University of Puerto Rico
College of Agricultural Sciences
Agronomy and Soils Department

DETERMINATION OF NUMERIC NUTRIENT TARGET CRITERIA IN LAKES AND RESERVOIRS OF PUERTO RICO

THIRD PROGRESS REPORT
ENCOMPASSING PERIOD FROM
JUNE 1ST, 2003 TO DECEMBER 25TH, 2004

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OBJECTIVE OF THE PROJECT

1. Develop numeric criteria for nutrients (nitrogen and phosphorus) in lakes of Puerto Rico.
2. Evaluate phytoplankton diversity in lakes of Puerto Rico.

DESCRIPTION OF PROGRESS

CHEMICAL ANALYSES OF WATER SAMPLES

A total of six sampling events have been completed to date (Table 1).

Table 1: Sampling events completed to this point:

Sampling event	Dates	Progress Reports
1	8/12/03 – 9/2/03	
2	11/7/03 – 12/10/03	11/25/03
3	2/23/04 – 3/18/04	5/29/04
4	6/7/04 – 6/21/04	
5	8/9/04 – 8/25/04	
6	11/16/04 – 12/9/04	12/27/2004

Results of the first three events have been presented in previous progress reports (November 25th 2003, and May 29th 2003). One hundred-fifty eight (158) samples were collected between sampling events 4 -6. All samples were analyzed for nutrients (TP, TKN), DOC and chlorophyll *a*, whereas one hundred-three (103) samples (sampling events 5 and 6) were submitted for periphyton diversity characterization.

Samples were analyzed for total kjedahl nitrogen (EPA method 351.2), dissolved reactive P and total P (EPA method 355.2). Estimates of the amount of dissolved organic carbon (DOC) were obtained by quantifying the absorbance (280nm) of filtrated samples (<0.45µm cellulose fiber

filter) on a DU-520 Beckman UV/VIS spectrophotometer after calibration with an organic carbon standard (Lab. Chem. Inc.).

LABORATORY RESULTS

A summary of the chemical analyses results for sampling events 4 - 6 is shown in Tables 2 -7 respectively (a full QA/QC report is available on request).

Table 2: Chemical analyses results of lake samples from the June (2004) sampling event.

PREQBs Sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	CHL a (ug/l)
Las Curias-01-sec 2 entrada	0.62	0.045	0.018	5.44	4.77
Las Curias-01-sec 2 entrada FD	0.60	0.043	0.010	5.43	5.02
Las Curias-02-sec 4 represa	0.48	0.049	0.010	5.42	8.75
La Plata-01-sec 1-entrada 44400	0.34	0.061	0.010	3.41	40.90
La Plata-01-sec 1FD-44400	0.36	0.053	0.010	3.33	34.64
La Plata-02-sec 3 - represa-44950	0.25	0.028	0.010	3.35	7.91
Carraizo-01-sec 2 57500	0.37	0.139	0.10	5.32	10.88
Carraizo-01-sec 2 FD 57500	0.38	0.151	0.10	5.30	5.76
Carite-01 sec 1 desemb rio plata 39900	0.40	0.010	0.010	2.35	6.57
Carite-01 sec 1 desemb rio plata 39900 FD	0.34	0.010	0.010	2.27	4.66
Carite-02 sec 4 represa 39950	0.31	0.011	0.010	2.15	5.56
Patillas entrada rio patillas	0.34	0.027	0.010	1.59	2.20
Patillas-02 sec 4-rio marín	0.43	0.018	0.010	1.58	2.21

Table 2 (cont.)

Patillas-03 sec 5-centro	0.39	0.020	0.010	1.59	2.66
Patillas-04 sec 7-represa	0.42	0.028	0.010	1.43	1.92
Guayo-01 sec 2 89004 near rio	0.50	0.026	0.010	2.33	6.04
Guayo-01 sec 2 89004 near rio	0.41	0.019	0.010	2.29	5.52
Guayo-02 sec 4 89005 center	0.28	0.053	0.010	2.14	6.74
Guayo Represa est 3 sec 6	0.19	0.019	0.010	1.94	10.07
Caonillas-01-sec 1	0.59	0.080	0.010	2.78	35.08
Caonillas-01-sec 1 FD	0.51	0.062	0.010	3.09	9.24
Caonillas-02-sec 3 centro	0.51	0.051	0.010	2.60	9.39
Caonillas-03 sec 5 represa	0.34	0.026	0.010	2.40	7.91
Matrullas-01 sec 2 entrada	0.39	0.024	0.010	2.33	8.72
Matrullas-01 sec 2 entrada FD	0.28	0.025	0.010	2.70	8.27
Matrullas-02 sec 3-represa	0.28	0.017	0.010	1.89	20.81
Cerrillo-01 entrada	0.33	0.044	0.013	1.98	13.54
Cerrillo-01FD entrada	0.43	0.045	0.013	1.99	99.09
Cerrillo 02 sec 5 centro	0.35	0.026	0.010	1.82	5.70
Cerrillo- 03 sec 8 represa	0.29	0.017	0.010	1.88	4.88
Cidra-01 sec 3-89029	0.64	0.061	0.010	5.87	30.26
Cidra-02 sec 4 center 89030	0.48	0.054	0.010	4.88	24.43
Cidra-03 sec 6 dam 89031	0.42	0.062	0.010	5.22	29.48
Guineo est 1 sec 1 desemb. Toro negro	0.28	0.027	0.014	2.92	4.19
Guineo est 1 sec 1 desemb. Toro negro	0.24	0.030	0.013	3.01	30.67

Table 2 (cont.)

Guineo est 2 sec 3 represa	0.16	0.014	0.010	2.61	3.91
Guajataca 10720 sec1 entrada	0.36	0.012	nd	2.49	5.07
Guajataca 10720 sec1 FD	0.33	0.013	nd	2.71	4.08
Guajataca 10790 sec3 represa	0.29	0.010	nd	2.43	3.18
Dos Bocas 25110sec3	0.24	0.062	0.020	1.66	46.08
Dos Bocas 25110sec3 FD	0.25	0.042	0.021	1.72	39.23
Dos Bocas 27090 sec6 represa	0.21	0.021	0.010	1.66	0.90
Melania sec 2 FD centro	0.49	0.037	0.011	3.50	4.39
Melania sec 2 centro	0.54	0.042	0.010	3.40	5.64
Loco represa 89021 sec2	0.34	0.048	0.010	3.04	7.37
Loco represa 89021 sec2	0.25	0.053	0.010	2.98	5.91
Luchetti 01 sec 1 entrada 89017	0.50	0.074	0.010	2.94	24.69
Luchetti 02 sec 3 centro 89018	0.46	0.060	0.010	2.74	44.47
Luchetti 03 sec 5 represa 89019	0.44	0.047	0.010	2.73	33.21
Garzas 20050 - sec 2	0.27	0.016	0.010	1.61	8.01
Garzas 20050 - sec 2FD	0.27	0.015	0.010	1.68	8.14
Guayabal est 2 sec 2 centro	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Guayabal est 2 sec 2 centroFD	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Guayabal est 3 sec 5 represa	Not sampled	Not sampled	Not sampled	Not sampled	Not sampled
Toa Vaca 01 sec 1 entrada	0.87	0.097	0.010	3.38	26.18
Toa Vaca 02 sec 3 centro	0.41	0.027	0.010	3.28	2.68
Toa Vaca 03 sec 5 frente represa	0.37	0.030	0.010	3.27	2.46
Toa Vaca 03 sec 5 frente represa	0.44	0.021	0.010	3.29	3.13

- Samples highlighted in red correspond to samples whose concentrations fall below our detection limit (0.01mg/L or 10 ppb in the case of phosphorus). Although a value was obtained we can not guarantee the accuracy of the result, and therefore the detection limit is reported as the result.
- nd refers to non detectable values.
- Samples highlighted in blue correspond to chlorophyll *a* filters whose filter bags were considerably wet when delivered. We can not establish how this could have affected the sample result.

Table 3: Descriptive Statistics for the June 04 sampling event

	TKN	TP	DP	DOC	Chl *a**
Median (mg/L)	0.37	0.030	0.007	2.70	7.31
Average(mg/L)	0.39	0.040	0.011	2.89	14.63
Std. Dev.	0.13	0.029	0.019	1.19	17.27
Max. (mg/L)	0.87	0.150	0.100	5.87	99.09
Min. (mg/L)	0.16	0.008	Nd	1.43	0.90

*µg/L

Table 4.: Results of samples from the August-September (2004) sampling event.

PREQBs Sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	CHL a (ug/l)
Las Curias-01-sec 3 entrada	0.40	0.035	nd	4.29	2.56
Las Curias-02-sec 4 represa	0.74	0.035	nd	3.99	2.35
La Plata-01-sec 1-entrada 44400	0.68	0.069	0.010	3.68	4.75
La Plata-01-sec 1FD-44400	0.61	0.100	0.010	3.60	4.58
La Plata-02-sec 3 – represa-44950	0.35	0.030	nd	3.20	2.43
Carraizo-01-sec 2 57500	1.01	0.234	0.24	5.03	1.80
Carraizo-02-58800 centro lago	0.72	0.097	0.048	5.39	82.87
Carite-01 sec 1 desemb rio plata 39900	0.24	0.022	nd	2.52	3.59
Carite-02 sec 4 represa 39950	0.26	0.020	nd	2.67	2.41
Patillas entrada rio patillas – 89022	0.30	0.026	nd	1.86	2.22

Table 4 (cont.)

Patillas entrada rio patillas - 89022 FD	0.20	0.027	nd	1.85	1.63
Patillas-02 sec 4-rio marin 89025	0.32	0.021	nd	1.95	0.77
Patillas-03 sec 5-centro 89023	0.27	0.025	0.010	1.97	3.27
Patillas-04 sec 7-represa	0.30	0.050	nd	1.75	2.14
Guayo-01 sec 2 89004 near rio	0.55	0.054	0.010	2.48	10.88
Guayo-01 sec 2 89004 near rio FD	0.51	0.045	0.010	2.50	10.64
Guayo-02 sec 4 89005 center	0.32	0.040	0.010	1.97	5.30
Guayo Represa est 3 sec 6	0.35	0.049	0.010	1.81	4.57
Caonillas-01-sec 189002	0.43	0.040	0.010	2.43	8.20
Caonillas-02-sec 3 centro	0.42	0.073	0.010	2.14	6.13
Caonillas-03 sec 5 represa	0.44	0.019	nd	1.91	4.95
Matrullas-01 sec 2 entrada	0.35	0.030	0.010	1.43	5.48
Matrullas-01 sec 2 entrada FD	0.33	0.024	0.010	1.76	6.15
Matrullas-02 sec 3-represa	0.70	0.021	0.010	1.52	7.16
Cerrillo-01 entrada 89032	0.73	0.060	0.010	2.24	9.30
Cerrillo-01FD entrada	0.67	0.055	0.010	2.19	7.54
Cerrillo 02 sec 5 centro 89033	0.50	0.045	nd	1.64	3.13
Cerrillo- 03 sec 8 represa 89034	0.39	0.029	nd	1.84	5.01
Cidra-01 sec 3-89029	0.50	0.036	nd	6.22	4.98
Cidra-01 sec 3-89029 FD	0.36	0.097	nd	4.99	6.36
Cidra-02 sec 4 center 89030	0.39	0.041	nd	4.14	4.81
Cidra-03 sec 6 dam 89031	1.12	0.035	nd	3.91	2.76

Table 4 (cont.)

Guineo est 1 sec 1 desemb. Toro negro	0.52	0.039	0.010	2.53	5.66
Guineo est 2 sec 3 represa	0.37	0.031	0.004	1.85	8.95
Guajataca 10720 sec1 entrada	0.45	0.028	nd	2.30	2.75
Guajataca 10790 sec3 represa	0.17	0.032	nd	2.31	1.38
Dos Bocas 25110sec3	0.27	0.213	0.14	2.87	62.53
Dos Bocas 25110sec3 FD	0.42	0.194	0.010	2.69	34.13
Dos Bocas 27090 sec6 represa	0.38	0.053	0.010	2.04	6.86
Melania sec 2 centro	0.55	0.047	0.010	3.99	3.51
Loco represa 89021 sec2	0.60	0.046	0.010	2.67	3.80
Luchetti 01 sec 1 entrada 89017	0.69	0.033	0.01	2.41	21.53
Luchetti 01 sec 1 entrada 89017 FD	0.49	0.023	0.010	2.29	5.20
Luchetti 02 sec 3 centro 89018	0.53	0.026	nd	2.15	9.93
Luchetti 03 sec 5 represa 89019	0.75	0.023	0.010	2.10	30.8
Garzas 20050 - sec 2	0.36	0.034	nd	2.61	3.50
Garzas 20050 - sec 2FD	0.45	0.038	nd	2.29	8.38
Toa Vaca 01 sec 1 entrada 89014	0.38	0.090	nd	3.13	5.78
Toa Vaca 01 sec 1 entrada 89014 FD	0.42	0.038	nd	3.10	4.21
Toa Vaca 02 sec 3 centro 89015	0.50	0.041	nd	2.87	2.14
Toa Vaca 03 sec 5 frente represa 89016	0.94	0.138	nd	2.92	1.28

- Samples highlighted in red correspond to samples whose concentrations fall below our detection limit (0.01mg/L or 10 ppb in the case of phosphorus). Although a value was obtained we can not guarantee the accuracy of the result, and therefore the detection limit is reported as the result.

-nd refers to non-detectable values.

Table 5: Descriptive Statistics for the August 04 sampling event

	TKN	TP	DP	DOC	Chl *a**
Median (mg/L)	0.43	0.038	0.001	2.43	4.95
Average(mg/L)	0.48	0.054	0.010	2.74	8.88
Std. Dev.	0.20	0.047	0.038	1.06	14.63
Max. (mg/L)	1.12	0.234	0.240	6.22	82.87
Min. (mg/L)	0.17	0.019	Nd	1.43	0.77

*µg/L

Table 6.: Chemical analyses results of lake samples from the November-December (2004) sampling event.

Sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	CHL a (ug/l)
Las Curias-01-sec 3 entrada	0.804	0.052	0.014	7.54	6.39
Las Curias-02-sec 4 represa	0.691	0.049	0.011	8.28	1.35
Las Curias-02-sec 4 represa FD	0.715	0.049	0.012	8.3	1.23
La Plata-01-sec 1- entrada 44400	0.351	0.06	0.025	2.35	19.22
La Plata-02-sec 3 -represa-44950	0.274	0.059	0.021	3.32	14.51
Carraizo-01-sec 2 57500	0.943	0.831	0.132	3.65	379.14
Carraizo-01-sec 2 57500 FD	1.219	1.139	0.168	3.55	480.18
Carraizo-02-58800 centro lago	0.281	0.047	0.015	3.59	11.72
Carite-01 sec 1 desemb rio plata 39900	0.251	0.015	0.010	2.14	1.24
Carite-01 sec 1 desemb rio plata 39900 FD	0.238	0.014	0.010	2.12	1.1
Carite-02 sec 4 represa 39500	0.22	0.014	0.010	2.01	2.12
Patillas entrada rio patillas – 89022	0.224	0.016	0.010	1.62	2.12
Patillas-02 sec 4-rio marin 89025	0.251	0.016	0.010	1.77	1.74
Patillas-03 dique-centro 89023	0.187	0.021	0.010	1.78	3.85
Patillas-04 sec 7- represa	0.283	0.015	0.010	1.78	2.31
Guayo-01 sec 2 89004 near rio	0.603	0.061	0.010	2.71	25.42
Guayo-02 sec 4 89005 center	0.572	0.044	0.010	2.82	23.13
Guayo Represa est 3 sec 6	0.485	0.034	0.010	2.92	19.48
Caonillas-01-sec 2 89002	0.721	0.073	0.012	3.05	25.08
Caonillas-01-sec 2 89002 FD	0.689	0.073	0.013	3.22	79.43
Caonillas-02-sec 5 centro	0.806	0.083	0.01	3.02	27.44
Caonillas-03 sec 8 represa	0.634	0.051	0.010	2.97	28.88
Matrullas-01 sec 2 entrada	0.375	0.049	0.02	1.74	7.99
Matrullas-02 sec 5-represa	0.371	0.038	0.010	1.84	5.83
Matrullas-02 sec 5-represa FD	0.419	0.043	0.010	1.69	37.04
Cerrillo-01 entrada 89032	0.271	0.026	0.010	1.95	3.47

Table 6 (cont.)

Cerrillo 02 sec 5 centro 89033	0.159	0.019	0.010	2.04	1.92
Cerrillo- 03 sec 8 represa 89034	0.171	0.016	0.010	1.86	4
Cerrillo- 03 sec 8 represa 89034 FD	0.193	0.016	0.010	1.9	4.15
Cidra-01 sec 3-89029	0.461	0.084	0.021	6.33	11.22
Cidra-02 sec 4 center 89030	0.786	0.088	0.036	6.85	5.43
Cidra-03 sec 6 dam 89031	0.631	0.076	0.037	7.26	1.81
Guineo est 1 89007 desemb. Toro negro	0.791	0.043	0.011	3.93	38.57
Guineo est 2 89008represa	0.265	0.021	0.010	3.09	3.65
Guajataca 10720 sec1 entrada	0.271	0.016	0.010	2.05	3.64
Guajataca 10720 sec1 entrada FD	0.275	0.015	0.010	2.08	3.41
Guajataca 10790 sec3 represa	0.263	0.015	0.010	2.03	3.92
Dos Bocas 25110sec3	0.449	0.121	0.024	1.07	30.97
Dos Bocas 27090 sec6 represa	0.337	0.041	0.010	1.3	7.16
Melania sec 2 centro	0.387	0.041	0.010	3.56	3.01
Melania sec 2 centro FD	0.40	0.035	0.010	3.44	2.08
Loco est 1 89020 sec 2	0.419	0.127	0.06	6.23	8.22
Loco represa 89021 sec5	0.341	0.029	0.010	1.98	7.02
Loco represa 89021 sec5 FD	0.394	0.031	0.010	1.84	8.78
Luchetti 01 sec 1 entrada 89017	0.693	0.097	0.014	2.13	45.11
Luchetti 02 sec 4 centro 89018	0.524	0.033	0.01	2.02	7.37
Luchetti 03 sec 7 represa 89019	0.46	0.026	0.010	1.84	7.06
Garzas 20050 - sec 2	0.306	0.028	0.010	1.13	7.38
Toa Vaca 01 sec 1 entrada 89014	0.408	0.033	0.011	3.25	6.21
Toa Vaca 02 sec 4 centro 89015	0.366	0.041	0.010	3.15	5.04
Toa Vaca 03 sec 7 frente represa 89016	0.352	0.034	0.014	3.11	3.9
Toa Vaca 03 sec 7 frente represa FD 89016	0.333	0.036	0.015	3.11	3.9

- Samples highlighted in red correspond to samples whose concentrations fall below our detection limit (0.01mg/L or 10 ppb in the case of phosphorus). Although a value was obtained we can not guarantee the accuracy of the result, and therefore the detection limit is reported as the result.
- Samples highlighted in blue correspond to chlorophyll *a* filters whose filter bags were considerably wet when delivered. We can not establish how this could have affected the sample result.

Table 7: Descriptive Statistics for the December 04 sampling event

	TKN	**TP**	**DP**	**DOC**	**Chl *a****
Median (mg/L)	0.38	0.040	0.008	2.53	6.30
Average(mg/L)	0.44	0.080	0.016	3.08	27.81
Std. Dev.	0.21	0.187	0.029	1.82	83.04
Max. (mg/L)	1.07	1.139	0.168	8.30	480.18
Min. (mg/L)	0.16	0.014	0.002	1.07	1.10

*µg/L

Graphical representations of **average** TP, TKN, DOC and chlorophyll *a* values for all lakes are shown in Figures 1 – 4. In general, most lakes follow a relatively consistent pattern throughout the sampling period (August 03 – December 04), this is confirmed by the median values for each of the descriptive parameters on events 4-6 which are remarkably similar (Tables 3, 5 and 7). It is important to recognize that important variations between stations do occur within a particular lake on different sampling events. Those differences are being properly identified through a statistical analysis and will be taken into account when establishing the final recommendations for the numeric nutrient criteria.

Preliminary estimates of the numeric nutrient criteria were computed at this stage to get a general notion on the range of values that may eventually be used to describe lake reference conditions in the island. Estimates were calculated based on the 25th percentile approach established by USEPA. Predicted values are close to reference values proposed in other regions of the US (Table 8). In the case of phosphorus, the resulting value (16.5ug/L) is substantially lower than the current water quality limit in the island (1,000 ug/L). It is imperative that sound criteria be adopted so that nutrient overenriched waters can be identified and corrective actions be implemented. In addition, numeric nutrient criteria for rivers, particularly those discharging into the lakes, must be adopted to reduce pollutant loadings at the point of entrance.

We must emphasize that our reference values are only preliminary in nature. Some modifications can be expected as data from the two remaining sampling events are considered, and as a throughout statistical evaluation is performed. However, the consistent behavior exhibited by the lakes up to this point provides some degree of confidence in the proposed values. Based on total P, TKN, and chlorophyll *a* concentrations, Carite, Patillas and Guajataca appear to be the cleanest lakes, with the rest of the lakes exhibiting various degrees of impact from antropogenic activities.

Table 8. Preliminary estimates of lake nutrient criteria Puerto Rico (analysis includes data from the December, 04 sampling event). Values for eight of the 14 USEPA ecoregions[1] are included for comparative purposes (refer to www.epa.gov for details on the ecoregions report).

Item	**PR[1]**	Eco II	Eco VI	Eco VII	Eco VIII	Eco IX	Eco XI	Eco XII	Eco XIII
TP ug/l	**16.5**	8.75	37.5	14.75	8.0	20.0	8.0	10.0	17.5
TN mg/l	**0.26**	0.10	1.68	0.66	0.24	0.36	0.46	0.52	1.27
Chl *a* ug/l	**3.59**	1.90	8.59	5.23	2.39	5.18	2.79	2.60	3.35

[1] – ER II (Western Forested Mountains); ER VI (Corn Belt and Northern Great Plains; ER VII (Mostly Glaciated Dairy Region); ER VIII (Nutrient Poor Largely Glaciated Upper Midwest and Northeast); ER IX (Southeastern Temperate Forested Plains and Hills); ER XI (Central and Eastern Forested Uplands); ER XII (Southern Coastal Plain); ER XIII (Southern Florida Coastal Plain).

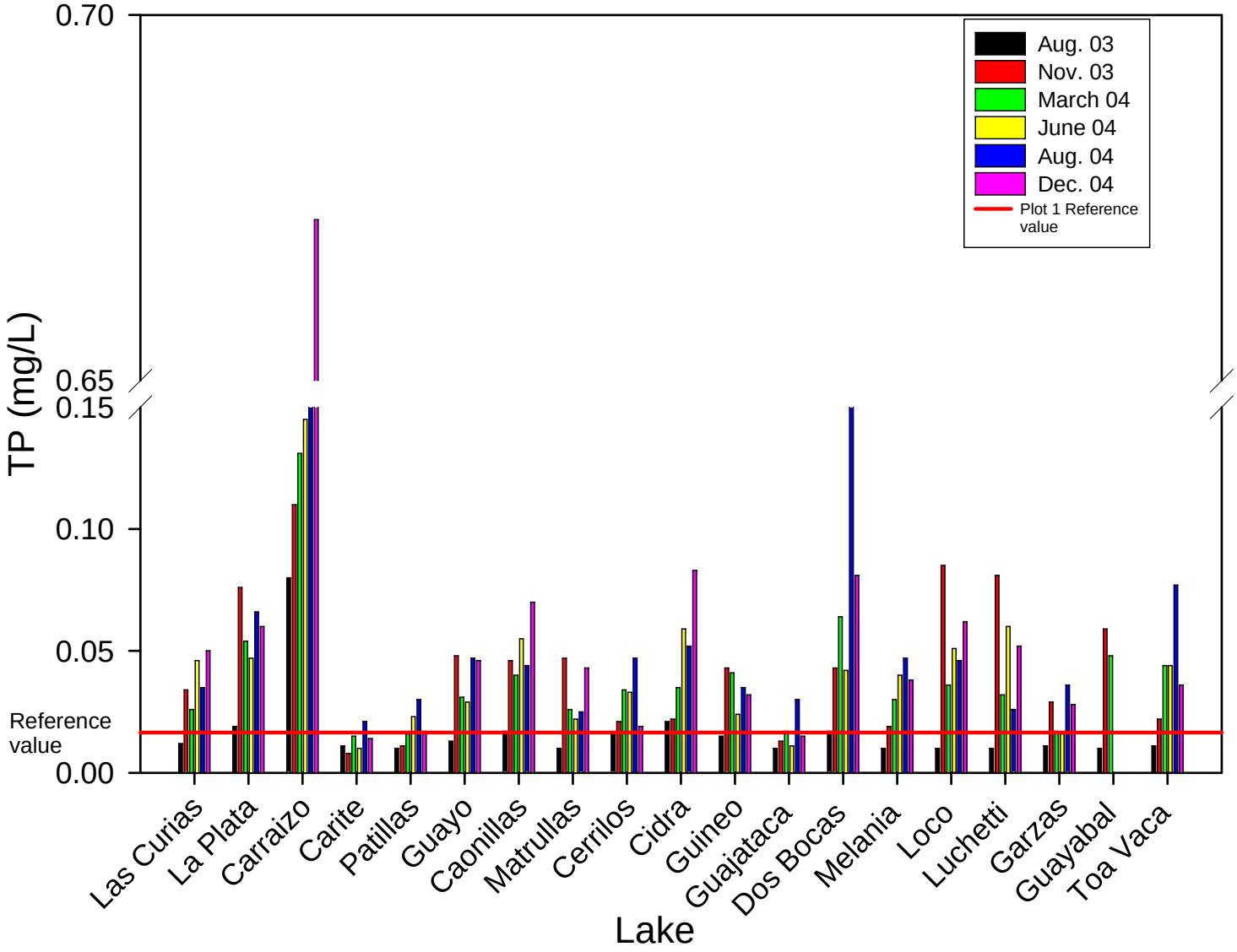

Figure 1. Average total phosphorus concentration in lakes of Puerto Rico (the preliminary reference value is included for illustrative purposes)

