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

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

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OBJECTIVE OF THE PROJECT

1. The objective of this study is to 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 three lake sampling events have been completed to date (Table 1).

Table 1: Dates for the sampling events completed at this point:

Sampling event	Dates
1	8/12/03 – 9/2/03
2	11/7/03 – 12/10/03
3	2/23/04 – 3/18/04

Results of the first sample event were presented in the first progress report. A total of 150 samples were collected in the second sampling event. A distribution of samples per analyses is as follows: nutrients (TKN, TP, DOC) - 51 samples, periphyton diversity- 51 samples, and chlorophyll a - 48 samples (samples for the Cerrillo lake were accidentally lost during the sampling trip). One hundred-seventy one (171) samples were colleted in the third sampling event, fifty-seven each for nutrients (TP, TKN), periphyton diversity, and chlorophyll *a* analysis respectively.

Samples were analyzed for total kjedahl nitrogen (EPA method 351.2), dissolved reactive P and total reactive 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.). As specified in our first progress report we recognize that this is not an EPA certified procedure. Results are used exclusively as reference values to make inferences on the potential limiting factors to biomass production.

LABORATORY RESULTS

A summary of the chemical analyses results for the second and third sampling is shown in Tables 2 and 3 respectively (A full QA/QC report is available upon request).

Second sampling event

Table 2: Chemical analyses results of lake samples from the second sampling event (November- December 03).

PREQBs sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	Chl a (μg/l)	NO_3-N (mg/L)	NH_4-N (mg/L)
Las Curias-01-sec 2 entrada	0.678	0.028	0.01	8.61	30.6	0.09	0.29
Las Curias-02-sec 5 represa	0.608	0.039	0.01	8.22	41.76	0.09	0.51
La Plata-01-sec 2 entrada	0.312	0.086	0.07	5.81	3.054	1.15	0.03
La Plata-02-sec 5 represa	0.383	0.065	0.01	6.35	60.3	0.46	0.02
Carraizo-01-sec 1 entrada	0.332	0.116	0.075	5.9	5.73	1.19	0.06
Carraizo-01-sec 1 entrada	0.334	0.104	0.072	5.85	7.89	1.45	0.06
Carite-01 sec1 cuenca R. la plata	0.172	0.01	nd	4.62	2.097	0.16	0.10
Carite-02 sec 4 represa	0.214	0.01	nd	5.32	3.051	0.15	0.08
Patillas-01 sec 2 entrada rio Patillas	0.18	0.012	0.01	5.87	5.79	0.03	0.09

Table 2 (cont.).

PREQBs sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	Chl a (µg/l)	NO_3-N (mg/L)	NH_4-N (mg/L)
Patillas-01 sec 2 entrada rio Patillas	**	0.011	0.01	2.58	6.96	0.04	0.07
Patillas-02 sec 4 entrada rio marin	0.177	0.013	0.01	2.73	2.17	0.05	0.09
Patillas-03 sec 8 represa derecha	0.207	0.01	0.01	2.4	6.18	0.03	0.04
Patillas-04 sec 11 represa AEE	0.161	0.01	0.01	2.38	7.62	0.03	0.06
Guayo-01 sec 2 entrada rio guayo	0.299	0.083	0.01	6.63	80.1	1.11	0.08
Guayo-02 sec 4 centro	0.221	0.031	nd	6.17	10.14	0.57	0.05
Guayo-03 sec 8 represa	0.229	0.03	nd	5.65	21.72	0.43	0.04
Caonillas-01-sec 1 rio caonillas	0.35	0.065	0.01	5.97	11.25	0.44	0.06
Caonillas-01-sec 1 rio caonillas	0.344	0.061	0.01	5.97	11.07	0.42	0.07
Caonillas-02-sec 4 centro	0.341	0.043	0.01	5.39	17.19	0.36	0.04
Caonillas-03 sec 7 represa	0.259	0.016	nd	6.1	19.08	0.45	0.04
Matrullas-1 sec1 entrada	0.068	0.052	0.04	1.63	0.354	0.70	0.05
Matrullas-1 sec 1 entrada	0.054	0.056	0.047	1.6	0.2187	0.69	0.04
Matrullas-2 sec 4 represa	0.108	0.032	0.017	3.6	2.043	0.54	0.03
Cerrillo-01 cerca rio	0.203	0.024	0.01	2.94	N/A	0.04	0.09
Cerrillo-01 cerca rio	0.218	0.024	nd	2.84	N/A	0.03	0.16
Cerrillo 02 sec 5 centro lago	0.178	0.016	nd	2.64	N/A	0.01	0.07
Cerrillo- 03 sec 8 represa	0.223	0.021	nd	2.44	N/A	0.00	0.07
Cidra-01 sec 1 Puente	0.457	0.015	0.01	8.37	9.12	0.16	0.18
Cidra-01 sec 1 Puente	0.457	0.018	0.012	8.43	8.901	0.14	0.06
Cidra-02 sec 4 center	0.4	0.023	0.03	8.17	43.56	0.18	0.09

Table 2 (cont.).

PREQBs sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	Chl a (µg/l)	NO_3-N (mg/L)	NH_4-N (mg/L)
Cidra-03 sec 7 represa	0.432	0.032	0.01	8.31	13.92	0.20	0.19
Guineo 1 sec 2 entrada	0.479	0.063	0.01	7.02	132.75	0.05	0.01
Guineo 2 sec 5 represa	0.208	0.023	0.01	5.58	4.74	0.09	0.04
Guajataca 1 sec 1 entrada rio	0.283	0.014	nd	4.02	7.68	0.08	0.07
Guajataca 1 sec 1 entrada rio	0.241	0.011	nd	3.95	11.22	0.07	0.09
Guajataca 2 sec 4 represa	0.23	0.014	nd	4.02	11.46	0.01	0.05
Dos Bocas 1 sec1	0.341	0.048	0.027	9.36	5.76	0.99	0.05
Dos Bocas 2 sec4 represa	0.224	0.038	0.025	10.17	6.57	0.91	0.07
Melania est 1 sec 1	0.442	0.019	0.035	4.91	5.67	0.03	0.05
Loco II represa sec 2	0.282	0.085	0.036	5.85	3.22	1.43	0.04
Luchetti 01 sec 1 entrada rio	0.248	0.07	0.01	3.31	7.59	1.25	0.06
Luchetti 01 sec 1 entrada rio	0.229	0.072	0.01	3.15	4.54	1.26	0.05
Luchetti 02 sec 4 centro	0.264	0.128	0.01	3.22	33.93	1.01	0.04
Luchetti 03 sec 7 represa	0.206	0.054	0.014	3.41	14.16	1.05	0.04
Garzas est 1 sec 2	0.142	0.03	nd	3.36	17.37	0.53	0.03
Garzas est 1 sec 2	0.124	0.027	nd	3.5	17.82	0.50	0.02
Guayabal II centro 1	0.268	0.051	0.04	4.84	18.99	0.71	0.06
Guayabal III represa sec 4	0.232	0.067	0.01	3.79	6.59	0.62	0.03
Toa Vaca 01 sec 2 entrada del rio	0.365	0.055	0.041	4.7	15.42	0.03	0.08
Toa Vaca 02 sec 6 centro	0.301	0.012	0.01	2.76	3.22	0.02	0.06

Table 2 (cont.).

PREQBs sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	Chl a (µg/l)	NO_3-N (mg/L)	NH_4-N (mg/L)
Toa Vaca 03 sec 9 represa	0.255	0.011	0.01	2.65	3.41	0.00	0.02
Toa Vaca 03 sec 6	0.34	0.011	nd	2.66	4.16	0.00	0.01

- **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. No difference between the laboratory blank and the sample was detected.**
- **** - This sample could not be analyzed for TKN. An accidental mix up occurred during sample preservation on which the preservative for phytoplankton was added to this sample.**
- **N/A – not applicable. The samples were accidentally lost during the sampling event.**

Table 3: Descriptive Statistics of results for the second sampling event

	TKN	TP	DP	DOC	Chl *a**	NO_3	NH_4
Median (mg/L)	0.255	0.030	0.006	4.77	7.79	0.19	0.057
Average(mg/L)	0.280	0.040	0.014	4.91	16.00	0.43	0.075
Std. Dev.	0.123	0.030	0.019	2.16	23.20	0.45	0.078
Max. (mg/L)	0.678	0.128	0.075	10.17	132.75	1.45	0.51
Min. (mg/L)	0.054	0.007	nd	1.6	0.22	0.003	0.007

***µg/L**

Third sampling event

Table 4: Chemical analyses results of lake samples from the second sampling event (February-March 04).

Sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	CHL a (µg/l)	NO_3-N (ppm)	NH_4-N (ppm)
Las Curias-01-sec 2 entrada	0.532	0.029	0.013	6.03	1.386	0.059	0.121
Las Curias-01-sec 2 entrada FD	0.539	0.027	0.01	5.71	6.318	0.078	0.044
Las Curias-02-sec 4 represa	0.385	0.023	0.012	5.57	3.588	0.059	0.052

Table 4 (cont.).

Sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	CHL a (µg/l)	NO_3-N (ppm)	NH_4-N (ppm)
La Plata-01-sec 3-entrada	0.616	0.062	0.019	16.53	41.79	0.130	0.065
La Plata-01-sec 3FD-	0.601	0.066	0.01	15.21	36.96	0.127	0.063
La Plata-02-sec 6 – represa	0.496	0.034	0.01	15.18	4.506	0.076	0.108
Carraizo-01-sec 3 57500	0.565	0.129	0.1	6.88	4.446	0.897	0.080
Carraizo-01-sec 3 FD 57500	0.659	0.133	0.096	6.49	8.208	1.160	0.077
Carite-01 sec 1 desemb rio plata	0.286	0.011	0.01	4.24	0.42	0.361	0.084
Carite-01 sec 1 desemb rio plata	0.294	0.018	0.01	3.66	0.492	0.416	0.103
Carite-02 sec 4 represa	0.286	0.015	0.01	3.96	1.218	0.290	0.084
Patillas entrada rio patillas	0.309	0.015	0.014	1.94	2.268	0.106	0.077
Patillas-02 sec 4-rio marin	0.271	0.017	0.016	2.04	2.724	0.161	0.134
Patillas-03 sec 6-frente dique	0.301	0.019	0.016	2.04	1.632	0.124	0.066
Patillas-04 sec 8-represa	0.431	0.014	0.011	2.06	1.548	0.168	0.117
Guayo-01 sec 3 89004 near rio	0.757	0.037	0.01	5.74	14.04	0.383	0.004
Guayo-02 sec 6 89005 center	0.484	0.025	0.01	4.19	10.54	0.567	0.191
Caonillas-01-sec 1	0.539	0.052	0.015	4.3	24.12	0.497	0.050
Caonillas-01-sec 1 FD	0.534	0.047	0.015	4.17	10.36	0.461	0.197
Caonillas-02-sec 4 centro	0.424	0.040	0.014	3.81	7.266	0.473	0.032
Caonillas-03 sec 7 represa	0.299	0.019	0.015	2.84	10.51	0.451	0.030
Matrullas-01 sec 2 entrada	0.354	0.027	0.011	1.34	21.96	1.955	0.036
Matrullas-01 sec 2 entrada FD	0.316	0.027	0.011	1.41	16.56	2.896	0.077
Matrullas-02 sec 5-represa	0.354	0.024	0.01	1.21	20.64	0.960	0.046

Table 4 (cont.).

Sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	CHL a (µg/l)	NO_3-N (ppm)	NH_4-N (ppm)
Cerrillo-01 entrada	0.485	0.052	0.014	3.33	6.18	0.129	N/A
Cerrillo-01FD entrada	0.481	0.049	0.014	3.3	17.76	0.134	0.055
Cerrillo 02 sec 5 centro	0.286	0.018	0.01	2.88	5.58	0.114	0.042
Cerrillo- 03 sec 8 represa	0.287	0.017	0.01	2.89	4.176	0.115	0.057
Cidra-01 sec 3 -89029	0.645	0.039	0.011	7.06	9.144	0.137	0.069
Cidra-01 sec 3FD-89029	0.600	0.041	0.011	7.48	4.884	0.143	0.054
Cidra-02 sec 6 center 89030	0.625	0.037	0.01	6.34	11.58	0.145	0.037
Cidra-03 sec 9dam 89031	0.551	0.024	0.01	6.16	3.048	0.178	0.051
Guineo est 1 sec 1 desemb. Toro negro	1.033	0.069	0.016	4.24	28.98	0.138	0.061
Guineo est 2 sec 4 represa	0.472	0.029	0.01	2.66	10.37	0.111	0.053
Guineo est 2 sec 4 represaFD	0.342	0.026	0.01	2.55	10.03	0.139	0.067
Guajataca 10720 sec3 entrada	0.336	0.016	0.01	2.6	2.124	0.308	0.111
Guajataca 10720 sec3 FD	0.375	0.019	0.01	2.44	2.58	0.252	0.093
Guajataca 10790 sec6 represa	0.316	0.017	0.01	2.22	2.64	0.120	0.070
Dos Bocas 25110sec3	0.564	0.075	0.04	2.63	2.292	0.916	0.116
Dos Bocas 25110sec3 FD	0.530	0.086	0.038	1.56	8.844	0.861	0.121
Dos Bocas sec6 represa	0.386	0.031	0.015	1.56	7.674	0.586	0.083
Melania sec 2 FD centro	0.679	0.032	0.017	6.4	1.644	0.061	0.078
Melania sec 2 centro	0.710	0.028	0.016	6.35	4.104	0.159	0.141
Loco entrada sec 2	0.304	0.036	0.019	3.16	4.356	1.175	0.049

Table 4 (cont.).

Sample I.D.	TKN (mg/L)	TP(mg/L)	DP (mg/L)	DOC (mg/L)	CHL a (µg/l)	NO_3-N (ppm)	NH_4-N (ppm)
Loco represa sec5	0.386	0.036	0.015	3.2	18.84	0.992	0.040
Luchetti 01 sec 2 entrada	0.006	0.032	0.012	11.6	9.924	N/A	0.086
Luchetti 01 sec 2 entradaFD	0.007	0.035	0.011	18.46	8.688	N/A	0.081
Luchetti 02 sec 5 centro	0.430	0.031	0.01	2.13	10.77	0.949	0.045
Luchetti 03 sec 8 represa	0.471	0.028	0.01	1.98	10.48	0.830	0.039
Garzas 20050 - sec 2	0.298	0.019	0.01	2.45	2.004	0.386	0.048
Garzas 20050 - sec 2FD	0.241	0.015	0.011	2.34	6.486	0.410	0.069
Guayabal est 2 sec 2 centro	0.481	0.044	0.017	2.7	27.6	0.157	0.078
Guayabal est 2 sec 2 centroFD	0.513	0.040	0.014	2.54	5.634	0.230	0.048
Guayabal est 3 sec 5 represa	0.697	0.059	0.017	2.79	24	0.326	0.041
Toa Vaca 01 sec 1 entrada	0.460	0.046	0.018	3.91	5.868	0.087	0.039
Toa Vaca 02 sec 4 centro	0.389	0.041	0.023	3.89	4.998	0.100	0.043
Toa Vaca 03 sec 7 frente represa	0.391	0.046	0.027	3.92	2.646	0.095	0.050

- **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.**
- **N/A – no sample available.**

Table 5. Descriptive Statistics of results for the third sampling event

	TKN	TP	DP	DOC	Chl *a**	NO_3	NH_4
Median (mg/L)	0.43	0.031	0.012	3.33	6.32	0.18	0.065
Average (mg/L)	0.45	0.037	0.016	4.67	9.46	0.42	0.072
Std. Dev.	0.17	0.024	0.017	3.79	9.13	0.51	0.037
Max. (mg/L)	1.03	0.133	0.1	18.46	41.79	2.90	0.20
Min. (mg/L)	0.006	0.011	0.006	1.21	0.42	0.058	0.0037

*µg/L

A detailed statistical analysis will be performed once the sampling protocol for this year (four sampling events) is completed (the next sampling event is scheduled for June, 2004). At that time comparisons will be performed to establish within lake, and between lake differences, as well as to identify any potential seasonal effect. At present only a preliminary assessment of the general quality status of the lakes will be established.

The distribution of average TKN, and TP values for each lake are shown on Figures 1 and 2. In general, TKN values show a continual increase with sampling date, with higher TKN concentrations obtained in the third sampling event (February-March 2004) in most lakes. Exceptions to that trend were Las Curias lake which exhibited a higher average TKN value on the second sampling event, and Matrullas and Cerrillos which exhibited higher TKN values in the first sampling event compared to the second sampling event (both lower than the third event). Carite and Patillas exhibited a similar behavior than the latter although the difference between the first and second sampling event was minimal. The behavior observed in terms of TKN values could be a reflection of the amount of precipitation occurring in the watershed areas of each lake. Increase nutrient loads from non-point sources can be expected as a transition from the dry season to the wet season of the island occurs. A specific evaluation on the connection between increase rainfall and the observed increases in nutrient concentrations for each lake can not be made at this time as data regarding the precipitation records on the watershed areas of each lake could not be assessed in time for this report. However, in general Puerto Rico has experienced continual higher precipitation in the months following the first sampling event, which may explain the results obtained.

Average total phosphorus concentrations (Fig. 2) exhibited a similar trend to TKN values in the sense that results for the first sampling event were generally lower than values obtained in the second and third event. Differences between the second and third event were much less uniform, with approximately half of the lakes exhibiting higher TP values in the second sampling relative to the third event, and the other have experiencing a reverse trend. Media values for TP concentrations for the second and third event were 0.030, and 0.031 mg/L a value twice as high as that obtained in the first sampling event (0.015 mg/L). Again, these results may be a reflection of an increase contribution from non-point sources as a result of increase precipitation in the watershed areas of each lake.

An evaluation of the TKN/TP ratio (molar basis) (fig. 3) suggests that in most lakes phosphorus would be the limiting nutritional factor controlling biomass growth (TKN/TP ratio > 16). For some lakes, (e.g., Loco, Luchetti, Garzas, Guayabal) nitrogen could prove limiting particularly during periods of low nutrient loads from external sources. There may also be some seasonal effects during which the limiting nutrient in a particular lake may alternate between nitrogen and phosphorus.

Even though the TKN/TP ratios suggest that in some lakes nitrogen could be the limiting factor for biomass growth, nitrate (NO_3) concentration results indicate that there is an additional pool of nitrogen (not accounted by TKN) available for biomass growth thus reducing the overall impact of nitrogen in controlling biomass growth. Curiously, those lakes which appear to be more prone to a nitrogen deficit (Loco, Luchetti, Garzas, Guayabal) exhibited substantial nitrate contributions during the second and third sampling event (fig. 4). These results stress the need of controlling non-point source contributions to the lakes as a means of restoring their natural nutritional status.

Average chlorophyll *a* values suggest that most of our lakes are mesotrophic with values that may reach eutrophic levels in particular zones of some lakes during different times of the year (Fig. 6). An accurate assessment of the trophic status of each lake can not be established based on average values due to the strong seasonal and within lake variability exhibited by most lakes. The results are only presented to substantiate the general condition of our lakes in terms of biomass growth. A detailed characterization of phytoplankton diversity within each lake, as well as an assessment of the nutrient-biomass growth relationships for a selected group of lakes will be established as part of this study (see phytoplankton diversity section). The results will enable us to provide an accurate assessment of the trophic status of our lakes and establish guidelines for the distinction between different trophic categories (i.e., oligotrophic, mesotrophic, eutrophic, hypertrophic).

An evaluation of the relationship between total phosphorus concentrations and chlorophyll *a* values was conducted to establish the role of this nutrient in controlling algae growth. To eliminate the effect of reduced light penetration on biomass growth only those samples with a Secchi depth > 1.0 m were included. As Secchi depth measurements from the third sampling had not been made available at the time of the writing of this report this criteria was only applied to samples from the second sampling event. That is, all samples from the third sampling event were included regardless of their Secchi depth measurement. In addition, due to its high eutrophic status samples from the Carraizo lake were not included. A positive correlation was observed between TP and chlorophyll *a* concentrations. That means that increases in phosphorus loading will translate into an increase in algae biomass productivity. The observed relationship is remarkably very similar to that reported by Martínez, 2002 for a limited data set of several lakes in the island. Is possible that the lower correlation coefficient obtained (R^2 = 0.27) in this study relative to that of Martínez 2002 (R^2 =0.79) could be due to the fact that samples from the third event could not be separated based on Secchi depth. Once the data is corrected for this effect the relationship may improve significantly. Despite this, the fact that a positive relationship between TP and Chl *a* is consistently observed in our lakes may indicate that, as observed in temperate regions, phosphorus could be the limiting nutrient for biomass growth.

A preliminary ranking of the trophic status of our lakes was performed by means of the trophic state index approach (Carlson, 1977). Trophic state index values for chlorophyll *a* and total P were calculated using the equations developed by Carlson for temperate regions lakes. This approach will be refined in the future by employing equations specifically derived from our data. The equations employed were:

1) $$TSI(Chl) = 10\left(6 - \frac{2.04 - 0.68 \ln Chl}{\ln 2}\right)$$

2) $$TSI(TP) = 10\left(6 - \frac{\ln \frac{48}{TP}}{\ln 2}\right)$$

A trophic state status is assigned based on the following categories (Table 6):

Table 6. Trophic state classification according to the trophic state index

Trophic State Category	Trophic State Index Value
Oligotrophic	< 30
Hypotrophic	30 – 40
Mesotrophic	40 – 50
Eutrophic	50 – 60
Hypertophic	> 70

Chlorophyll a and TP trophic state index values were calculated for each data point. An analysis of the distribution of values between the different categories was conducted. Tables 7 and 8 show the category distribution for each lake based on the frequency values. Results from this exercise confirms the previous statement that most of our lakes would fall into the mesotrophic category with conditions that promote eutrophic behavior in particular zones of a significant number of lakes during different times of the year

Table 7. TSI(Chl) values of lakes of Puerto Rico. Except for Guineo only those categories with higher than 30% of the individual values are identified.

Trophic State Index Categories						
Lake name	< 30	30 - 40	40 -50	50 – 60	60 - 70	>70
Caonillas				X		
Carite		X	X			
Carraizo			X			
Cerrillo		X	X			
Cidra			X			
Curias		X	X			
Dos Bocas			X	X		
Garzas			X			
Guajataca		X	X	X		
Guayabal			X			
Guayo			X	X		
Guineo		X	X	X	X	X
La Plata			X	X		
Loco				X		
Luchetti				X		
Matrulla			X	X		
Melania			X			
Patillas		X	X			
Toa Vaca			X			

Table 8. TSI(TP) values of lakes of Puerto Rico. Only those categories with higher than 30% of the individual values are identified.

