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Nutrient enrichment studies of natural phytoplankton populations in the Lower Hillsborough River conducted on October 10 - 13, 2005

The City of Tampa Wastewater Department

NUTRIENT ENRICHMENT STUDIES OF NATURAL PHYTOPLANKTON POPULATIONS IN THE LOWER HILLSBOROUGH RIVER CONDUCTED ON OCTOBER 10 - 13, 2005

BY

THE CITY OF TAMPA
WASTEWATER DEPARTMENT
BAY STUDY GROUP

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INTRODUCTION

The Bay Study Group (BSG) conducted nutrient enrichment studies (bioassays) on natural phytoplankton population samples collected at four locations in the Lower Hillsborough River on October 10, 2005. Test site HR1 was located approximately 320m upriver of the Rowlett Park Blvd. bridge (Lat: 28^{o} 01.368'N; Lon: 82^{o} 25.914'W), test site HR2 was located approximately 200m upriver of the Nebraska Ave. bridge (Lat: 28^{o} 01.174'N; Lon: 82^{o} 26.960'W), test site HR3 was located approximately 280m downriver of the Florida Ave. bridge (Lat: 28^{o} 01.257'N; Lon: 82^{o} 27.740'W), and test site HR4 was located approximately 20m upriver of the Sligh Ave. bridge (Lat: 28^{o} 00.655'N; Lon: 82^{o} 27.897'W).

METHODS

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

Samples for nutrient analyses were collected and preserved by the BSG, however, the analyses were kindly provided by the Hillsborough County Environmental Protection Commission (HCEPC). Nutrient samples were collected at the time of sampling from the four test sites, from all treatment containers following the treatment additions prior to the start of the experiments, and from all treatment containers at the termination of the experiments.

Chlorophyll-a was analyzed using both spectrophotometric methods and an extracted whole water fluorometric method. Samples for spectrophotometric chlorophyll-a analyses were collected from the ambient surface water of the four test sites and from all treatment containers at the termination of the experiments. Fluorometric chlorophyll-a samples were collected from the ambient surface samples and from all treatment containers on day 1, 2, and 3 of the experiments.

The taxonomic composition of the ambient phytoplankton population of the four test sites and the population composition present in the HR1 and HR4 treatment containers at the termination of the experiments, were identified and enumerated to major taxonomic groups.

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, 1992b and 1999; and Fisher and Gustafson 2003). A summary of the method used by the BSG is provided here.

A large volume of surface water was used to provide the nutrient treatments listed below. Further, each treatment consisted of duplicate three-liter samples contained in four-liter capacity cubitainers:

- Control (no nutrient addition),
- Nitrogen (N) addition (25uM of NH3-N added as ammonium chloride salt),
- Phosphorous (P) addition (10uM of PO4-P added as potassium phosphate salt), and
- N+P addition (combination of the N and P addition).

The respective treatment concentrations at the start of the experiments are shown in Table 3.

The bioassay treatment containers were incubated outside under natural sunlight in a tap water fed deck incubator. The incident radiation reaching the treatment containers 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 maintained at approximately 29C. Experiments for all four sites started at 19:22 on October 10, 2005. All experiments were terminated on October 13, 2005. The tests for sites HR1and HR4 were ended at 09:35, the HR3 test was ended at 12:06, and the HR2 test was ended at 13:20.

BIOASSAY RESPONSE DECISION APPROACH

Changes of algal biomass, measured as chlorophyll-a, were used to determine the response of the natural phytoplankton community to the different nutrient addition treatments.

A treatment was considered significantly greater than the Control if the chlorophyll-a was >120% of the Control. Analyses of bioassay data by Fisher and Gustafson (2003) have shown that cumulative experimental errors approach 20%.

The possible response categories of treatments that were significantly greater than the Control are listed below (see Fisher and Gustafson 2003). A conceptual model illustrating these response categories is shown in Figure 1. The possible categories are:

- Exclusive Nitrogen limitation,
- Primary Nitrogen limitation,
- Balanced Nitrogen and Phosphorus limitation,
- Primary Phosphorus limitation, and
- Exclusive Phosphorus limitation.

Bioassays in which no significant responses to nutrient additions occurred would be classified as “No Response”. Further, bioassay results would be deemed “Inconsistent” if chlorophyll-a in two or more replicate containers in any treatment are < 75% of the Control. Other potential bioassay results not conforming to the conceptual model would be classified as “Inconsistent.”

Bioassays classified as “No Response” have been interpreted by Fisher and Gustafson (2003) as an indication of nutrient saturation and/or light limitation. Ambient light limitation may also be indicated when chlorophyll-a concentrations in the Control show a significant increase in

biomass above the ambient concentrations at the onset of the experiment.

RESULTS

Ambient water quality conditions and field observations at the four river sample locations on October 10, 2005 are shown in Table 1.

Table 1. Ambient water quality conditions and observations at the four Lower Hillsborough River sample locations on October 10, 2005

Parameter	**Test Site**			
	HR1	**HR2**	**HR3**	**HR4**
Time (hhmm)	1311	1359	1459	1529
Surface temp. (C)	28.6	28.0	28.1	28.2
Surface DO (mg/l)	7.6	5.3	5.1	4.9
Surface salinity (PSU)	0.1	0.3	1.2	1.8
pH	7.5	7.3	7.2	7.2
Secchi depth (m)	>Water column depth	1.6	1.8	1.6
Water column depth (m)	1.5	3.1	4.1	3.1
Apparent water color	Clear brown	Clear brown	Clear brown	Clear brown
Water color (PCU; 345uM)	53.4	53.5	49.8	45.6
Surface flow (m/s)	0.18 Downstream	0.12 Downstream	0.22 Downstream	0.15 Downstream
Turbulence	None observed	None observed	None observed	None observed
Turbidity (NTU)	2.2	1.2	1.2	1.3
Percent light transmission (660nm; 10cm)	82.4	88.5	87.9	86.9
Chlorophyll-a (ug/l; trichromatic-spectrophotometric)	10.71	3.54	3.10	3.83
Chlorophyll-a (ug/l; fluorometric)	12.28	4.90	5.45	6.07

Ambient surface nutrient concentrations at the four river sample locations are shown in Table 2. Nutrient analyses were provided by the HCEPC.