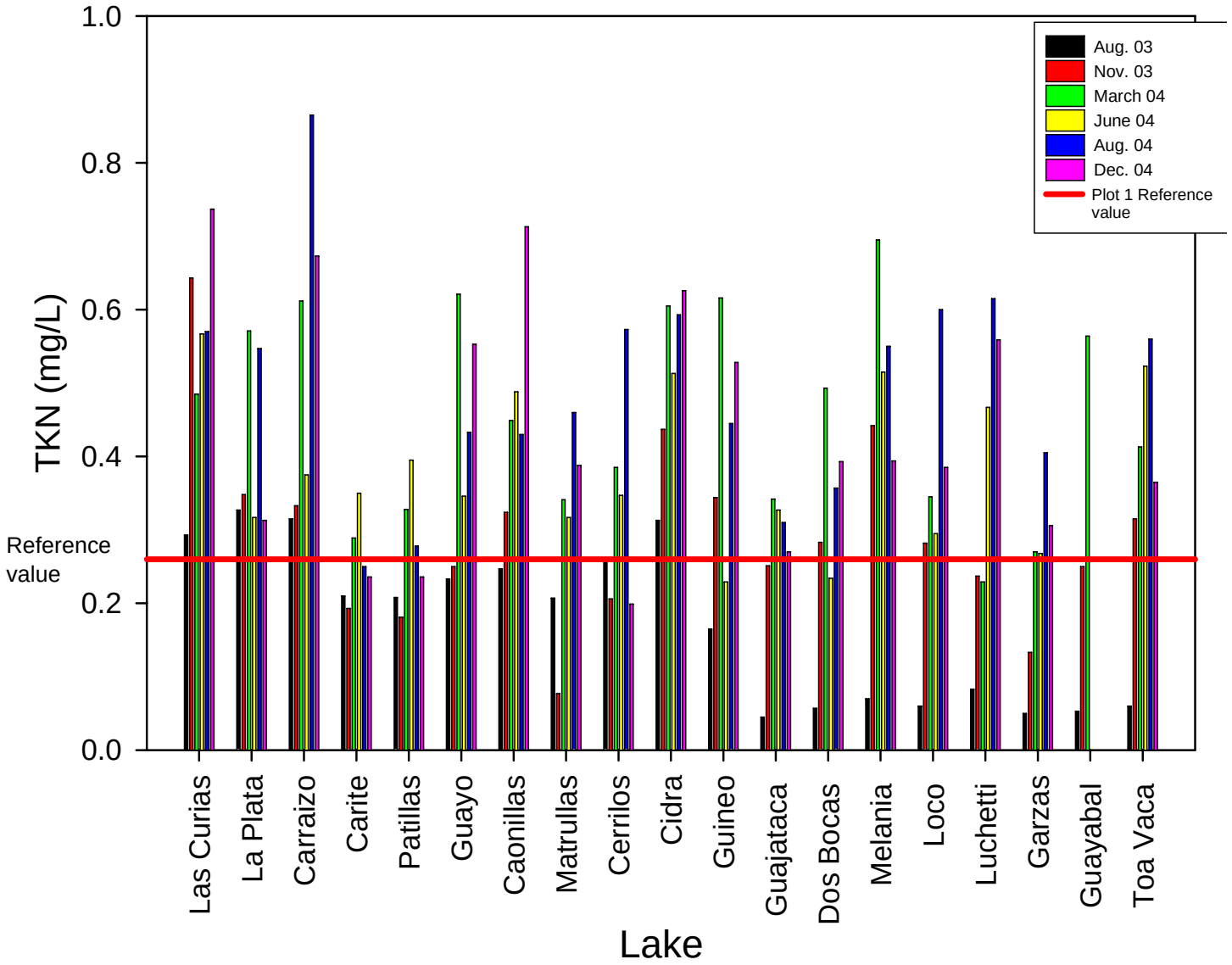

Figure 2. Average TKN concentration in lakes of Puerto Rico (the preliminary reference value is included for illustrative purposes).

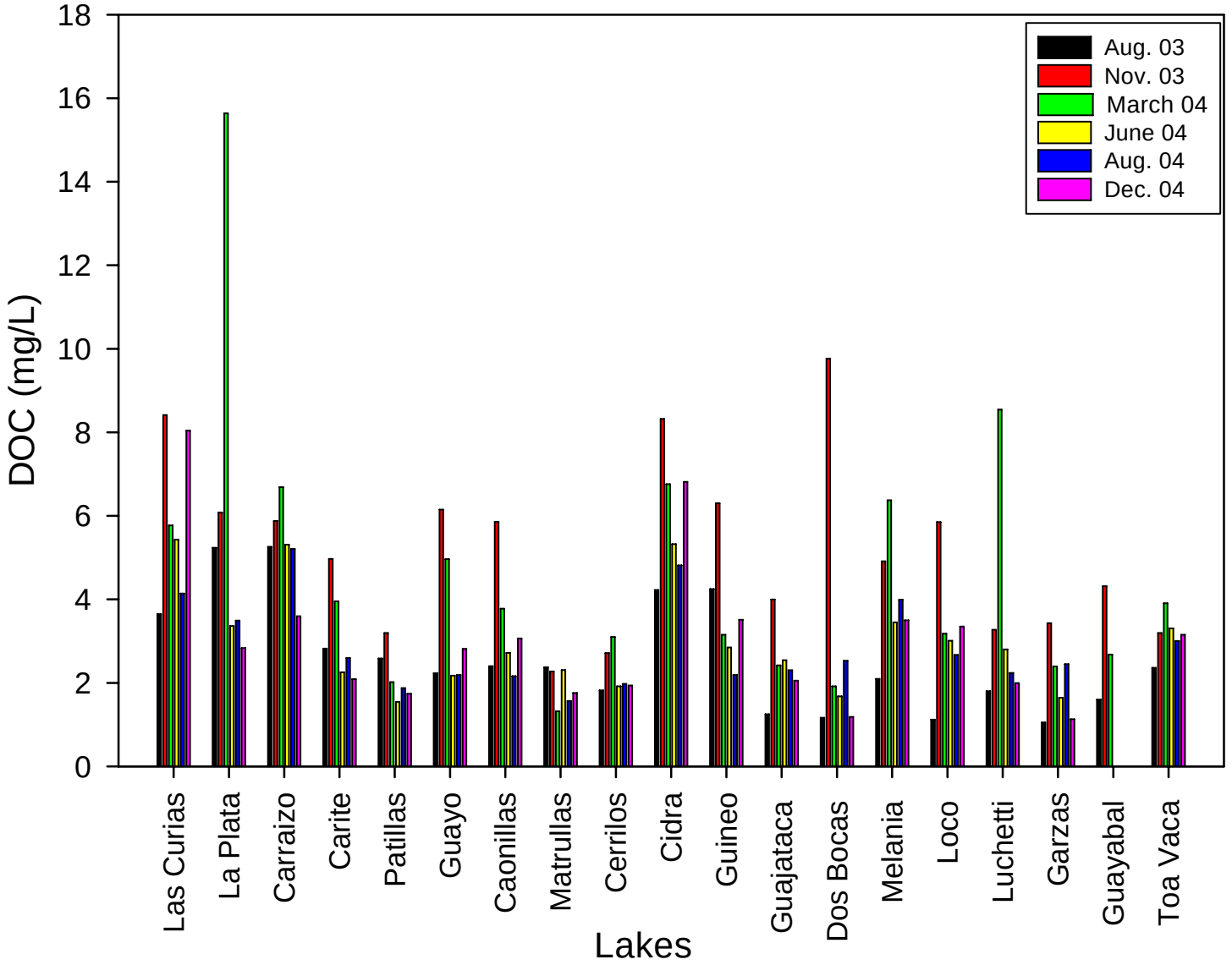

Figure 3. Average DOC concentration in lakes of Puerto Rico (data from this study)

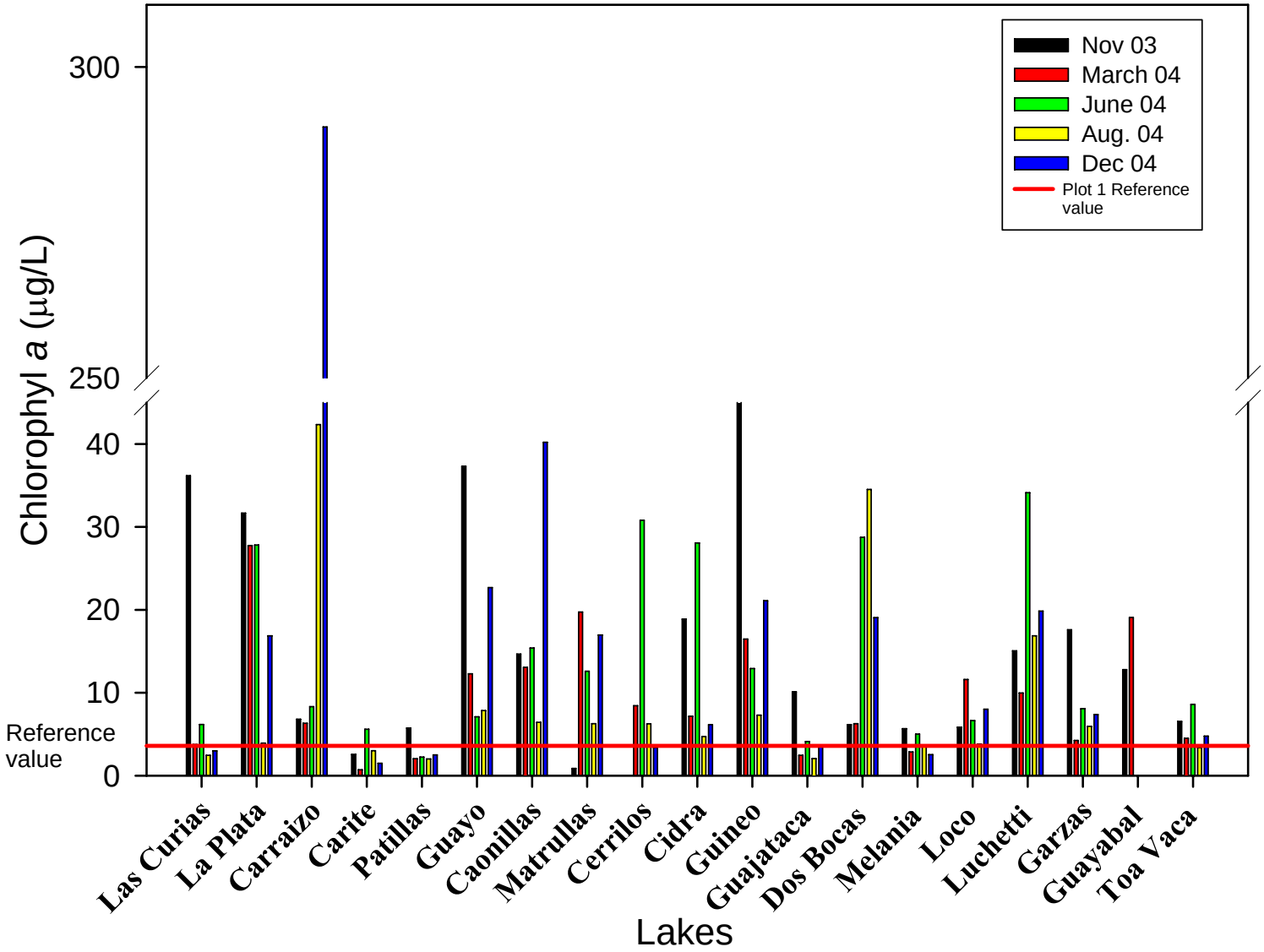

Figure 4. Chlorophyll *a* concentration in lakes of Puerto Rico (the preliminary reference value is included for illustrative purposes).

A statistical analysis was performed to identify within lake, and between lake differences, and evaluate potential correlations between different chemical, biological and/or physical parameters. The analysis included data from the first five sampling events (data for the sixth event was not included). A non-parametric test with paired data (Wilcoxon Signed Rank) was used to establish differences between stations 1 (entrance) and 3 (dam), and 2 (center) and 3 (dam) since ANOVA residuals were not uniform. A summary of that analysis will be discussed next (the complete statistical report is shown in Appendix A). Significant differences between stations 1 (lake entrance) and 3 (lake dam) were observed for: pH, Secchi depth, DO, TP, TKN, DP, DOC, N/P ratio and TSITP respectively (Table 9).

Table 9. Wilcoxon Signed Rank sample sizes and P values for median differences. P-value < 0.05 indicate significant differences between stations 1 and 3.

Parameter	**Sample size**	**P-value**
pH	43	2.8e-2
Secchi Depth	65	1.15e-12
DO	38	3.0e-3
TP	71	2.15e-8
TKN	71	2.0e-3
DP	70	2.6e-3
DOC	71	5.53e-9
Chl a	55	1.797e-1
N/P	71	1.28e-3
TSI Chl a	55	6.10e-2
TSI TP	71	7.92e-9

As expected the lake entrance exhibited a higher nutritional status (TP, TKN, DOC) than water at the dam. Consequently, chlorophyll *a* values were higher, and dissolved oxygen and Secchi depth values lower at the entrance than near the dam.

Spearman correlation coefficients for selected variables (see Appendix 1 for complete data) are shown on Table 10 – 17. When considering all lake data available (Table 10) results point out to a relatively good positive correlation between TP and Chl *a*. In other words, as total P loadings into the lakes increase, there will be an increase in algae biomass density. As expected increases in algae biomass (as measured by Chl *a*) diminish the light penetration depth in the lake profile (SD). This is evidenced by the negative correlation coefficient obtained between these parameters. In fact, both Chl *a* and TP are negatively correlated with SD.

Reductions in SD values may be caused either by an increase in algae biomass concentration or by inorganic (sediment) particles coming into the lake as a result of runoff events. High sediment loads will mask the effect of nutrients on aquatic biomass development which must be taken into consideration for the development of numeric criteria. To evaluate the potential interference effect of sediment loads on our relationships a separate analysis was conducted excluding samples with SD less than 1. A significant improvement in the TP- Chl *a*, TKN - Chl *a*, and TKN - TP relationships is observed when excluding those data (Table 11).

The interfering effect of sediments appears to be more significant at the lake entrance (station 1). Mirroring the results obtained with the complete data set, results for station 1 (entrance), evidence a significant improvement in the Spearman correlation coefficients for the TP- Chl *a*, TKN - Chl *a*, and TKN - TP relationships when data with SD < 1 is excluded (Tables 12 and 13).

A different result is obtained at the center (second) and dam (third) station (Tables 14 – 17). The exclusion of data with SD < 1 causes a reduction in the TP- Chl *a* Spearman correlation coefficient. This suggests that contrary to the entrance, SD at the center and near the dam is more reflective of algae biomass behavior and is not significantly influenced by an inorganic component. At both stations, SD was negatively correlated to Chl *a* as expected.

Table 10: Spearman partial correlation coefficients between *specific* descriptive parameters of lake trophic status; **all sampling stations combined** (analysis excludes data from December 04 sampling event).

	TP	**TKN**	**SD**
Chl *a*	0.50	0.29	-0.27
TP	1.00	0.44	-0.40

Table 11: Spearman partial correlation coefficients between *specific* descriptive parameters of lake trophic status. Data for **all sampling stations with Secchi Depth values ≥ 1m** (analysis excludes data from December 04 sampling event).

	TP	**TKN**	**SD**
Chl *a*	0.63	0.46	-0.35
TP	1.00	0.62	-0.31

Table 12: Spearman partial correlation coefficients between *specific* descriptive parameters of lake trophic status; **data from first station (lake entrance) only** (analysis excludes data from December 04 sampling event).

