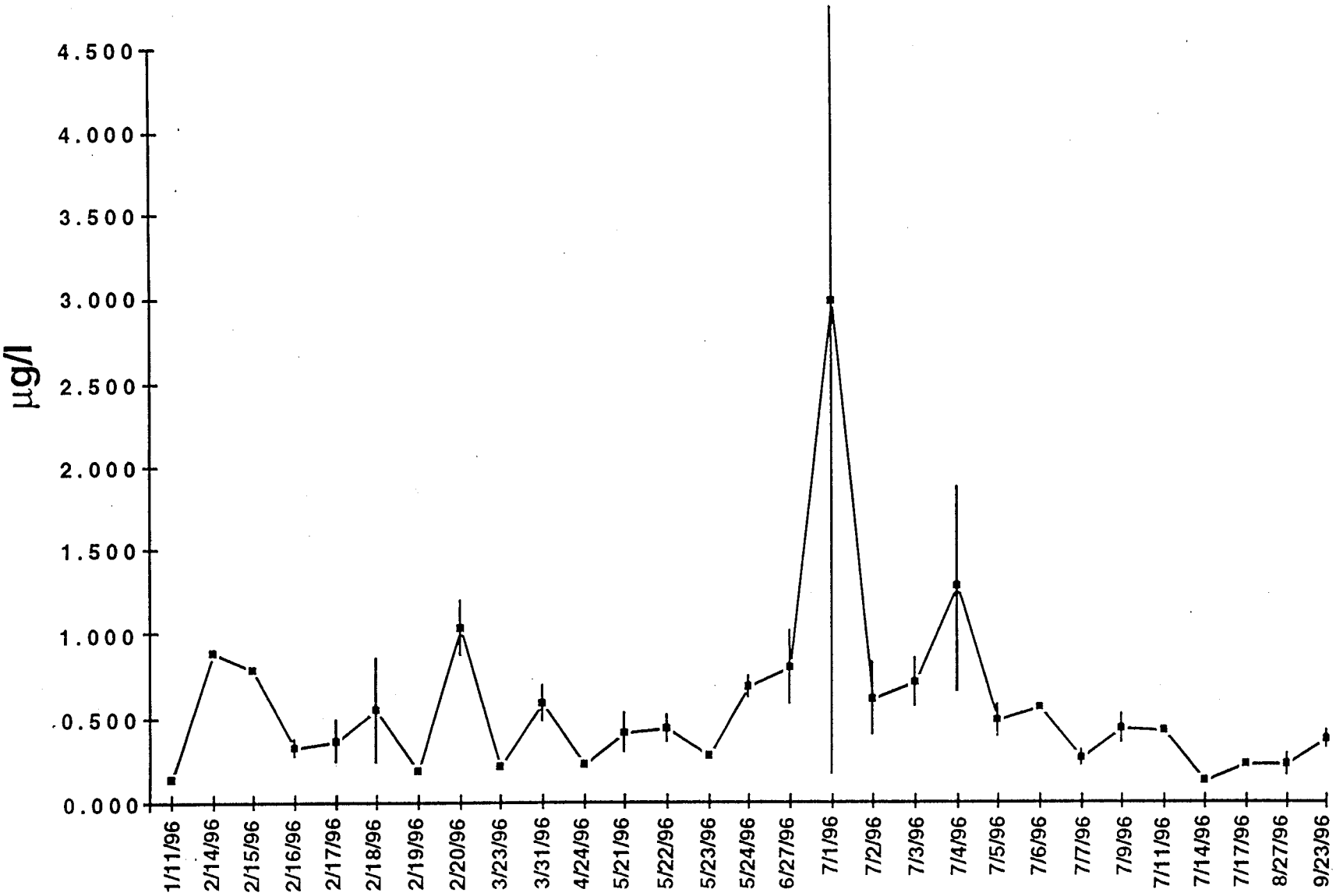


Figure 12

CHLOROPHYLL-a

LOOE KEY (LK)

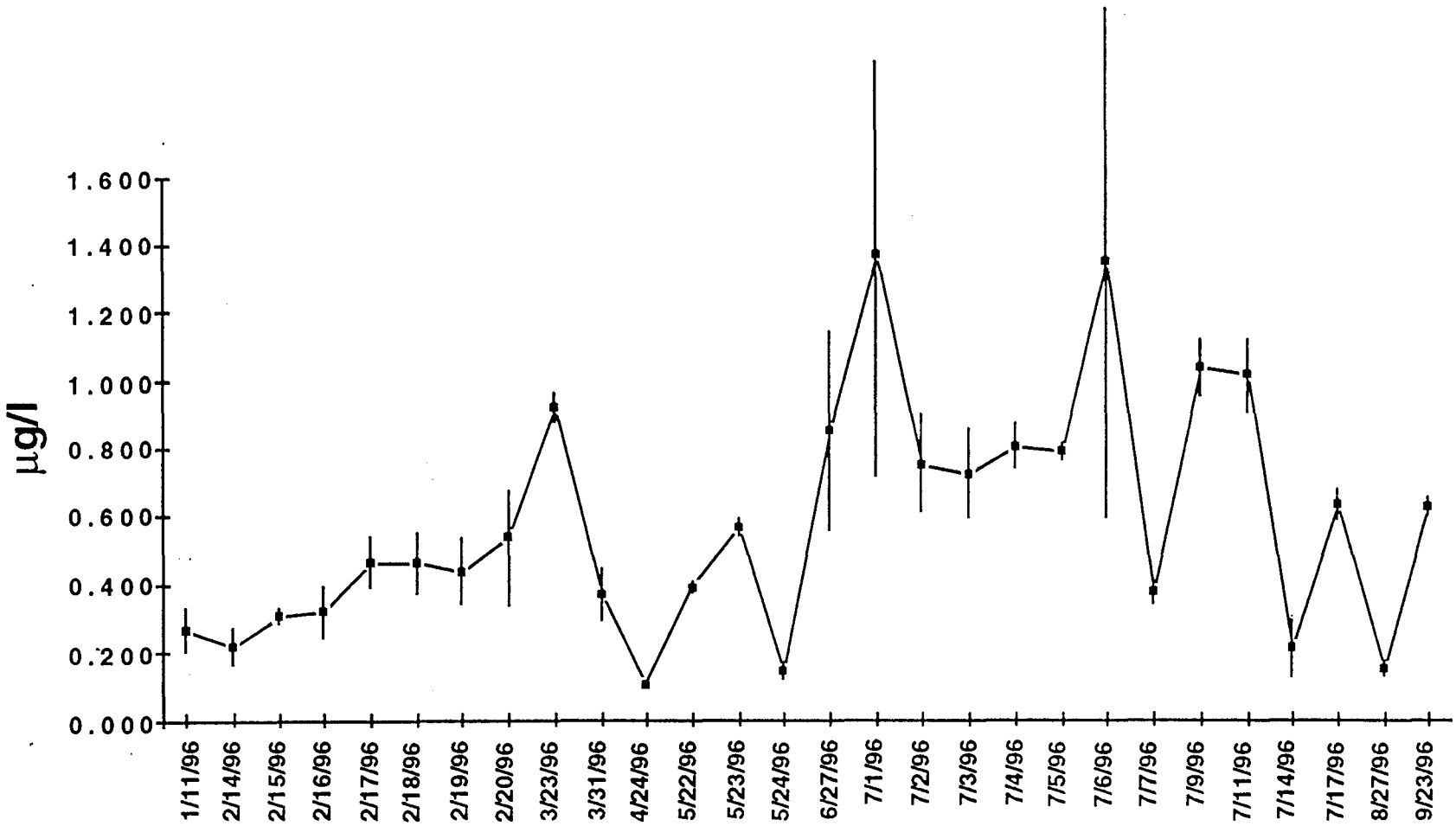


Figure 13

MACROALGAL BIOMASS

(FLESHY)

AVENUE J (AJ)

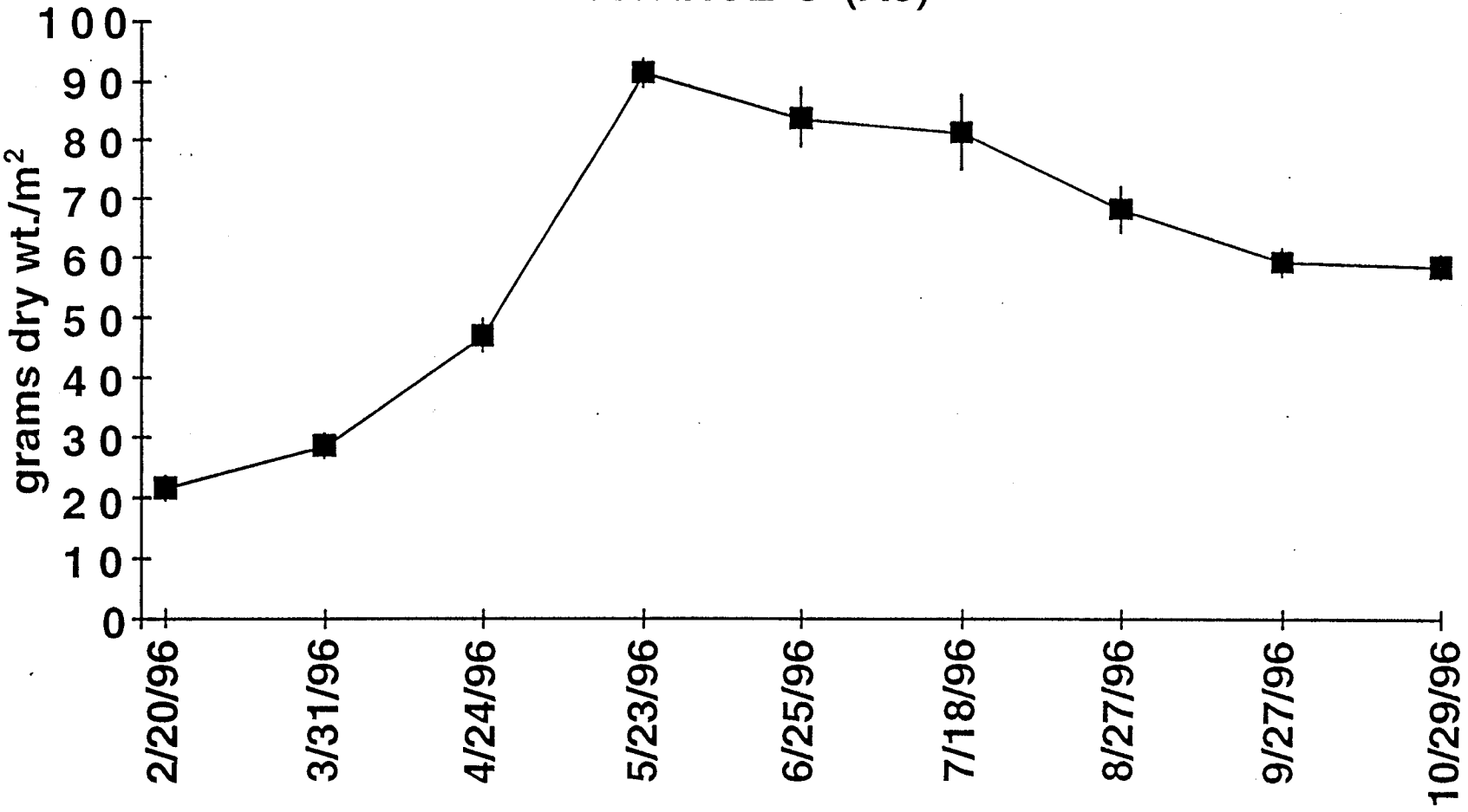


Figure 14

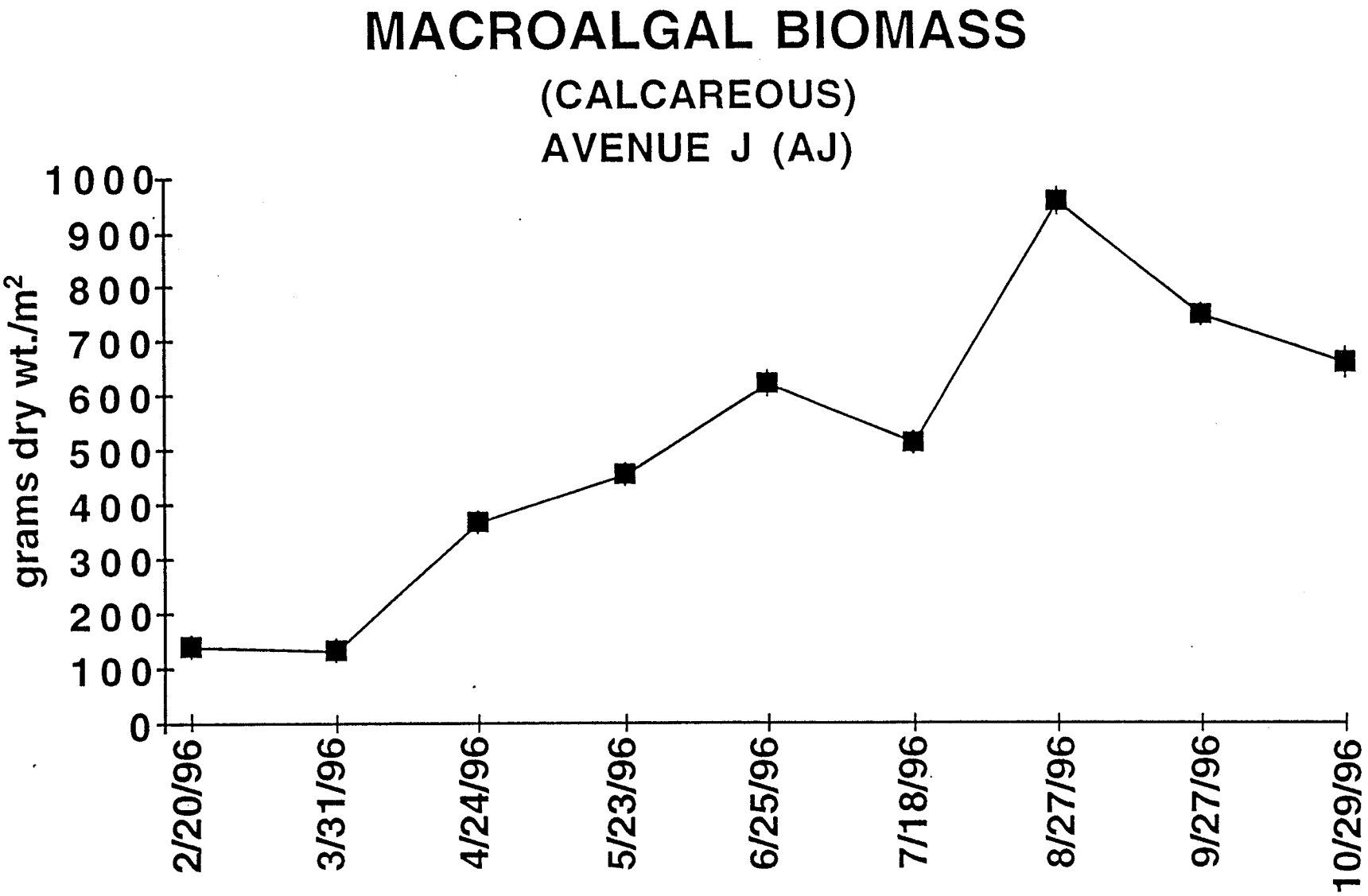

MACROALGAL BIOMASS
(CALCAREOUS)
AVENUE J (AJ)
grams dry wt./m2
1000
900
800
700
600
500
400
300
200
100
0
2/20/96
3/31/96
4/24/96
5/23/96
6/25/96
7/18/96
8/27/96
9/27/96
10/29/96

Figure 15

MACROALGAL BIOMASS

(FLESHY)

PATCH REEF (PR)

