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Summary of nutrient enrichment studies of natural phytoplankton populations in the Lower Hillsborough River and the Palm River conducted on February 1 - 4, 2005

The City of Tampa Wastewater Department

SUMMARY OF NUTRIENT ENRICHMENT STUDIES OF NATURAL PHYTOPLANKTON POPULATIONS IN THE LOWER HILLSBOROUGH RIVER AND THE PALM RIVER CONDUCTED ON FEBRUARY 1 - 4, 2005

THE CITY OF TAMPA
WASTEWATER DEPARTMENT
BAY STUDY GROUP

FEBRUARY 23, 2005

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INTRODUCTION

The Bay Study Group (BSG) conducted nutrient enrichment studies (bioassays) on natural phytoplankton population samples collected in the lower Hillsborough River and the Palm River on February 1, 2005. The Hillsborough River test site was located approximately 200m upriver of the Nebraska Avenue bridge (Lat: 28^{o} 01.171'N; Lon: 82^{o} 27.025'W) and the Palm River test site was located approximately 300m downstream from the barrier at Structure-160 (Lat: 27^{o} 57.308'N; Lon: 82^{o} 22.200'W).

METHODS

The bioassays were performed on the natural phytoplankton populations collected from surface waters of the two test sites. All water samples were collected from a small boat near the center of each stream. General observations were recorded at the sampling sites and ambient water quality conditions were measured in the field and the laboratory (see Table 1).

The bioassay method used was similar to a method that has been used in Tampa Bay and Chesapeake Bay waters (see City of Tampa 1992; Fisher et al. 1992a and 1992b). A summary of the method used by the BSG is provided here.

A large volume of surface water was used to provide the following nutrient treatments. Each treatment was conducted in duplicate on three-liter samples:

- Control (C; no nutrient addition).
- Nitrogen (N) addition (ammonium chloride added to reach the final treatment concentration shown in Table 3).
- Phosphorous (P) addition (potassium phosphate added to reach the final treatment concentration shown in Table 3).
- N+P-addition (combination of the N-addition and P-addition).

The treatment samples were incubated outside under natural sunlight in a tap water fed deck incubator. The incident radiation was reduced by approximately 40 percent by a neutral density screen during clear skies. The screen was removed during cloudy conditions. The water temperature in the incubator was relatively constant at approximately 20C. The incubation of all samples started at 12:15pm on February 1, 2005. The Palm River experiment was ended on February 3, 2005 at 08:15am and the Hillsborough River experiment on February 4, 2005 at 08:10am.

The growth response of the natural phytoplankton community to the different treatments was determined through measurements in changes of algal biomass, measured as chlorophyll-a.

Chlorophyll was analyzed using both a trichromatic spectrophotometric method and an extracted whole water fluorometric method.

Paired t-test statistics were used to help interpret the bioassay results and to classify the growth response to the nutrient additions into the following possible response categories:

- Exclusive N limitation: (1) the addition of P induced no response relative to the control, and (2) the addition of N alone had virtually the same effect as the addition of N+P.
- Primary N limitation: (1) the addition of P alone induced little response relative to the control, (2) the addition of N alone induced a significant response, and (3) the addition of N+P induced the largest response.
- Balanced NP limitation: (1) the addition of N and P alone induced no response relative to the control, (2) the addition of N+P induced a large response.
- Exclusive P limitation: (1) the addition of N induced no response relative the control, and (2) the addition of P alone had virtually the same effect as the addition of N+P.
- Primary P limitation: (1) the addition of N alone induced little response relative to the control, (2) the addition of P alone induced a significant response, and (3) the addition of N+P induced the largest response.
- No response to any nutrient addition, indicating nutrient saturation, light limitation, and/or insufficient incubation time.

RESULTS

Ambient water quality conditions and field observations at the two river sampling locations on February 1, 2005 are shown in Table 1.

Table 1. Ambient water quality conditions and observations at the two river sample locations on February 1, 2005.

Parameter	**Station**	
	Hillsborough River	**Palm River**
Time (hhmm)	0844	1033
Surface temp. (C)	19.4	17.1
Surface DO (mg/l)	5.8	5.5
Surface salinity (PSU)	2.2	24.3
PH	7.6	7.9
Secchi depth (m)	>water column depth	1.4
Water column depth (m)	2.3	5.7
Apparent water color	Light brown	Gray green
Water color (PCU)	21.7	8.9
Surface flow (m/s)	0.17 downstream	0.13 downstream
Turbulence	None observed	None observed
Turbidity (NTU)	0.5	3.5
Percent light transmission (660nm; 10cm)	93.1	68.8
Chlorophyll-a (ug/l; spectrophotometric)	2.46	26.05
Chlorophyll-a (ug/l; fluorometric)	3.38	39.46

Ambient surface nutrient concentrations at the two river sample locations are shown in Table 2. The Environmental Protection Commission of Hillsborough County kindly provided the analyses.