Trophic State Index Categories						
Lake name	< 30	30 - 40	40 -50	50 – 60	60 - 70	>70
Caonillas			X	X		
Carite		X	X			
Carraizo						X
Cerrillo			X			
Cidra			X	X		
Curias			X	X		
Dos Bocas				X		
Garzas		X	X	X		
Guajataca			X			
Guayabal		X			X	
Guayo			X		X	
Guineo			X		X	
La Plata			X		X	
Loco		X		X		
Luchetti		X		X		
Matrulla			X	X		
Melania		X	X	X		
Patillas	X	X				
Toa Vaca		X		X		

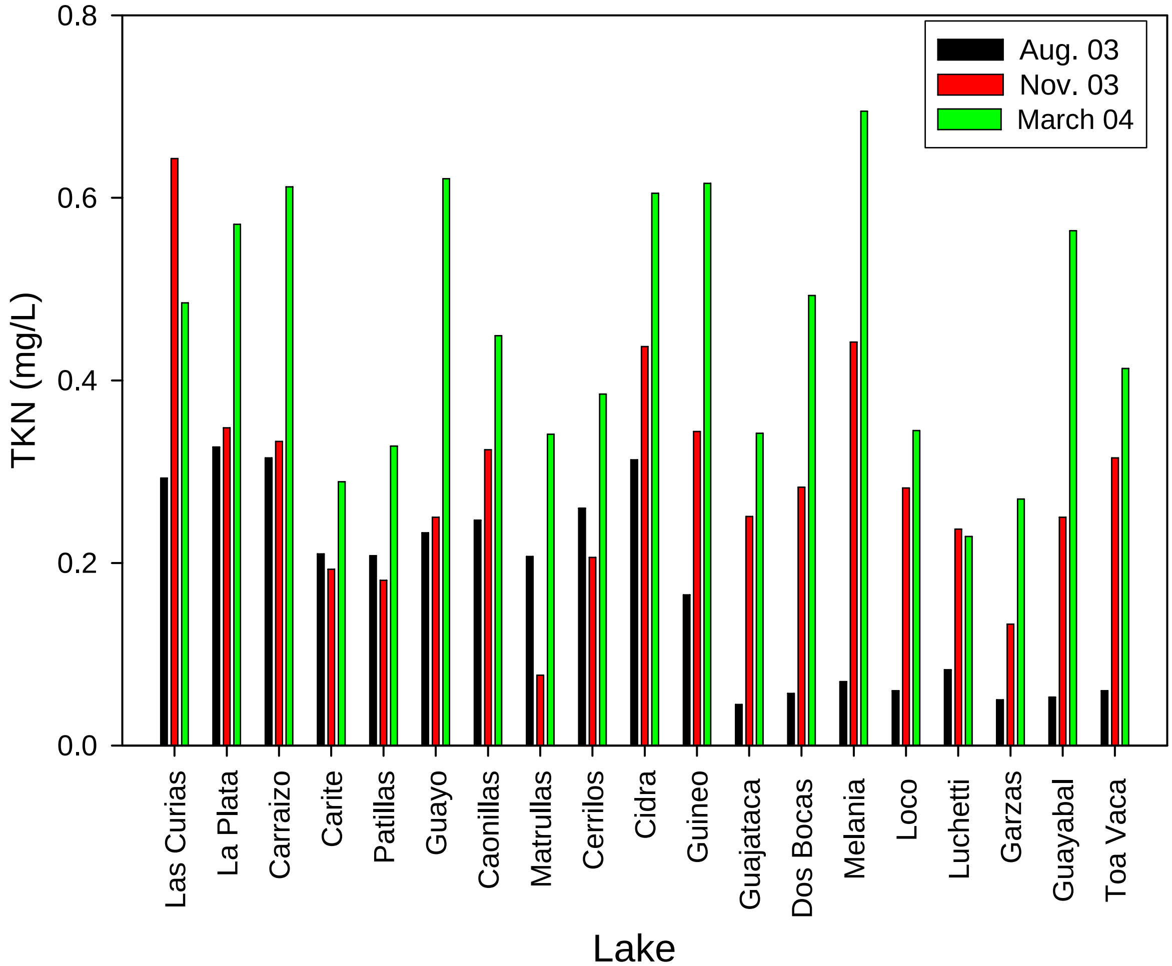

Figure 1: Average TKN concentrations of Puerto Rican lakes at different sampling dates.

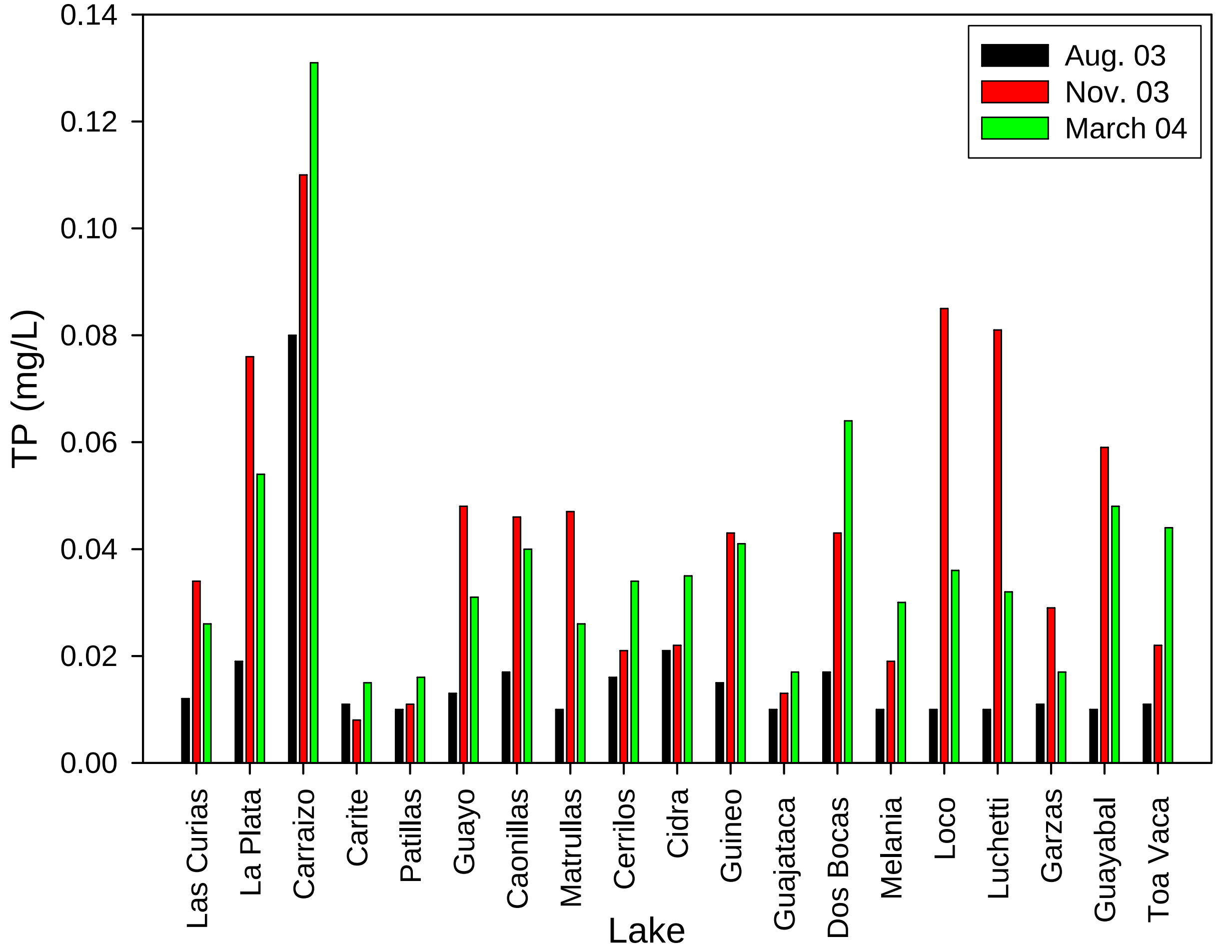

Figure 2: Average TP concentrations of Puerto Rican lakes at different sampling dates.

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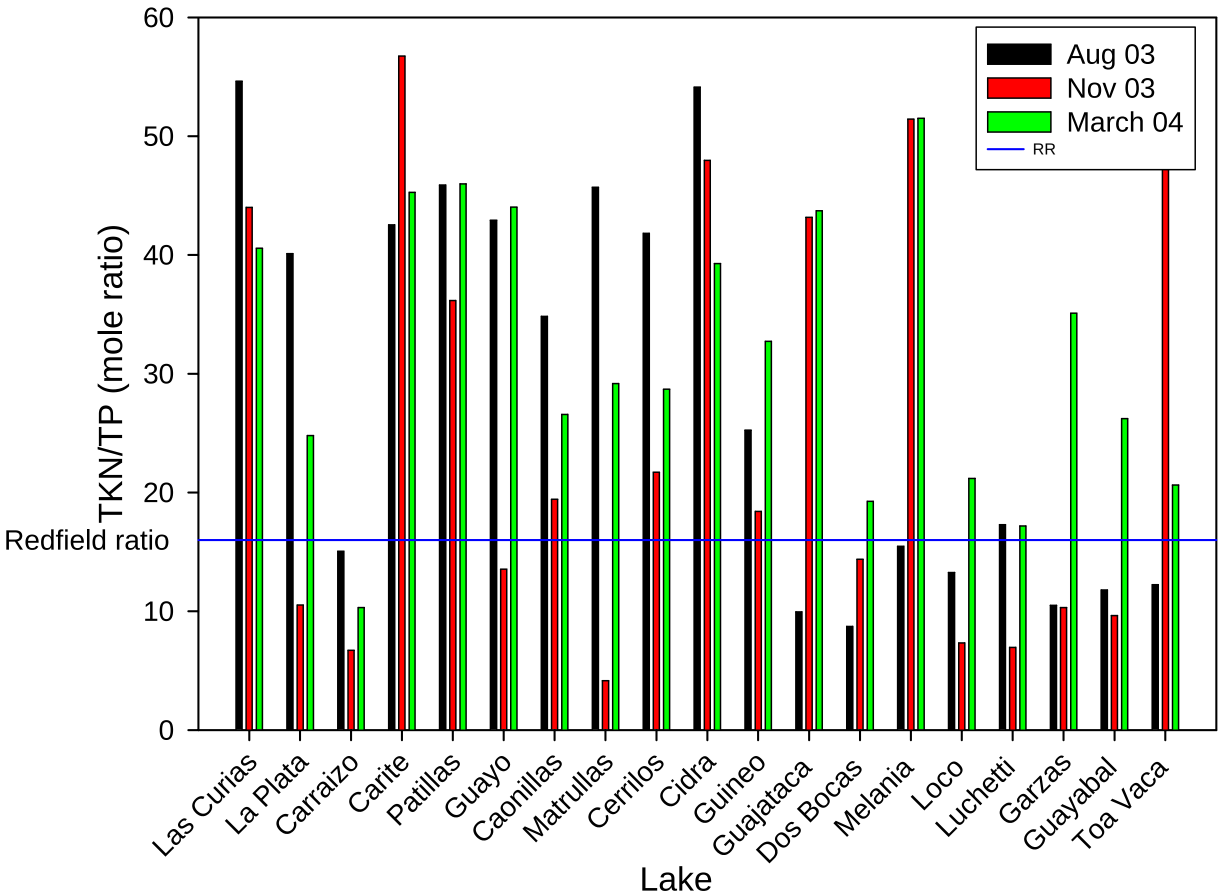

Figure 3: Average TKN/TP molar ratios for Puerto Rican lakes at different sampling events.

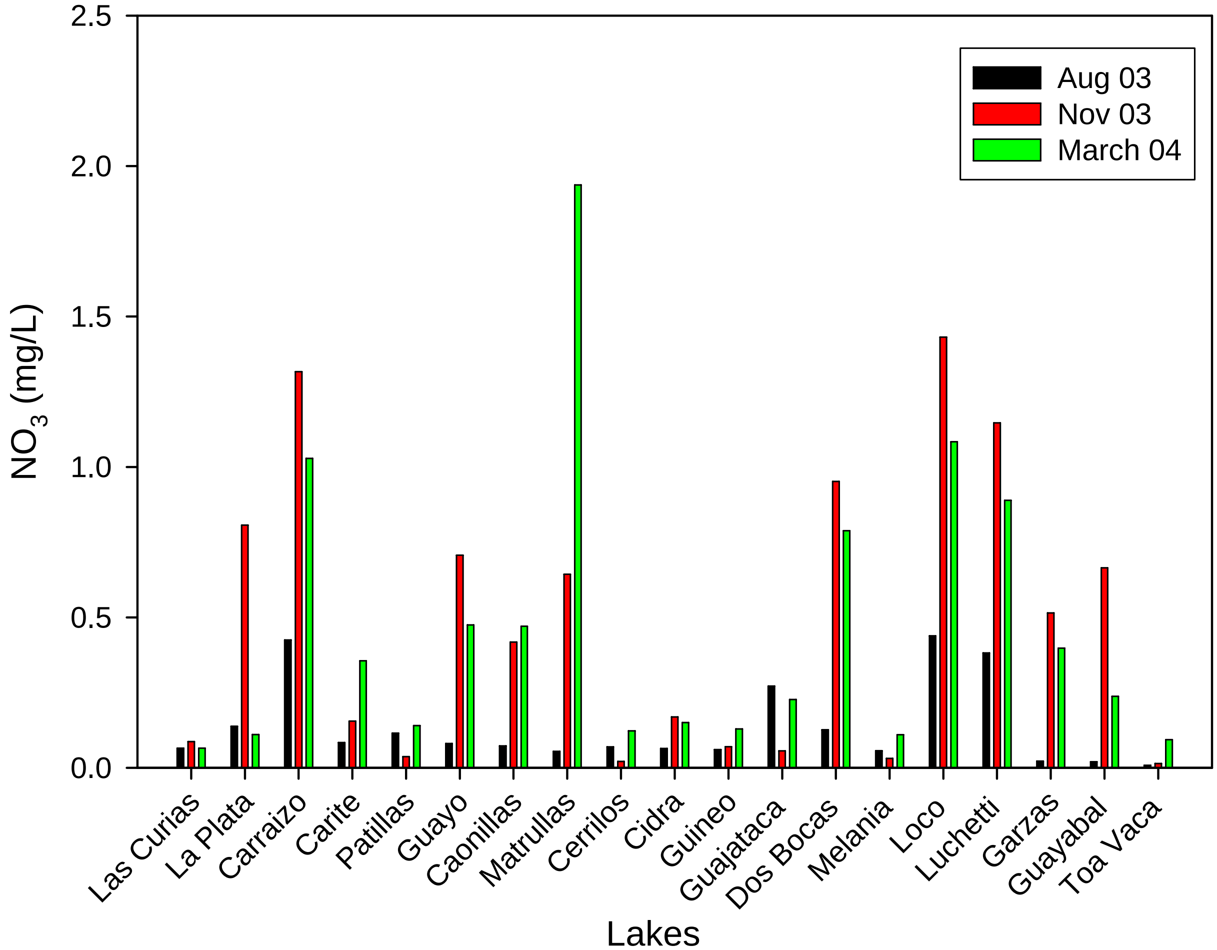

Figure 4: Average NO_3 levels for Puerto Rican lakes at different sampling events