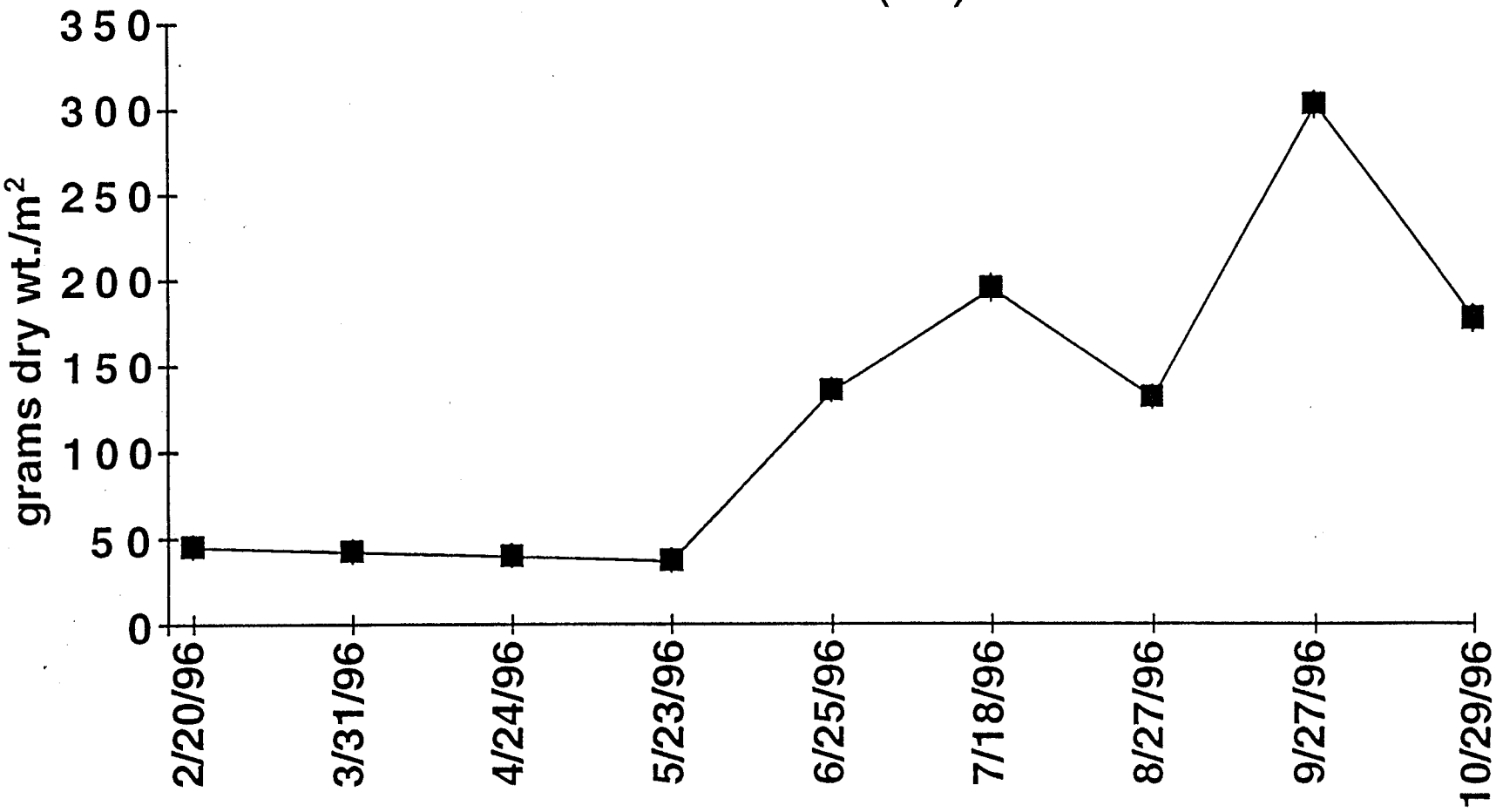


Figure 16

MACROALGAL BIOMASS

(CALCAREOUS)

PATCH REEF (PR)

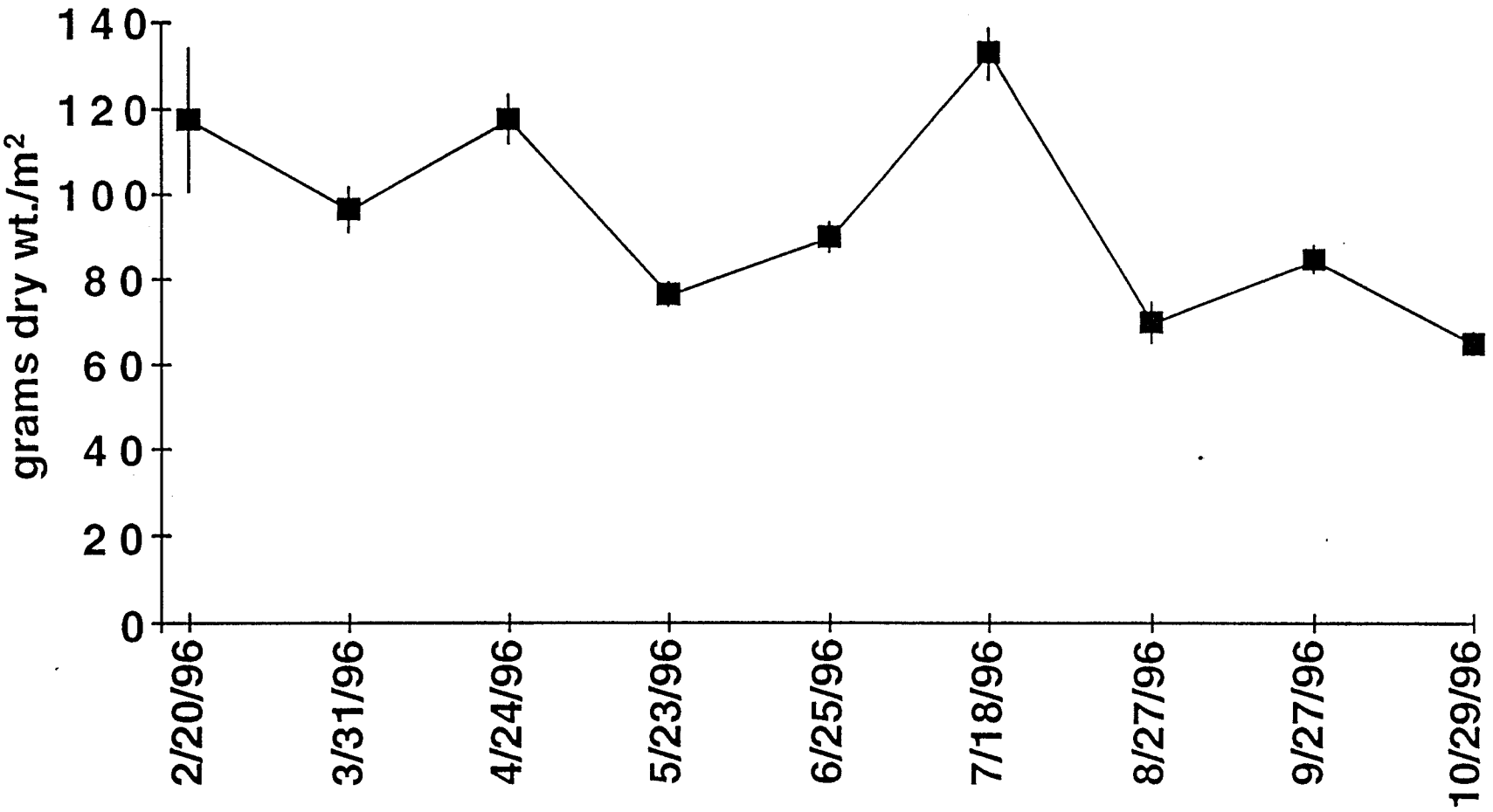


Figure 17

MACROALGAL BIOMASS

(FLESHY)

LOOE KEY (LK)

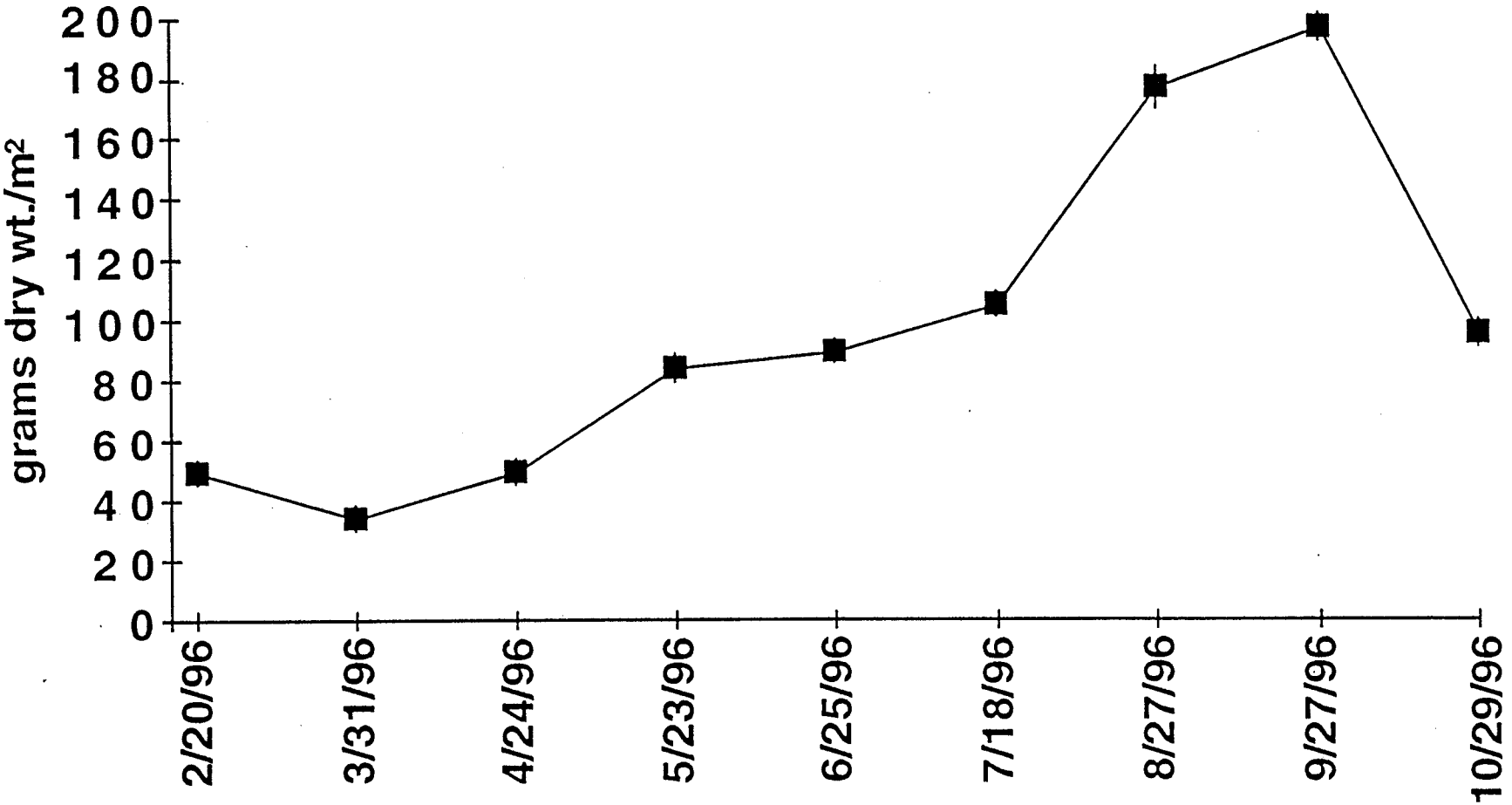


Figure 18

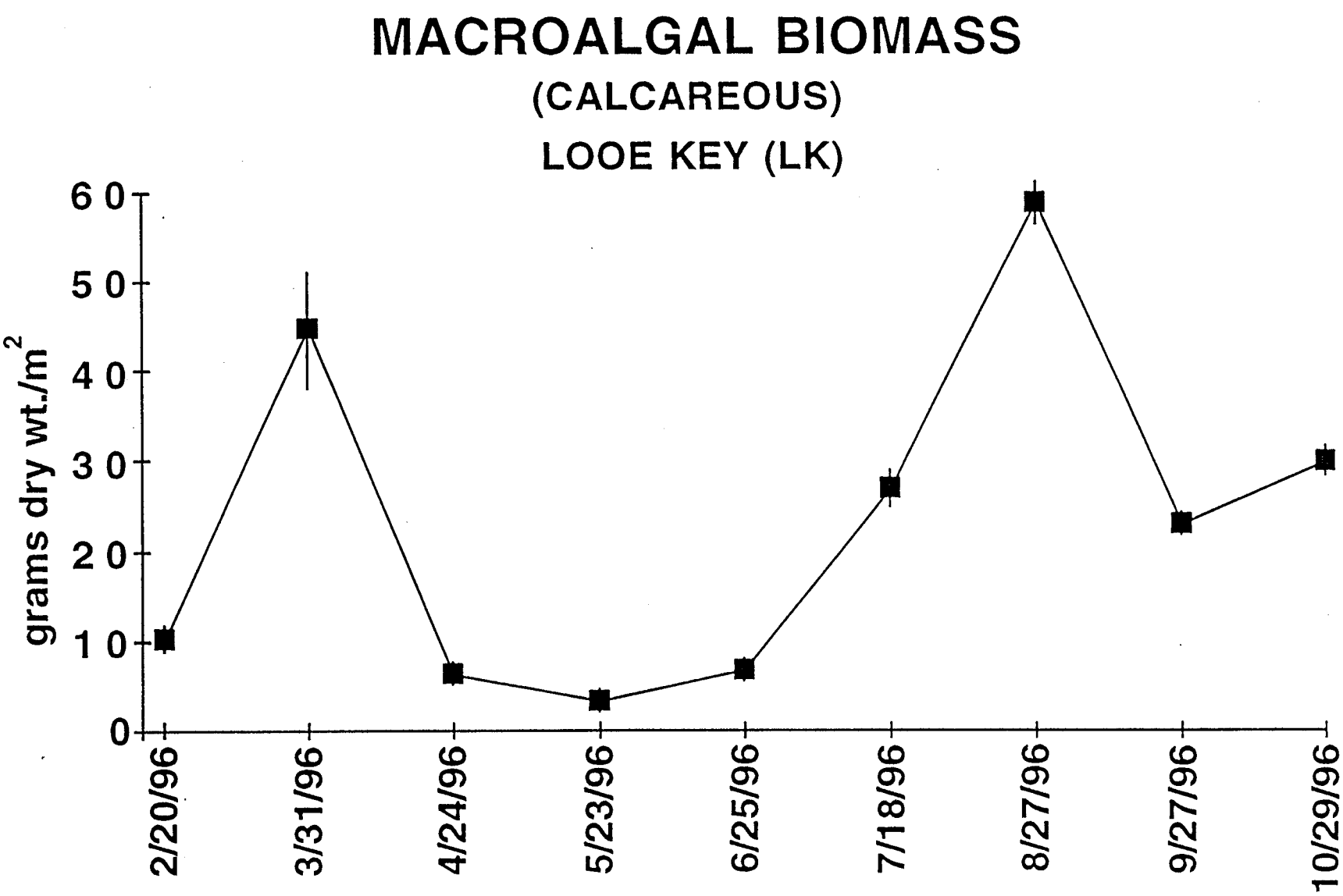

MACROALGAL BIOMASS
(CALCAREOUS)
LOOE KEY (LK)
grams dry wt./m2
60
50
40
30
20
10
0
2/20/96
3/31/96
4/24/96
5/23/96
6/25/96
7/18/96
8/27/96
9/27/96
10/29/96