Table 2. Ambient surface nutrient concentrations at the four Lower Hillsborough River sample locations on October 10, 2005.

Measured Nutrient Parameter (uM)	**Test Site**			
	HR1	**HR2**	**HR3**	**HR4**
NH3	5.7	6.4	6.4	6.4
NO3+NO2	3.2	6.6	8.9	13.0
TKN	52.1	52.1	47.9	45.7
TN	55.4	58.7	56.7	58.7
PO4	5.2	6.5	6.5	6.5
TP	6.8	8.1	8.1	8.1

All treatment containers were sub-sampled for nutrient analyses following the nutrient additions and just prior to the start of the experiments. The average concentrations of the duplicate containers for each treatment are shown in Table 3. To further illustrate Table 3 and to explain the four bioassay treatment combinations: (1) The Control treatment containers received no nutrient additions and nutrient concentrations in these containers should be similar to the ambient nutrient concentrations shown in Table 2: (2) The N treatment containers contained the NH3 addition in addition to the ambient nutrient concentrations: (3) The P treatment containers contained the PO4 addition in addition to the ambient nutrient concentrations: (4) The N+P treatment containers contained both the NH3 and the PO4 additions plus the ambient nutrient concentrations.

Table 3. Concentrations of nutrients in treatment containers of the four Lower Hillsborough River sample locations following nutrient additions and just prior to the start of incubation on October 10, 2005.

Measured Nutrient Parameter (uM)	**Test Site HR1 Nutrient Treatments**			
	Control	**N**	**P**	**N+P**
NH3	5.0	28.9	4.6	23.9
NO3+NO2	3.4	3.4	3.4	2.9
TKN	50.7	78.6	52.5	79.3
TN	54.1	81.9	55.9	82.1
PO4	6.3	6.5	16.3	16.5
TP	8.5	8.5	19.2	19.4
	Test Site HR2 Nutrient Treatments			
	Control	**N**	**P**	**N+P**
NH3	6.4	30.4	5.7	25.4
NO3+NO2	7.0	7.0	7.0	5.9
TKN	49.3	78.6	51.1	80.7
TN	56.3	85.5	58.1	86.6
PO4	6.8	6.0	16.9	16.8
TP	8.1	6.9	19.4	19.8
	Test Site HR3 Nutrient Treatments			
	Control	**N**	**P**	**N+P**
NH3	7.5	30.0	7.1	31.1
NO3+NO2	10.0	9.7	9.9	9.9
TKN	50.7	77.5	50.0	72.1
TN	60.8	87.2	59.9	82.0
PO4	6.6	6.8	16.6	16.9
TP	9.0	8.2	19.0	18.1
	Test Site HR4 Nutrient Treatments			
	Control	**N**	**P**	**N+P**
NH3	7.5	30.4	6.8	31.4
NO3+NO2	13.6	13.2	13.3	13.1
TKN	40.7	65.4	41.4	67.1

TN	54.4	78.5	54.7	80.2
PO4	6.1	6.5	17.4	16.9
TP	6.8	6.8	16.6	17.1

The nutrient concentrations of the bioassay treatment containers at the termination of the experiments are shown in Table 4. The concentrations shown are averages of the duplicate containers.

Table 4. Nutrient concentrations at the four Lower Hillsborough River sample locations at the time of bioassay termination October 13, 2005.

Measured Nutrient Parameter (uM)	**Test Site HR1 Nutrient Treatments**			
	Control	**N**	**P**	**N+P**
NH3	1.1	0.0	0.0	0.4
NO3+NO2	0.5	0.2	0.1	0.2
TKN	49.3	75.7	50.7	75.0
TN	49.8	75.9	50.8	75.2
PO4	3.1	3.1	14.8	14.5
TP	6.0	6.9	17.6	17.9
	Test Site HR2 Nutrient Treatments			
	Control	**N**	**P**	**N+P**
NH3	0.4	0.0	0.0	0.0
NO3+NO2	0.1	0.1	0.1	0.1
TKN	50.0	75.7	52.9	77.9
TN	50.1	75.9	63.3	77.9
PO4	4.2	3.4	15.6	12.9
TP	7.1	6.1	17.4	16.3
	Test Site HR3 Nutrient Treatments			
	Control	**N**	**P**	**N+P**
NH3	0.4	0.0	0.0	0.0
NO3+NO2	0.1	0.1	0.1	0.1
TKN	50.0	75.7	52.9	77.9
TN	50.1	75.9	63.3	77.9
PO4	4.2	3.4	15.6	12.9
TP	7.1	6.1	17.4	16.3
	Test Site HR4 Nutrient Treatments			
	Control	**N**	**P**	**N+P**
NH3	1.4	3.6	1.8	3.6
NO3+NO2	0.1	0.1	0.2	0.1
TKN	54.6	76.1	51.1	73.6
TN	54.8	76.2	51.3	73.7
PO4	3.4	1.3	12.1	8.7
TP	6.6	6.1	16.8	16.1