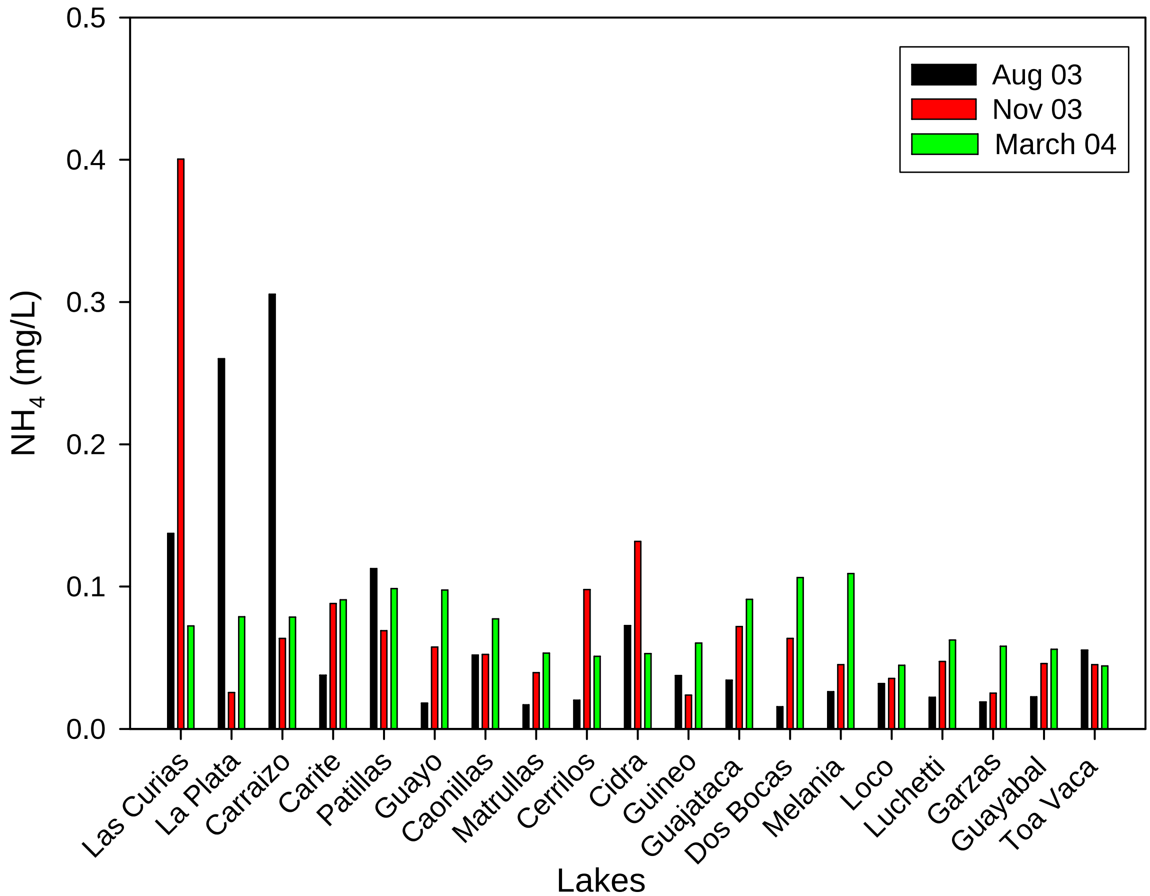

Figure 5: Average NH_4 levels for Puerto Rican lakes at different sampling events

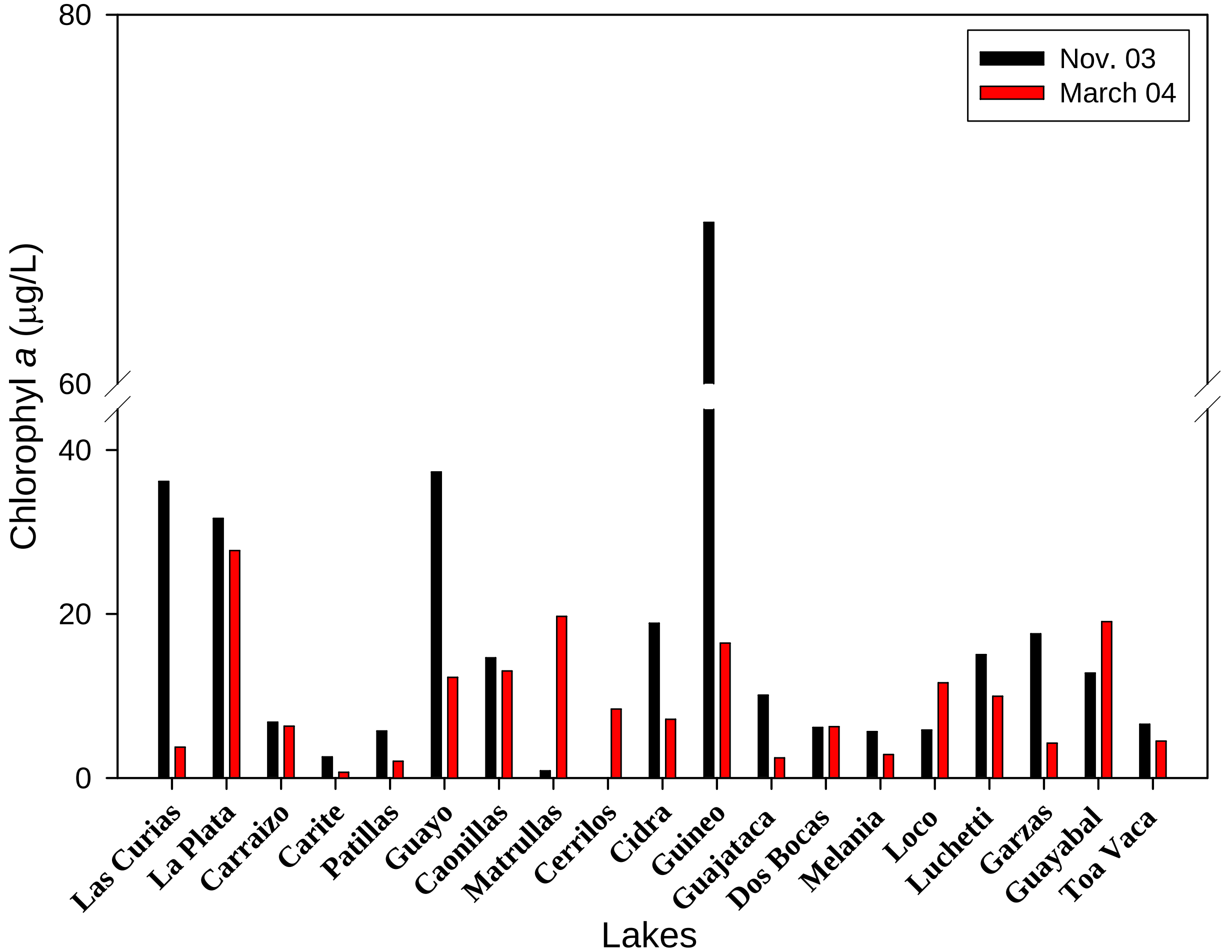

Figure 6: Average chlorophyll *a* concentrations for Puerto Rican lakes sampled at different times throughout the year.

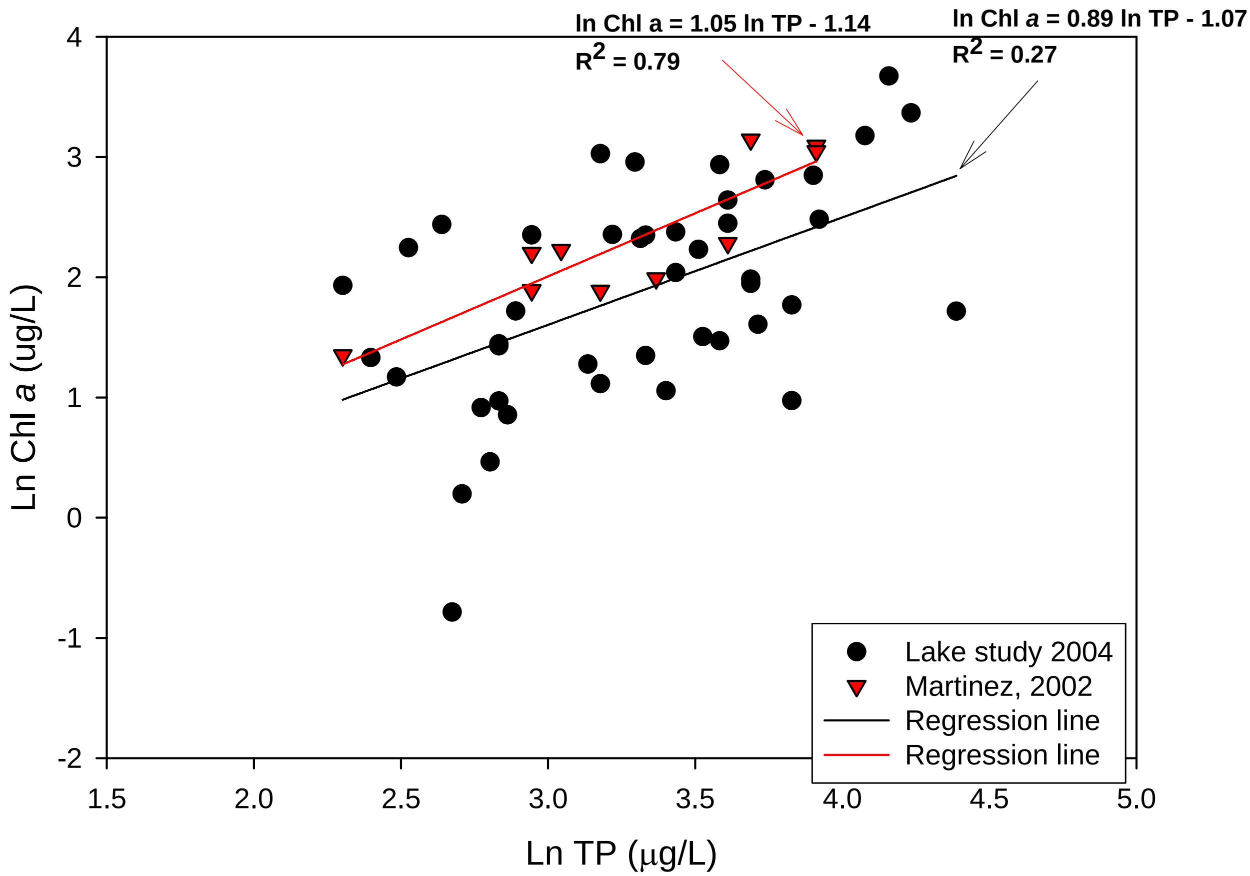

Figure 7. Relationship between total phosphorus and chlorophyll a concentrations in lakes of Puerto Rico. Data corresponds exclusively to samples from the second and third sampling events. Samples from the second sampling event with less than 1 meter Secchi Depth were excluded. Results from Carraizo (second and third sampling event) are not included. Data from Martínez, 2002. J Agric. Univ. of P.R. 86(3-4): 139 – 144.