	TP	**TKN**	**SD**
Chl *a*	0.35	0.25	-0.25
TP	1.00	0.58	-0.30

Table 13: Spearman partial correlation coefficients between *specific* descriptive parameters of lake trophic status; **data from first station (lake entrance) with Secchi depths values ≥ 1m** (analysis excludes data from December 04 sampling event).

	TP	**TKN**	**SD**
Chl *a*	0.68	0.49	-0.26
TP	1.00	0.73	-0.35

Table 14: Spearman partial correlation coefficients between *specific* descriptive parameters of lake trophic status; **data from second station (center of lake) only** (analysis excludes data from December 04 sampling event).

	TP	**TKN**	**SD**
Chl *a*	0.41	-0.007	-0.57
TP	1.00	0.51	-0.25

Table 15: Spearman partial correlation coefficients between *specific* descriptive parameters of lake trophic status; **data from second station (center of lake) with Secchi depths values ≥ 1m** (analysis excludes data from December 04 sampling event).

	TP	**TKN**	**SD**
Chl *a*	0.16	0.13	-0.40
TP	1.00	0.64	-0.11

Table 16: Spearman partial correlation coefficients between *specific* descriptive parameters of lake trophic status; **data from third station (lake dam) only** (analysis excludes data from December 04 sampling event).

	TP	**TKN**	**SD**
Chl *a*	0.16	0.09	-0.38
TP	1.00	0.55	-0.34

Table 17: Spearman partial correlation coefficients between *specific* descriptive parameters of lake trophic status; **data from third station (lake dam) with Secchi depths values ≥ 1m** (analysis excludes data from December 04 sampling event).

	TP	**TKN**	**SD**
Chl *a*	0.13	0.12	-0.53
TP	1.00	0.64	-0.30

PERIPHYTOMETER STUDIES

A trial was conducted to evaluate the effect of different exposure times on chlorophyll *a* response at Lake Cerrillos, Ponce, Puerto Rico. Three reactions times were evaluated, namely: 7 days, 10 days, 14 days. A detailed experimental protocol for the experiment follows: The floating rack contained 42 bottles in randomized complete block arrangement (Table 18), with the bottles placed perpendicular to the expected current flow. The bottles were randomly assigned to one of two treatments which were blank (distilled water) and a nutrient solution (2 ppm of phosphorus and 14 ppm of nitrogen). The N solution was prepared with NH_4NO_3, and the P solution was prepared with NaH_2PO_4. Bottles from each of the two treatments/ replications were harvested at 7, 10, and 14 days after initiation of the experiment (Table 18). The floating rack was placed approximately at the center of the lake.

Table 18: Schematic distribution of the periphytometer study

: Arrangement by Bottles

Block	I	II	III	IV	V	VI	VII
Bottle	4	7	13	21	26	36	38
	5	9	17	20	27	35	40
	1	10	15	23	30	34	39
	2	11	14	24	25	31	41
	6	8	18	22	29	32	42
	3	12	16	19	28	33	37

Table 3: Harvest Days Arrangement

Block	I	II	III	IV	V	VI	VII
Harvest Day	7	7	7	14	10	14	10
	10	14	10	10	14	10	7
	7	7	14	10	14	7	14
	10	10	10	14	7	7	10
	14	10	14	7	10	10	14
	14	14	7	7	7	14	7

Table 4: Solutions Arrangement

Block	I	II	III	IV	V	VI	VII
Solutions	B	A	A	A	A	B	A
	B	A	B	A	A	B	B
	A	B	A	B	B	B	A
	A	B	A	B	A	A	B
	B	A	B	B	B	A	B
	A	B	B	A	B	A	A

Solution A = Nutrient solution containing 2 PPM of phosphorus and 14 PPM of nitrogen

Solution B = Distilled water

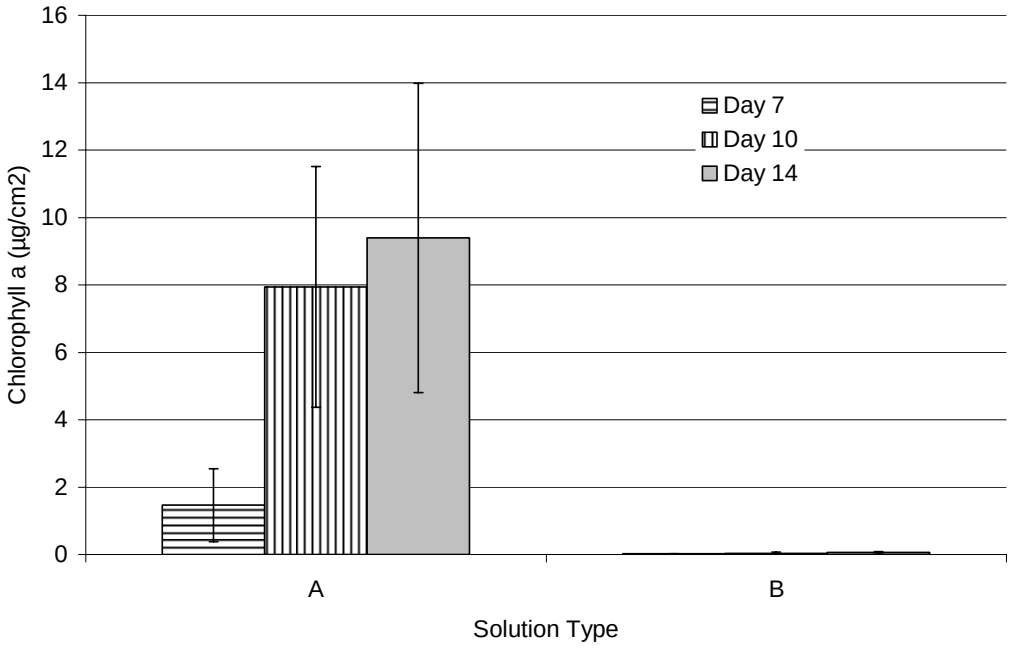

Figure 5: Effect of time and solution concentration on Chlorophyll *a* concentrations at Lake Cerrillos.

Results indicate that a 10 day reaction time is adequate to characterize aquatic biomass response to nutrients by the Matlock periphytometer approach (Figure 5). On the basis of these results a 10-day reaction time was chosen for subsequent trials.

Evaluation of limiting nutrient effect on aquatic biomass growth

Once the adequate reaction time was established a series of trials were conducted to evaluate aquatic biomass response to nutrient additions.

1) july 23, 2004 – august 2, 2004.

The trial was conducted at lake Guajataca (18°22'45" N y 66°55'33" O, Puerto Rico) following the same experimental set up described in the previous experiment. The objective was to

identify the nutrient (N and/or P) most limiting to aquatic biomass growth. Results from temperate regions suggest that phosphorus is the controlling factor for aquatic biomass growth on lakes. Treatments evaluated were:

Treatment Number	Treatment
1	-N, -P
2	+N, -P
3	-N, +P
4	+N, +P

where: +N = 14 mg N/L
+P = 2 mg P/L
-N-P = distilled water

I	*II*	*III*	*IV*	*V*	*VI*	*VII*
T1 2	T2 5	T2 10	T3 14	T3 20	T3 24	T2 28
T2 1	T3 8	T3 11	T4 16	T1 18	T2 23	T1 26
T4 3	T4 6	T4 9	T1 13	T2 17	T4 22	T3 25
T3 4	T1 7	T1 12	T2 15	T4 19	T1 21	T4 27

Figure 6. Schematic representation of treatment distribution in the Matlock periphytometer for trial on nutrient effect on primary production.

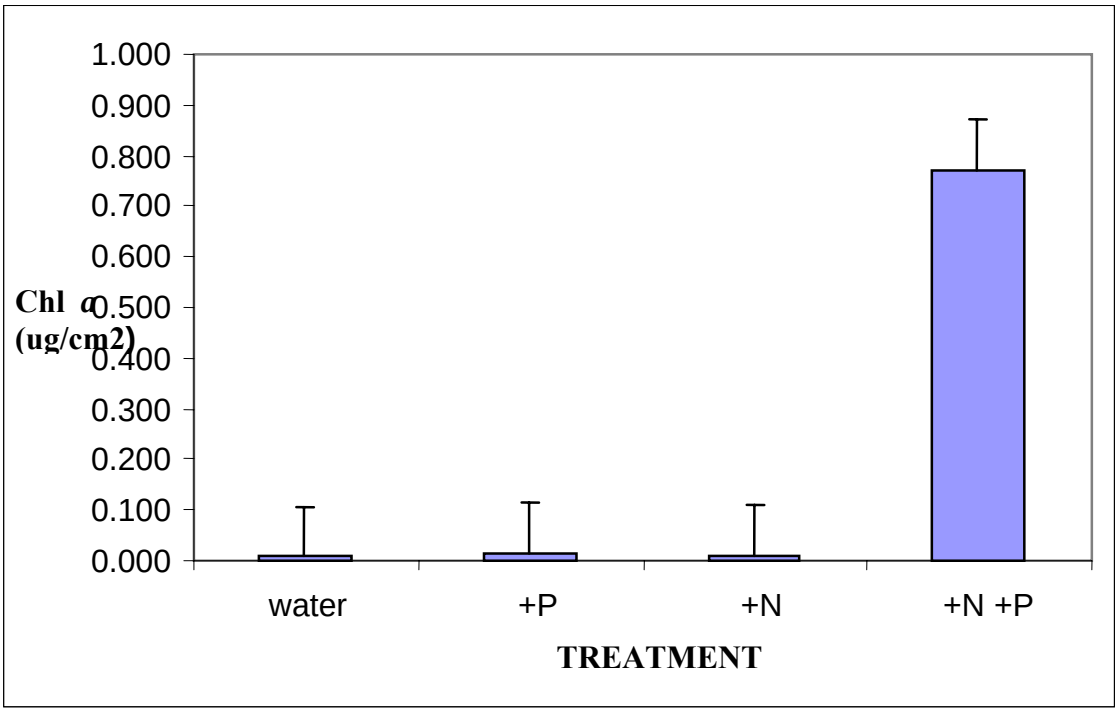

Figure 7. Effect of N, P and N+P on primary production in Guajataca Lake.

Table 19. Summary of treatment results for trial on nutrient effect on primary production.

TREATMENT	MEDIA (Chl. a µ/cm^2)	Std. Dev.
Distilled water	0.008	0.0042
+ P	0.014	0.0142
+ N	0.009	0.0058
+ N, + P	0.771	0.5706

Results (Table 19, Figure 7) indicate that both N, and P are limiting factors to aquatic biomass growth on this lake. Similar results had been previously obtained at lake Cerrillos (Figure 8), although in that case the lost of several experimental units prior to the trial harvest precluded the results to be conclusive. The response pattern observed in our lakes contrasts with results obtained in temperate lakes, where P has been identified as the sole controlling factor to algae growth (Vollenweider, R.A., 1976; Correl, 1998).

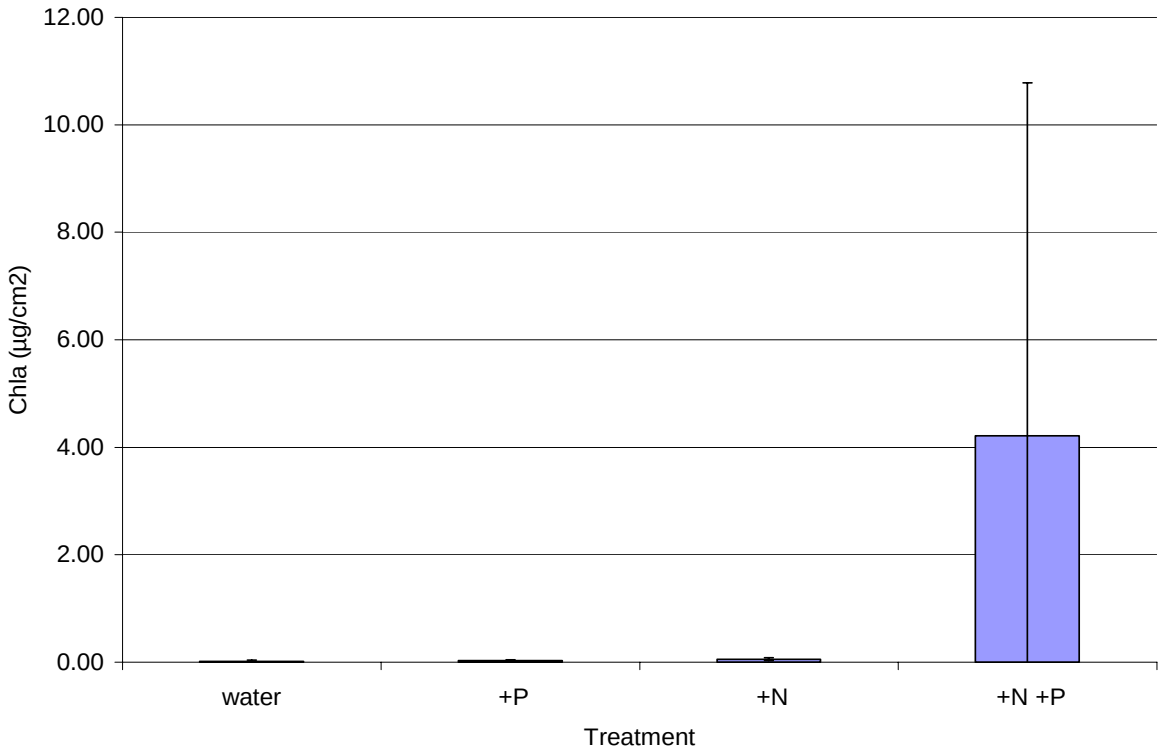

Figure 8. Effect of N, P and N+P on primary production in Lake Cerrillos

Following these trails we decided to conduct a series of response curve studies to ascertain if a concentration threshold for each limiting nutrient (N and P) could be established. Initially a trial was conducted to evaluate the effect of variations in P concentrations under non-limiting N conditions.

The first of these trials was conducted on *october 8, 2004* at Lake Guajataca. Treatments and schematic description of the experimental set up are presented in Table 20 and Figure 9. Results are shown in Table 21 and Figure 10.