A comparison of nutrient concentrations between the start and the termination of the experiments

shows that virtually all dissolved inorganic nitrogen (DIN; NH3 and NO3+NO2) was utilized in the N and N+P treatments for test sites HR1, HR2, and HR3. Minor amounts of NH3 remained in these treatments for site HR4. Further, only small amounts of PO4 were utilized in the P and N+P treatments for test sites HR1, HR2, and HR3. The small amounts of PO4 utilized in the P and N+P treatments for these test sites was similar to that utilized in the Controls. The P and N+P treatments for test site HR4 indicated a somewhat higher PO4 utilization above that of the Control. However, as will be shown below, the biomass response of the phytoplankton for this test site clearly indicates that nitrogen was the limiting nutrient and that no significant biomass increase occurred in the P treatment above that of the Control (see Table 5 and Figure 5).

Results from the daily measurements of fluorometric phytoplankton biomass (chlorophyll-a) of all treatment containers during the progress of the experiments are shown in Figures 2A, 3A, 4A and 5A. Ambient chlorophyll-a concentrations and concentrations at the termination of the experiments, for both the fluorometric and spectrophotometric techniques, are shown in Figures 2B and 2C, 3B and 3C, 4B and 4C, and 5B and 5C. Further, the mean fluorometric chlorophyll-a concentrations of the six replicates for each treatment and study site at the termination of the experiments are shown in Table 5.

Table 5. The mean and one standard deviation of the fluorometric chlorophyll-a concentrations (n=6) for each treatment at the termination of the experiments for the four Lower Hillsborough River bioassay experiments.

Test Site	**Chlorophyll-a treatment response (ug/l)**			
	Control	**N**	**P**	**N+P**
HR1	20.30 (0.30)	104.0 (3.9)	21.68 (1.53)	106.9 (3.4)
HR2	32.06 (0.98)	105.3 (3.9)	33.50 (0.80)	107.5 (2.0)
HR3	49.79 (1.82)	155.8 (1.6)	52.19 (2.67)	161.2 (3.4)
HR4	52.51 (19.89)	144.7 (18.1)	44.44 (17.51)	177.4 (9.5)

Phytoplankton samples were analyzed for taxonomic composition and abundance of major taxonomic groups (diatoms, phytoflagellates, blue-greens, and green algae) for all ambient samples collected on October 10, 2005. Further, the same analyses were conducted on all treatment containers for test sites HR1 and HR4 at the termination of the experiments.

Phytoflagellates and diatoms were the most abundant groups identified in the ambient river samples. The relative abundance of phytoflagellates was smallest at the upstream HR1 test site and increased downstream, and the relative abundance of diatoms was greatest at test site HR1 and decreased downstream. Blue-greens and green algae were relatively rare at all sites. These groups combined did not comprise more than 6 percent of the total population at any site. Further, test site HR1 had by far the highest total phytoplankton abundance of all sites.

Phytoflagellates and diatoms were also the dominant phytoplankton groups present at the termination of the experiments for test sites HR1 and HR4. For test site HR1 all nutrient treatments indicated a loss in abundance of both major groups. For test site HR4 all nutrient treatments indicated a minor increase in abundance of diatoms and a substantial increase in phytoflagellates.

INTREPRETATION OF BIOASSAY RESULTS

The percent changes in fluorometric and spectophotometric chlorophyll-a concentrations relative the control at the termination of the bioassay experiments were calculated and used to interpret to the phytoplankton biomass responses according to the decision rules outlined above. The calculated percentages are shown in Table 6.

Table 6. Average percent change in fluorometric (n=6) and spectrophotometric (n=2) chlorophyll-a concentrations relative to the Control treatments at the termination of the experiments for the four Lower Hillsborough River bioassay experiments.

Test Site	**Percent change in fluorometric chlorophyll-a concentrations relative to the Control treatment**		
	N	**P**	**N+P**
HR1	412.4	6.8	426.9
HR2	228.4	4.5	235.4
HR3	213.0	5.0	224.0
HR4	175.6	-15.4	237.8
	Percent change in spectrophotometric chlorophyll-a concentrations relative to the Control treatment		
	N	**P**	**N+P**
HR1	433.8	2.0	437.6
HR2	231.9	-0.6	228.6
HR3	231.5	13.4	239.9
HR4	171.7	-14.5	219.5

The fluorometric and spectrophotometric chlorophyll-a analyses resulted in identical assignments to response categories. All N and N+P treatments had a >120 percent increase in chlorophyll-a above the Control and all P treatments had a < 120 percent increase in chlorophyll-a above the Control.

Based on changes in chlorophyll-a and interpretation of the conceptual model illustrating possible bioassay response categories shown in Figure 1, nutrient limitation at the four test sites of the Lower Hillsborough River were classified as shown in Table 7.

Table 7. Summary of bioassay response results from chlorophyll-a concentrations at the termination of the experiments for the four Lower Hillsborough River bioassay experiments

Test Site	**Incubation time (hr)**	**Response**
HR1	62	Exclusive Nitrogen limitation
HR2	66	Exclusive Nitrogen limitation
HR3	65	Exclusive Nitrogen limitation
HR4	62	Exclusive Nitrogen limitation

The response of the phytoplankton for the four tests sites clearly indicate that nitrogen was the limiting nutrient at all sites. Based on interpretation of the conceptual model (Figure 1), all test sites were categorized as being exclusively limited by nitrogen.