Figure 19

% NITROGEN

Cladophora fuliginosa

LOOE KEY (LK)

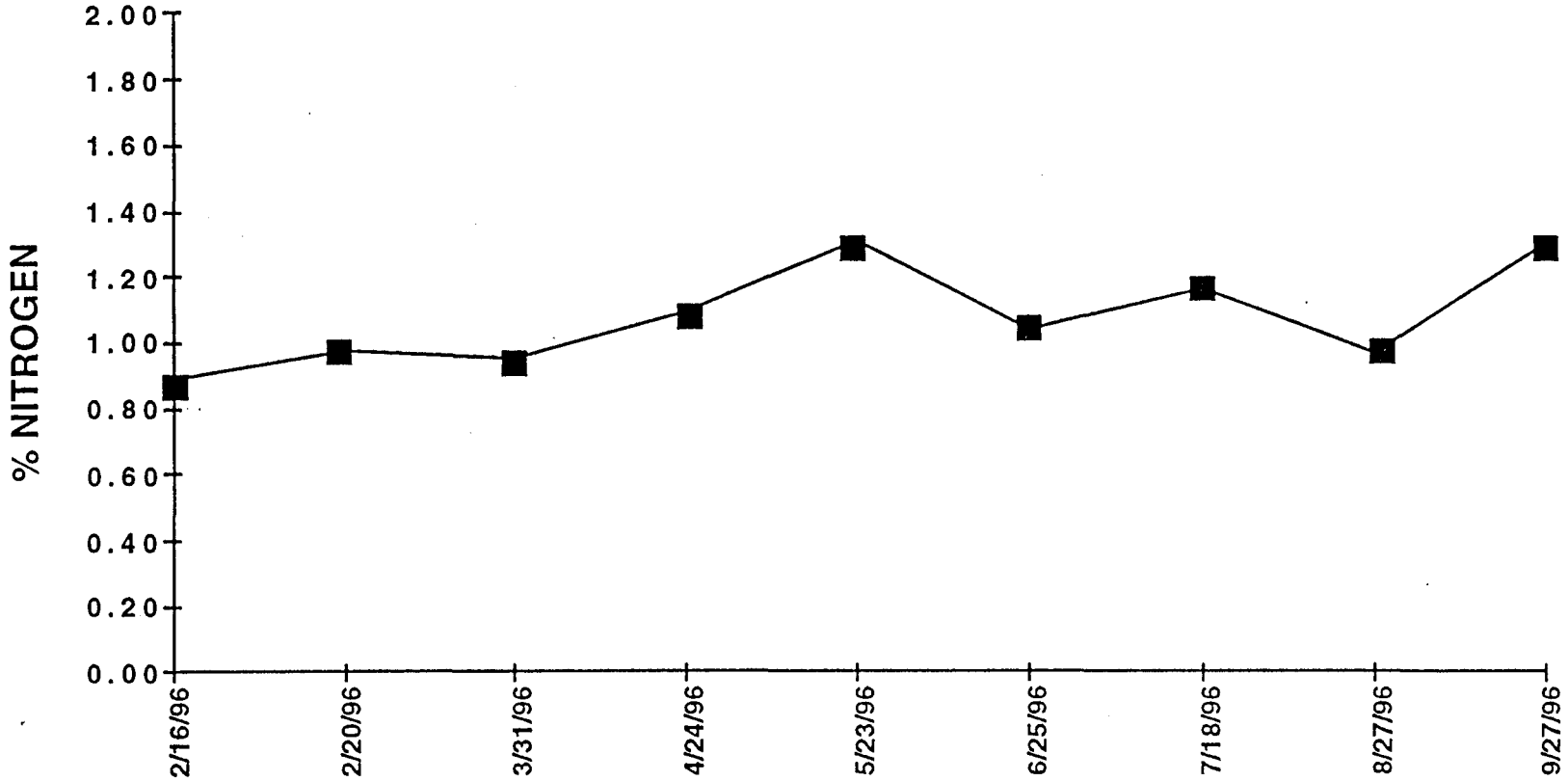


Figure 20

% PHOSPHORUS
Cladophora fuliginosa
LOOE KEY (LK)

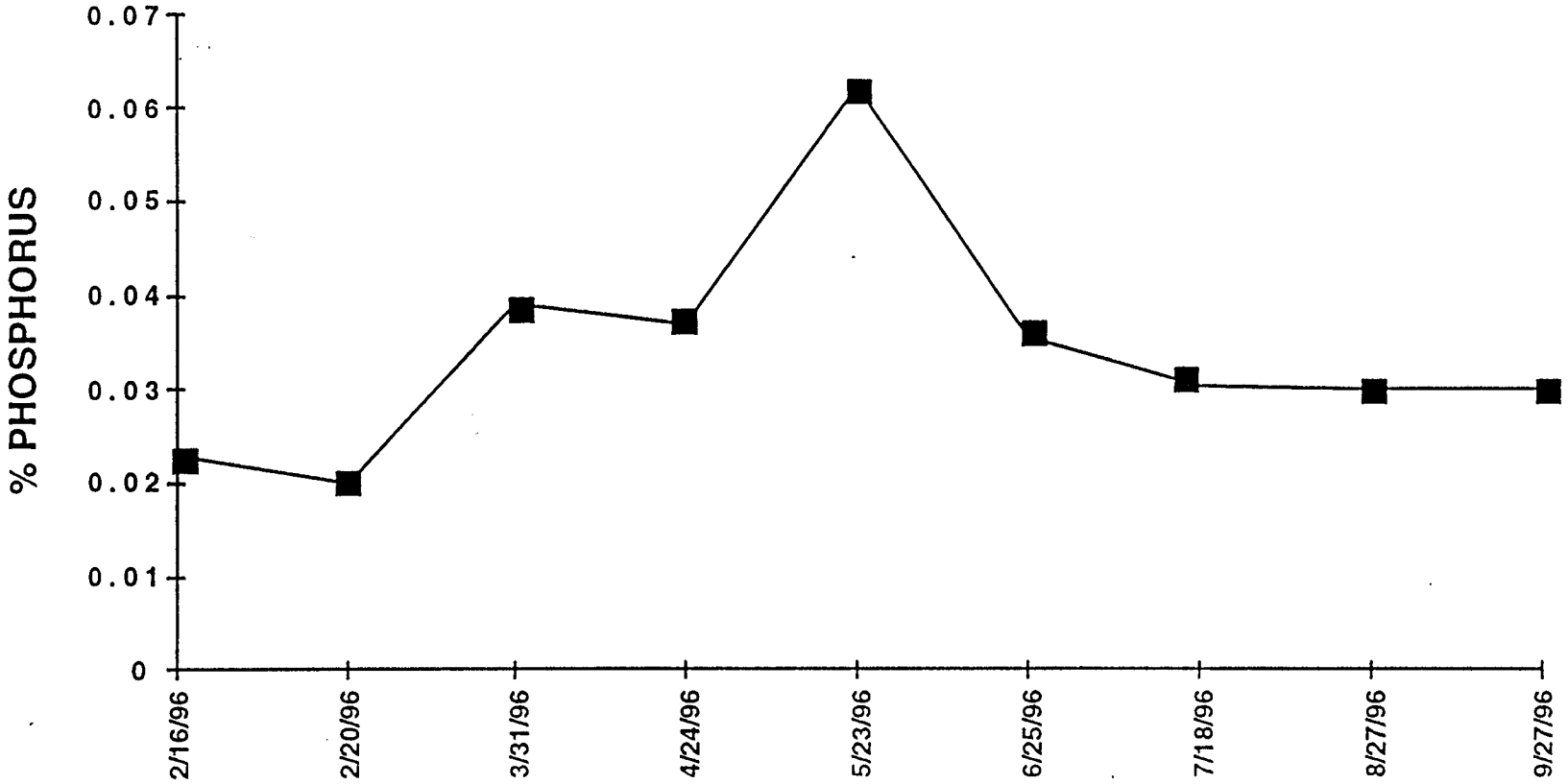


Figure 21

ALKALINE PHOSPHATASE

Cladophora fuliginosa

LOOE KEY (LK)

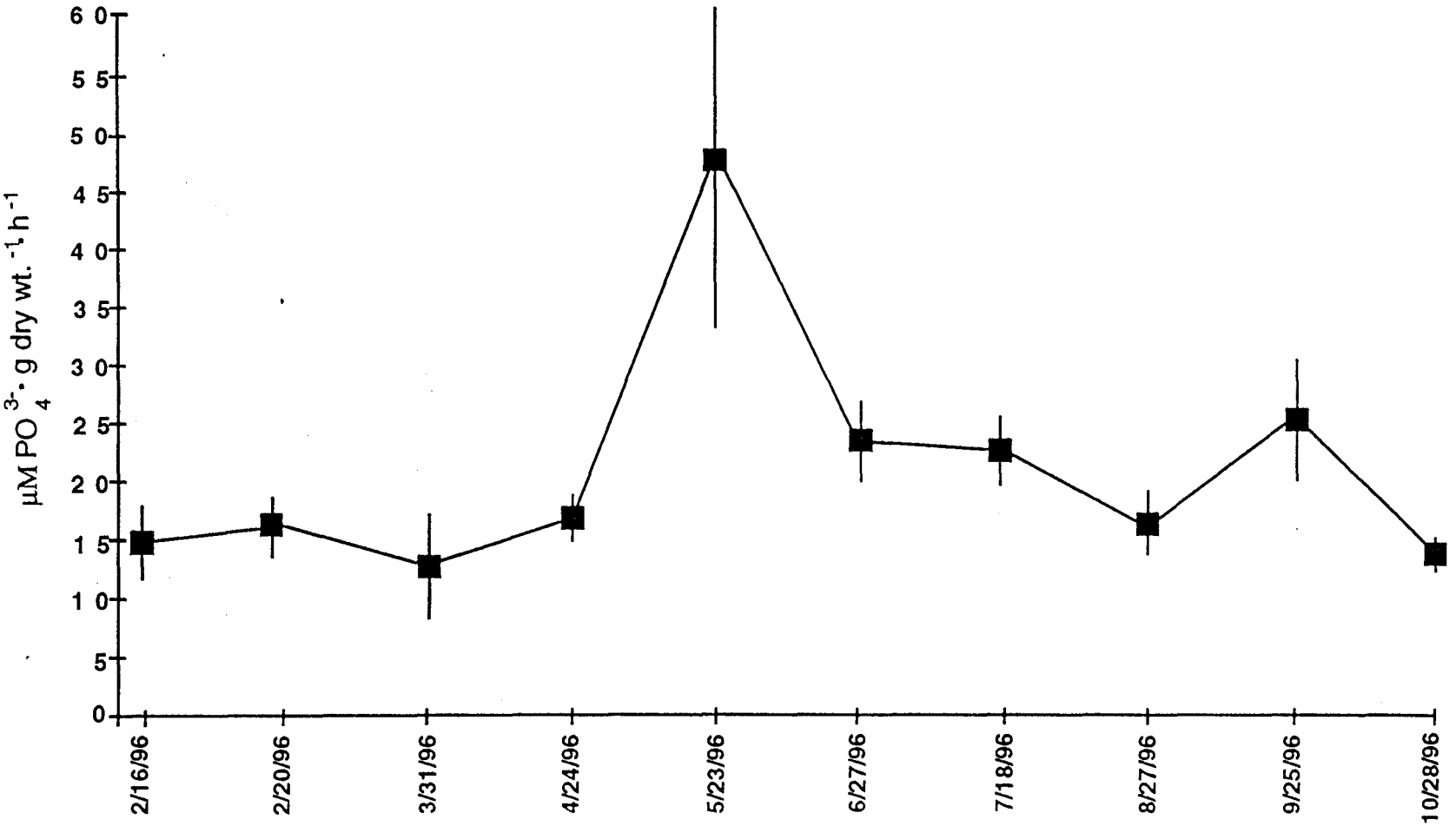


Figure 22

ALKALINE PHOSPHATASE

Laurencia poiteaui

AVENUE J (AJ)

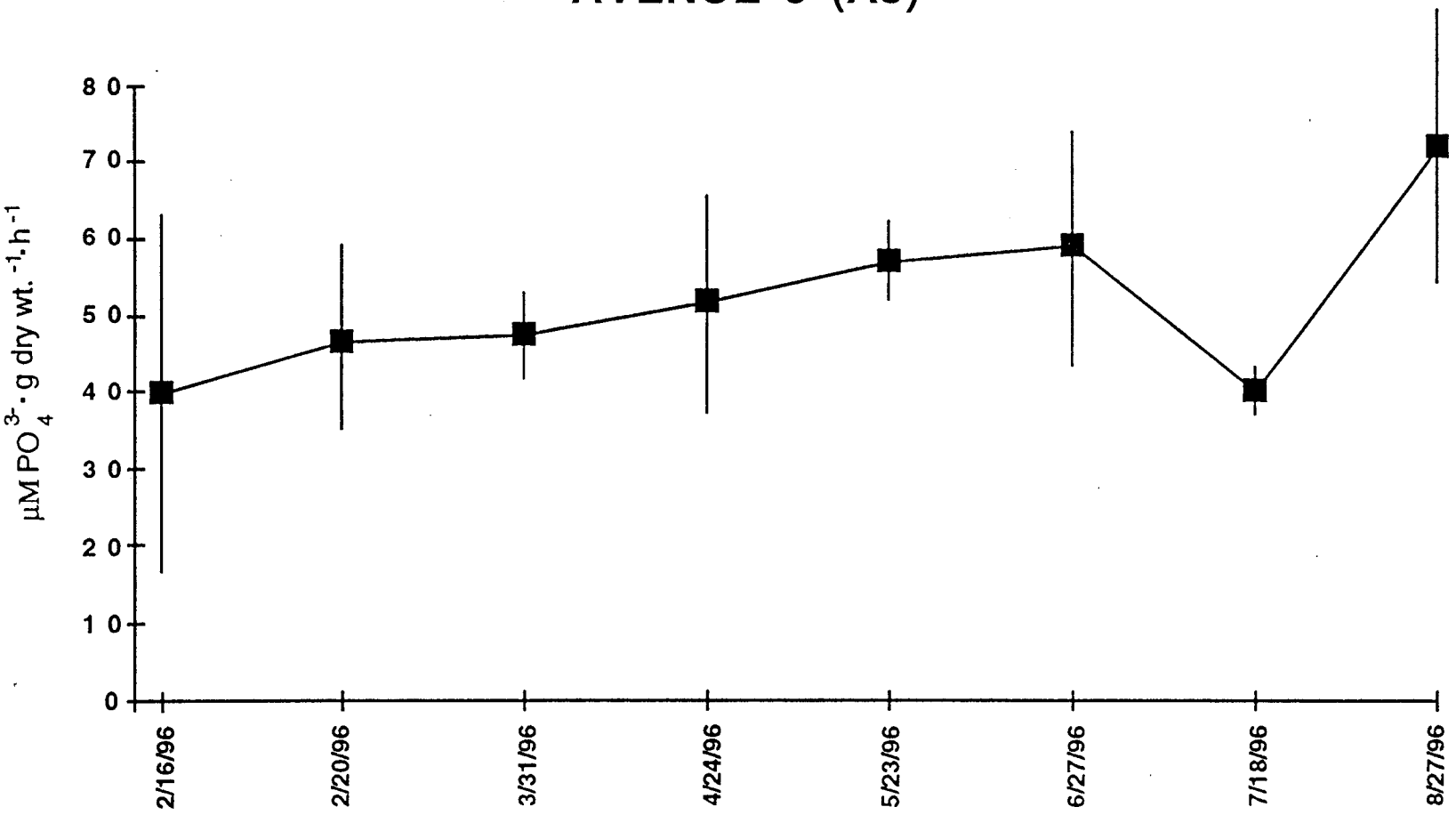


Figure 23

ALKALINE PHOSPHATASE

Laurencia poiteaui

PATCH REEF (PR)