Table 20. Treatment description of P response study.

Treatment No.	P (mg/L)	N (mg/L)
1	0	14
2	0.02	14
3	0.1	14
4	0.5	14
5	1.0	14
6	2.0	14
7	4.0	14

I	*II*	*III*	*IV*	*V*	*VI*	*VII*
5 T 5	22 T 1	7 T 7	2 T 2	23 T 2	25 T 4	33 T 5
34 T 6	12 T 5	31 T 3	17 T 3	13 T 6	11 T 4	3 T 3
15 T 1	35 T 7	27 T 6	28 T 7	16 T 2	1 T 1	14 T 7
18 T 4	4 T 4	29 T 1	26 T 5	20 T 6	21 T 7	9 T 2
6 T 6	10 T3	8 T 1	32 T 4	24 T 3	19 T 5	30 T 2

Figure 9. Schematic representation of treatment distribution in the P response study.

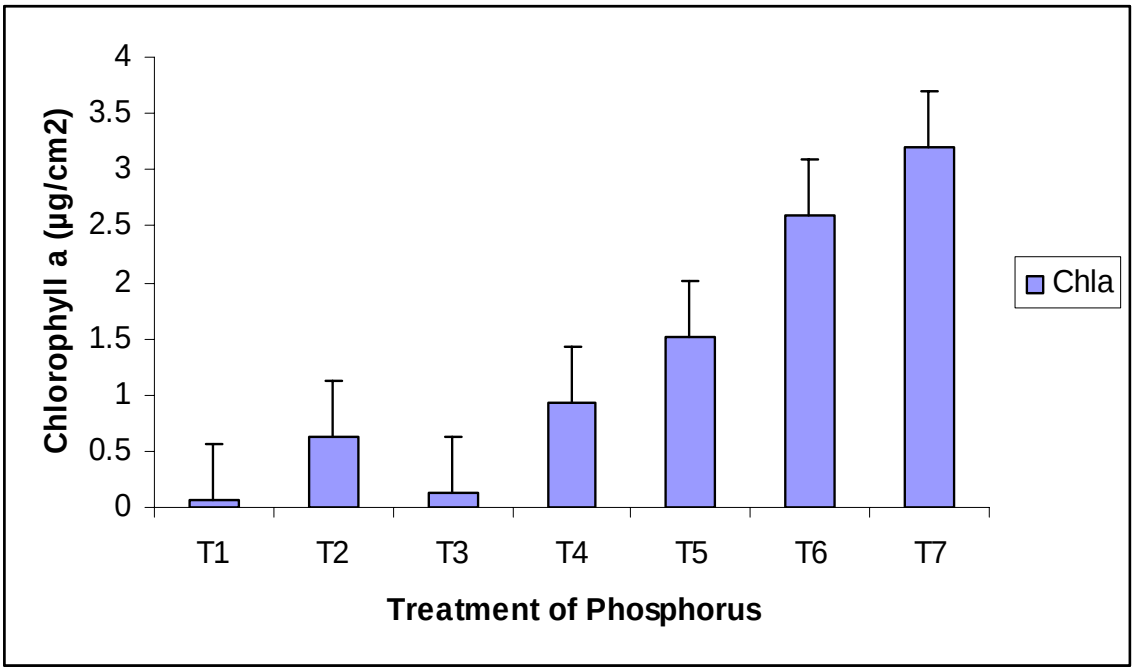

Figure 10. Effect of variations in phosphorus concentrations on primary production in Guajataca Lake (trial A).

Table 21. Descriptive statistics of experimental results (trial A) for P response study.

TREATMENT	MEDIA Chl *a* µg/cm2	Std. Dev.
T 1	0.061466	0.051
T 2	0.625395	0.92
T 3	0.132815	0.066
T 4	0.920377	1.16
T 5	1.503996	0.51
T 6	2.589180	2.05
T 7	3.202709	1.13

The experiment was repeated immediately (october 20, 2004) to acquire a sense on the reproducibility of the results. Relatively good reproducibility between trials was obtained (Table 22, Figure 11). A relative response curve suggests that it might be possible to identify a

concentration threshold with this approach (Figure 12). These are very encouraging results, however, we are still at a very preliminary stage in terms of defining a P response threshold based on this approach.

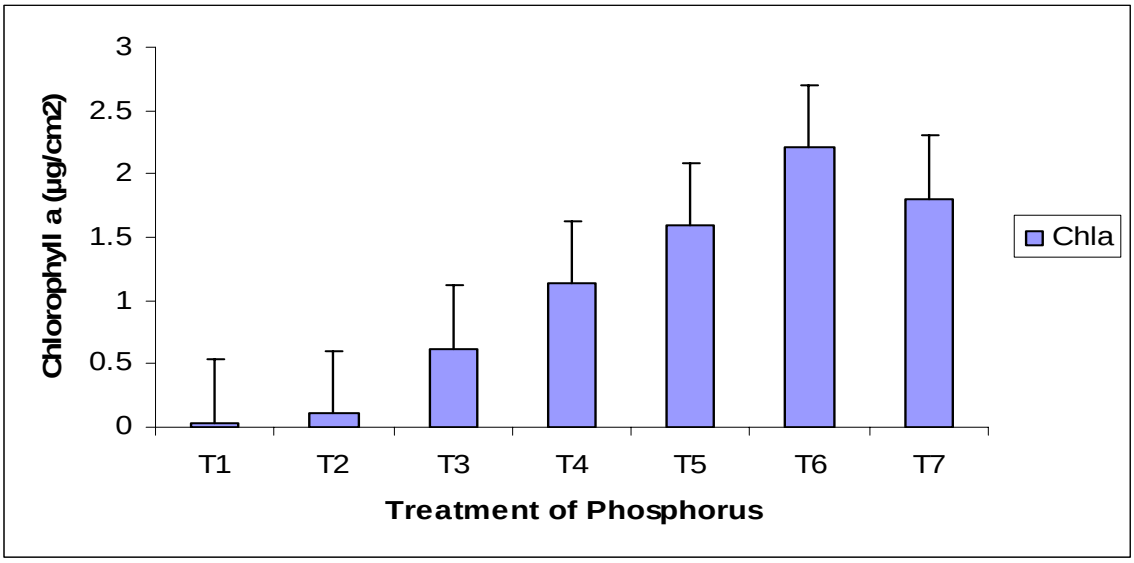

Figure 11. Effect of variations in phosphorus concentrations on primary production in lake Guajataca (trial B).

Table 22. Descriptive statistics of experimental results for trial B of P response study

TREATMENT	MEDIA Chl *a* µg/cm2	Std. Dev.
T 1	0.033755	0.005
T 2	0.104922	0.05
T 3	0.614639	0.34
T 4	1.130329	0.37
T 5	1.141134	0.93
T 6	2.207593	0.75
T 7	1.802546	0.43

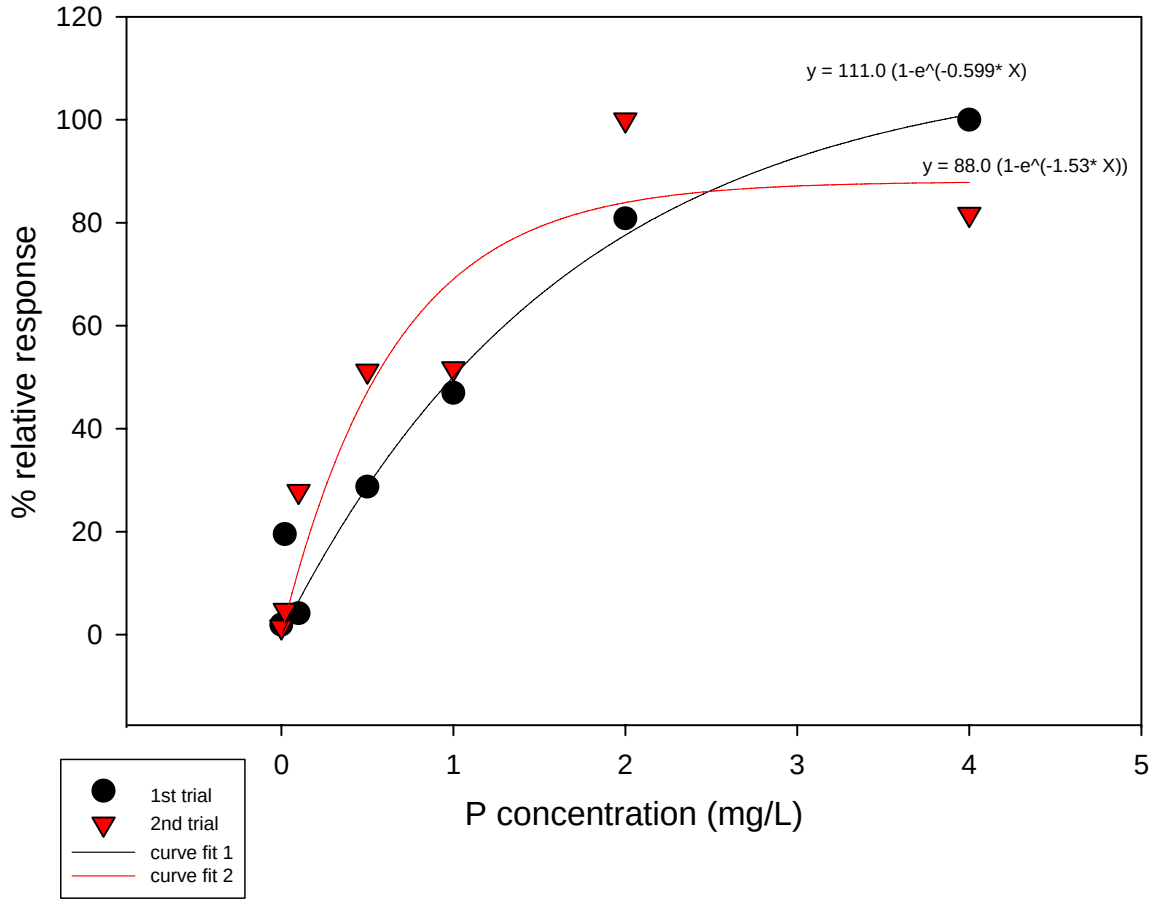

Fig 12. Relative response curve of algae growth to P additions. Lines represent empirical descriptions of the experimental points obtained through a curve fitting procedure.

A similar trial was conducted with nitrogen, that is, we evaluated aquatic biomass response to variations in N under non-limiting P conditions. The trial began on November 20, at Guajataca. A description of the treatments used is presented in Table 23. Results are presented in Table 24, and Figure 13.

Table 23. Description of treatments for the N response trial

Treatment No.	P (mg/L)	N (mg/L)
1	2	0
2	2	0.25
3	2	1
4	2	2
5	2	5
6	2	10
7	2	14

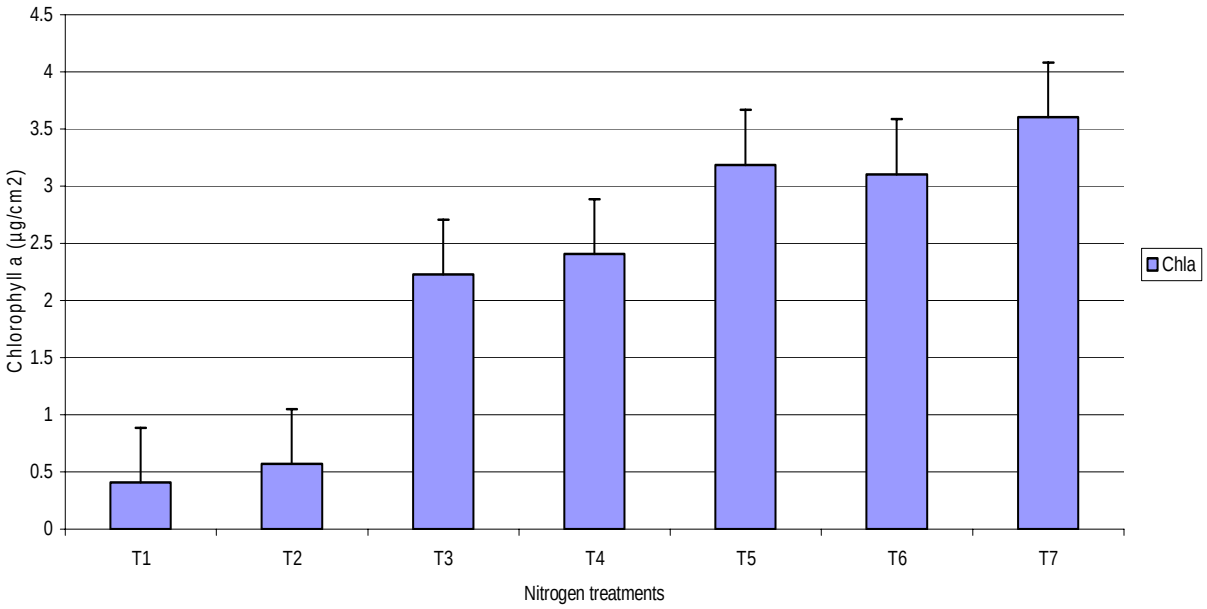

Figure 13. Matlock periphytometer study on the effect on nitrogen additions on algae biomass growth at Guajataca.

Table 24. Descriptive statistics of N response study.

Treatment ID	Chl *a* mean	Std. Dev.
T1	0.405684	0.120068
T2	0.57	0.17167
T3	2.226095	0.44613
T4	2.407194	0.77734
T5	3.186945	0.376465
T6	3.10524	1.080697
T7	3.604056	0.898862

Similarly to the case of P, a strong response to N additions was observed (Fig. 14). These results accentuate the need to control nutrient loadings (both N and P) in our lakes and underscore the importance of establishing numeric nutrient criteria as a means of protecting the integrity of our waters.