CONCLUSION

The results presented in this report clearly indicate that the natural phytoplankton community present at all four test sites of the Lower Hillsborough River were limited by nitrogen. There was no indication that the phytoplankton population was limited by phosphorus.

REFERENCES

City of Tampa. 1992. Nutrient enrichment studies of Tampa Bay phytoplankton. A plan of study submitted to the Florida Department of Environmental Regulation, Southwest District. 6p.

Fisher, T.R., A.B. Gustafson, K.G. Sellner, and R.B. Lacouture.1992a. Nutrient bioassays in Chesapeake Bay to assess nutrients limiting algal growth. Progress Rep. MD Dept. of the Environment, Jan. 1992, Baltimore, MD.

Fisher, T.R., E.R. Peele, J.W. Ammerman, and L.W. Harding, Jr. 1992b. Nutrient limitation of phytoplankton in Chesapeake Bay. Mar. Ecol. Prog. Ser., 82:51-63.

Fisher, T.R. A. B. Gustafson, K. Sellner, R. Lacouture, L. W. Haas, R. L. Wetzel, R. Magnien, D. Everitt, B. Michaels, R. Karrh. 1999. Nutrient limitation of phytoplankton in Chesapeake Bay. Marine Biology 133:763-778.

Fisher, T. R. A. B. Gustafson. 2003. Progress Report: Aug 1990-Dec 2002. Nutrient-Addition Bioassays in Chesapeake Bay to Assess Resources Limiting Algal Growth. Report to the Maryland Department of Natural Resources. July 2003.

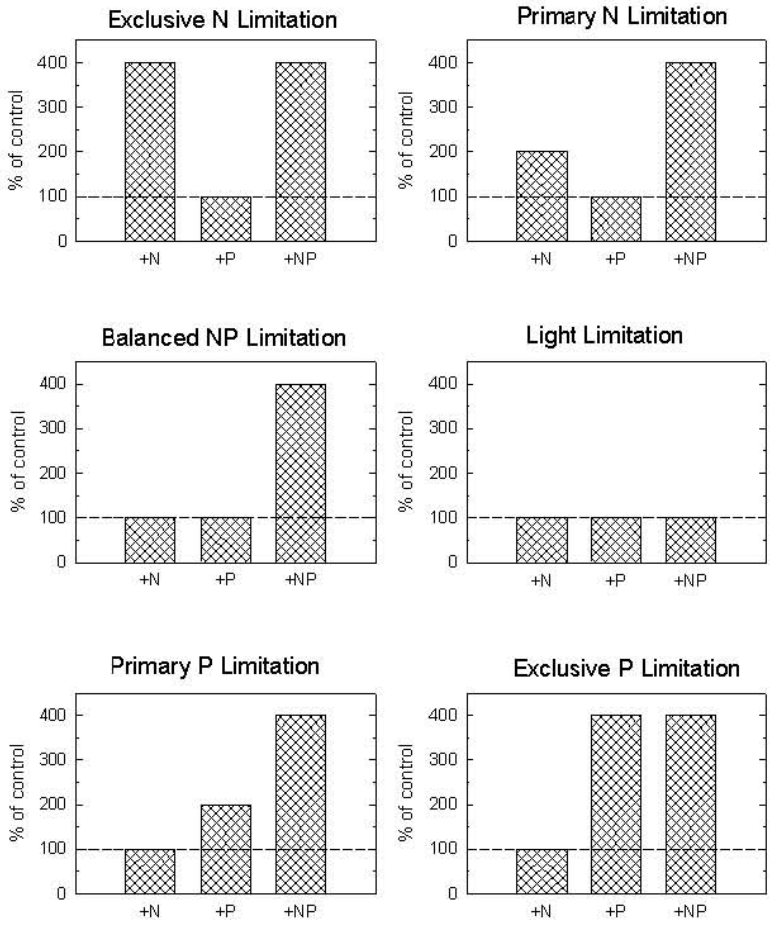
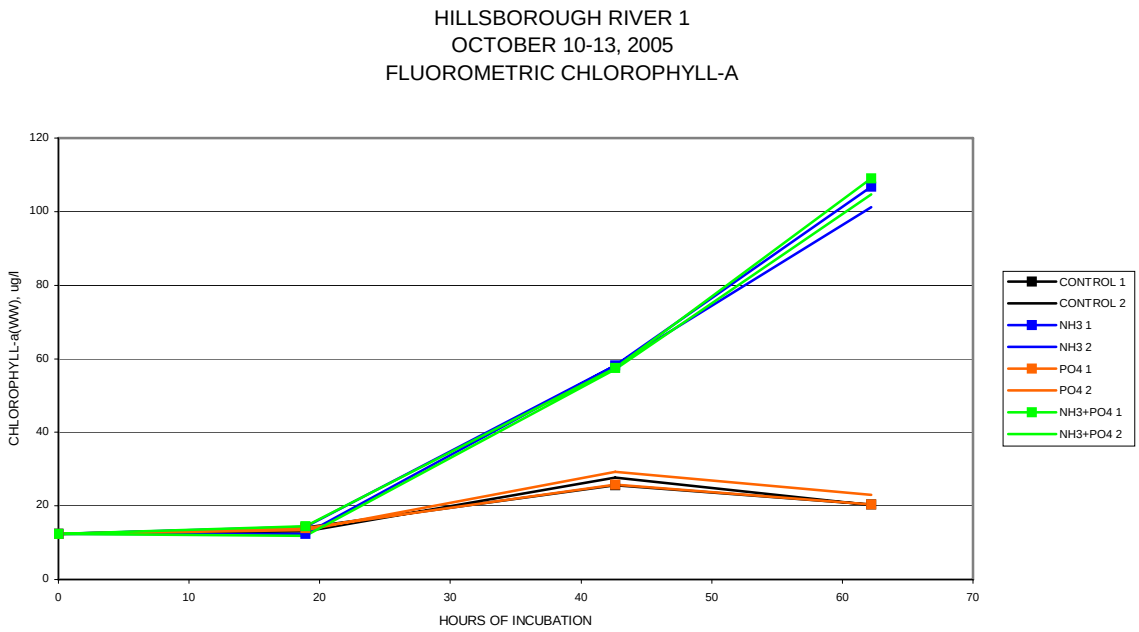


Figure 1. Conceptual model for classification of nutrient addition bioassays (From Fisher and Gustafson 2003).