Table 2. Ambient surface nutrient concentrations at the two river sample locations on February 1, 2005.

Parameter (uM)	**Station**	
	Hillsborough River	**Palm River**
NH3	7.1	4.3
TKN	33.6	43.6
NO3+NO2	24.2	0.6
TN	57.9	44.3
PO4	1.0	3.5
TP	1.0	5.8
SiO2	229.3	28.2

The measured nitrogen (NH3) and phosphorous (PO4) additions to the bioassay treatment sample containers are shown in Table 3. The table also shows the measured final nutrient concentration in the treatments, which includes the nutrient addition plus the ambient nutrient concentrations.

The Hillsborough River sample will be used as an example to further illustrate Table 3 and the four bioassay treatment combinations: (1) Duplicate control sample containers received no nutrient additions and these containers only contained the ambient nutrient concentrations shown in Table 2. (2) Duplicate containers received the NH3 treatment in addition to the ambient nutrient concentrations. (3) Duplicate containers received the PO4 treatment in addition to the ambient nutrient concentrations. (4) Duplicate containers received both the NH3 treatment and the PO4 treatment in addition to the ambient nutrient concentrations.

Table 3. Concentrations of measured nutrient additions to the two river surface samples and the measured final concentrations in respective treatments prior to incubation on February 1, 2005.

Parameter (uM)	**Station**	
	Hillsborough River	**Palm River**
NH3 addition	21.5	20.0
NH3 final treatment conc.	28.6	24.3
PO4 addition	8.7	8.3
PO4 final treatment conc.	9.7	11.8

Phytoplankton biomass (chlorophyll-a) was measured in the bioassay containers during the progress of the experiments using the fluorometric technique (see Figures 1A and 2A). The final chlorophyll-a concentrations, following the incubation periods shown in Table 5, were measured using both the fluorometric and spectrophotometric techniques (see Figures 1B, 1C, 2B and 2C). The final mean fluorometric chlorophyll-a concentrations of the six replicates for each treatment and river are shown in Table 4.

Table 4. The mean and one standard deviation of the final fluorometric chlorophyll-a concentrations (n=6) for each treatment at the termination of the experiments for the Hillsborough River and the Palm River bioassay experiments.

Station	Chlorophyll-a treatment response (ug/l)			
	Control	NH3	PO4	NH3+PO4
Hillsborough River	26.83 (0.34)	33.94 (0.77)	27.93 (0.59)	31.17 (0.99)
Palm River	25.26 (0.56)	107.8 (11.37)	25.34 (0.81)	114.2 (3.63)

The final fluorometric chlorophyll-a concentrations were used in t-test analyses to help interpret the bioassay results. The results of the t-test are shown in Table 5.

Table 5. Results of t-test analyses comparing the fluorometric chlorophyll-a concentrations at the termination of the experiments for the Hillsborough River and the Palm River bioassay experiments.

Station	Incubation time (hr)	T-test results of nutrient addition treatments vs. controls			Response
		NH3 > control	PO4 > control	NH3+PO4 > control	
Hillsborough River	68	**	*	**	Exclusive nitrogen limitation (1)
Palm River	44	**	NS	**	Exclusive nitrogen limitation

** Significant difference; $p<0.01$

* Significant difference; $p<0.05$

NS: No significant difference

All tests 5df

(1) Response most closely resembled exclusive nitrogen limitation (see Table 4 and Figure 2), however, the PO4 addition may have caused a small increase in chlorophyll-a concentration above the control.

DISCUSSION AND CONCLUSION

Chlorophyll-a concentrations measured at the termination of the bioassay experiments indicated that the phytoplankton populations of the lower Hillsborough River and the Palm River, present at the time of sampling on February 1, 2005, were apparently limited by nitrogen (see Tables 4 and 5, and Figures 1 and 2).

The Palm River treatments with NH3 and NH3+PO4 additions increased quickly in chlorophyll-a concentrations following the start of the experiments (Figure 1A). These treatments reached a final chlorophyll-a concentration at the termination of the experiment that was substantially higher than both the control and the PO4 treatments. Additions of phosphate alone to samples from this river did not increase phytoplankton biomass significantly above the control treatments. Nor did the combined additions of NH3+PO4 increase biomass significantly above the nitrogen alone treatments. Therefore, nitrogen was clearly the most limiting nutrient for the Palm River sample and the sample appeared to be “exclusively” limited by nitrogen, as defined by the possible bioassay response categories listed above. Further, the Palm River sample responded to the bioassay additions in a manner that is most often typical of estuarine Tampa Bay samples with a relatively high salinity and phytoplankton biomass (see Table 1).