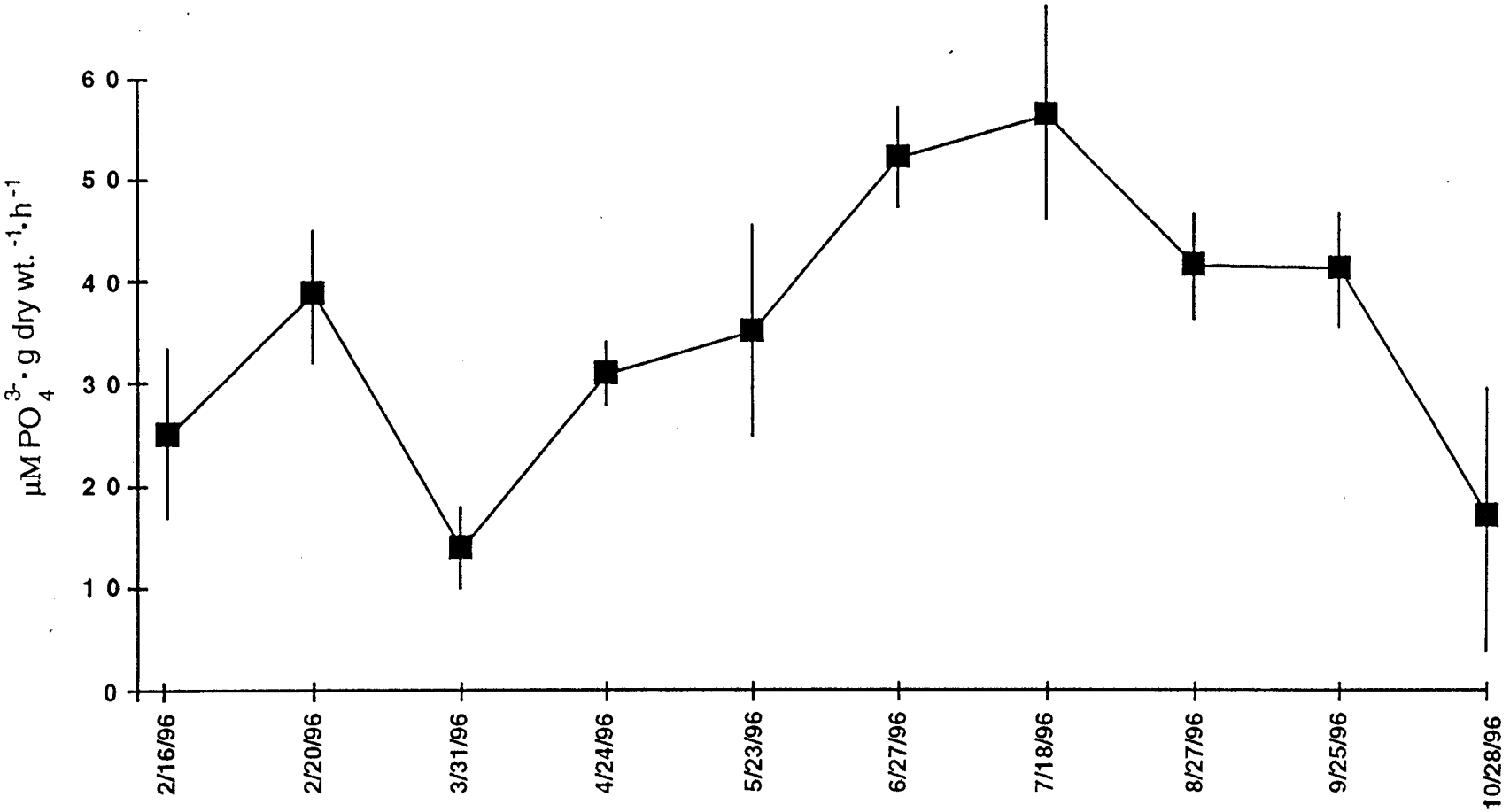


Figure 24

ALKALINE PHOSPHATASE

Laurencia poiteaui

LOOE KEY (LK)

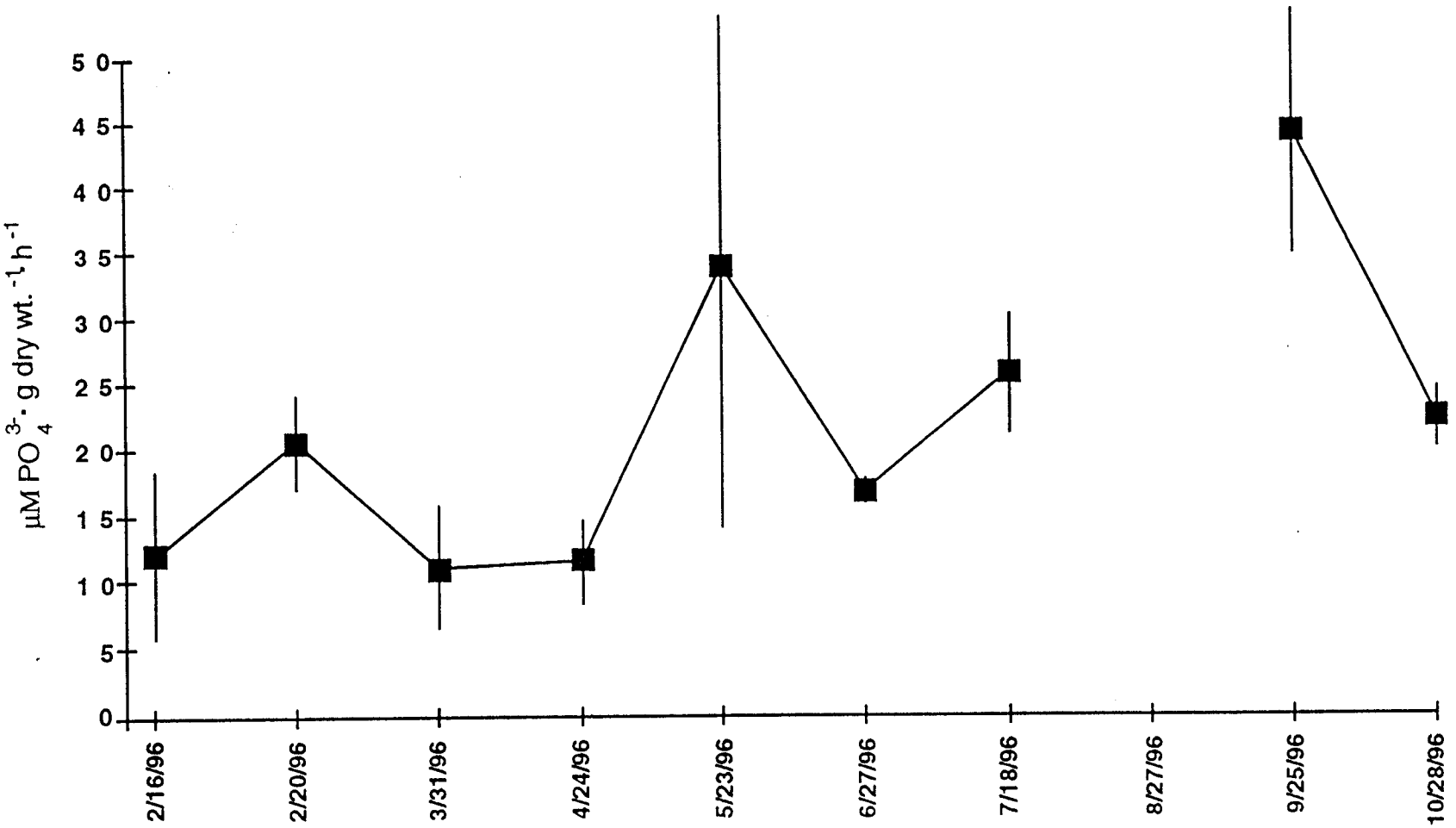


Figure 25

δ^{15} N

Laurencia poiteaui

AVENUE J (AJ)

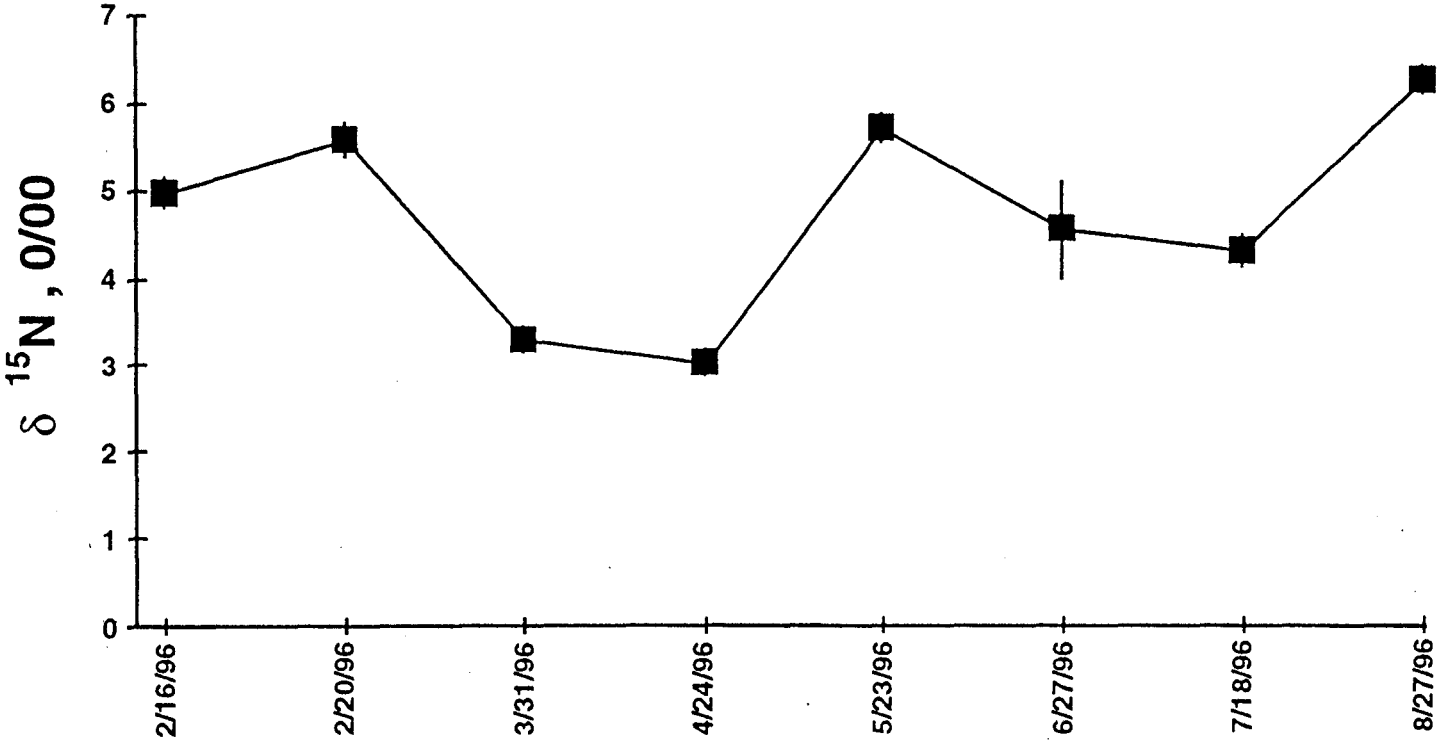


Figure 26

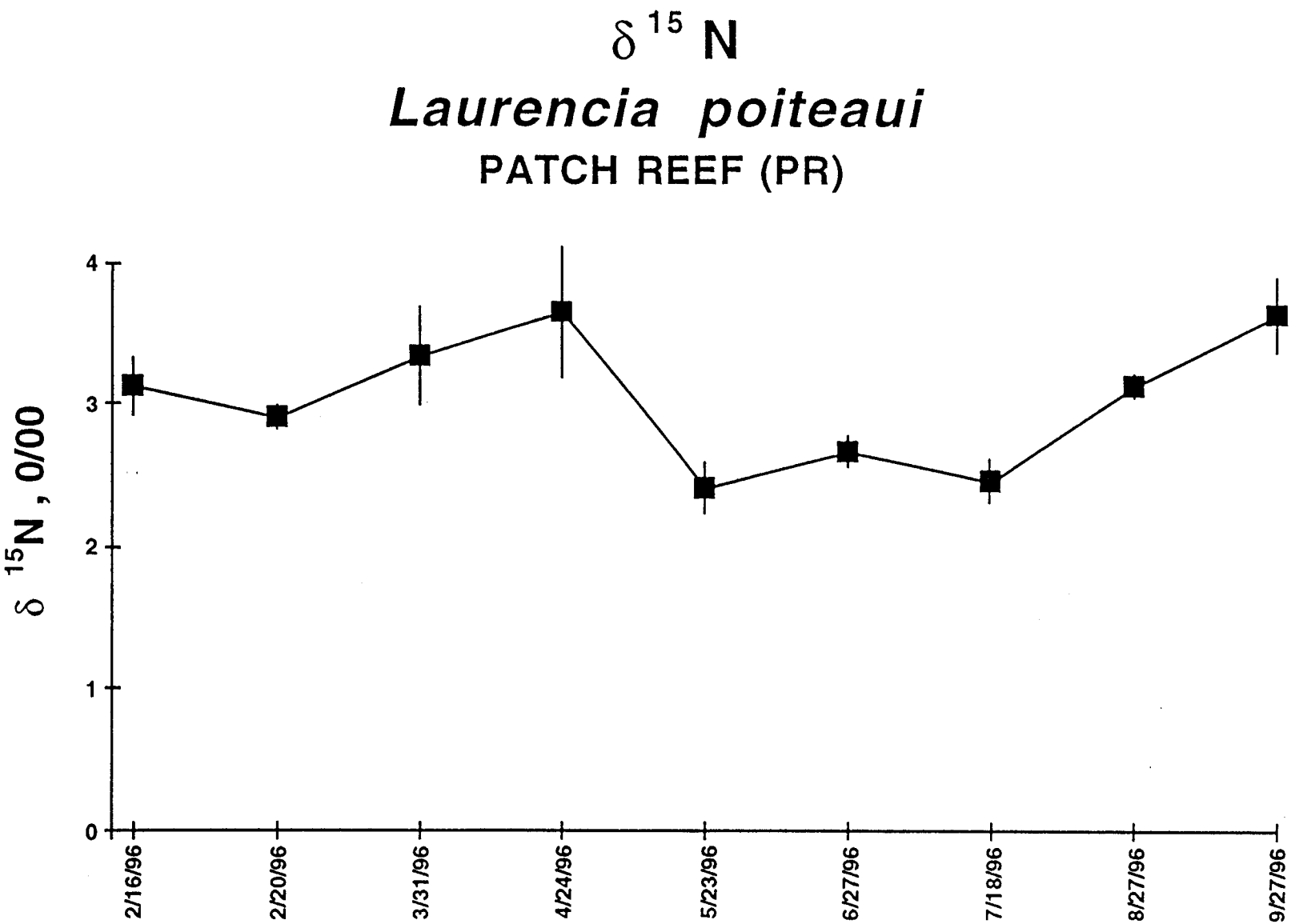

δ 15 N
Laurencia poiteaui
PATCH REEF (PR)
δ 15N , 0/00
4
3
2
1
0
2/16/96
2/20/96
3/31/96
4/24/96
5/23/96
6/27/96
7/18/96
8/27/96
9/27/96