A



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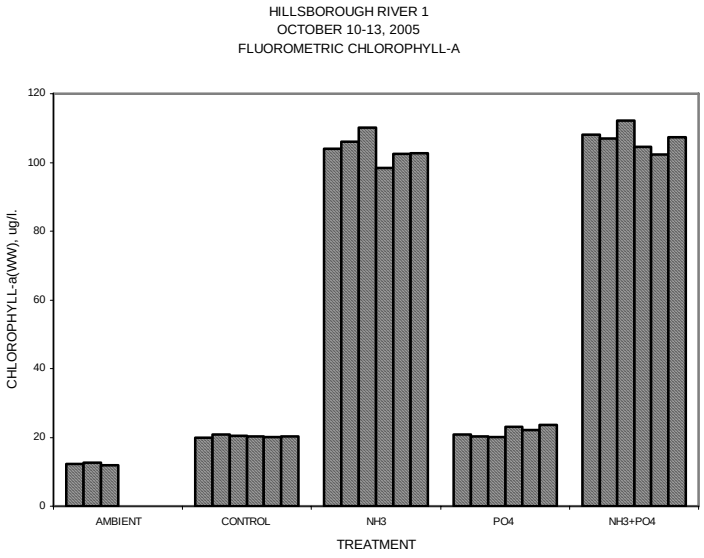

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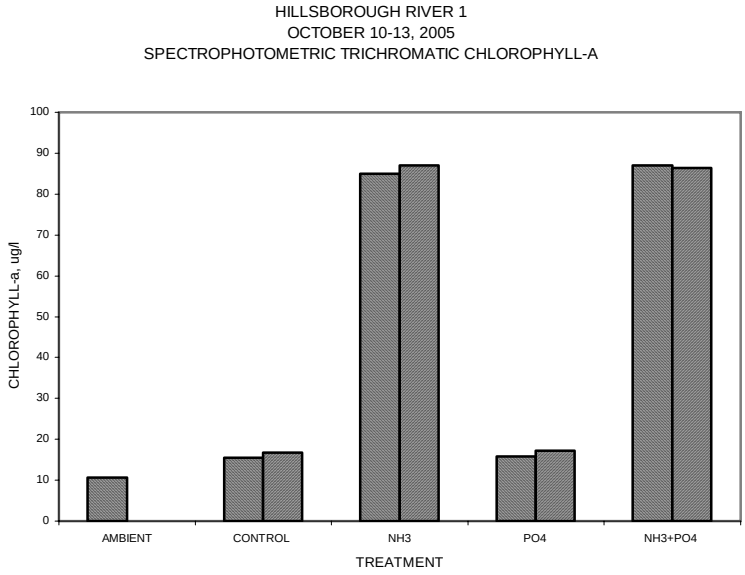
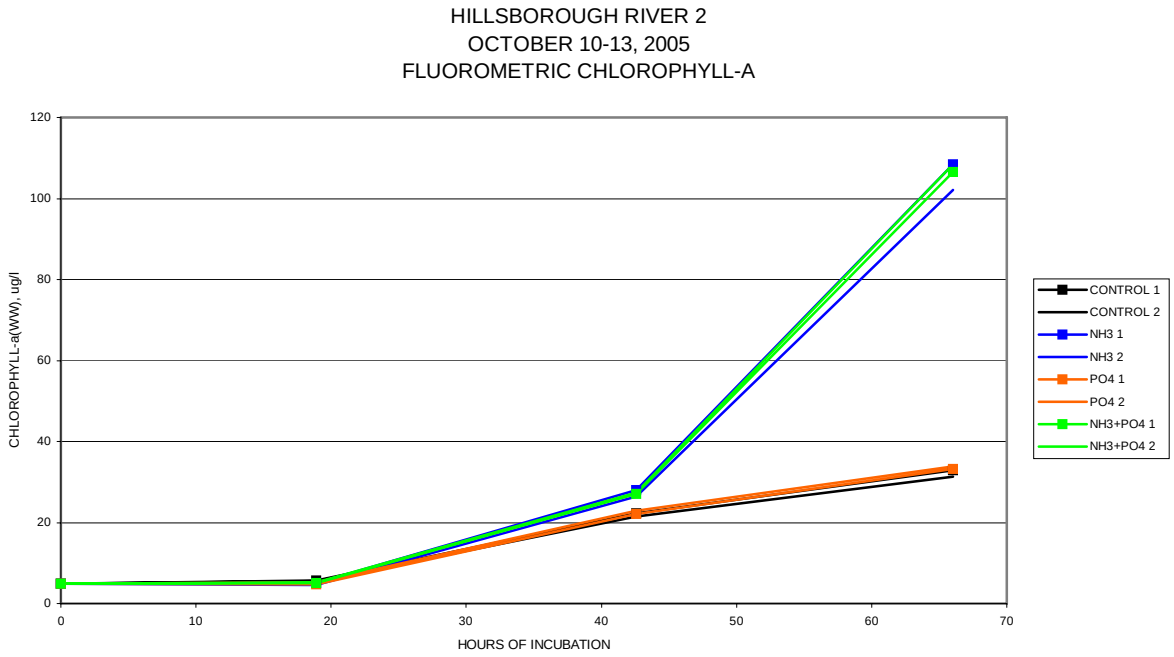