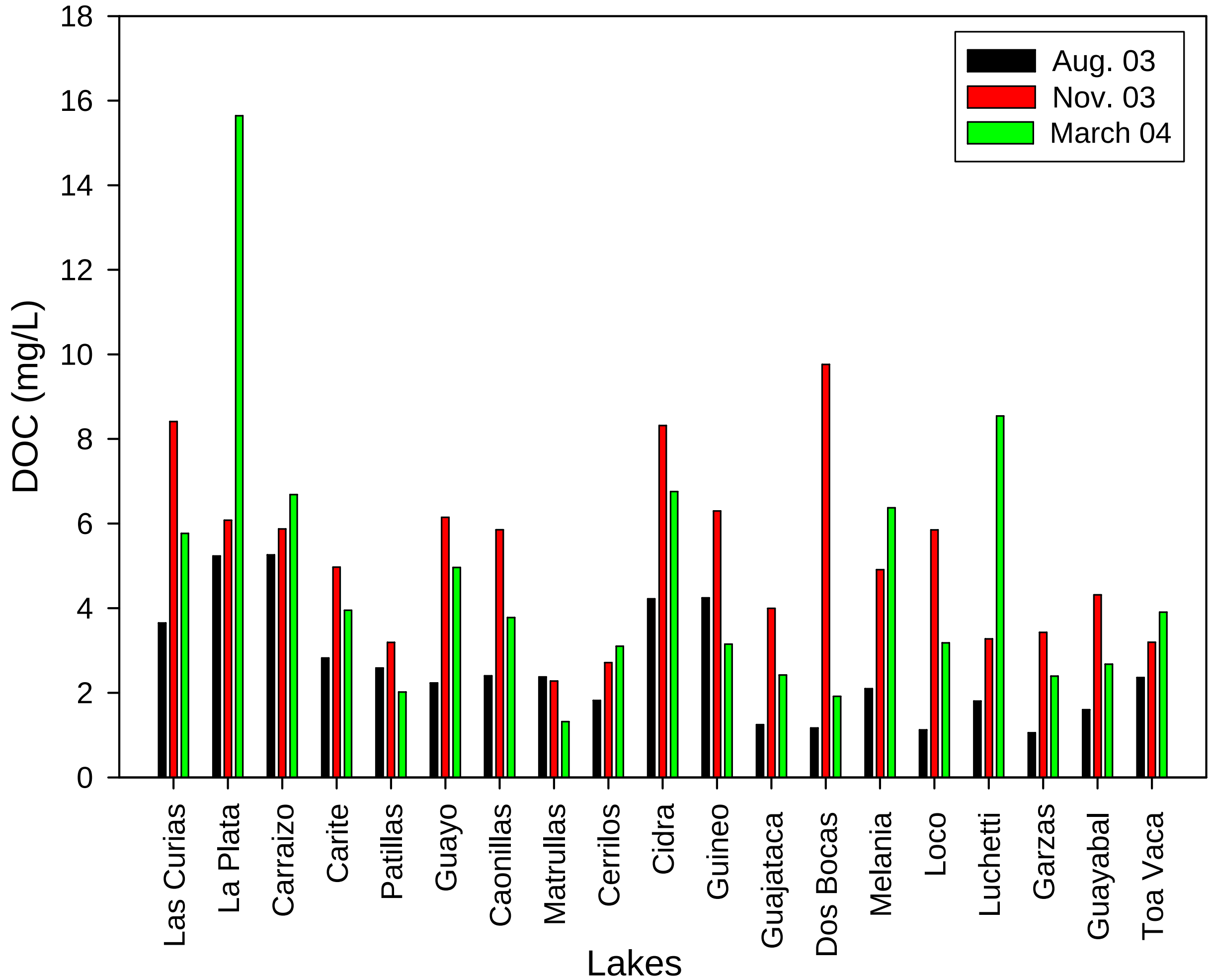

Figure 8: Distribution of average DOC concentrations for Puerto Rican lakes sampled at different times throughout the year.

PERIPHYTOMETER STUDY

A series of *in-situ* assays have been conducted to evaluate the effectives of the "Matlock periphytometer" as a tool to identify the limiting nutrient for phytoplankton growth on our lakes (Figures 9, 10 and 11) (Table 9).

Table 9. Periphytometer studies

Trial Date	Locality
2/4/04	Cerrillos lake, Ponce
3/24/04	Irrigation Pond Isabela Agricultural Experimental Station
5/3/04	Cerrillos lake, Ponce

Initially, in addition to evaluating the adequacy of our version of the Matlock periphytometer and acquiring experience dealing with field conditions, the preliminary trials were used to evaluate the effect of different exposure times on chlorophyll *a* response. Three reactions times were evaluated, namely: 7 days, 10 days, 14 days. A detailed experimental protocol for these experiments follows:

Experiment Description:

The Matlock periphytometer floating rack was constructed using 7.62-cm (diameter) polyvinyl chloride (PVC) pipes and wire mesh (5 x 10 cm). The floating rack dimensions were 2.7 m (length) x 1.5 m (width) (Figure 9 and 10). The floatability of the rack was tested during a 12-hour period in a freshwater lagoon.

The experimental unit (periphytometer) consisted of a 1-L narrow-mouth bottle (Nalgene LDPE Narrow-Mouth, Fisher no. 02-9254-6F). A 2.54 cm diameter hole was drilled within the bottle, which permitted enclosing a 0.45 μm nylon membrane filter (Cole Palmer A-02916-44, which served as biofilter) and glass fiber filter (Whatman glass microfiber filter, Fisher 934 AH) as the growth substrate (Figure 11).

The floating rack contained 42 bottles in randomized complete block arrangement (Table 10 and Figure 8), 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 (See Table 3). The floating rack was placed approximately at the center of the lake.

Solutions were prepared as follow:

Ammonium Nitrate Certified ACS
NH_4NO_3 FW 80.04 g/ mol
Fisher Scientific Lot Number 015219
Catalog Number A676-500

Quantity of NH_4NO_3/ L of 14 PPM solution =

$$\frac{\text{(Molecular weight of } NH_4NO_3\text{) (1 g) (Solution concentration mg/ L)}}{\text{Molecular weight of N (1000 mg)}}$$

$$\frac{\text{(80.04 g/ mol/ 2 mol) (1 g) (14 mg/ L)}}{\text{14.01 g/ mol (1000 mg)}} = 0.0399 \text{ g } NH_4NO_3\text{/ L}$$

= 0.0399 g NH_4NO_3/ Lt (10 L) = 0.399 g NH_4NO_3/ 10 L

Quantities of NH_4NO_3 weighted: 0.3975 g, 0.3976 g and 0.3983 g

Sodium Phosphate Dibasic GR
$Na_2HPO_4 \cdot 7H_2O$ FW 268.07 g/ mol
EM Science Lot Number 36030630
Catalog Number SX0715-1 500g

Quantity of $Na_2HPO_4 \cdot 7H_2O$/ lt of 2 PPM solution =

$$\frac{(\text{Molecular weight of } Na_2HPO_4\cdot 7H_2O)\ (1\ g)\ (\text{Solution concentration mg/ L})}{\text{Molecular weight of P (1000 mg)}}$$

$$\frac{(268.07\ g/\ mol)\ (1\ g)\ (2\ mg/\ L)}{30.97\ g/\ mol\ (1000\ mg)} = 0.0173\ g\ Na_2HPO_4\cdot 7H_2O/\ L$$

= 0.0173 g $Na_2HPO_4\cdot 7H_2O$ / Lt (10 L) = 0.173 g NH_4NO_3/ 10 L

Quantities of $Na_2HPO_4\cdot 7H_2O$ weighted: 0.1735 g, 0.1747 g and 0.1743 g

Table 10: 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

Results:

On our first trial (February 2004, Cerrillos) 10 of the bottles became loose probably as a result of inadequate strapping. That prevented us from obtaining a representative number of samples to evaluate the effect of the different reaction times on biomass growth. A second trial was conducted under much more controlled conditions in an irrigation lagoon at the Agricultural Experimental Station in Isabela. No significant differences between the different reaction times were observed (figure 12). This suggests that 7 days may be an appropriate reaction time for evaluating the impact of different nutrients on algae growth. A third trial was recently conducted at the Cerrillos lake to evaluate this in a lake setting. Results of that trial were not available at the time of writing this report.

Figure 9. Deployment of the Matlock periphytometer at the Cerrillos lake (2/4/04).

Figure 10. Transport of the Matlock periphytometer to the deployment site at the Cerrillos lake (2/4/04).

Figure 11. Overview of the algae growth media of different treatments after 7 days of reaction in the Cerrillos lake.

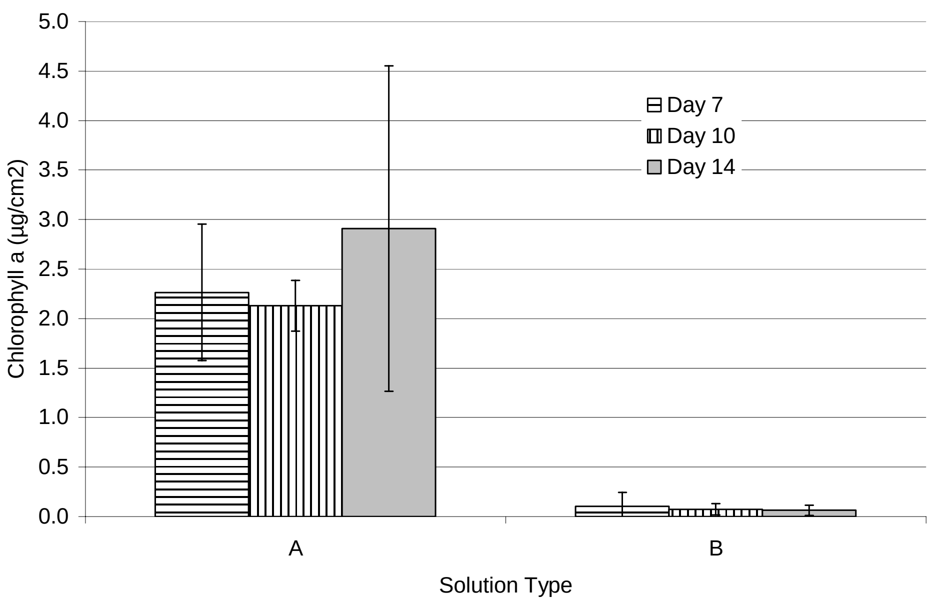

Figure 12. Effect of the reaction time on chlorophyll *a* concentrations (periphytometer study). Results from an experimental trial conducted at the Isabela Agricultural Experimental Station (lagoon water chemical status - TKN = 0.39 mg/L, TP = 0.027 mg/L).

SURVEY OF PHYTOPLANKTON DIVERSITY IN LAKES OF PUERTO RICO

Collaborators: Carlos J. Santos, Diana Gualtero, Jezabel Pagán, and Fernando Pantojas

INTRODUCTION

The most-complete survey on the water chemistry and plankton abundance in the reservoirs of Puerto Rico was produced by Jobin *et al*. (1979) on data collected in 1977. Martínez (1979) conducted a comparative study based on water quality for 10 major reservoirs. The results of these two studies demonstrated the advanced eutrophic state of the major reservoirs, and the Puerto Rico Environmental Quality Board produced a preliminary trophic classification and priority ranking for the restoration of these reservoirs (PEQB, 1984).

The best-studied reservoir is Lake Carraízo, also known as Lake Loíza, for which the U.S.G.S. published a comprehensive treatise (Quiñones-Márquez, 1980). This reservoir has also been studied by de Jesús (1979), Candelas (1981) and Candelas *et al*. (1992), PRASA (1992, 1995a-b), Montañez (1997), and Gellis *et al*. (1999). García and Tilly (1982, 1983) conducted a one-year study on the dynamics of phosphorus and nitrogen in La Plata reservoir, while Ramos-Ginés (1997) recently produced a model for the total nitrogen and total phosphorus budgets of Lake Cidra. The other Puerto Rican reservoirs are under-investigated, and little is known about their plankton composition.