Figure 27

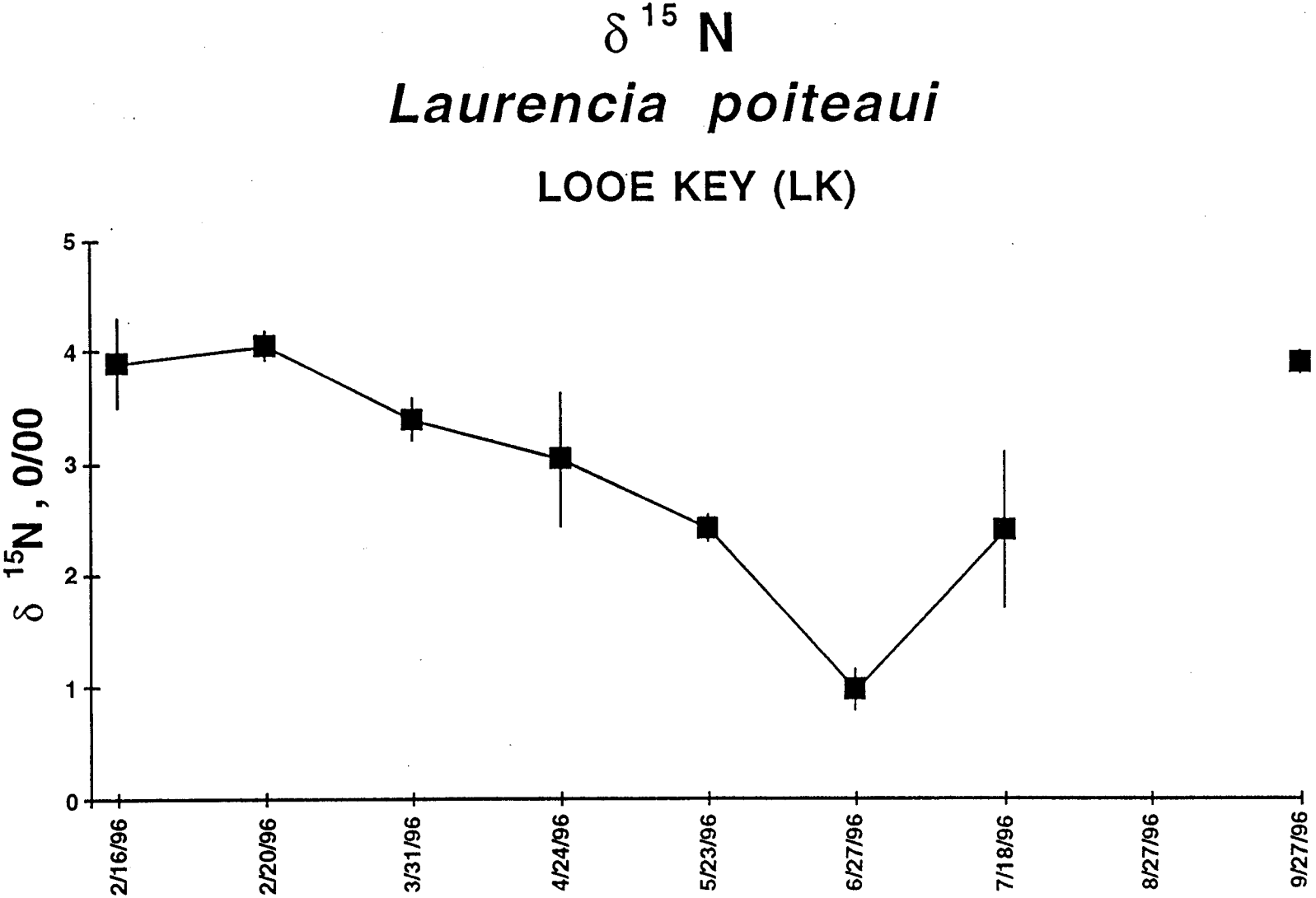

δ 15 N
Laurencia poiteaui
LOOE KEY (LK)
δ 15N , 0/00
5
4
3
2
1
0
2/16/96
2/20/96
3/31/96
4/24/96
5/23/96
6/27/96
7/18/96
8/27/96
9/27/96

Figure 28

DIN vs δ^{15} N
Cladophora fuliginosa
LOOE KEY (LK)

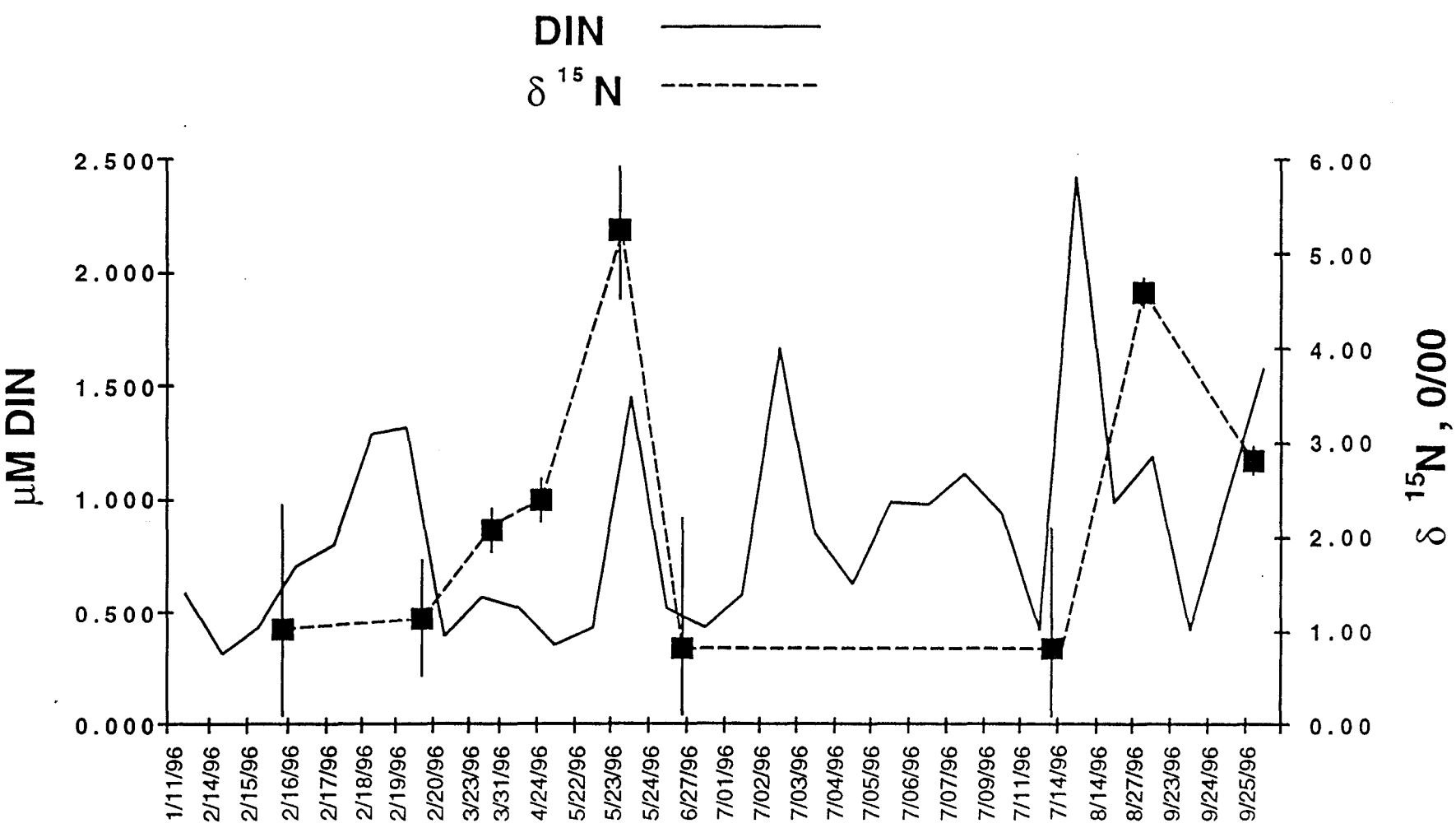


Figure 29

EPIPHYTE:SEAGRASS BLADE RATIO

AVENUE J (AJ)

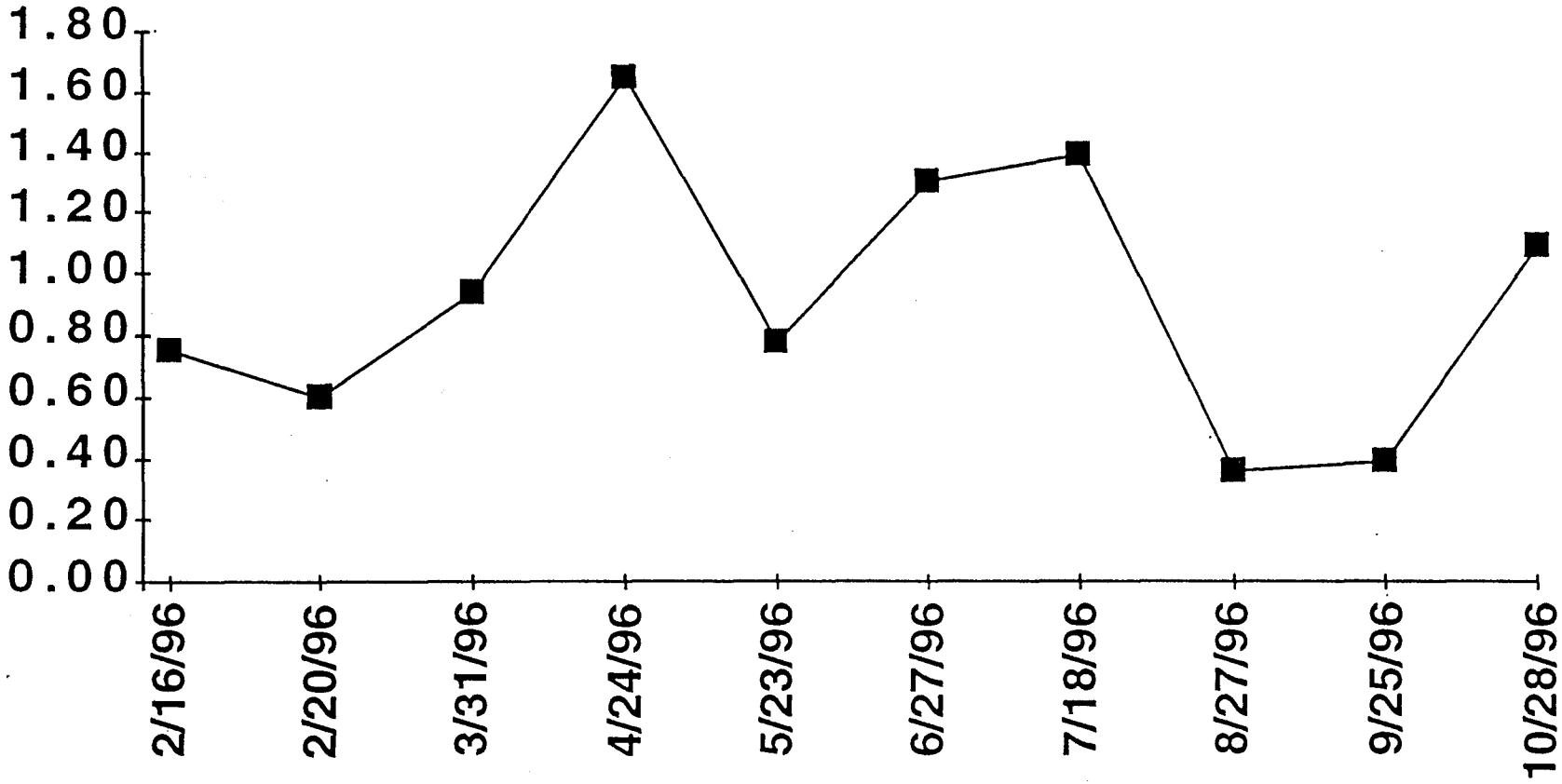


Figure 30

EPIPHYTE:SEAGRASS BLADE RATIO

PATCH REEF (PR)

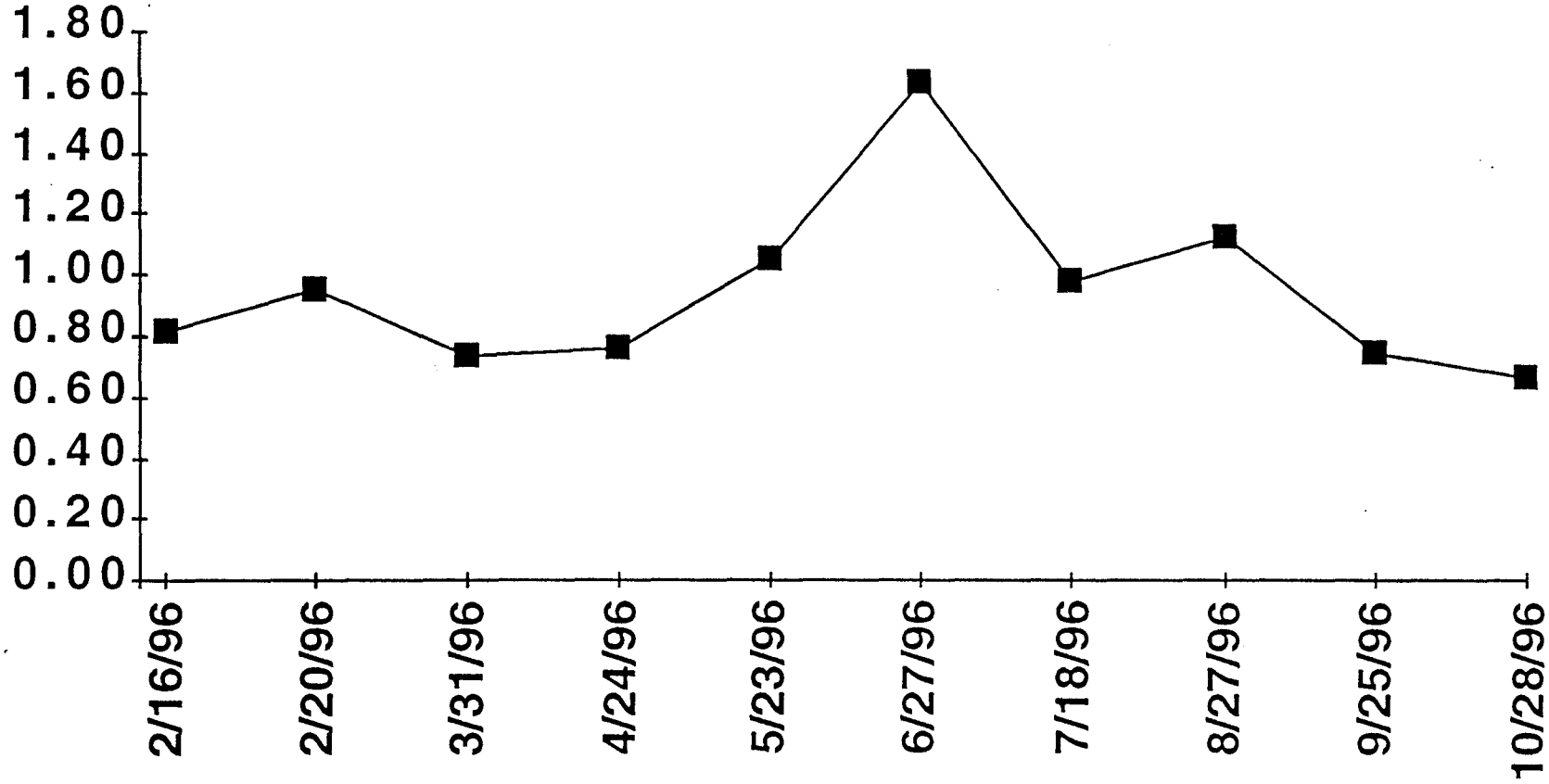


Figure 31

EPIPHYTE:SEAGRASS BLADE RATIO

LOOE KEY (LK)