Figure 2. Graph A shows fluorometric chlorophyll-a concentrations measured during the progress of the Lower Hillsborough River site HR1 bioassay experiment. Each data point is the average of three measurements. Graph B shows all measurements of fluorometric chlorophyll-a at the start of the HR1 bioassay experiment (ambient) and at the termination of the experiment. Graph C shows all measurements of spectrophotometric chlorophyll-a at the start of the HR1 bioassay experiment (ambient) and at the termination of the experiment.

A



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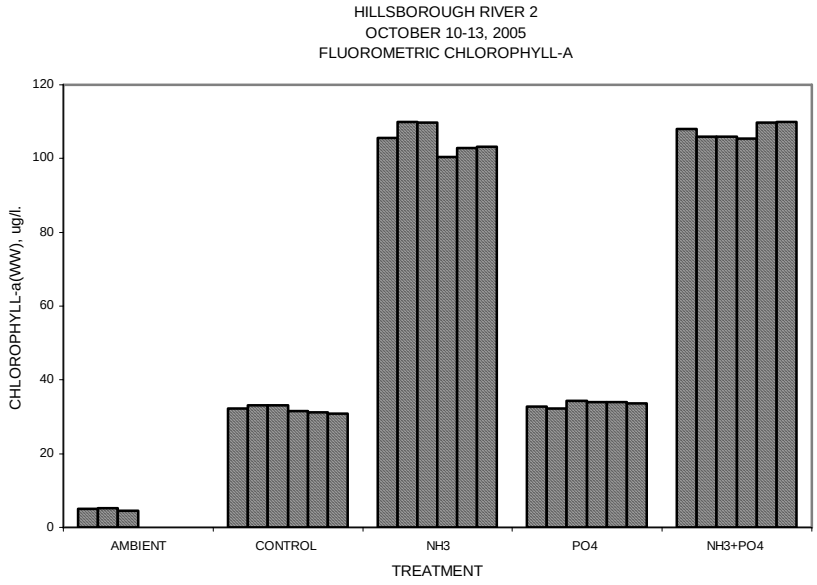

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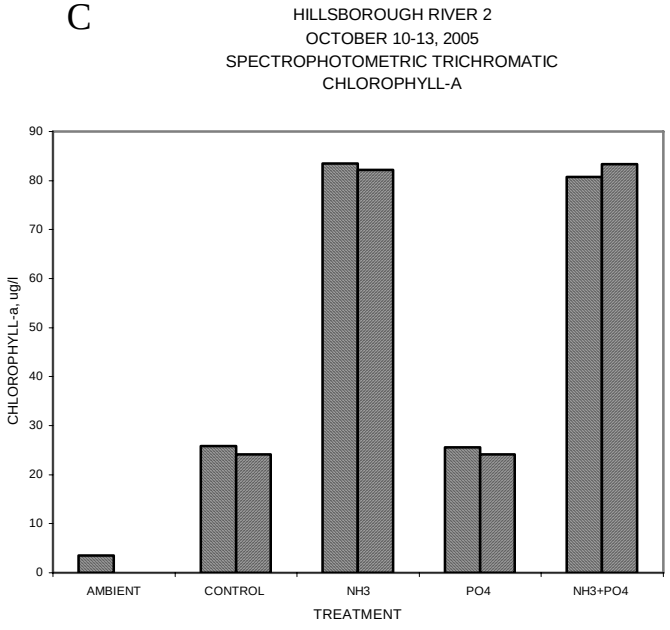
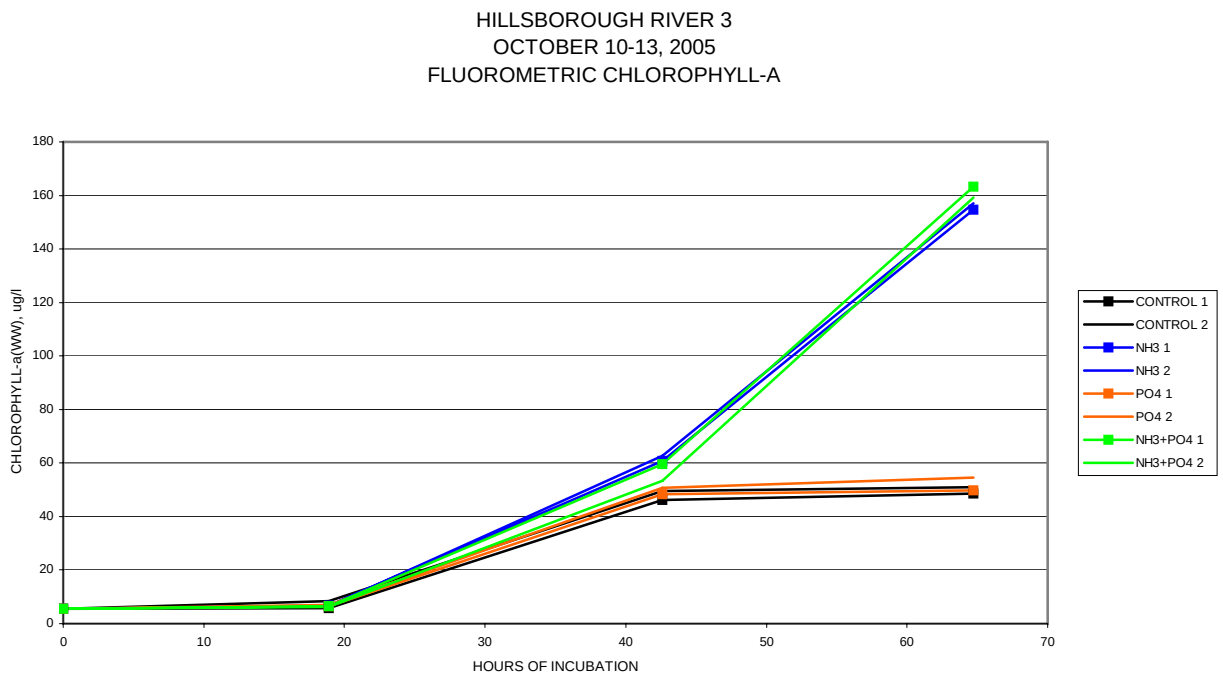