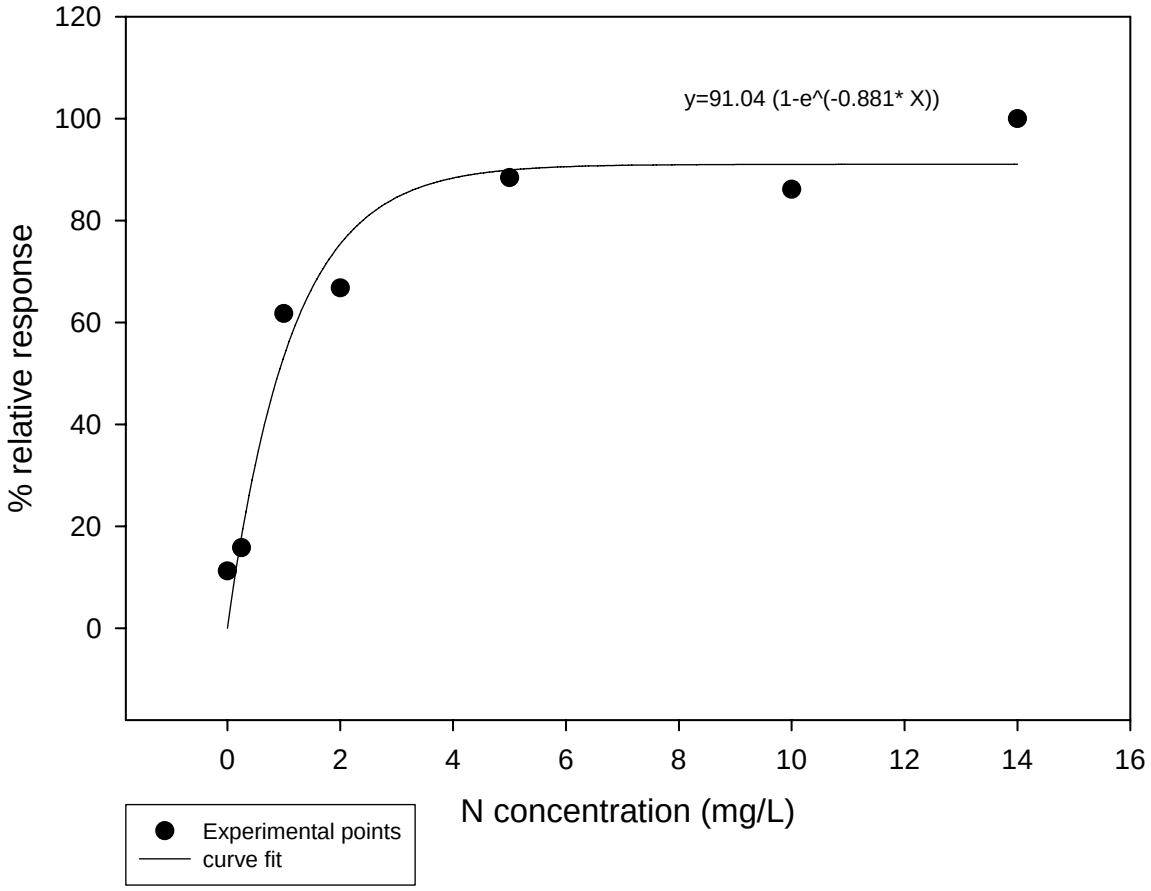

Fig 14. Relative response curve of algae growth to N additions. Lines represent empirical descriptions of the experimental points obtained through a curve fitting procedure.

We are extremely pleased with the results obtained through this experimental approach. In the coming months we will intensify our experimental trials and will particularly concentrate on documenting algae response at the low concentration ends of both curves (N and P). We will also attempt to establish an algae counting procedure that, together with the Matlock periphytometer studies, will hopefully enable us to make inferences on concentration response thresholds for both nutrients. To accomplish this we have decided to modify our original plans of repeating the basic nutrient response trials in lake Carite and concentrate our efforts on documenting in detail algae response dynamics on lake Guajataca.

CHARACTERIZATION OF PHYTOPLANKTON DIVERSITY IN LAKES OF PUERTO RICO

During this period we have established the protocol for the evaluation of phytoplankton specimens with the Scanning Electron Microscope and the Phase Contrast Microscope (Nomarsky).

Methodology (APHA_AWWA, 1998)

Phytoplankton specimens are concentrated by centrifugation at 1000 g for 20 minutes. Samples are filtrated through a 0.45µm membrane filter, then, the filter is placed over a specimen slide to which two drops of immersion oil are added, and then covered with a glass microcover slip. Phytoplankton counting was performed at low magnification (200x) using a Sedwick-Rafter camera.

Results

Samples from the following lakes have been analyzed in detail: Guayo, Toa Vaca, Garzas, Patillas, Las Curias, and Guineo.

Guayo

Eleven genera belonging to 5 algae divisions were detected at this lake (Table 25). The species *Pediastrum simplex* var. *duodenarium* and *Peridiniopsis* sp. were the most abundant in the samples evaluated. These species have been associated with eutrophication conditions and can generate objectionable taste and color when abundant. *Pediastrum simplex* is an indicator of the presence of compounds rich in salts, particularly those containing sodium sulfates and chlorides (Pinilla, 1998; Ramírez, 2000). *Merismopedia* sp. was the least abundant species, however, this species is an indicator of contaminated waters. The predominant zooplankton organisms were rotifera from the generus: *Keratella*, *Brachionus, Polyarthra, Trichocerca*, and *Lecane*; the first three are indicators of meso-eutrophic conditions with the presence of organic matter and alkaline pH (Pinilla, and Gabriel, 1998).

Table 25. Phytoplankton genera at Guayo lake

Division	**Class**	**Order**	**Family**	**Genera**
Euglenophyta	Euglenophyceae	Euglenales	Euglenaceae	*Strombomonas*
Cyanophyta	Cyanophyceae	Chroococcales	Chroococcaceae	*Chroococcus*
				Merismopedia
Chlorophyta	Chloropyceae	Tetrasporales	Oocystaceae	*Monoraphidium*
				Chlorella
		Chlorococcales	Hydrodictyaceae	*Pediastrum*
		Zygnematales	Zygnemataceae	*Euastrum*
Pyrrophyta	Dinophyceae	Peridiniales	Peridiniaceae	*Peridinium*
				Peridiniopsis
Bacillariophyta	Bacillariophyceae	Pennales	Fragilariaceae	*Fragilaria*
			Naviculaceae	*Navicula*

Toa Vaca

The list of genera observed on samples from this lake is shown in Table 26. On this lake however, there was a variation in the species detected depending on the lake station. There were 16 species at the lake entrance, 12 at the center, and 11 species at the dam. Eight of the species detected at the entrance belonged to the Bacillariophyta division, namely; *Amphora* sp, *Anomoeoneis* sp, *Gomphonema* sp1, *Gomphonema* sp2, *Synedra* sp, *Gyrosigma* sp, *Navicula* sp1 and *Nitzschia* sp. According to Pinilla and Gabriel (1998), and Páramo and Pinilla (1994) diatomea species are associated with waters that are neutral to slightly acidic, and have a high N/P ratio.

Prevalent species at the dam belonged to the Cyanophyta division of the genera: *Aphanocapsa, Microcystis*, *Chroococcus, and* an unidentified *genera.* Next in abundance was the Pyrrophyta division with the *Peridinium* and the *Peridiniopsis* genera. The Bacillariophyta division diminished in numbers, with *Synedra*, and *Navicula* the only genera present. The Chorophyta division was represented by the *Ulothrix*, and the *Spirogyra* genera, but in slight numbers. The predominance of Cyanophytas at the dam could be indicative of a decay in the quality of the water at this lake, since this group has been associated with eutrophic and N limiting conditions (Duque and Donato, 1992).

At the center of the lake we observed a similar situation with a predominance of *Aphanocapsa* sp. and an unidentified species. The *Peridiniopsis sp.* species was in greater numbers than at the dam. Among the Diatomea the most prevalent species were *Anomoeoneis* sp, *Synedra* sp

and *Fragilaria* sp. The Chlorophyta species detected at this station were: *Pediastrum simplex* , *Monoraphidium* sp and *Ulothrix* sp.

Table 26. Phytoplankton genera at Toa Vaca

Division	Class	Order	Family	Genera
Euglenophyta	Euglenophyceae	Euglenales	Euglenaceae	*Trachelomonas*
Cyanophyta	Cyanophyceae	Chroococcales	Chroococcaceae	*Chroococcus*
				Microcystis
				Merismopedia
				Aphanocapsa
Chlorophyta	Chlorophyceae	Ulotrichales	Ulothrichaceae	*Ulothrix*
		Zygnematales	Zygnemataceae	*Spirogyra*
		Chlorococcales	Hydrodictyaceae	*Pediastrum*
		Tetrasporales	Oocystaceae	*Monoraphidium*
Pyrrophyta	Dinophyceae	Peridiniales	Peridiniaceae	*Peridinium*
				Peridiniopsis
Bacillariophyta	Bacillariophyceae	Pennales	Fragilariaceae	*Fragilaria*
				Synedra
			Naviculaceae	*Navicula*
				Gyrosigma
				Anomoeoneis
			Nitzschiaceae	*Nitzschia*
			Cymbellaceae	*Amphora*
			Gomphonemaceae	*Gomphonema*

Garzas

Twelve genera belonging to 5 divisions were observed (Table 27). The predominant specie was *Aphanothece* sp., followed by *Fragilaria* sp1 y *Dinobryon* sp. The Chlorophyceas were in less numbers represented by *Staurastrum* sp, *Pediastrum simplex* var. *duodenarium*,

Pediastrum simplex, *Mougeotia* sp and *Monoraphidium* sp. Among the Pirrophytas we identified *Peridinium* sp and *Peridiniopsis* sp, with very few organisms.

A large number of the cyanobacter species (*Microcystis, Aphanothece*, etc.) have been detected in eutrophic waters. It seems that these organisms are more prevalent under these conditions than in oligotrophic conditions (Margalef, 1983). This may be due to their capacity to develop in low CO_2 waters, typical of systems with high algae density as a result of nutrient enrichment. In addition their photosynthetic pigments make them highly efficient in terms of light use reducing the penetration of light to deeper profiles where chlorophyceaes may develop (Peinador, 1999). However, Pinilla and Gabriel (1998) associated the presence of *Aphanothece* to oligothophic conditions in template waters.

The *Fragilaria* genera has been associated with mesotrophic systems and when present in great numbers can generate a soil-like smell (Ramírez, 2000). In general, the Crysophyceas are associated with oligotrophic conditions in neutral to slightly alkaline waters. The genera *Dinobryon* in particular has been detected in media undergoing a change from eutrophic to oligotrophic conditions (Pinilla, and Gabriel 1998). Ramírez (2000) states that this genera can deteriorate the quality of the waters producing strong fish odors even when present in small numbers.

Table 27. Phytoplankton genera at Garzas

Division	Class	Order	Family	Genera
Cyanophyta	Cyanophyceae	Chroococcales	Chroococcaceae	*Aphanothece*
				Microcystis
Chlorophyta	Chlorophyceae	Chlorococcales	Hydrodictyaceae	*Pediastrum*
		Zygnematales	Desmidiaceae	*Staurastrum*
			Zygnemataceae	*Mougeotia*
		Tetrasporales	Oocystaceae	*Monoraphidium*
Pyrrophyta	Dinophyceae	Peridiniales	Peridiniaceae	*Peridinium*
				Peridiniopsis
Bacillariophyta	Bacillariophyceae	Pennales	Fragilariaceae	*Fragilaria* *Synedra*
			Naviculaceae	*Navicula*
Chrysophyta	Chrysophyceae	Ochromomadales	Dinobryaceae	*Dinobryon*

Patillas

Two phytoplankton genera were identified: *Microcystis* and *Peridiniopsis, with Microcystis* sp occurring in largest numbers (Table 28). The samples exhibited high amounts of particulate material which made more difficult the visualization of the species. In addition, the zooplankton community occurred in greater numbers than the phytoplankton community, dominated by copepods, and rotifers of the *Keratella, Polyarthra, Trichocerca* and Cladocers genera. As mentioned previously, cyanobacters of the *Microcystis* genera are indicators of water pollution and some species are potentially hazardous to humans. Copepodes have been associated with eutrophic systems, alkaline pH and lake stratification. Cladocers are have been associated with meso-eutrophic , and some have been associated with oxygenated waters.

Table 28. Phytoplankton genera at Patillas

Division	Class	Order	Family	Genera
Cyanophyta	Cyanophyceae	Chroococcales	Chroococcaceae	*Microcystis*
Pyrrophyta	Dinophyceae	Peridiniales	Peridiniaceae	*Peridiniopsis*

Curias

The dominating species was *Pediastrum simplex* var. *duodenarium* and *P. simplex*, followed by *Peridinium* and *Aphanocapsa* (Table 29). The *Pediastrum* genera occurs in surface waters and can produce a fishy smell and taste when in abundance. The *P. simplex* specie is an indicator of eutrophic conditions, as well as the presence of residues containing sodium sulfates and chlorides (Ramírez, 2000).

Table 29. Phytoplankton genera at Curías

Division	Class	Order	Family	Genera
Cyanophyta	Cyanophyceae	Chroococcales	Chroococcaceae	*Aphanocapsa*
Chlorophyta	Chloropyceae	Chlorococcales	Hydrodictyaceae	*Pediastrum*
Pyrrophyta	Dinophyceae	Peridiniales	Peridiniaceae	*Peridinium*

Guineo

The predominant specie was *Peridinium* sp, followed by *Synedra* sp, *Staurastrum* sp and *Asterococcus* sp (Table 30). According to Ramírez (2000), algae of the *Peridinium* genera produce bad smell, that intensifies as the pH diminishes. This is the most abundant dinoflagelate and is indicative of eutrophic conditions (Pinilla, and Gabriel 1998; Ramírez, 2000).