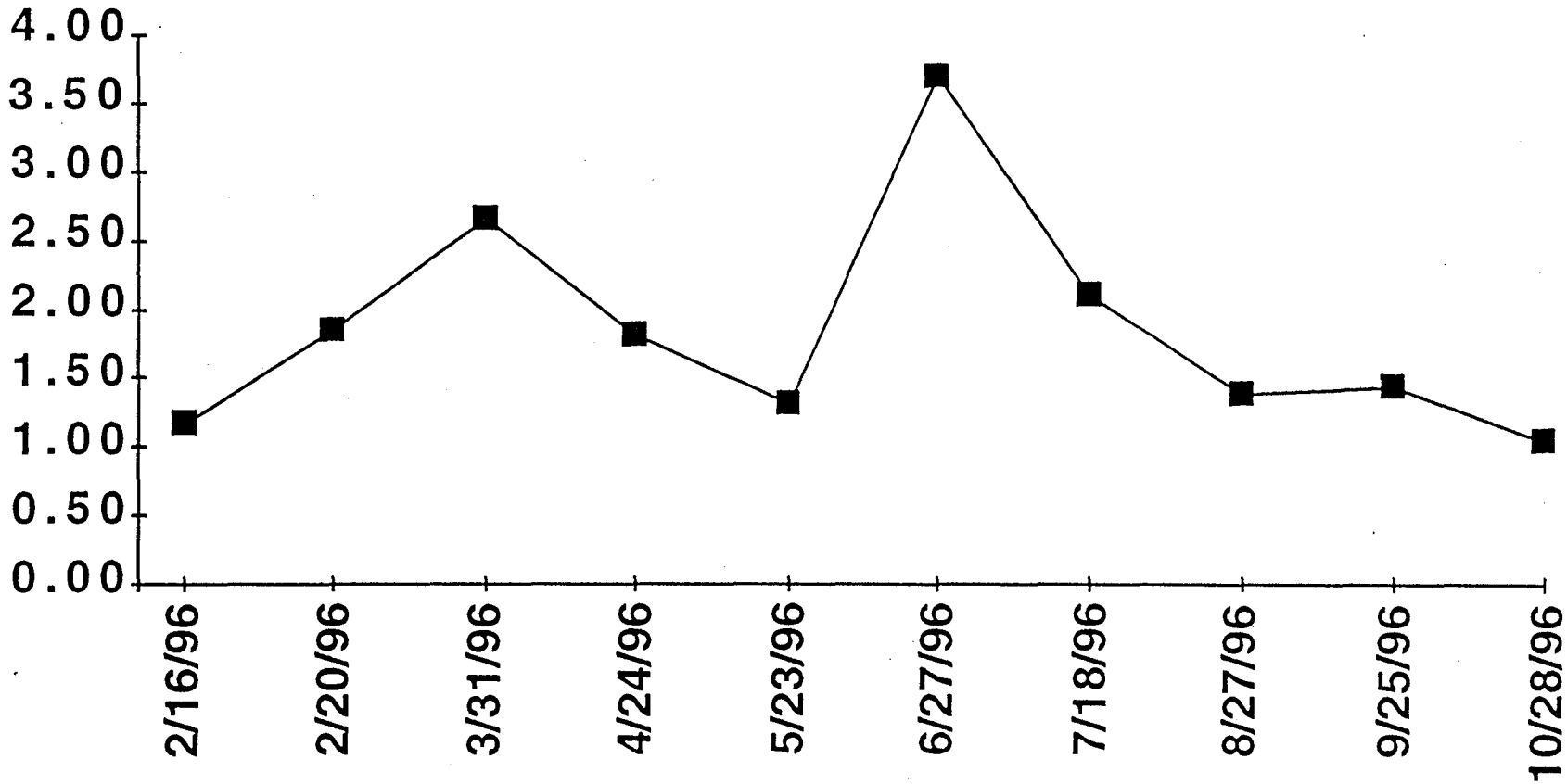


Figure 32

δ^{15}N

Thalassia testudinum

AVENUE J (AJ)

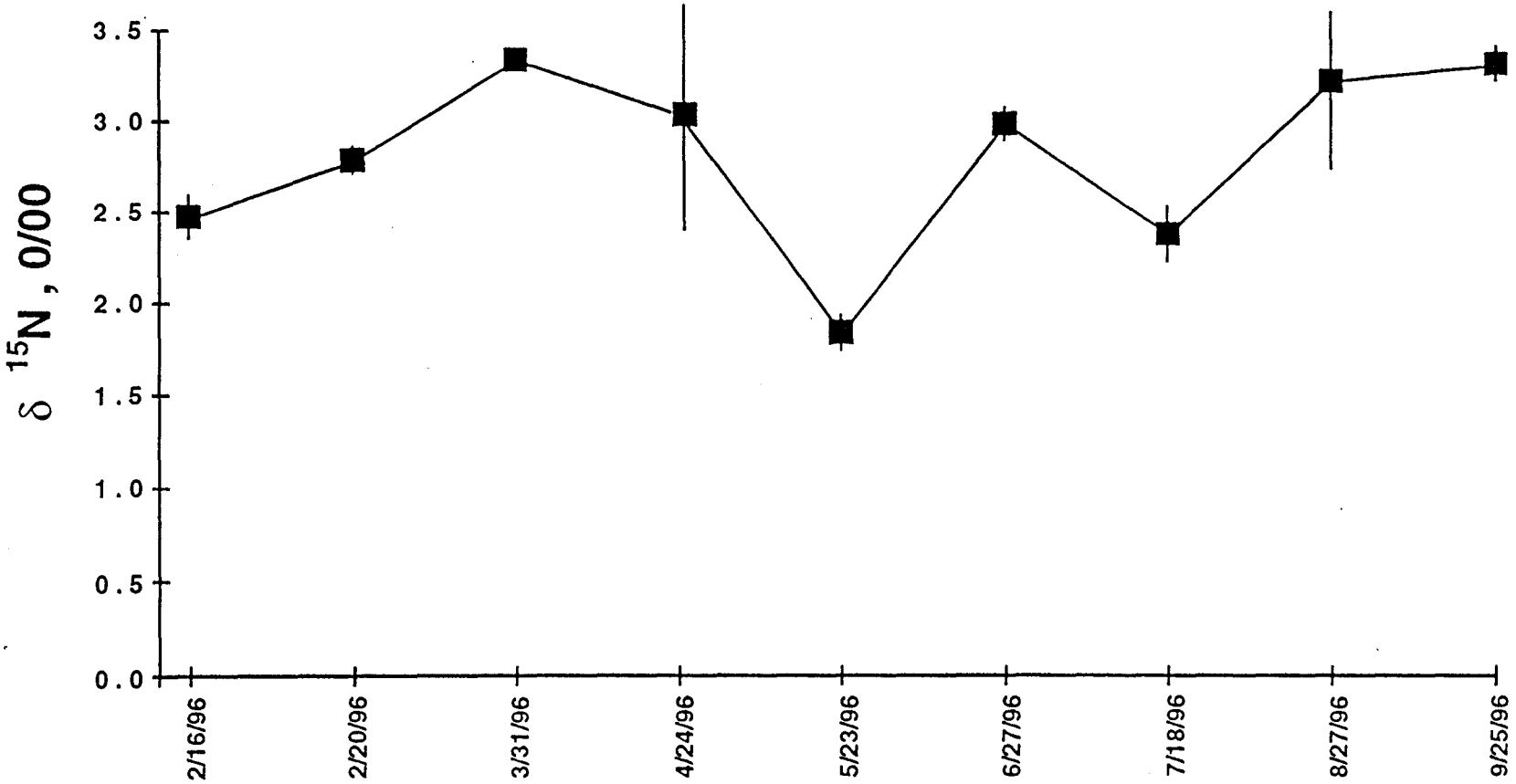


Figure 33

δ^{15} N

Thalassia testudinum

PATCH REEF (PR)

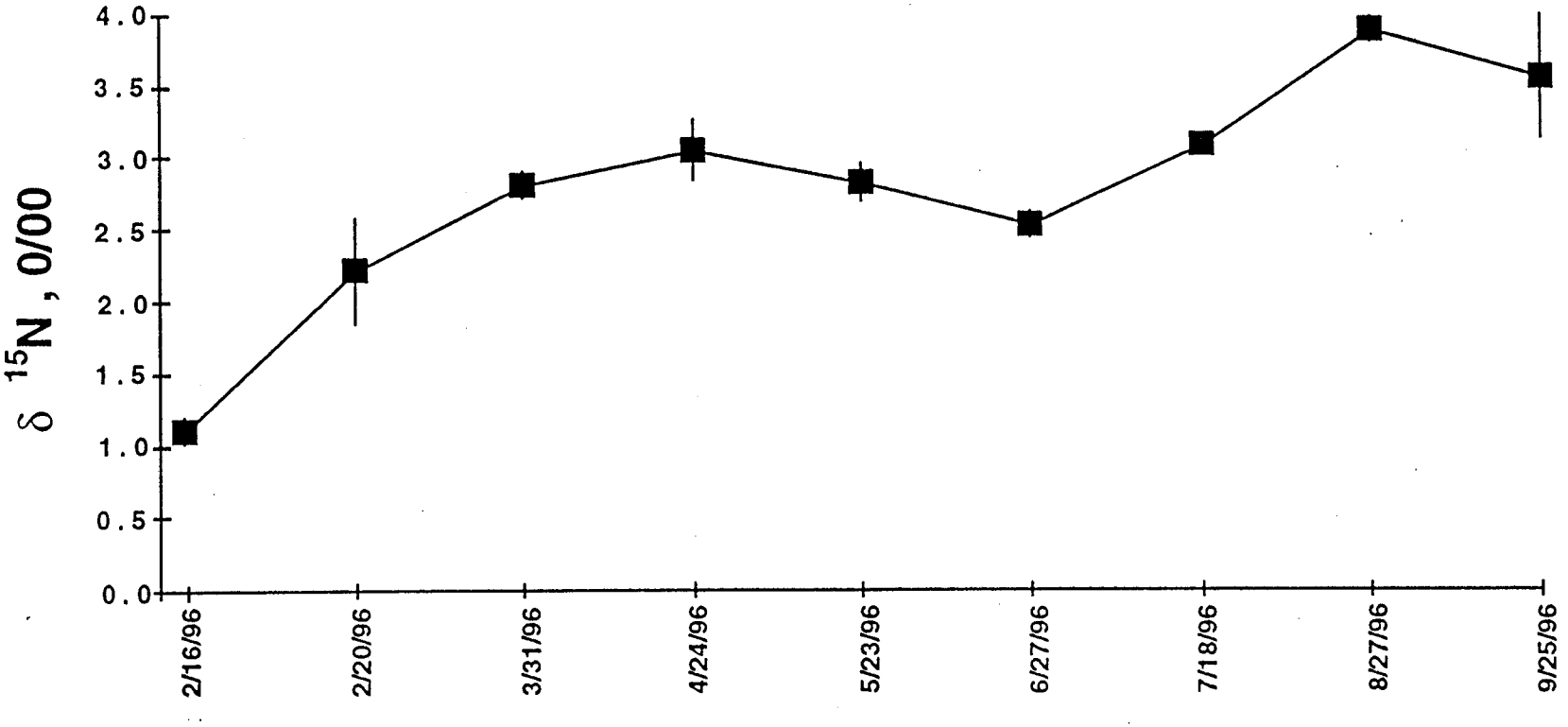


Figure 34

δ^{15} N

Thalassia testudinum

LOOE KEY (LK)

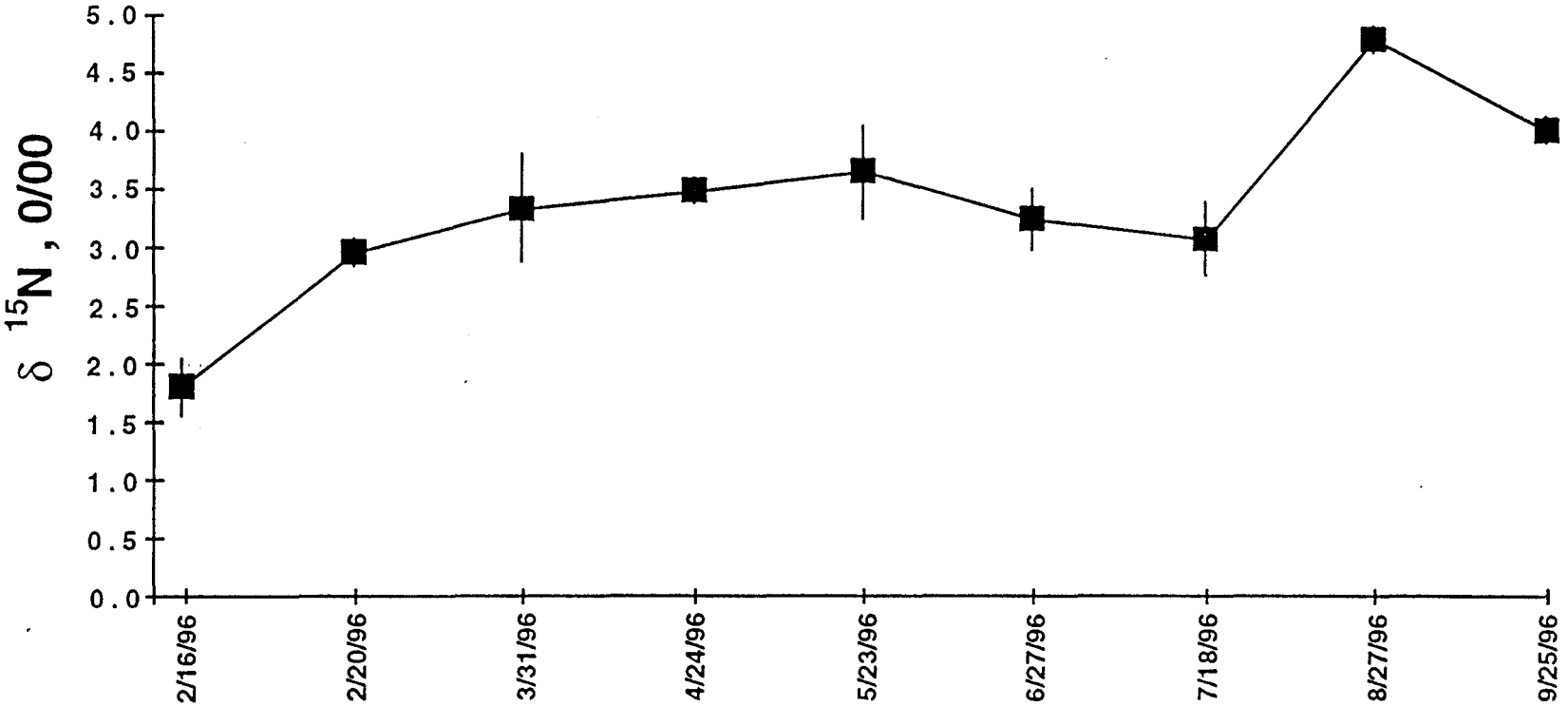


Figure 35

δ^{15} N

Thalassia testudinum

EPIPHYTES

AVENUE J (AJ)