Figure 3. Graph A shows fluorometric chlorophyll-a concentrations measured during the progress of the Lower Hillsborough River site HR2 bioassay experiment. Each data point is the average of three measurements. Graph B shows all measurements of fluorometric chlorophyll-a at the start of the HR2 bioassay experiment (ambient) and at the termination of the experiment. Graph C shows all measurements of spectrophotometric chlorophyll-a at the start of the HR2 bioassay experiment (ambient) and at the termination of the experiment.

A



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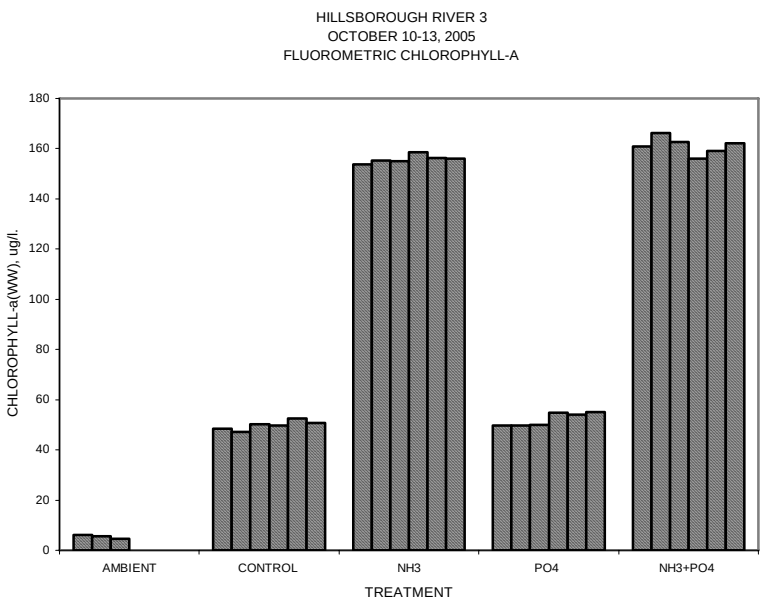

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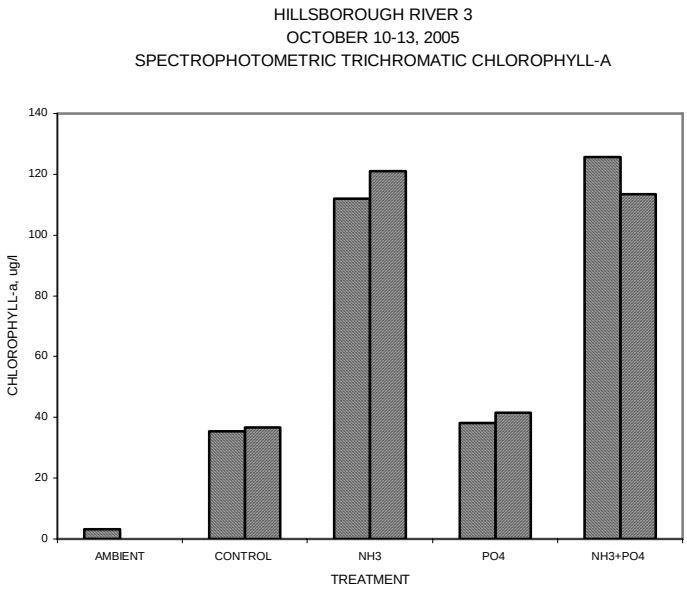
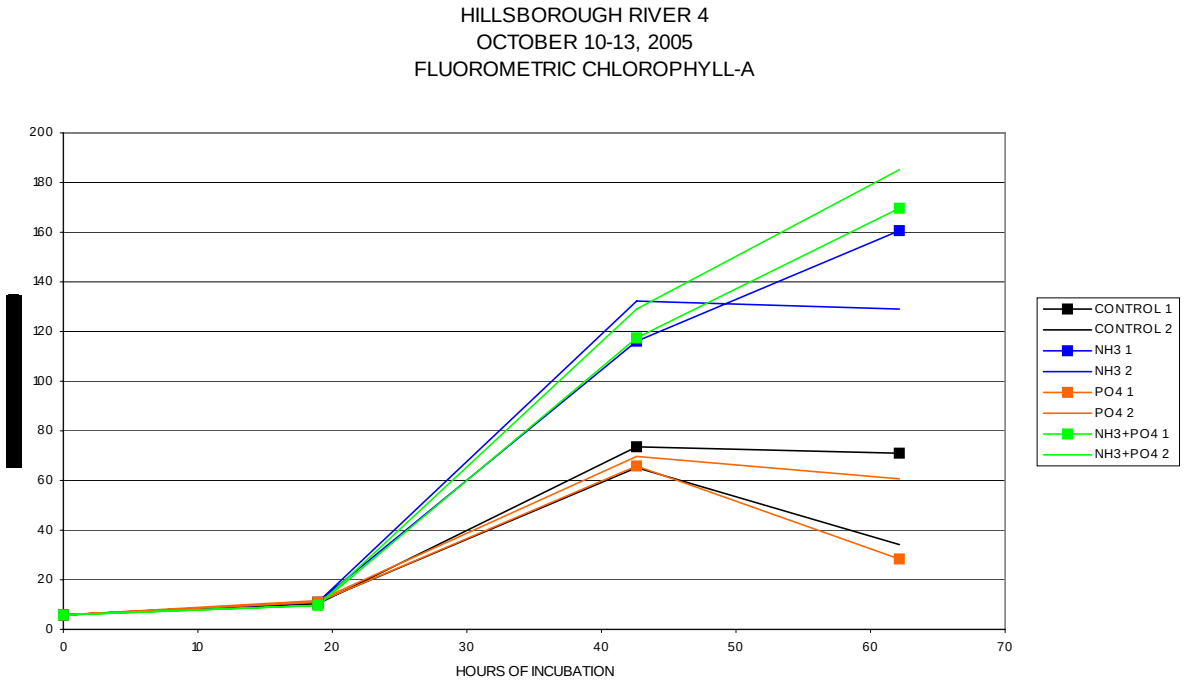