None of the above-mentioned works considered the plankton diversity of the reservoirs. The only comprehensive study on the freshwater plankton of Puerto Rico was conducted over 47 years ago (Candelas, 1956), is taxonomically outdated, and was based on limited sampling effort. Even in Candelas' work the main objective was to determine the chemical and physical conditions of the artificial reservoirs of Puerto Rico in relation to plankton production rather than a determination of plankton diversity.

Any comprehensive reservoir management system should include the monitoring of planktonic organisms, to provide information on the general functioning of the reservoir ecosystem. The identification of indicators of changes in water quality would be valuable for managing plans. Shifts in the structure of communities may give more valuable information upon variations in water quality than would changes in the abundance of a single species.

Although the Puerto Rico Water Authority has some data sets available on phytoplankton of the major reservoirs, the identifications usually are only to genus-level. A preliminary survey in the summer of 1997 revealed that as many as 30 species of the desmid genus *Cosmarium* can be collected in a single reservoir (Santos-Flores, pers. obs.). This result indicated that the identification of algae only to genus has clearly underestimated the plankton diversity of the Puerto Rican freshwaters. The situation is even worst for the zooplankton because recent species-lists are not available.

Since the mid 1950's, use of algal indicators of environmental conditions has flourished based on the environmental sensitivities and tolerances of individual taxa and species composition of assemblages (Stevenson and Smol, 2003). Nutrient stimulation of algal growth made algae part of the problem in the eutrophication of lakes; therefore, the trophic status of lakes was also characterized by the amount of algae (Vollenweider, 1976; Carlson, 1977). Thus, characterization of algal assemblages has been important in environmental assessment, both in indicating changes in environmental conditions that impair or threaten ecosystem health. Algae are particularly valuable in environmental assessments because they are the base of most aquatic food chains, are important in biogeochemical cycling, and serve as habitat for many other aquatic organisms (Stevenson and Smol, 2003).

In the proposed study, we will conduct collections of phytoplankton in the main reservoirs of Puerto Rico to determine the plankton richness at the species-level (when possible) and to correlate the species richness of different groups (i.e. Chlorophyta, Bacillariophyta, Cyanophyta) with several physical-chemical parameters of the reservoirs. This survey will be coupled to an ongoing project which will take advantage of periphytometers to measure algal responses to nutrient additions.

METHODOLOGY

At each sampling site, three consecutive tows were conducted with phytoplankton nets (Wisconsin style) with mesh size of 63 µm. The volume of water collected after every three tows in the nets varied from 300 and 500 ml. The samples collected were transferred to 500 ml bottles and fixed with gluraraldehyde. Enough glutaraldehyde was added to each bottle to bring the glutaraldehyde concentration down to 2%.

For microscopic examination, 1 ml aliquots from each sample were centrifuge at 6000 rpm for 2 min. Slides were scanned under a Nikon Optiphot microscope at 300X and 600X. Algae will be enumerated on a Sedgewick-Rafter chamber, until at least 15 fields and 300 identified cells have been counted.

Most species will be identified to infra-generic or species-level. Not all nanoplanktonic species can be identified because of the lack of keys for tropical areas. Some species, particularly in the diatoms and some unicellular cyanobacteria, can be identified only to genus unless molecular techniques are employed.

Keys used to identify the chlorophytes are summarized in Santos-Flores (2001). For the identification of cyanobacteria (Cyanophyta) and diatoms (Bacillariophyta), the following works will be used: Bourrelly and Manguin (1952), Desikachary (1959), Drouet (1968, 1973, 1978, 1981), Drouet and Daly (1939, 1952), Gomont (1892, 1893), Koster (1960, 1963), Maldonado-González and Genes Dueñas (1986), Prescott (1962), Thomasson (1971), Toledo-Iser (1989, 1992a-b), Toledo-Iser *et al*. (1997a-b), and Yacubson (1980).

RESULTS

Samples from all lakes have been examined, but six lakes have been studied in more detail than the others due to time constrains. These lakes are: Dos Bocas, Melania, Guajataca, Cidra, Loíza and Patillas. All samples from Lake Dos Bocas and Lago Melania that have been screened for algae are presented herein (Tables 13-16).

Table 13 and 14 show the diversity and occurrence of the main algal genera found during the three samplings conducted in Lago Melania. Three genera: *Microcystis, Pediastrum,* and *Botryococcus*, all related to eutrophic conditions, were present in all. *Microcystis* cf. *aeruginosa*, *Pediastrum simplex* and *P. duplex* compose the bulk (over 70%) of the algae found in Lake Melania. Most species in the phytoplankton of Lake Melania belong to the green algae (Division Chlorophyta), order Chlorococcales. Two species of dinoflagellates (*Peridinium* spp.) were also common.

Lake Dos Bocas' phytoplankton samples contained at least 10 genera, belonging to three algal divisions (see Tables 15 and 16). At least 30% of the algae are diatoms (Bacillariophyceae), and these were the most diverse algae at genus level (about 7 genera). The green algae *Botryococcus* and *Pediastrum* and the diatom *Synedra* were the most abundant algae.

Lake Dos Bocas has a considerable number of benthic diatoms in the water column. This observation suggests a high rate of sedimentation, with riparian sediments colonized by algae entering the water column.

PRELIMINARY REMARKS

1. Complete screening of samples for phytoplankton species has been achieved only for Lake Dos Bocas and Lake Melania, and it is noticeable the prolific growth of species of green algae *Pediastrum simplex*, *P. duplex* and *P. duodenarium* in both lakes. When abundant, *Pediastrum* species are known to produce fishy flavors to the water. Similarly, the dinoflagellate *Peridinium* is responsible for some odors and distasteful flavors even at low densities.

2. *Microcystis* spp. were found in most lakes, but were abundant in Dos Bocas, Loíza and Melania. Only one colony of *Microcystis* was found in the samples form Lake Guajataca. *Microcystis* is widely used as an indicator of eutrophic conditions and is a well-known **toxigenic** bloom-forming genus.

3. The most abundant algae found in Lake Melania, Dos Bocas and Loíza are of concern from the sanitary point of view.

4. Lake Guajataca has low numbers (less than 10 cells/ml) of chlorococcalean green algae. The zooplankton of this lake is mainly conformed by rotifers that prey upon small algae.

Table 11. Algal concentrations in some Puerto Rican Lakes in 1977 and 1998. (Data for 1977 from Jubin et al.; data for 1998 from Santos-Flores, 2001)

Lake	Sample	Date	Total Algae (cells/ ml)	Type and % of Alga in Highest Concentration	% Blue-Green Algae	% Diatoms	% Other Algae
Carraízo	1	8-1977	18.2	*Actinastrum* (31%)	6	37	57
	2	8-1977	18.1	*Stephanodiscus* (32%)	18	53	29
	3	8-1977	23.1	*Cylindrospermum* (23%)	26	38	36
"	1	8-1998	201.2	*Anabaena* (42%)	59	10	31
	2	8-1998	156.7	*Scenedesmus* (51%)	10	36	54
	3						
Dos Bocas	1	12-1977	19.8	*Staurastrum* (35%)	3	27	70
	2	12-1977	13.6	*Staurastrum* (20%)	5	55	40
	2	12-1977	8.8	*Staurastrum* (20%)	4	35	61
	4	12-1977	9.1	*Stephanodiscus* (26%)	6	47	47
	1	12-1977	15.9	*Navicula* (50%)	0	63	37
"	2	12-1998	101.5	*Scenedesmus* (24%)	7	61	32
	3	12-1998	65.9	*Staurastrum* (37%)	12	49	39

Table 12. Some algal values of 19 Puerto Rican reservoirs calculated for the period between 1997 and 2004.

Lake	Algal Richness	Number of Chlorophyte Species	Number of [1]Cyanophyte Species
Adjuntas	99	77	8
Caonillas	117	95	8
Carite	143	116	10
Carraizo	195	158	17
Cidra	176	139	15
Dos Bocas	115	92	12
Garzas	100	75	10
Guajataca	109	78	11
Guayabal	98	74	10
Guayo	106	88	7
Guineo	112	87	7
Las Curias	38	25	6
Loco	54	41	7
Lucchetti	104	81	10
Matrullas	138	107	11
Patillas	138	102	15
Toa Vaca	129	101	11
Toro	79	60	8

1= blue-green algae

Table 13. Phytoplankton genera in Lake Melania (2003-2004).

Division	**Class**	**Order**	**Family**	**Genus**
Euglenophyta	Euglenophyceae	Euglenales		*Phacus*
Cyanophyta		Chroococcales	Chroococcaceae	*Microcystis*
		Oscillatoriales	Oscillatoriaceae	*Oscillatoria*
Chrysophyta	Bacillariophyceae	Pennales	Fragillariaceae	*Synedra*
			Naviculaceae	*Navicula*
			Nitzschiaceae	*Pinnularia*
Pyrrophyta	Dinophyceae	Peridiniales	Peridiniaceae	*Peridinium*
Chlorophyta	Chlorophyceae	Chlorococcales	Hydrodictyaceae	*Pediastrum*
			Dictyosphaeriaceae	*Botryococcus*
			Palmellaceae	*Sphaerocystis*
			Oocystaceae	*Monoraphidium*
		Oedogoniales		*Oedogonium*
	Charophyceae	Zygnematales	Desmidiaceae	*Closterium*
	Ulvophyceae	Ulotrichales		*Uronema*

Table 14. Occurrence of the main genera in Lake Melania (2003-2004).

Sampling 1= 26/08/03; Sampling 2= 7/11/03; sampling 3= 24/02/04			
P= present A= absent			
Genus	**Sampling 1**	**Sampling 2**	**Sampling 3**
Phacus	P	P	A
Microcystis	P	P	P
Oscillatoria	A	A	P
Synedra	A	A	P
Navicula	A	P	P
Pinnularia	A	A	P
Peridinium	P	P	A
Pediastrum	P	P	P
Botryococcus	P	P	P
Sphaerocystis	P	P	A
Monoraphidium	P	P	P
Oedogonium	A	P	A
Closterium	A	P	A
Uronema	A	P	P

Table 15. Phytoplankton genera in Lake Dos Bocas (2003-2004).

Division	**Class**	**Order**	**Family**	**Genus**
Cyanophyta		Chroococcales	Chroococcaceae	*Microcystis*
Chrysophyta	Bacillariophyceae	Pennales	Fragillariaceae	*Synedra*
				Fragilaria
			Naviculaceae	*Navicula*
				Anomoeoneis
			Nitzschiaceae	*Nitzschia*
			Nitzschiaceae	*Pinnularia*
			Surirellaceae	*Gyrosigma*
			Gomphonemaceae	*Gomphonema*
Chlorophyta	Chlorophyceae	Chlorococcales	Hydrodictyaceae	*Pediastrum*
			Dictyosphaeriaceae	*Botryococcus*

Table 16. Occurrence of genera in Lake Dos Bocas (2003-2004).

Sampling 1= 25/08/03; Sampling 2= 4/12/03; Sampling 3= 10/03/04			
Genus	**Sampling 1**	**Sampling 2**	**Sampling 3**
Microcystis	A	P	A
Synedra	P	P	P
Fragilaria	A	P	A
Navicula	P	P	P
Anomoeoneis	A	P	A
Pinnularia	A	P	A
Gyrosigma	A	P	P
Gomphonema	A	P	A
Pediastrum	P	P	P
Botryococcus	P	P	P

Table 17. Preliminary list of species in the plankton of Lake Guajataca.-

TAXA	SPECIES
PHYTOPLANKTON	
Cyanophyta	*Microcystis* sp. (non *aeruginosa*)
Chlorophyta	
Chlorococcales	*Pediastrum simplex*
Chlorococcales	*Pediastrum clatharatum*
Chlorococcales	*Asterococcus superbus*
Chlorococcales	*Scenedesmus* sp.
Chrysophyta	
Bacillariophyceae	*Stauroneis* sp.
	Synedra sp.
	Unidentified species
ZOOPLANKTON	
Rotifera	*Aneuropsis* sp.
	Keratella cochlearis
	Lecane cf. *monostyla*
	Platyas quadricornis
Cladocera	*Sarsilatona* sp. (juveniles)

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OTHER ACTIVITIES RELATED TO THE PROJECT

A meeting between the PREQB Water Quality division and our research group was held on (April 15th). Minutes of that meeting are included in Appendixes B.