Table 30. Phytoplankton genera at Guineo

Division	Class	Order	Family	Genera
Chlorophyta	Chloropyceae	Zygnematales	Desmidiaceae	*Staurastrum*
		Tetrasporales	Gloeocystaceae	*Asterococcus*
Pyrrophyta	Dinophyceae	Peridiniales	Peridiniaceae	*Peridinium*
Bacillariophyta	Bacillariophyceae	Pennales	Fragilariaceae	*Synedra*

Cerrillo

The dominant species were *Pandorina* sp, *Fragilaria* sp3 y *Synedra* sp (Table 31). Some Chlorophyceas have been associated with eutrophic waters having high calcium content and a high N/P ratio (Pinilla, and Gabriel 1998). The *Pandorina* genera is an indicator of low mineralization, it is usually present in meso-hypereuthophic environments. The diatomeas (*Fragilaria*, *Synedra*, *Anomoeoneis* and *Gomphonema*) are indicadors of eutrophic conditions with neutral pH, and a high N/P ratio (Pinilla, and Gabriel 1998). We observed another *Peridinium* specie characterized by having sharp spines in the hypoteca, which we believe is *P. quadridens.*

Table 31. Phytoplankton genera at Cerrillo

Division	Class	Order	Family	Genera
Cyanophyta	Cyanophyceae	Chroococcales	Chroococcaceae	*Microcystis*
Chlorophyta	Chlorophyceae	Volvocales	Volvocaceae	*Pandorina*
Pyrrophyta	Dinophyceae	Peridiniales	Peridiniaceae	*Peridinium*
				Peridiniopsis
Bacillariophyta	Bacillariophyceae	Pennales	Fragilariaceae	*Synedra*
				Fragilaria
			Naviculaceae	*Anomoeoneis*
				Pinnularia
			Gomphonemaceae	*Gomphonema*
Chrysophyta	Chrysophyceae	Ochromomadales	Dinobryaceae	*Dinobryon*

General observations

Some of the most common species encountered in the majority of the lakes evaluated (*Peridinium* sp, *Pediastrum simplex*, *Microcytis*, *Aphanocapsa*) are indicators of meso - eutrophic conditions. It is important to follow up closely the cyanophyceas since they can quickly deteriorate the quality of the waters and affect the development of other phytoplankton species that are important to the biological integrity of the waters.

As part of this project we will not only identify the algae present but will establish species abundance, relative frequency and species richness. This will enable us to evaluate several ecological indexes: a) Nygaard index, b) Shannon and Weaver (1949) ecological diversity index, and the species richness index (Margalef (1980)).

Nygaard Index

The Nygaard index establishes relations between dominant species and accidental species to determine the trophic state of the system.

1) Cyanophyceae index = number of Cyanophyceae taxa/ number of Desmidiaceae taxa

2) Diatomea index = number of central diatomea taxa/ number of penal diatomeas taxa

3) Euglenophyta index = number of Euglenophyta taxa/ number of Cyanophyceae + Chlorophyceae taxa

4) Composite index = number of Cyanophyceae + Chlorococcales + central diatomea + Euglenophyta taxa / number of Desmidiaceae taxa.

These indexes are specific to the phytoplankton communities. The composite index offers a clear separation between highly productive systems and less productive systems. Lakes with composite index values <1 are classifies as oligotrophic whereas those with values higher than 3 as eutrophic. Intermediate values represent mesotrophic or weakly eutrophic conditions (Ramírez, 2000). These indexes have yet to be validated in tropical lakes.

Shannon – Weaver diversity index

$H' = - \sum p_i \ln p_i$

Where:

$p_i = n_i / n$

n_i = number of individuals at the i^{th} taxa

n = total individuals in sample $\sum n_i$

According to Margalef (1983), the diversity index can be significantly less than 1 in highly eutrophic environments and greater than 5 in oligotrophic environments.

Species Richness Index (Margalef)

Measures the number of species per sample unit. A reduction on the number of species is indicative of system disturbance.

$R = s-1 / \ln n$

Where:

s = number of toxons registered

n = number of individuals in sample

Characterization of algae response to nutrient additions by the Matlock periphytometer

As part of the periphytometer studies we intend to characterize the algae present on the different nutrient treatments. The filters used as substrate for algae growth (glass fiber)

precluded the use of the light microscope to characterize the systems, thus we had to use a scanning electron microscope (SEM). However, the sample processing with the SEM is very laborious and time consuming, so we are currently evaluating other alternatives to achieving our objectives. Figures 15 to 24 show some of the images collected with the SEM and the Nomarski microscope.

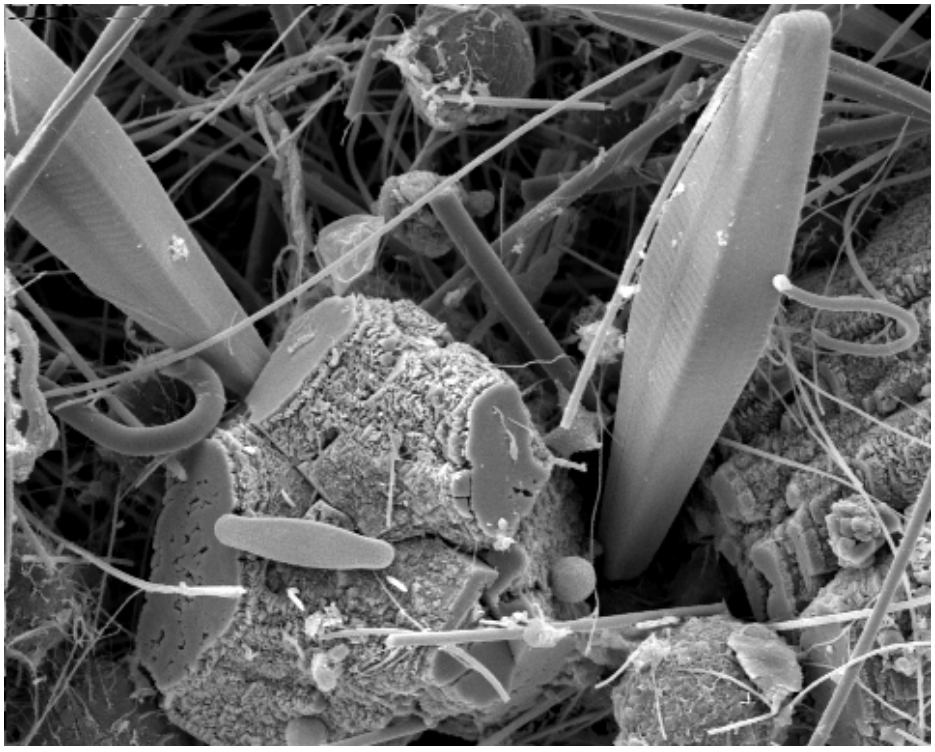

Figure 15. SEM image of *Diatomeas* present in fiber glass samples obtained from the Matlock periphytometer.

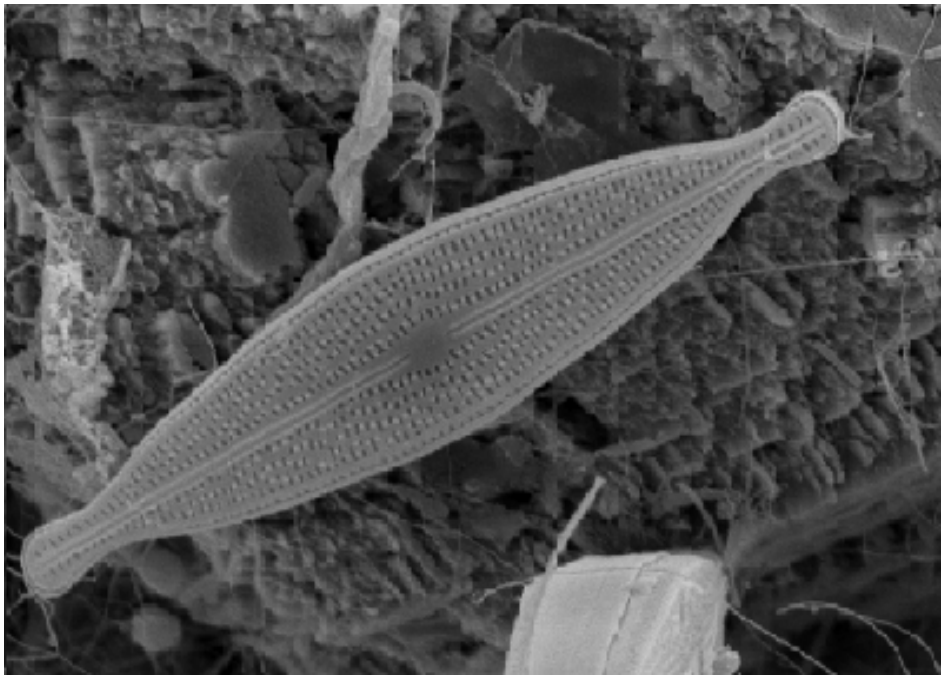

Figure 16. SEM image of *Navicula* sp in a fiber glass sample obtained from the Matlock periphytometer.

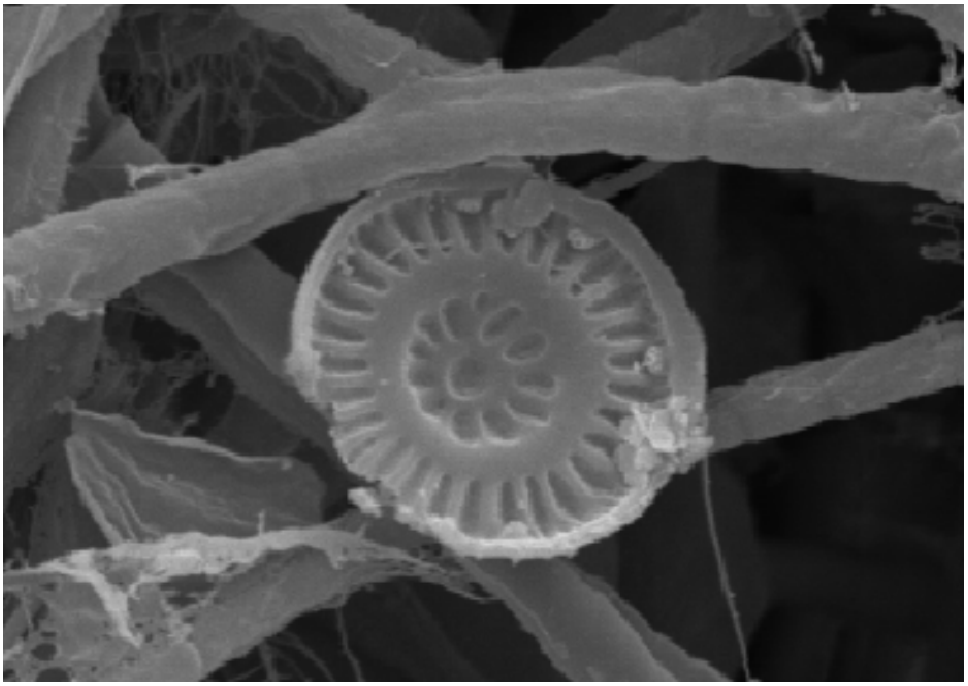

Figure 17. SEM image of *Cyclotella* (a centrical Diatomea) present in a fiber glass sample obtained from the Matlock periphytometer.

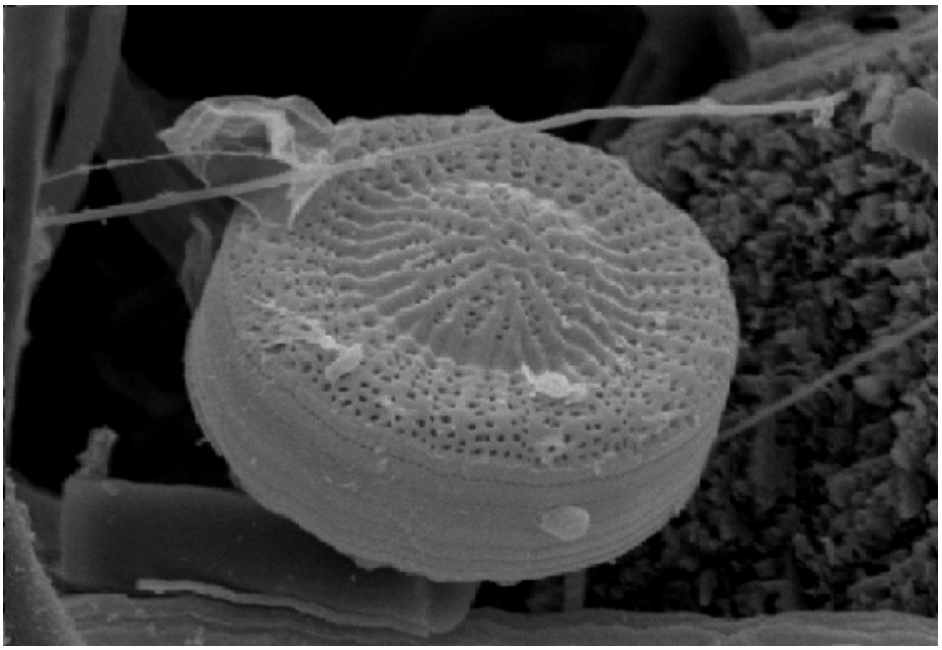

Figure 18. SEM image of *Cyclotella* (a centrical Diatomea) present in a fiber glass sample obtained from the Matlock periphytometer.