The Hillsborough River sample had a low ambient chlorophyll-a concentration and all bioassay treatments remained near ambient levels for more than 24hr following the start of the experiments (Figure 2A). Following this initial period, chlorophyll-a concentrations increased substantially in all treatment containers, including the control. However, at the termination of the experiment, the NH3 and NH3+PO4 additions had the highest chlorophyll-a concentrations (Table 4, and Figures 2B and 2C), indicating that nitrogen was the most limiting nutrient for the Hillsborough River sample. This sample also appeared to most closely respond as “exclusively” limited by nitrogen, as defined by the possible response categories listed above. Although, the PO4 treatment had a slightly higher mean chlorophyll-a concentration than the control at the termination of the experiment (see Table 4), it was clearly the nitrogen addition that caused the greatest response in chlorophyll-a.

REFERENCES

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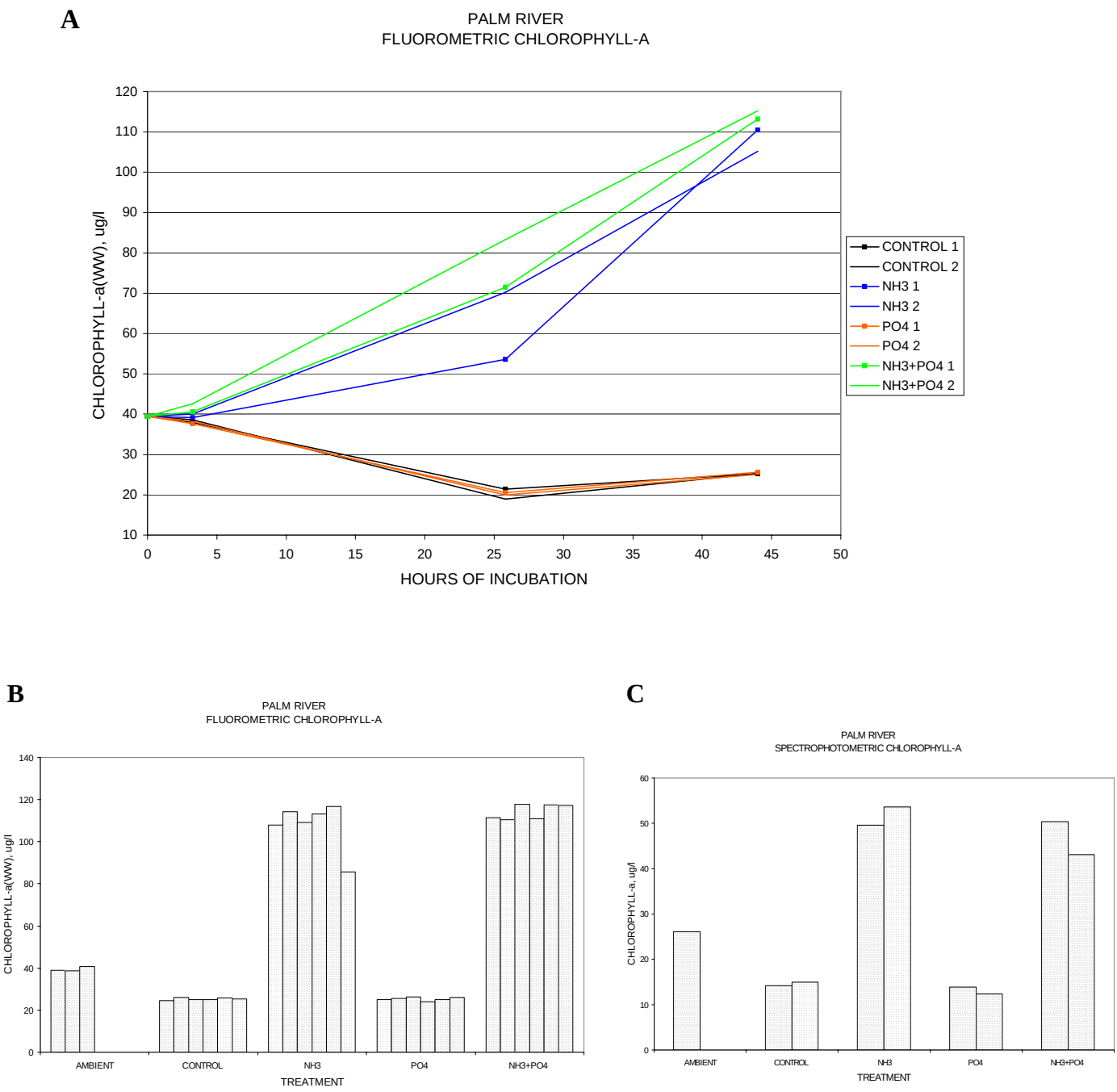

Figure 1. Graph A shows fluorometric chlorophyll-a concentrations measured during the progress of the Palm River bioassay experiment. Each data point is the average of three measurements. Graph B shows all measurements of fluorometric chlorophyll-a at the start of the Palm River bioassay experiment (ambient) and at the termination of the experiment. Graph C shows all measurements of spectrophotometric chlorophyll-a at the start of the Palm River bioassay experiment (ambient) and at the termination of the experiment.