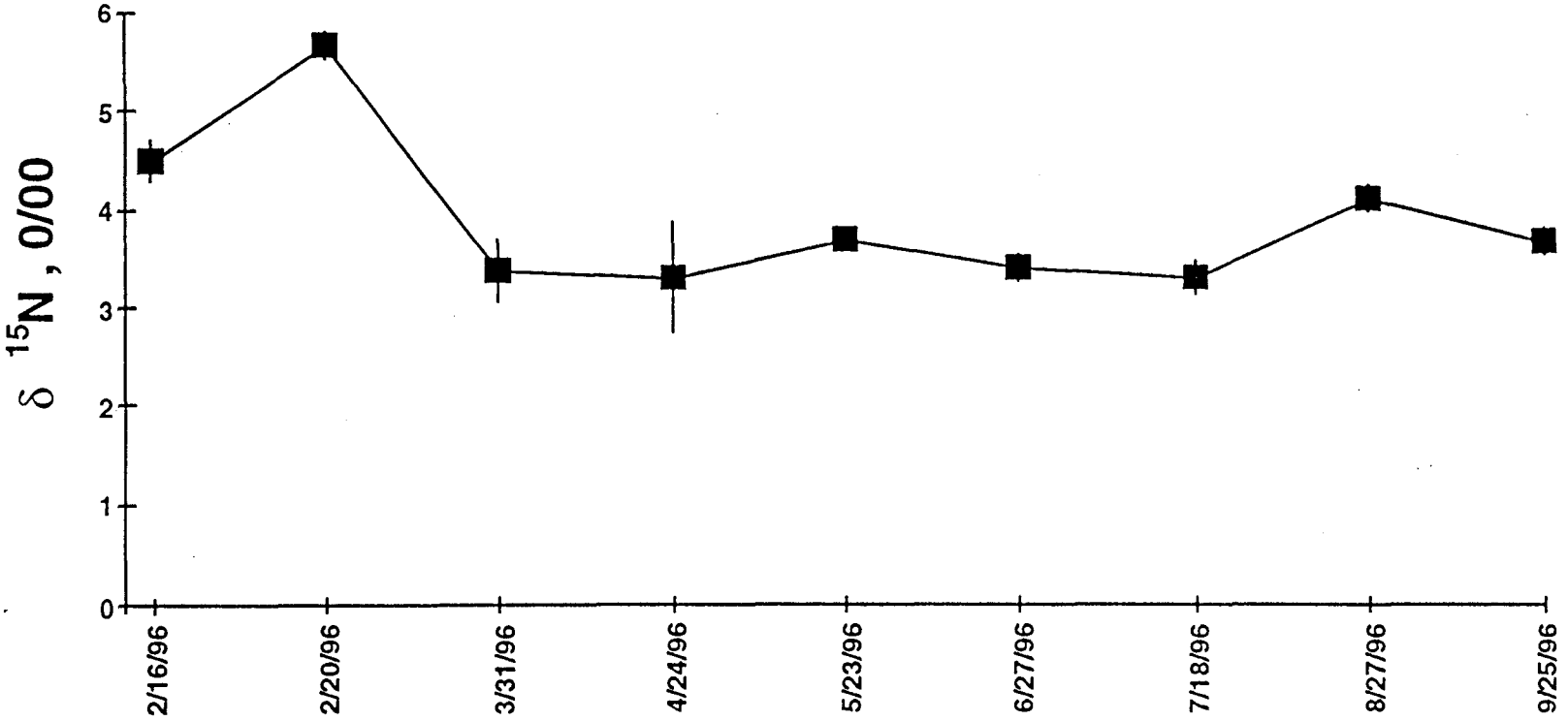


Figure 36

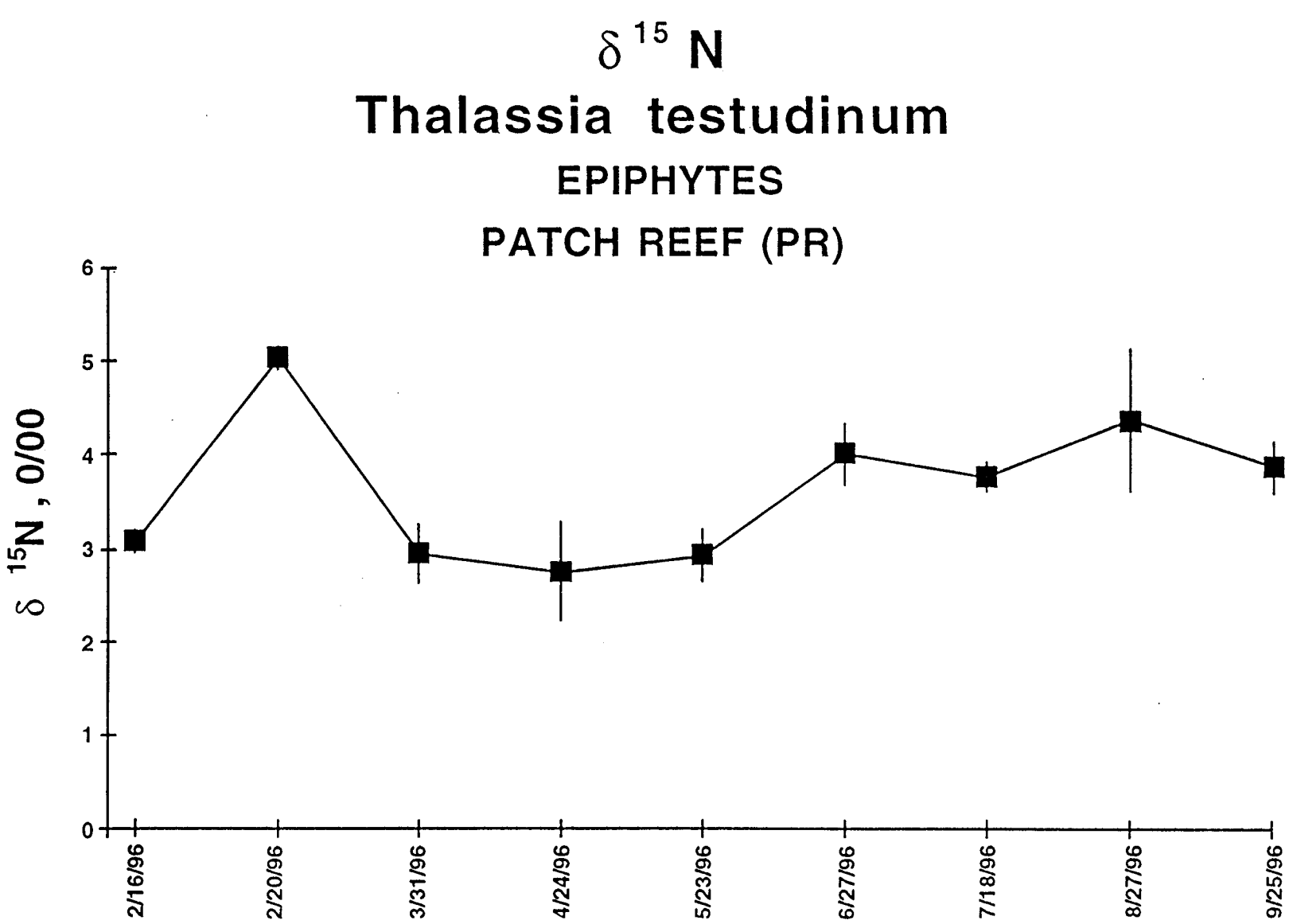

δ 15 N
Thalassia testudinum
EPIPHYTES
PATCH REEF (PR)
δ 15N, 0/00
6
5
4
3
2
1
0
2/16/96
2/20/96
3/31/96
4/24/96
5/23/96
6/27/96
7/18/96
8/27/96
9/25/96

Figure 37

$\delta^{15}N$

Thalassia testudinum

EPIPHYTES

LOOE KEY (LK)

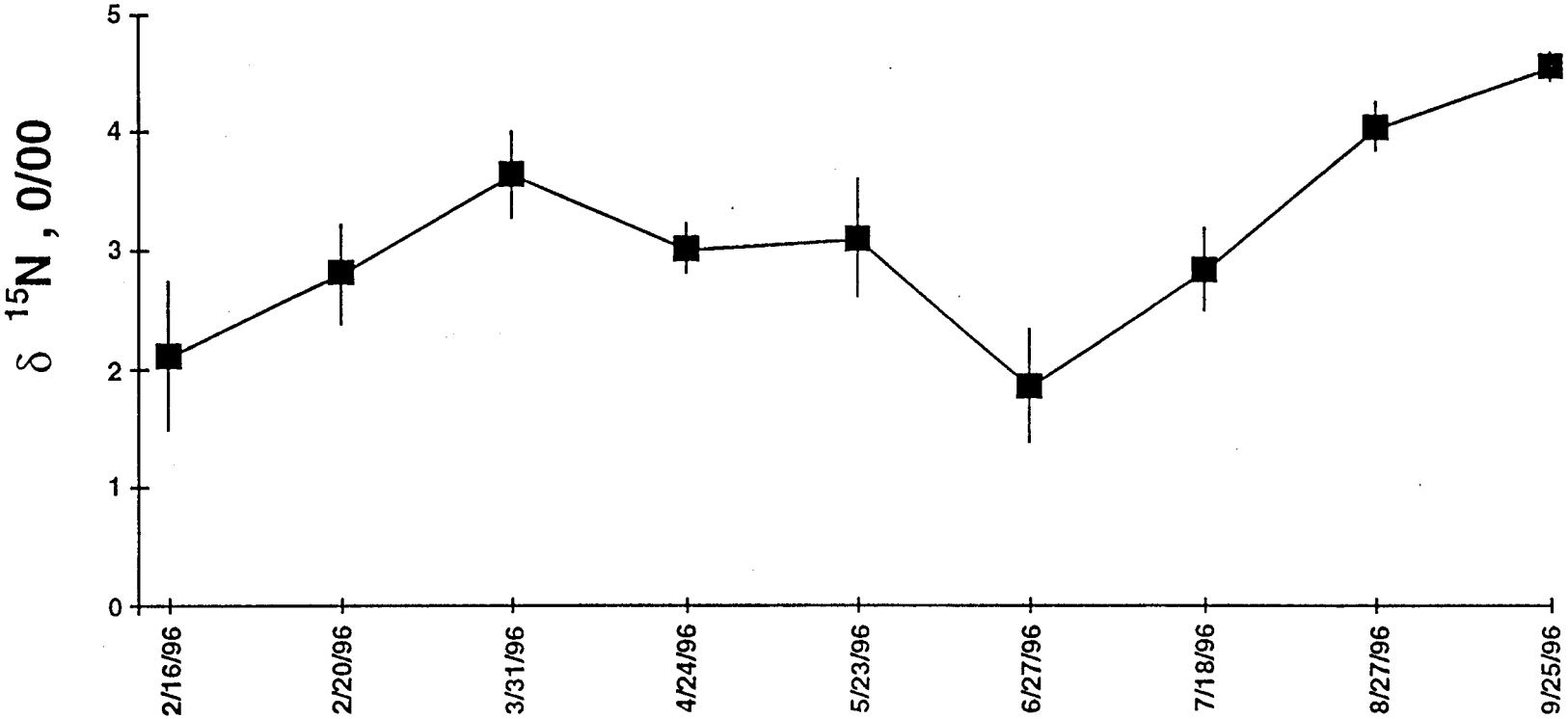


Figure 38

CHLOROPHYLL-a

LOOE KEY (LK)

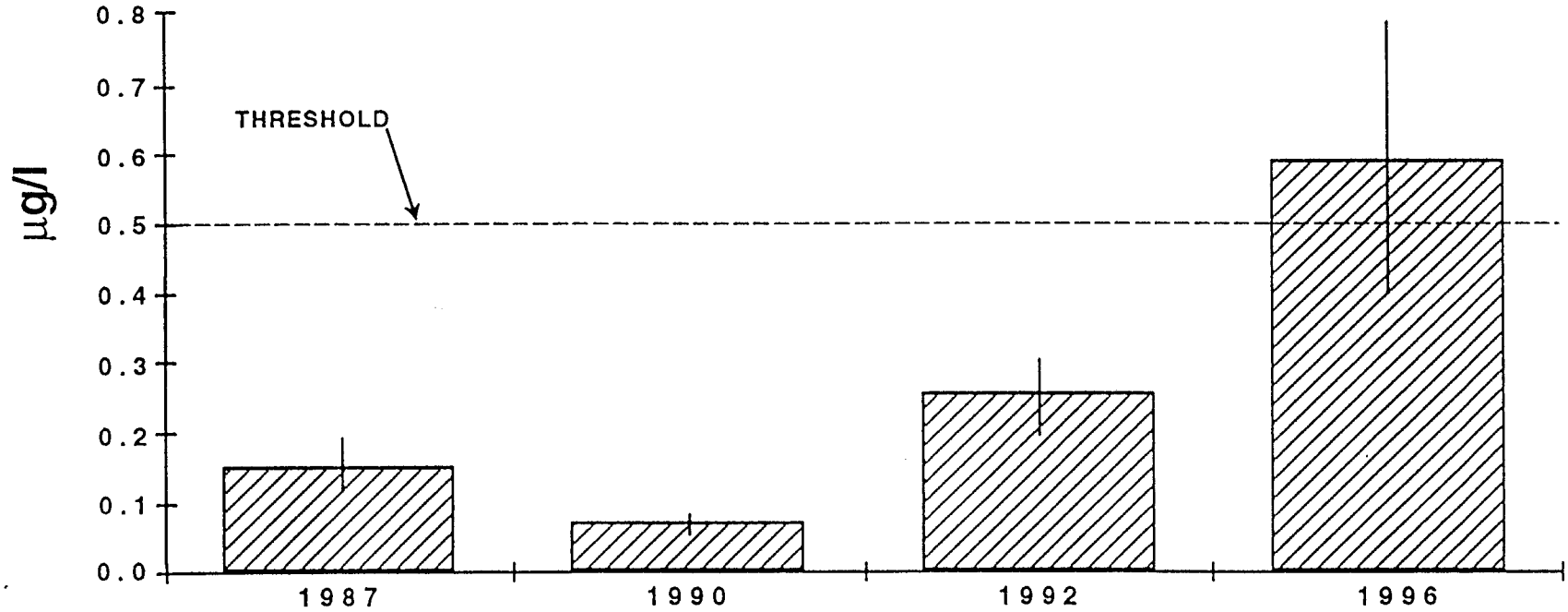


Figure 39

HARBOR BRANCH
ENVIRONMENTAL LABORATORY

5600 U.S. 1 NORTH
FORT PIERCE, FLORIDA 34946

(561) 465-2400, Ext. 285 or 448
FAX (561) 467-1584

APPENDIX I

HIGH FREQUENCY MONITORING OF WASTEWATER NUTRIENT DISCHARGES AND THEIR ECOLOGICAL EFFECTS IN THE FLORIDA KEYS MARINE SANCTUARY

QUALITY ASSURANCE SUMMARY
NUTRIENT ANALYSIS

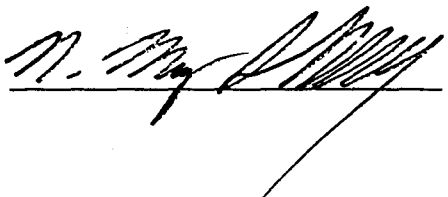
PRESENTED TO:

BRIAN LAPOINTE, Ph.D.
HARBOR BRANCH OCEANOGRAPHIC INSTITUTION, INC.

ANALYSIS BY:

HARBOR BRANCH ENVIRONMENTAL LABORATORY

LABORATORY DIRECTOR:
N. MYRON GUNSALUS, JR.



August 12, 1997

OVERVIEW

Harbor Branch Environmental Laboratory (HBEL) is committed to producing high quality data that is legally defensible, scientifically sound and fulfills the usability requirements for our clients' application in terms of analytical accuracy, precision, and completeness. HBEL has implemented the QA/QC program to ensure that the generation of monitoring and analytical measurement data are of known adequate quality to meet the requirements of each project's statement of work.

The Harbor Branch Environmental Laboratory pursues excellence in environmental testing and conducts applied research to understand and protect the natural environment.

PROJECT GOALS

Samples were collected during and following meteorological events that exceed normal weather conditions and held on ice in the dark until arrival in the laboratory where they were frozen. The samples were then thawed and analyzed for NH_4, NO_3, NO_2, and SRP at Harbor Branch Environmental Laboratory by methods derived from widely accepted chemistries used in previously published studies in the determination of low level nutrients in surface waters. The results obtained from these analyses will be evaluated to determine the development of phytoplankton and macroalgal blooms as a result of nutrient enrichment following episodic events.