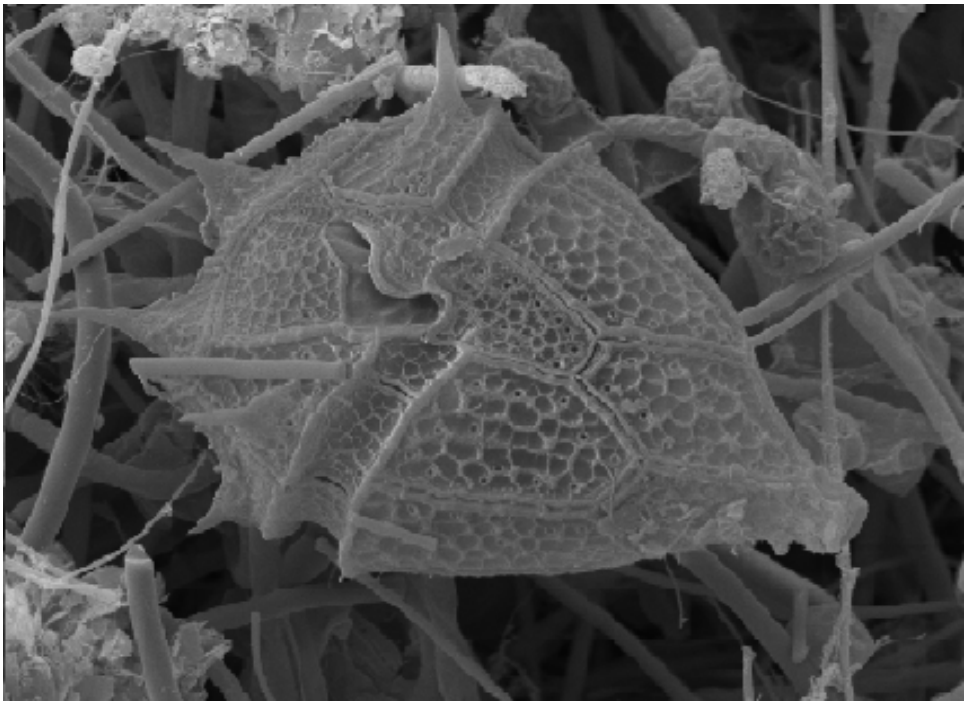

Figure 19. SEM image of *Peridiniopsis* sp present in a fiber glass sample obtained from the Matlock periphytometer.

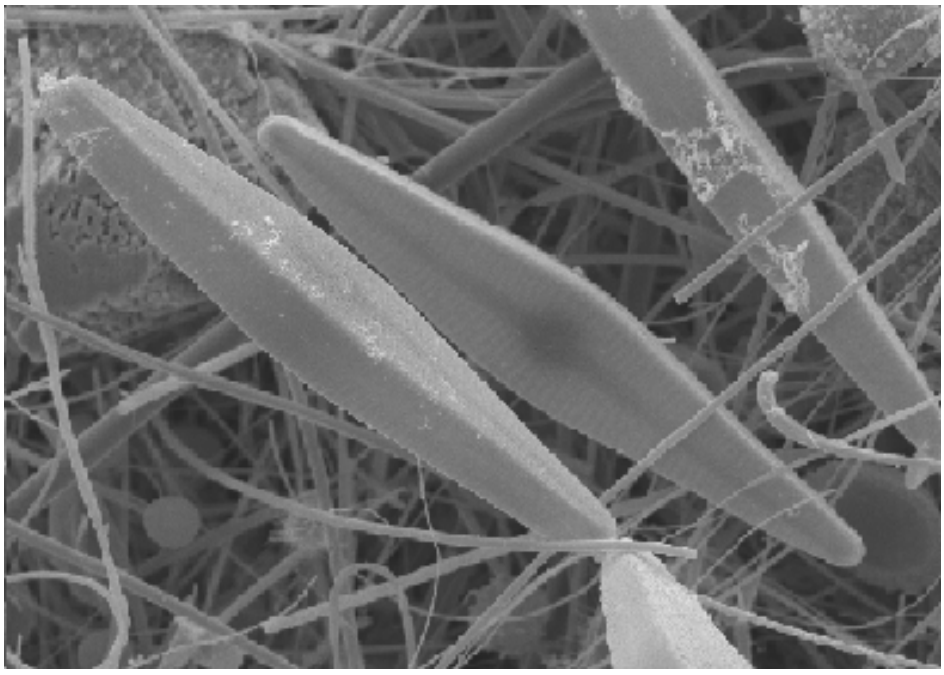

Figure 20. SEM image of *Gomphonema* present in a fiber glass sample obtained from the Matlock periphytometer.

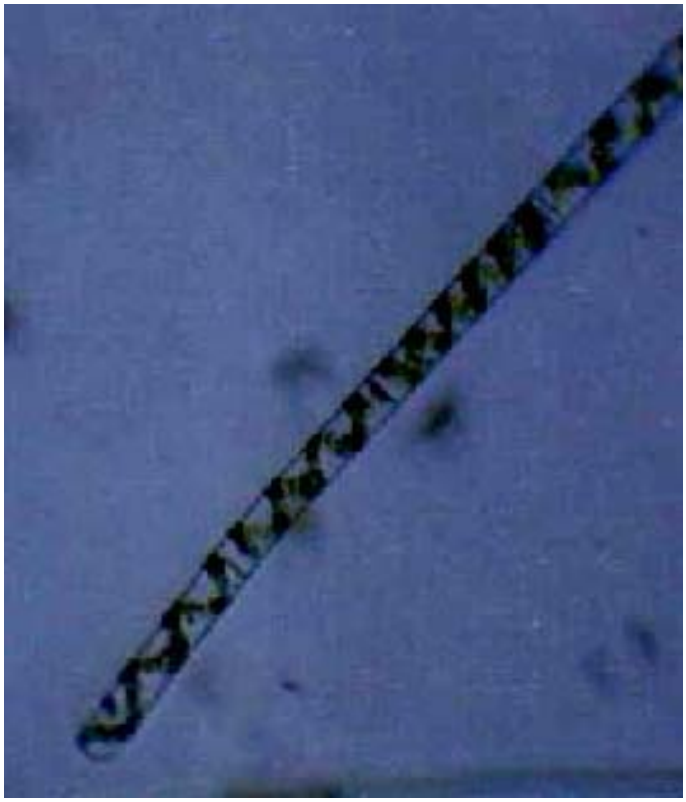

Figure 21. *Spirogyra* sp. Image obtained with the Nomarski microscope.

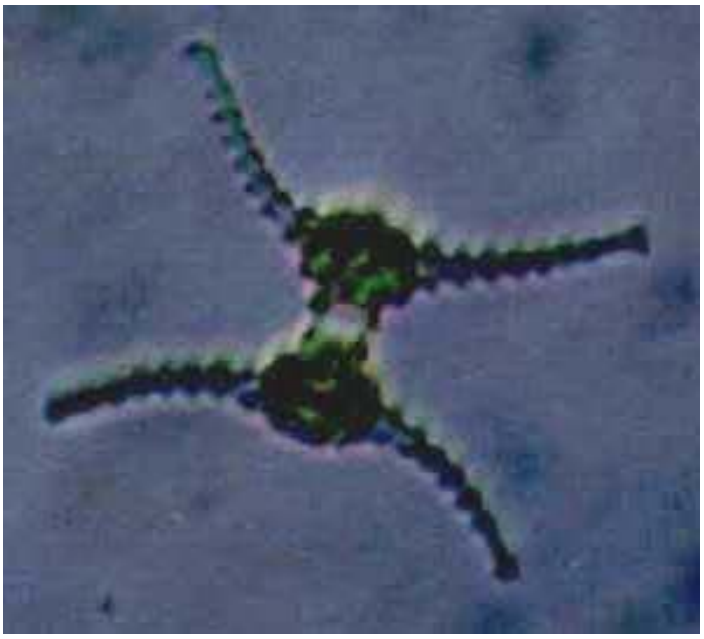

Figure 22. *Staurastrum* sp. Image obtained with the Nomarski microscope.

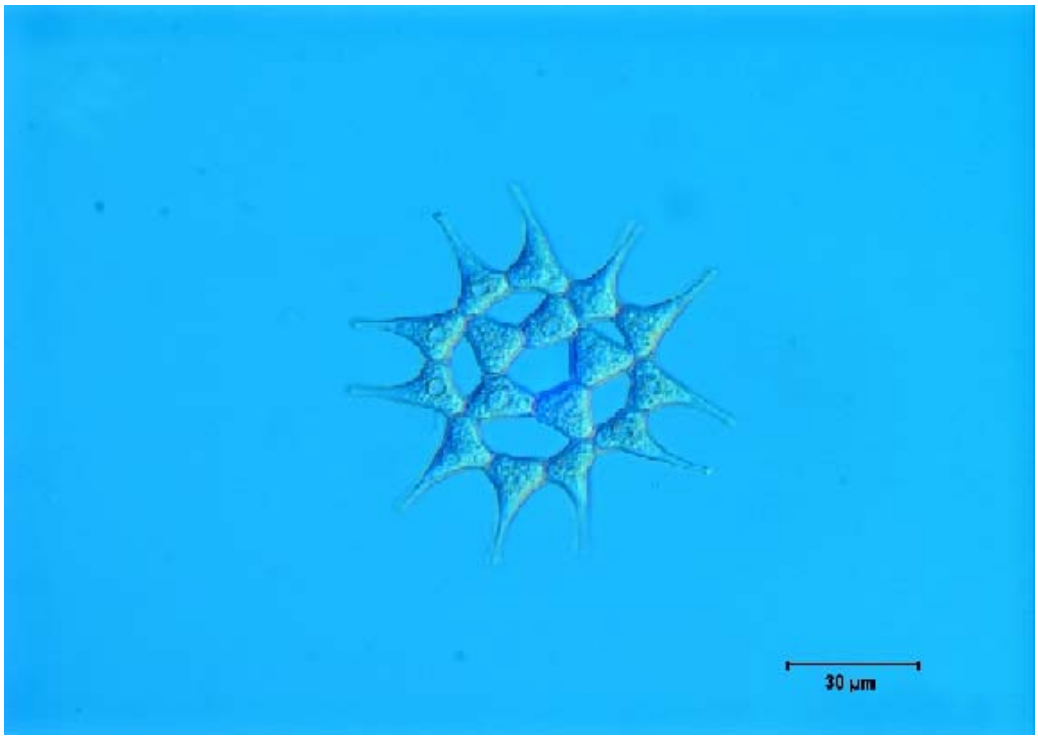

Figure 23. *Pediastrum simplex*. Image obtained with the Nomarski microscope.

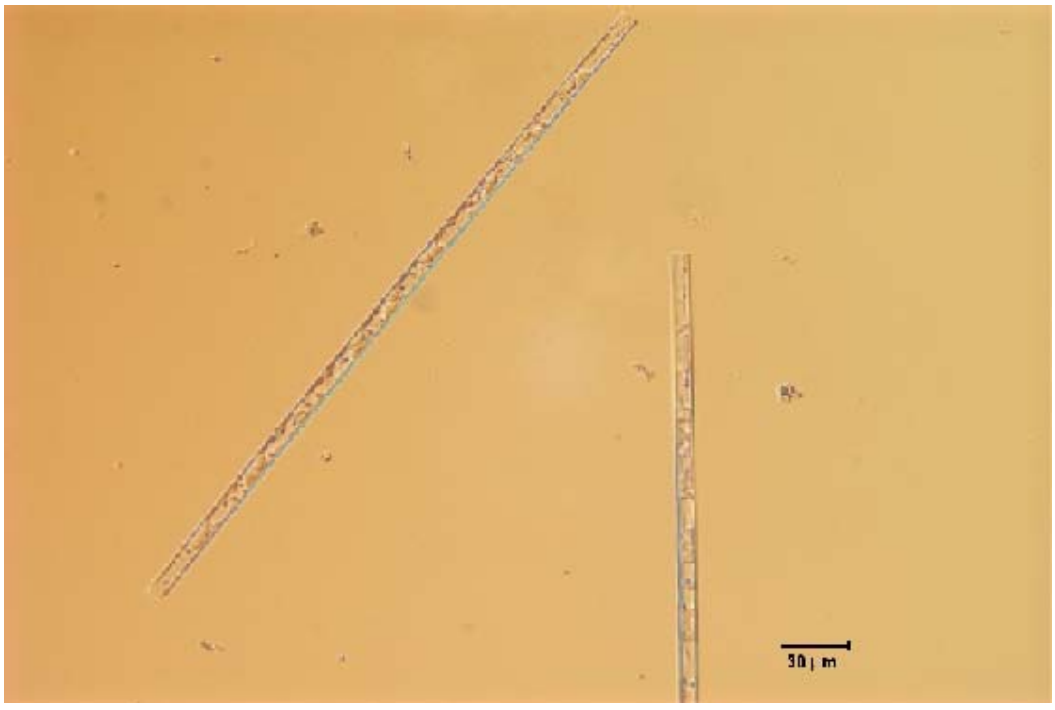

Figure 24. *Synedra* sp. Image obtained with the Nomarski microscope.

OTHER ACTIVITIES RELATED TO THE PROJECT

An estimate of the professional hours devoted to the different tasks associated with this project since our last report (May 2004) is shown in Tables 32 - 34.

Table 32. Professional hours devoted to the chemical characterization portion of the study.

Tasks	Professional hours	Total hours since last report*
Simple pick up at EQBs laboratory	8 hrs/ sampling event	24
Filtering of simples for DP, and DOC determination	8 hrs/ sampling event	24
Digestion – TKN	16 hrs/ sampling event	48
Digestion –TP	12 hrs/ sampling event	36
Cleaning glassware	40 hrs/ sampling event	120
DOC analysis	3 hrs/ sampling event	9
TP, DP analysis	8 hrs/ sampling event	24
TKN analysis	8 hrs/ sampling event	24
Preparation of laboratory Report	16 hrs/ sampling event	48
Clorophyll analysis - Extraction, and sample measurement	24 hrs/ sampling event	72
Data evaluation- (e.g., preparation of graphs, data manipulation)	24 hrs/ sampling event	72
Preparation of purchase orders for materials, and supplies	24 hrs	24
Financial report preparation	10 hrs	10
Statistical Analyses	16 hrs	16
Preparation of Biannual report (Dic. 04)	40 hrs	40
Total hours		**591**

* A total of 3 sampling events have been conducted since our last report (May, 2004).

Table 33. Professional hours devoted to the periphytometer studies.

Other tasks related to the project	Professional hours	Total hours since last report*
Periphytometer studies	144 hrs/trial (3 persons @ 48 hrs each/trial)	**1,152**

-Eight trials have been conducted since our last report (May 04).

Table 34. Professional hours devoted in the phytoplankton characterization study:

Number of lakes	Number of samples per lake	Number of visits	Number of slides scanned per sample	Time /slide (including species identification)
11-12	**2-3**	**3**	**6**	**1.5-2 hrs.**
			TIME EFFORT	**594-1296 hours (25-54 straight days)**

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