As requested an estimate of the amount of professional hours devoted to the different tasks associated with this project was computed. Results for the nutrient and phytoplankton diversity portions of this project are shown separately on tables 18 - 20.

Table 18. Professional hours devoted to the chemical characterization portion of the study.

Tasks by Sampling event	Professional hours
Simple pick up at EQBs laboratory	8 hrs
Filtering of simples for DP, and DOC determination	8 hrs
Digestion – TKN	16 hrs
Digestion –TP	12 hrs
Claning glassware	40 hrs
DOC analysis	3 hrs
TP, DP analysis	8 hrs
TKN analysis	8 hrs
Preparation of laboratory Report	16 hrs
Clorophyll analysis - Extraction, and simple measurement	24 hrs
Data evaluation	25 hrs
Preparation of purchase orders for materials, and supplies	20 hrs
Financial report preparation	10 hrs
Total hours per sampling event*	**198**

* To calculate the total number of professional hours devoted to the study multiply the number of hours (198) times the number of sampling events. As of May 26, 2004, that would amount to: **594** hours.

Table 19. Professional hours devoted to peripheral tasks related to the project

Other tasks related to the project	Professional hours
Development of electronic data base	288 hrs
Periphytometer construction	48 hrs
Field trial at Cerrillos – February 2004	57 hrs
Field trial at the Isabela AES	52 hrs
Field trial at Cerrillos – May 2004	42 hrs
Total	**487**

Table 20. Time effort required to complete the phytoplankton survey:

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)**

Appendix A

Algal species found in the reservoirs of Puerto Rico (1998-2004):

1. *Gonatozygon monotaenium*
2. *Netrium digitus*
3. *Mougeotia tumidula*
4. *Pleurodiscus borinquenae*
5. *Spirogyra decimina*
6. *Spirogyra discreta*
7. *Spirogyra emilianensis*
8. *Spirogyra farlowii*
9. *Spirogyra fluviatilis*
10. *Spirogyra hatillensis*
11. *Spirogyra hyalina*
12. *Spirogyra minor*
13. *Spirogyra neglecta*
14. *Spirogyra weberi*
15. *Actinotaenium costatum*
16. *Actinotaenium cucurbita*
17. *Actinotaenium inconspicuum*
18. *Actinotaenium subglobosum*
19. *Actinotaenium wollei*
20. *Arthrodesmus convergens*
21. *Arthrodesmus incus*
22. *A. longispinus*
23. *A. octocornis*
24. *Arthrodesmus subulatus* f. *Americana*
25. *Bambusina brebissoni var. brebissoni*
26. *Closterium acerosum* var. *acerosum*
27. *Closterium acerosum* var. *borgei*
28. *Closterium a.* var. *elongatum*
29. *Closterium cornu* var. *cornu*
30. *C. costatum* var. *costatum*
31. *C. costatum* var. *subcostatum*
32. *Closterium dianae* var. *dianae*
33. *Closterium ehrenbergii* var. *ehrenbergii*
34. *Closterium gracile* var. *gracile*
35. *Closterium lanceolatum* var. *lanceolatum*
36. *Closterium leibleinii* var. *leibleinii*
37. *Closterium lineatum* var. *lineatum*
38. *Closterium lunula* var. *lunula*
39. *C. parvulum* var. *parvulum*
40. *Closterium promum*
41. *Closterium ralfsii* var. *hybridum*
42. *Closterium rostratum*
43. *Closterium setaceum*
44. *C. tumidulum* var. *tumidulum*
45. *Closterium turgidum* var. *turgidum*
46. *Closterium turgidum* var. *borgei*
47. *C. turgidum* var. *giganteum*
48. *Cosmarium* cf. *americanum*
49. *Cosmarium angulare* var. *angulare*
50. *Cosmarium annulatum* var. *annulatum*
51. *Cosmarium arctoum* var. *arctoum*
52. *Cosmarium biauritum*
53. *Cosmarium bicuneatum*
54. *Cosmarium bireme* var. *bireme*
55. *Cosmarium bireme* var. *barbadense*
56. *Cosmarium blytti* var. *novae-sylvae*
57. *Cosmarium brebissonni* var. *brebissonni*

58. *Cosmarium candianum* var. *candianum*
59. *Cosmarium candianum* var. *minutum*
60. *Cosmarium connatum* var. *connatum*
61. *C. contractum* var. *contractum*
62. *C. costatum* var. *costatum*
63. *Cosmarium cucumis*
64. *Cosmarium decedens* var. *decedens*
65. *Cosmarium exiguum* var. *exiguum*
66. *Cosmarium granatum* var. *granatum*
67. *Cosmarium humile* var. *substriatum*
68. *Cosmarium longiense* var. *longiense*
69. *Cosmarium lundellii* var. *ellipticum*
70. *Cosmarium mamilliferum* var. *mamilliferum*
71. *Cosmarium margaritaceum* var. *margaritaceum*
72. *Cosmarium margaritaceum* var. *margaritaceum f. minor*
73. *Cosmarium meneghinii* var. *meneghinii*
74. *Cosmarium moniliforme* var. *moniliforme*
75. *Cosmarium obliquum* var. *obliquum*
76. *C. obsoletum* var. *obsoletum*
77. *C. pachydermum* var. *pachydermum*
78. *C. phaseolus* var. *phaseolus*
79. *Cosmarium phaseolus* f. *elevatum*
80. *Cosmarium pokoryanum* var. *pokoryanum*
81. *Cosmarium porrectum*
82. *C. portianum* var. *portianum*
83. *C. protractum* var. *protractum*
84. *Cosmarium pseudoconnatum* var. *pseudoconnatum*
85. *Cosmarium pseudoexiguum* var. *pseudoexiguum*
86. *Cosmarium pseudonitidulum* var. *pseudonitidulum*
87. *Cosmarium pseudopyramidatum* var. *pseudopyramidatum*
88. *Cosmarium pyramidatum* var. *pyramidatum*
89. *Cosmarium pyramidatum* var. *stenonotum*
90. *C. pyriforme* var. *subpyriforme*
91. *Cosmarium quadratum* var. *quadratum*
92. *Cosmarium quadrum* var. *quadrum*
93. *Cosmarium ralfsii* var. *ralfsii*
94. *Cosmarium rectangulare* var. *rectangulare*
95. *Cosmarium regnesi* var. *regnesi*
96. *Cosmarium stichocondrum*
97. *C. subcostatum* var. *subcostatum*
98. *C. subcucumis* f. *subcucumis*
99. *C. subprotumidum* var. *subprotumidum*
100. *Cosmarium subtumidum* var. *subtumidum*
101. *Cosmarium subtumidum* var. *pachydermum*
102. *Cosmarium tumidum* var. *tumidum*
103. *Cosmarium venusutum* var. *excavatum*
104. *Desmidium aequale* f. *aequale*
105. *Desmidium elegans*

106. *D. gracilipes*
107. *Desmidium swartzii* var. *swartzii*
108. *Docidium undulatum* var. *undulatum*
109. *Euastrum abruptum* var. *abruptum*
110. *Euastrum abruptum* var. *lagoense*
111. *Euastrum ansatum* var. *ansatum*
112. *Euastrum attenuatum* var. *attenuatum*
113. *E. bidentatum* var. *bidentatum*
114. *Euastrum crassicole* var. *crassicole*
115. *Euastrum crassum*
116. *Euastrum crassum var. scrobiculatum*
117. *Euastrum cuneatum*
118. *Euastrum denticulatum* var. *denticulatum*
119. *Euastrum denticulatum* var. *rectangulare*
120. *E. divaricatum* var. *divaricatum*
121. *E. elegans* var. *elegans*
122. *Euastrum gemmatum* var. *gemmatum*
123. *Euastrum incavatum*
124. *Euastrum insulare* var. *silesiacum*
125. *Euastrum sibiricum* var. *sibiricum*
126. *Euastrum* sp.
127. *Hyalotheca laevicincta*
128. *Hyalotheca mucosa*
129. *M. abrupta*
130. *Micrasterias alata*
131. *Micrasterias arcuata* var. *arcuata*
132. *Micrasterias arcuata* var. *expansa* f. *expansa*
133. *Micrasterias laticeps* var. *laticeps*
134. *Micrasterias radiata* var. *radiata*
135. *Micrasterias rotata* var. *rotata*
136. *M. rotata* var. *rotata* f. *evoluta*
137. *Micrasterias truncata* var. *pusilla*
138. *Onychonema filiforme*
139. *Onychonema laeve* var. *latum*
140. *Penium margaritaceum* var. *margaritaceum*
141. *Penium multicostatum*
142. *Penium phymatosporum*
143. *Penium silvae-nigrae* f. *silvae-nigrae*
144. *Penium silvae-nigrae* f. *minus*
145. *Penium spinulosum*
146. *Pleurotaenium caldense* var. *cristatum*
147. *Pleurotaenium coronatum* var. *coronatum*
148. *Pleurotaenium nodosum* var. *nodosum*
149. *Pleurotaenium ovatum*
150. *Pleurotaenium trabecula* var. *trabecula*
151. *Staurastrum bacillare*
152. *Staurastrum brasiliense* var. *brasiliense*
153. *Staurastrum clepsydra*
154. *Staurastrum cosmarioides* var. *cosmarioides*
155. *S. crenulatum* var. *crenulatum*
156. *S. cuspidatum* var. *cuspidatum*
157. *S. dejectum* var. *dejectum*
158. *Staurastrum denticulatum*
159. *Staurastrum dickei* var. *dickei*
160. *Staurastrum dickei* var. *circulare*
161. *S. dilatatum*

162. *S. furcatum* var. *furcatum*
163. *Staurastrum gladiosum*
164. *Staurastrum gracile* var. *gracile*
165. *Staurastrum leptacanthum*
166. *Staurastrum leptocladum* var. *leptocladum*
167. *Staurastrum l.* var. *africanum*
168. *Staurastrum longipes* var. *evolutum* f. *gracilius*
169. *S. margaritaceum* var. *margaritaceum*
170. *Staurastrum micron*
171. *S. muticum* var. *muticum*
172. *Staurastrum polymorphum*
173. *S. punctulatum var. punctulatum*
174. *Staurastrum radians* var. *radians*
175. *Staurastrum rotula*
176. *Staurastrum trifidum* var. *glabrum*
177. *S. turgescens* var. *turgescens*
178. *Staurodesmus cuspidatus(m)*
179. *Teilingia excavata*
180. *T. granulata*
181. *Tetmemorus brebissonii* var. *brebissonii*
182.*T. granulatus* var. *granulatus*
183. *Xanthidium antilopaeum* var. *antilopaeum*
184. *Xanthidium a.* var. *antilopaeum* f. *javanicum*
185. *Xanthidium armatum* var. *armatum*
186. *Xanthidium concinnum* var. *concinnum*
187. *X. concinnum* var. *boldtianum*
188. *Xanthidium fasciculatum*
189. *Treubaria setigera*
190. *Treubaria triappendiculata*
191. *Acanthosphaera* sp.
192. *Actinastrum hantzchii var.subtile*
193. *Actinastrum cerastioides*
194. *Ankistrodesmus bernardii*
195. *Ankistrodesmus braunii*
196. *Ankistrodesmus convolutus*
197. *Ankistrodesmus falcatus*
198. *Ankistrodesmus fusiformis*
199. *Ankistrodesmus gracilis*
200. *Ankistrodesmus spiralis*
201. *Botryococcus braunii*
202. *B. protuberans*
203. *Chlorella minutissima*
204. *Chlorella vulgaris*
205. *Chlorolobion guamense*
206. *Coelastrum astroideum*
207. *Coelastrum cruciatum*
208. *Coelastrum intermedium*
209. *Coelastrum microsporum*
210. *Coelastrum morus*
211. *Coelastrum morus* var. *capense*
212. *Coelastrum proboscideum*
213. *Coelastrum pulchrum*
214. *C. reticulatum* var. *reticulatum*
215. *Coenocystis asymmetrica*
216.*Coenocystis micrococca*
217. *Coenocystis subcylindrica*
218. *Coenocystis tapasteana*