For the duration of the project, a previously developed Quality Assurance Project Plan (QAPP) consisting of quality control goals was in place to ensure accurate reproducible data and to meet quality expectations for data usability. The data quality goals included target method detection limits, recommended holding times, and five quality assurance parameters: precision, accuracy, representativeness, comparability, and completeness. When quality objectives were not met for all samples, they were noted with corrective actions and narrated in the report.

METHOD DETECTION LIMIT (MDL)

Method Detection Limits were performed on "bluewater," saltwater matrix in accordance to CFR. 40 Part 136 Appendix B. These studies demonstrated achievable detection limits below the QAPP specified MDLs. The project detection limits were utilized instead of the statistically derived MDLs.

HOLDING TIMES

The QAPP recommended holding time of twenty-eight days was exceeded for several sample sets in this project. These exceedances and the performance of several analytical series for the various sample sets provided an opportunity for observation of the stability of these nutrients upon freezing and thawing as noted in the following discussion. Exceedances were noted in the final analytical report.

DISCUSSION OF ANALYTE STABILITY

In the course of work performed, the following informal observations on analyte stability were made: Nitrate, nitrite and soluble reactive phosphorous(SRP) are fairly stable throughout the freezing and thawing process when frozen for longer periods of time than the recommended holding time specified in the QAPP. Ammonium appears to be far less stable, with potential for loss or gain in the freezing and thawing process. Also, although ambient lab conditions can adversely affect any data collected, Ammonium and SRP were noticeably more sensitive to lab environmental conditions.

Soluble Reactive Phosphate (SRP)

For SRP care must be taken to avoid phosphate contamination of the analytical aliquot through reagent impurities or ambient laboratory conditions. This was accomplished through the monitoring of the reagent supply, locating the nutrient auto analyzer in the most phosphate free section of the laboratory, and accomplishing the analytical run as quickly as possible once the analytical aliquot was poured for analyses.

Ammonium

For ammonium, control of the loss or gain of ammonium from the analytical aliquot and the sample was of concern and more difficult than SRP to control. Great care was taken to minimize loss by immediate analyses upon opening of the sample bottle after the sample had been thawed and gently mixed. Air space in the bottle upon collection could have an affect on final results. Also, atmospheric lab ammonium can be absorbed into the analytical aliquot and the sample itself. Minimizing exposure to air during handling and analyses reduced ammonium absorption. Without these kind of procedures, NH_4 can be lost or gained.

From our observations, it appears the most reliable way to produce ammonium results which are representative of the sample upon collection is to avoid the complications of freezing and ambient conditions. It is recommended that fresh, unfrozen samples collected with no air space be analyzed as soon as possible on the day of collection.

ACCURACY

The following table was used to define the data quality objectives for accuracy and precision. These are different than the goals outlined in the QAP. These are derived from data points obtained from the lab.

LCL = Lower Control Limit
UCL = Upper Control Limit
MS = Matrix Spike
MSD = Matrix Spike Duplicate

Analyte		*Achieved recovery limits*		*Target Recovery Limits*
		LCL	*UCL*	
NH_4	MS	69.18	127.10	90-110
	LCS	74.44	121.77	
NO_3	MS	77.70	114.90	90-110
	LCS	79.11	116.36	
NO_2	MS	73.43	121.94	90-110
	LCS	82.72	116.06	
SRP	MS	71.37	117.20	90-110
	LCS	83.30	108.37	

Continuing Calibration - +/- 10% and run after every 10 samples	Continuing Calibration +/- 10% and after every 10 samples
Linearity $R^2 \geq 0.995$	Linearity $R^2 \geq 0.995$

PRECISION

Achieved	*Target*
≤ 20 % Relative Percent Difference	≤ 10 % Relative Percent Difference

REPRESENTATIVENESS

Samples were collected in triplicate from the test sites and analyzed separately. Also, please refer to the section on accuracy and precision of the analytical results.

COMPARABILITY

Harbor Branch Environmental Laboratory participated in a number of split studies and round robin analyses monitoring the comparability of data generated by the nutrient auto analyzer department.

INTERLABORATORY SPLIT SAMPLE STUDIES

Low Level Marine Nutrient Split - Attachment A

Samples were split with Ron Jones' Laboratory at Florida International University. Please see attachment A for comparability spread sheet.

The calculations used to determine comparability are:

%RSD – Percent Relative Standard Deviation

$$\%\,RSD = \frac{|A - B| \times 2 \times 100}{A + B \times \sqrt{2}}$$

Where: A = concentration in sample A
B = concentration in sample B

RPD – Relative Percent Difference

$$RPD = \frac{|A - B| \times 200}{A + B}$$

Where: A = concentration in sample A
B = concentration in sample B

I – Industrial Statistic

$$I = \frac{|A - B|}{A + B}$$

Where: A = concentration in sample A
B = concentration in sample B

This data indicates that overall interlaboratory comparability is acceptable for the QC goals and objectives of this project. The split study demonstrated that dissolved inorganic nutrients were determined to be at the same relative levels of concentration by both laboratories.

For individual samples, Nitrite and Nitrate exhibited a good level of reproducibility between the two laboratories, Ammonium exhibited an acceptable level of reproducibility, and SRP exhibited a weaker level of reproducibility. Possibilities for this pattern of reproducibility are numerous; including the consistency of distribution in the environment, stability in the freezing process, stability of the analyte in ambient laboratory conditions, low level reagent impurities, and the robustness of the chemistries employed in the analytical processes at these very low levels of analyte concentration.

The comparability of values for each sampling site per event is even greater when averages of the triplicate samples sets collected for each site are considered.

FDEP Surface Water Study for Total Phosphate - Attachment B

During the time frame of this project's duration, HBEL participated in two Florida Department of Environmental Protection (FDEP) round robin surface water studies for the evaluation of Total Phosphate. Compared accuracy and data reproducibility for HBEL was of a consistently high quality.

COMPLETENESS

Documentation of the extent to which the database fulfills QAPP quality control objectives has been provided for each criterion in the final analytical report and this quality assurance summary.

ATTACHMENT A

INTERLABORATORY SPLIT SAMPLE STUDIES

HFM-EPA

DATE	SITE CODE	HBOI NO2	*FIU NO2*	%RSD	RPD		HBOI NO3	*FIU NO3*	%RSD	RPD	
9/23/96	*AJA*	0.208	*0.270*	18.3	25.9	.13	0.674	*0.970*	25.5	36	.18
9/23/96	*AJB*	0.154	*0.230*	28	39.6	.2	0.464	*0.560*	13.3	18.8	.09
9/23/96	*AJC*	0.222	*0.220*	.64	.89	.005	0.326	*0.420*	17.9	25.3	.127
9/23/96	*PRA*	0.152	*0.210*	22.7	32	.16	1.057	*2.100*	46.7	66.1	.33
9/23/96	*PRB*	0.143	*0.160*	7.9	11.2	.06	0.994	*1.310*	19.4	27.4	.14
9/23/96	*PRC*	0.164	*0.170*	2.5	3.6	.02	1.287	*1.180*	6.1	8.7	.04
9/23/96	*LKA*	0.066	*0.100*	28.9	40.8	.2	0.328	*0.750*	55.4	78.4	.39
9/23/96	*LKB*	0.037	*0.060*	33	46.7	.23	0.156	*0.170*	6.1	8.7	.04
9/23/96	*LKC*	0.052	*0.040*	18.6	26.3	.13	0.100	*0.250*	60.8	86	.43
9/24/96	*AJA*	0.146	*0.100*	26.4	37.4	.19	0.244	*0.230*	4.2	6	.03
9/24/96	*AJB*	0.124	*0.060*	49.2	69.6	.35	0.097	*0.110*	8.8	12.5	.06
9/24/96	*AJC*	0.067	*0.080*	12.3	17.4	.09	0.087	*0.000*	141.4	200	1
9/24/96	*PRA*	0.089	*0.080*	7.8	11.1	.06	0.540	*0.450*	12.9	18.2	.09
9/24/96	*PRB*	0.086	*0.080*	5.4	7.6	.04	0.601	*0.340*	39.3	55.5	.28
9/24/96	*PRC*	0.108	*0.080*	21.1	29.8	.15	0.381	*0.420*	6.9	9.7	.05
9/24/96	*LKA*	0.121	*0.110*	6.7	9.5	.05	0.722	*0.740*	1.7	2.4	.01
9/24/96	*LKB*	0.106	*0.080*	19.8	28	.14	0.523	*0.550*	3.6	5.1	.03
9/24/96	*LKC*	0.089	*0.070*	17.1	24.1	.12	0.424	*0.410*	2.3	3.3	.02
9/25/96	*AJA*	0.184	*0.210*	9.3	13.2	.07	0.420	*0.400*	3.4	4.8	.02
9/25/96	*AJB*	0.121	*0.150*	15.1	21.4	.11	0.168	*0.250*	27.9	39.5	.2
9/25/96	*AJC*	0.106	*0.090*	11.5	16.3	.08	0.147	*0.170*	10.2	14.4	.07
9/25/96	*PRA*	0.195	*0.050*	83.7	118.4	.59	0.323	*0.440*	21.6	30.6	.15
9/25/96	*PRB*	0.065	*0.050*	18.6	26.2	.13	0.153	*0.260*	36.6	51.7	.26
9/25/96	*PRC*	0.083	*0.050*	34.7	49.1	.25	0.157	*0.280*	39.8	56.4	.28
9/25/96	*LKA*	0.150	*0.120*	15.7	22.2	.11	1.370	*1.500*	6.4	9.1	.05
9/25/96	*LKB*	0.118	*0.110*	5	7	.04	1.145	*1.550*	21.3	30.1	.15
9/25/96	*LKC*	0.105	*0.110*	3.3	4.7	.02	1.204	*1.600*	19.9	28.2	.14
	averages	**0.121**	**0.116**	**19.378**	**27.405**	**0.137**	**0.522**	**0.645**	**24.429**	**34.548**	**0.173**

HFM-EPA

DATE	SITE CODE	HBOI NH4	*FIU NH4*	%RSD	RPD	I	HBOI SRP	*FIU SRP*	%RSD	RPD	I
9/23/96	***AJA***	2.380	*2.940*	14.9	21.1	.11	0.026	*0.140*	97.3	137.6	.69
9/23/96	***AJB***	2.540	*2.810*	7.1	10.1	.05	0.025	*0.070*	67	94.7	.47
9/23/96	***AJC***	2.490	*2.710*	5.98	8.5	.042	0.000	*0.060*	141.42	200	1
9/23/96	***PRA***	0.392	*0.500*	17.1	24.2	.12	0.000	*0.060*	141.41	200	1
9/23/96	***PRB***	0.469	*0.400*	11.2	15.9	.08	0.017	*0.020*	12.3	17.4	.09
9/23/96	***PRC***	0.254	*0.410*	33.2	47	.23	0.040	*0.030*	19.5	27.6	.14
9/23/96	***LKA***	0.204	*0.380*	42.6	60.3	.3	0.000	*0.040*	141.4	200	1
9/23/96	***LKB***	0.218	*0.270*	15.1	21.3	.11	0.034	*0.010*	76.8	108.7	.54
9/23/96	***LKC***	0.107	*0.310*	68.8	97.4	.49	0.080	*0.010*	110	155.6	.78
9/24/96	***AJA***	3.668	*5.200*	24.4	34.6	.17	0.068	*0.070*	1.7	2.5	.01
9/24/96	***AJB***	3.500	*4.620*	19.5	27.6	.14	0.095	*0.040*	57.3	81	.41
9/24/96	***AJC***	4.680	*6.220*	20	28.3	.14	0.078	*0.080*	1.4	2	.01
9/24/96	***PRA***	0.454	*0.550*	13.5	19.1	.1	0.049	*0.000*	141.4	200	1
9/24/96	***PRB***	0.107	*0.370*	78	110.3	.55	0.000	*0.000*	0	0	0
9/24/96	***PRC***	0.292	*0.400*	22.1	31.2	.16	0.000	*0.010*	141.4	200	1
9/24/96	***LKA***	0.422	*0.710*	36	50.9	.25	0.015	*0.030*	48.4	68.5	.34
9/24/96	***LKB***	0.266	*0.540*	48.1	68	.34	0.034	*0.010*	76.7	108.5	.54
9/24/96	***LKC***	0.281	*0.470*	35.6	50.3	.25	0.011	*0.000*	141.4	200	1
9/25/96	***AJA***	1.350	*1.650*	14.1	20	.1	0.017	*0.280*	125.7	177.7	.89
9/25/96	***AJB***	1.420	*1.650*	10.6	15	.07	0.017	*0.190*	118.6	167.7	.84
9/25/96	***AJC***	2.440	*1.710*	24.9	35.2	.18	0.022	*0.170*	109.1	154.4	.77
9/25/96	***PRA***	0.324	*0.300*	5.4	7.7	.04	0.000	*0.020*	141.4	200	1
9/25/96	***PRB***	0.020	*0.270*	121.9	172.4	.86	0.027	*0.000*	141.4	200	1
9/25/96	***PRC***	0.029	*0.260*	113	159.9	.8	0.000	*0.010*	141.4	200	1
9/25/96	***LKA***	0.355	*0.530*	28	39.5	.2	0.117	*0.050*	56.7	80.2	.4
9/25/96	***LKB***	0.089	*0.400*	89.8	127	.63	0.019	*0.020*	4	5.7	.03
9/25/96	***LKC***	0.207	*0.390*	43.4	61.3	.31	0.016	*0.020*	15.7	22.2	.11
	averages	**1.073**	**1.369**	**35.718**	**50.512**	**0.253**	**0.030**	**0.053**	**87.352**	**123.534**	**0.618**

HFM-EPA

DATE	SITE CODE	HBOI DIN	FIU DIN	%RSD	RPD	I
9/23/96	AJA	3.26	4.18	17.4	24.7	.12
9/23/96	AJB	3.16	3.60	9.3	13.1	.07
9/23/96	AJC	3.04	3.35	6.92	9.8	.049
9/23/96	PRA	1.60	2.81	38.8	54.8	.27
9/23/96	PRB	1.61	1.87	10.7	15.2	.08
9/23/96	PRC	1.70	1.76	2.2	3.2	.02
9/23/96	LKA	0.60	1.23	48.9	69.2	.35
9/23/96	LKB	0.41	0.50	13.8	19.5	.1
9/23/96	LKC	0.26	0.60	56.2	79.5	.4
9/24/96	AJA	4.06	5.53	21.7	30.7	.15
9/24/96	AJB	3.72	4.79	17.8	25.1	.13
9/24/96	AJC	4.83	6.30	18.6	26.3	.13
9/24/96	PRA	1.08	1.08	.24	.34	.002
9/24/96	PRB	0.79	0.79	.41	.58	.003
9/24/96	PRC	0.78	0.90	10	14.2	.07
9/24/96	LKA	1.27	1.56	14.8	20.9	.1
9/24/96	LKB	0.89	1.17	18.9	26.7	.13
9/24/96	LKC	0.79	0.95	12.7	17.9	.09
9/25/96	AJA	1.95	2.26	10.3	14.5	.07
9/25/96	AJB	1.71	2.05	12.8	18.2	.09
9/25/96	AJC	2.69	1.97	21.9	31	.16
9/25/96	PRA	0.84	0.79	4.5	6.4	.03
9/25/96	PRB	0.24	0.58	59.1	83.5	.42
9/25/96	PRC	0.27	0.59	53	74.9	.37
9/25/96	LKA	1.88	2.15	9.7	13.7	.07
9/25/96	LKB	1.35	2.06	29.4	41.5	.21
9/25/96	LKC	1.52	2.10	22.8	32.3	.16
	averages	1.72	2.13	20.103	28.430	0.142