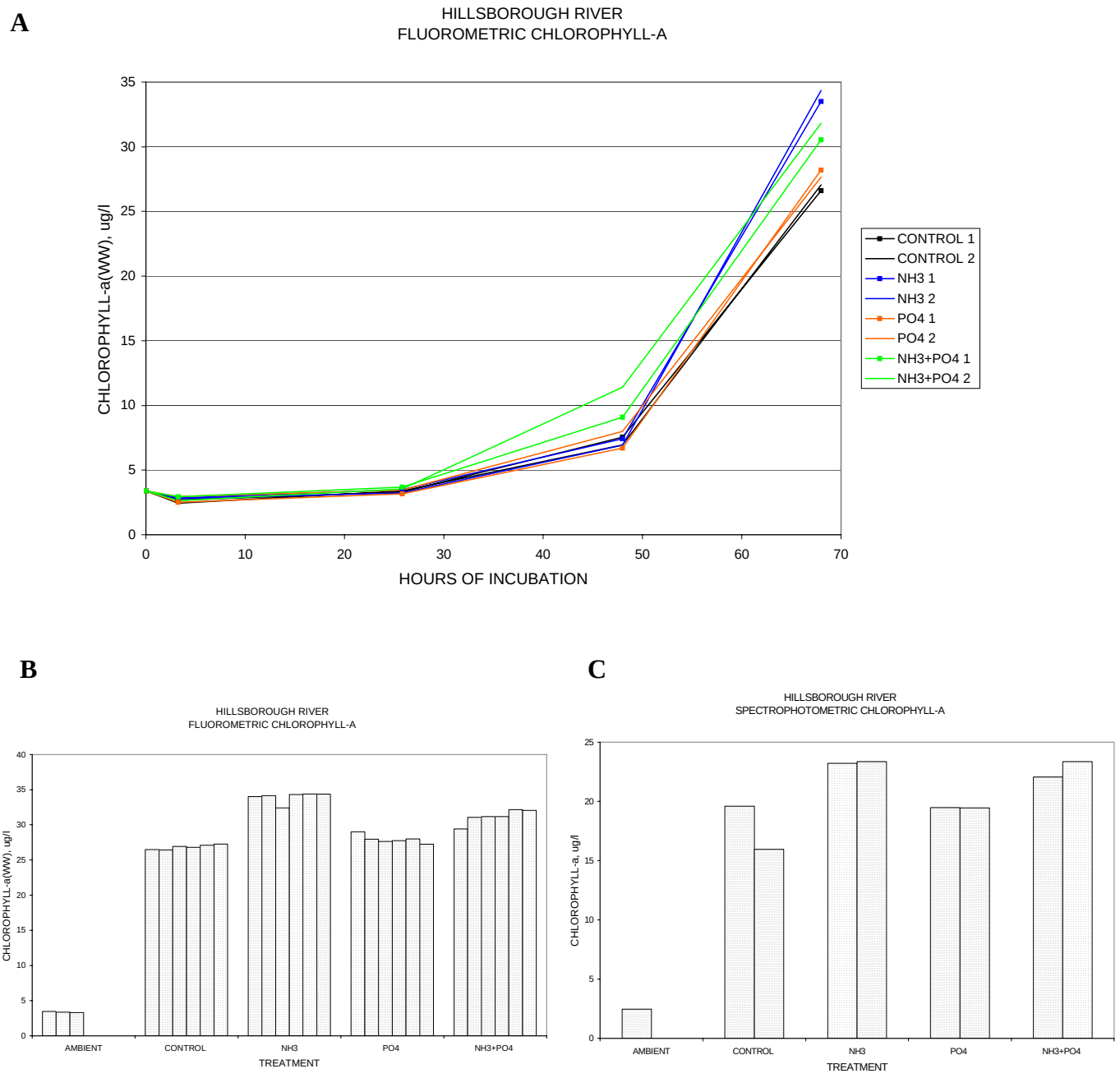

Figure 2. Graph A shows fluorometric chlorophyll-a concentrations measured during the progress of the Hillsborough River bioassay experiment. Each data point is the average of three measurements. Graph B shows all measurements of fluorometric chlorophyll-a at the start of the Hillsborough River bioassay experiment (ambient) and at the termination of the experiment. Graph C shows all measurements of spectrophotometric chlorophyll-a at the start of the Hillsborough River bioassay experiment (ambient) and at the termination of the experiment.