219. *Crucigenia mucronata*
220. *Crucigenia tetrapedia*
221. *Crucigenia triangularis*
222. *Crucigeniella apiculata*
223. *Crucigeniella pulchra*
224. *Crucigeniella rectangularis*
225. *Crucigeniella* cf. *rectangularis*
226. *Crucigeniella saguei*
227. *Dictyosphaerium botrytella*
228. *Dictyosphaerium tetrachtomum* var. *fallax*
229. *D. pulchellum*
230. *Didymocystis* cf. *comasii*
231. *Pseudodidymocystis fina*
232. *Dimorphococcus lunatus*
233. *Ecdysichlamys obliqua*
234. *Eutetramorus fottii*
235. *Eutetramorus tetrasporus*
236. *Franceia javanica*
237. *Gloeotaenium loitlesbergerianum*
238. *Hydrodictyon reticulatum*
239. *Kirchneriella dianae*
240. *Kirchneriella contorta*
241. *Kirchneriella irregularis*
242. *K. lunaris*
243. *Kirchneriella mayori*
244. *Kirchneriella obesa*
245. *Kirchneriella pseudoaperta*
246. *Lagerheimia ciliata*
247. *Micractinium pusillum*
248. *Monoraphidium arcuatum*
249. *Monoraphidium caribeum*
250. *Monoraphidium contortum*
251. *Monoraphidium convolutum*
252. *Monoraphidium dybowskii*
253. *Monoraphidium griffithii*
254. *Monoraphidium minutum*
255. *Monoraphidium tortile*
256. *Monoraphidium* sp.
257. *Nephrochlamys subsolitaria*
258. *Nephrochlamys willeana*
259. *Nephrocytium obesum*
260. *Oocystis bispora*
261. *Oocystis ecballocystiformis* var. *americana*
262. *Oocystis marsonii*
263. *Oocystis parva*
264. *Oocystis solitaria*
265. *Oocystis tainoensis*
266. *Oocystis* sp.
267. *Oonephris palustris*
268. *Pediastrum argentiniense*
269. *Pediastrum angulosum*
270. *Pediastrum boryanum* var. *brevicorne*
271. *Pediastrum duplex* var. *duplex*
272. *Pediastrum duplex* var. *subgranulatum*
273. *Pediastrum orbitale*
274. *Pediastrum simplex*
275. *P. simplex* var. *duodenarium*
276. *Pediastrum tetras*
277. *Pediastrum tetras* var. *tetraodon*
278. *Quadrigula* sp.

279. *Rhombocystis complanata*
280. *Rhombocystis lacryma*
281. *Schroederia antillarum*
282. *Selenastrum bibraianum*
283. *Selenastrum bibraianum* var. *gracile*
284. *Scenedesmus abundans*
285. *S. acuminatus* var. *acuminatus*
286. *Scenedesmus a.* var. *tetradesmoides*
287. *Scenedesmus acunae*
288. *Scenedesmus acutiformis*
289. *Scenedesmus acutus*
290. *Scenedesmus alternans*
291. *Scenedesmus antillarum*
292. *S. arcuatus* var. *platydisca*
293. *Scenedesmus armatus*
294. *Scenedesmus bijuga*
295. *Scenedesmus bijuga* var. *alternans*
296. *Scenedesmus brasiliensis*
297. *Scenedesmus brasiliensis* var. *norvegicus*
298. *Scenedesmus calyptratus*
299. *Scenedesmus caribeanus*
300. *S. denticulatus*
301. *Scenedesmus dimorphus*
302. *Scenedesmus disciformis*
303. *S. ecornis*
304. *Scenedesmus hystrix*
305. *Scenedesmus incrassatulus* var. *mononae*
306. *Scenedesmus intermedius*
307. *Scenedesmus obliquus*
308. *Scenedesmus* cf. *obliquus*
309. *Scenedesmus opoliensis*
310. *Scenedesmus perforatus*
311. *Scenedesmus polyglobulus*
312. *S. quadricauda*
313. *Scenedesmus quadricauda* var. *maximum*
314. *Scenedesmus semipulcher*
315. *Scenedesmus smithii*
316. *Scenedesmus* cf. *spinosus*
317. *Scenedesmus* cf. *subspicatus*
318. *Scenedesmus velitaris*
319. *Sorastrum americanum*
320. *S. spinulosum*
321. *Tetraedron caudatum*
322. *Tetraedron* (*Chlorotetraedron*) *incus*
323. *Tetraedron minimum*
324. *Tetraedron minimum* f.
325. *Tetraedron minimum tetralobulatum*
326. *Tetraedron muticum*
327. *T. pentaedricum*
328. *T. regulare*
329. *Tetraedron regulare var. torsum*
330. *T. triangulare*
331. *Tetraedron trigonum*
332. *Tetrastrum elegans*
333. *Tetrastrum heteracanthum*
334. *Tetrastrum staurogeniaeforme*
335. *Tetrastrum triangulare*
336. *Westella botryoides*
337. *Chlamydocapsa ampla*

338. *Chlamydomonas spp.*

339. *Gonium pectorale*

340. *Pandorina morum*

341. *Gloeocystis ampla*

342. *Sphaerocystis schoeteri*

343. *Microspora tumidula*

344. *Microspora* sp.

345. *Ulothrix zonata*

346. *Cloniophora capitellata*

347. *Cloniophora willei*

348. *Cladophora glomerata*

349. *Pithophora mooreana*

350. *P. oedogonia*

351. *Rhizoclonium fontanum*

352. *Rhizoclonium hieroglyphicum*

353. *Bulbochaete elatior*

354. *Oedocladium operculatum*

355. *Oedogonium abbreviatum*

356. *Oedogonium capitelliforme*

357. *Oedogonium crispum*

358. *O. flexuosum*

359. *Oedogonium howei*

360. *Oedogonium intermedium*

361. *Oedogonium macrandrium* var. *macrandrium*

EUGLENOPHYTA

362. *Euglena acus*

363. *E. ehrenbergii*

364. *Euglena fusca*

365. *Euglena gaumei*

366. *Euglena mutabilis*

367. *Euglena oxyuris* cf. f. *charcowiensis*

368. *Euglena oxyuris* f. *playfairii*

369. *Euglena spirogyra*

370. *Euglena tripteris* var. *klebsii*

371. *Euglena variabilis*

372. *Euglena* sp. *1*

373. *Euglena* sp. *2*

374. *Euglena* sp. *3*

375. *Euglena* sp. *4*

376. *Lepocinclis fusiformis*

377. *Phacus longicauda*

378. *Phacus tortus*

379. *Trachelomonas granulosa var. subglobosa*

380. *Trachelomonas hispida*

381. *T. volvocina*

OCHROPHYTES

(Includes Diatoms)

382. *Centritrarcus belanophorus*

383. *Dinobryon sertularia*

384. *Mallomonas* spp.

385. *Amphiprora* spp.

386. *Amphora proteus*

387. *Anomoeoneis sphaerophora*

388. *Asterionella* spp.

389. *Coratoneis arcus*

390. *Cyclotella meneghiniana*

391. *C. lanceolata*

392. *Cymbella cymbiformis*

393. *Cymbella pusilla*
394. *Cymatopleura gibba*
395. *Dipleoneis* cf. *elliptica*
396. *Eunotia paralella*
397. *Fragillaria* cf. *crotonensis*
398. *Fragillaria* spp.
399. *Gomphonema affine*
400. *Gomphonema spp.*
401. *Homocladia* (*Nitzchia*) cf. *anuphioxys*
402. *Homocladia* (*Nitzchia*) *fasciculata*
403. *Homocladia* (*Nitzchia*) *linearis*
404. *H.* (*Nitzchia*) spp.
405. *Mastogloia smithii*
406. *Mastogloia smithii var. lacustris*
407. *Melosira granulata*
408. *Melosira granulata var. angustissima*
409. *Navicula braunii*
410. *Navicula circumtexta*
411. *Navicula decurrens*
412. *Navicula inaculata*
413. *Navicula lanceolata*
414. *Navicula palpebralis*
415. *Navicula pseudosentiformis*
416. *Navicula pupula var. capitata*
417. *Navicula radiosa*
418. *Navicula scutum*
419. *Navicula viridis*
420. *Navicula spp.*
421. *Neidium dubium*
422. *Nitzschia* spp.
423. *Pinnularia rupestris*
424. *Pinnularia* spp.1
425. *Surirella* spp.
426. *Synedra acus*
427. *Synedra delicatissima*
428. *Synedra fasciculata*
429. *S. minuscula*
430. *Synedra tabulata*
431. *Synedra ulna*
432. *Synedra* spp.
433. *Stauroneis anceps*
434. *Stephanodiscus hantzchii*
435. *Tabellaria* spp.
436. *Terpsinoe musica*

CYANOBACTERIA

437. *Anabaena catenula*
438. *Anabaena tortulosa var. tenuis*
439. *A. variabilis*
440. *Anacystis dimidiata*
441. *Anacystis cyanea*
442. *Aphanizomenon cf. tropicalis*
443. *Aphanothece clathrata*
444. *Aphanothece* spp.
445. *Arthrospira gomontiana*
446. *A. khanuae*
447. *Borzia trilocularis*
448. *Chroococcus limneticus*
449. *Chroococcus* spp.
450. *Coelosphaerium spp.*
451. *Cylindrospermopsis raciborski*
452. *Dactylococcopsis acicularis*
453. *Eucapsis* spp.

454. *Gloeocapsa* spp.
455. *Gloeothece* spp.
456. *Gomphosphaeria* spp.
457. *Johanesbaptista pellucida*
458. *Lyngbya limnetica*
459. *Merismopedia tenuissima*
460. *Microcystis aeruginosa*
461. *Oscillatoria forosa*
462. *Oscillatoria acutissima*
463. *Oscillatoria geminata*
464. *O. limnetica*
465. *Oscillatoria princeps*
466. *Oscillatoria tortuosa*
467. *Phormidium mucicola*
468. *Phormidium subfuscum*
469. *Spirulina princeps*
470. *Spirulina* sp.
471. *Synechococcus* spp.

APPENDIX B

MINUTA
REUNIÓN PROYECTO NUTRIENTES

Fecha:	16 de abril 2004
Lugar:	Oficina del Director, Área de Calidad de Agua Junta de Calidad Ambiental, Hato Rey, PR
Personas Presentes:	Dr. Gustavo Martínez Estación Experimental Agrícola Universidad de Puerto Rico
	Ing. Ángel Meléndez Equipo TMDL Área Calidad de Agua Junta de Calidad Ambiental
	Sra. Angela A. García Fernández Equipo TMDL

Resumen Asuntos Discutidos y Acuerdos

1. El Dr. Gustavo Martínez presentó el estatus de los trabajos realizados hasta el presente y puntualizó la importancia de establecer la recopilación de datos por medio del perifitómetro en varios lagos para solidificar la investigación.

2. El Dr. Martínez nos entregó un CD con el primer informe de progreso corregido conforme a los señalamientos que hiciera la Junta de Calidad Ambiental.

3. Se discutió como mejorar la coordinación entre el área de Finanzas del Instituto y el área científica para que se logre incluir en el reporte de progreso la información que le corresponde suministrar a Finanzas.

4. Se discutió la posibilidad de celebrar una reunión con el Director del Laboratorio de la JCA para que el Dr. Martínez pueda recibir los resultados de los muestreos, de manera que se pueda establecer una comparación entre los resultados obtenidos del laboratorio de la JCA y el del Dr. Martínez. Esto tiene como propósito validar los resultados de la investigación y establecer un grado de confiabilidad de los mismos.

5. El día en que se efectuó esta reunión se estaba llevando a cabo un muestreo de clorofila en Mayagüez, según expresado por el Dr. Martínez.

6. Al presente conforme a la información del Dr. Martínez se han realizado dos rondas de muestreo y un tercero se está realizando al presente. De otra parte, se realizará un cuarto muestreo en el mes de junio.

7. Para establecer el criterio de nutrientes los datos de Caraizo se ha considerado descartarlos, debido a que el resultado obtenido es muy alto y pudiera arrojar en la etapa final un criterio alejado de la realidad.

Acuerdos

1. Para la próxima reunión el Dr. Martínez nos entregará el Informe de Progreso Enmendado con los cambios solicitados