Figure 4. Graph A shows fluorometric chlorophyll-a concentrations measured during the progress of the Lower Hillsborough River site HR3 bioassay experiment. Each data point is the average of three measurements. Graph B shows all measurements of fluorometric chlorophyll-a at the start of the HR3 bioassay experiment (ambient) and at the termination of the experiment. Graph C shows all measurements of spectrophotometric chlorophyll-a at the start of the HR3 bioassay experiment (ambient) and at the termination of the experiment.

A



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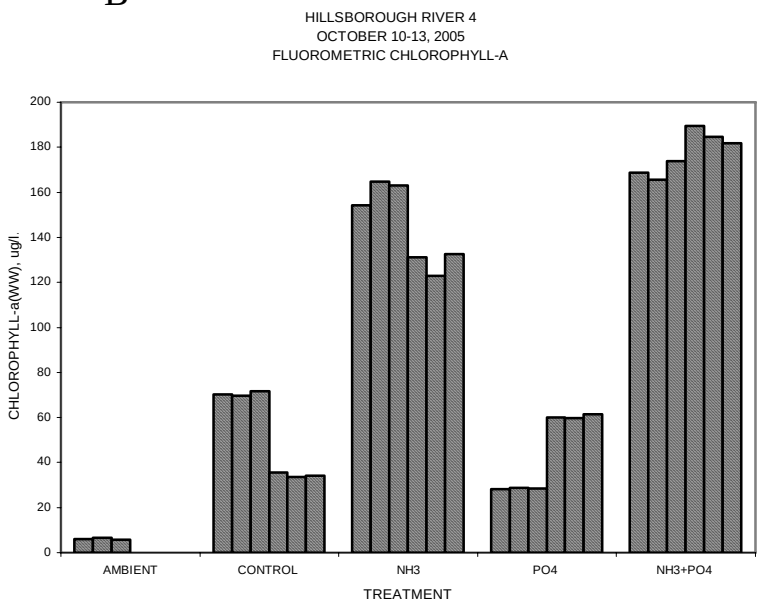

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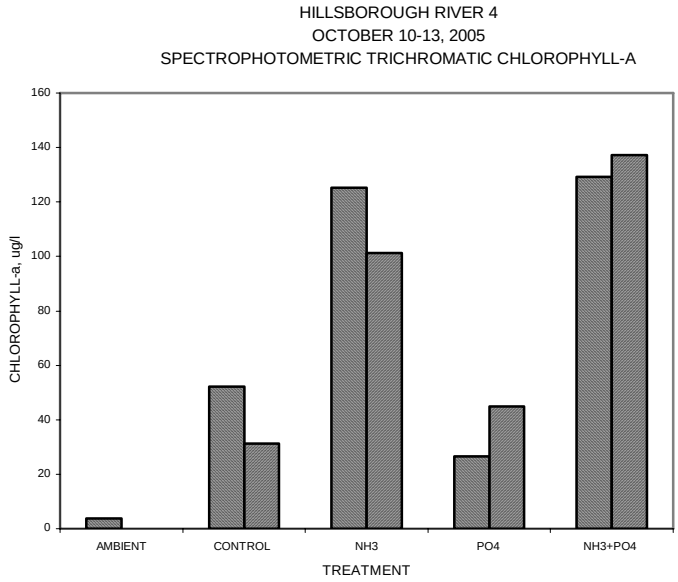

Figure 5. Graph A shows fluorometric chlorophyll-a concentrations measured during the progress of the Lower Hillsborough River site HR4 bioassay experiment. Each data point is the average of three measurements. Graph B shows all measurements of fluorometric chlorophyll-a at the start of the HR4 bioassay experiment (ambient) and at the termination of the experiment. Graph C shows all measurements of spectrophotometric chlorophyll-a at the start of the HR4 bioassay experiment (ambient) and at the termination of the experiment.