Monitoring the Effectiveness of Treating Reid Park Lake with “Beneficial” Bacteria to Alleviate Algae Blooms and Eutrophication

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Executive Summary

We sampled from Reid Park Lake from late July to late October 2005 to test the effectiveness of supplementing the lake with “beneficial” bacteria in alleviating problems associated with excess algal growth and general eutrophication. Sampling consisted of 2 samplings pre- and 3 samplings post-treatment. Suites of general chemistries, nutrients, and physico-chemical variables, in addition to levels of algae and bacteria were monitored during each sampling. We found that overall numbers of bacteria were significantly higher during the pre-treatment rather than the post-treatment and that algae growth was much higher during the post- rather than the pre-treatment period. Additionally, algal type went from one dominated initially by green algae (“Chlorophyta”) to one dominated by cyanobacteria, the latter being generally more problematic than the former. The lake responded temporally similar to what would be expected with no treatment *i.e*. with algal biomass increasing greatly during the summer and into fall. Based upon our findings, there was no detectable benefit whatsoever of treating the lake with “beneficial” bacteria.

Methods

We sampled from 2 sites within the lake, one very close to the eastern shore where reclaimed water delivered to the lake (RP1) and another just to the east of the most-westerly aerator (RP2). Lake depths were typically around 1 m. at RP1 and 3 m. at RP2. Reid Park Lake has two large “fountains” within it which serve to mix and aerate water. During the course of the study, there was no sign of any stratification within Reid Park Lake and physico-chemical variables were, more or less, homogenous from top to bottom.

Physico-chemical variables were taken with a pre- and post-calibrated Hydrolab Surveyor 4 MiniSonde and data display. Analysis included measurements of dissolved, oxygen (mg/L and % saturation), temperature, pH, specific conductance, and oxygen-reduction potential (mV), and were taken every 0.5 m throughout the water column. Turbidity was measured on a calibrated Hach Corp. model 2100P turbidimeter. Secchi disk depth was measured with a standard, 20 cm in diameter, black and white disk.

Samples submitted for chemical analysis to Transwest Geochem were collected in a 4-liter, messenger-activated, beta-bottle. All samples, besides algae, chlorophyll *a* and bacteria, were delivered to the lab within 1-2 hours after collection. Chemical analytes included total suspended solids (TSS), chloride, sulfate, ammonia, total nitrogen, nitrate, nitrite, orhto-P, total-P, biological oxygen demand (BOD), chemical oxygen demand (COD), total alkalinity, dissolved organic carbon (DOC), and total organic carbon (TOC).

Algae and chlorophyll *a* samples were collected from approximately 0.2 m. below the surface in 4-liter, amber, plastic, wide-mouth bottles and preserved with a 2-3% solution of glutaraldehyde. Algae were enumerated and identified using a Sedgewick-Rafter counting cell and ocular micrometer at magnifications of between 250 and 400X on an Olympus model BH2, phase contract microscope. Analysis of chlorophyll *a* was performed on a Turner Designs, TD-700 laboratory fluorometer using EPA Method 445.0.

Bacterial samples were collected from just below the water surface in sterilized glass containers and placed in a cooler with cold packs for transport to the Environmental Research Lab. The following day, we performed heterotrophic plate counts (HPC) using R2A agar medium. This medium is commonly used for enumerating heterotrophic microorganisms in treated potable water. Samples were serially diluted to extinction in a sterile 0.85 % saline solution. Each 10-fold dilution was aseptically plated onto triplicate R2A agar plates and allowed to incubate for 1 week at 22^{o}C prior to enumerating the number and type of colonies per dilution.

Pellets of the"beneficial bacterial" inoculum were supplied by the manufacturer for analysis of numbers, colony morphology, and diversity. Pellets were suspended by vortexing c.a. 0.25 g in 10 mL of 0.85% sterile saline and then aseptically plated using the exact same technique used for environmental samples.

Results

Bacterial numbers in samples collected from the lake actually decreased during the post-treatment period (Figs. 1 and 2). Site RP2, farthest from the reclaimed water/bacterial inlet, had slightly higher mean numbers than did RP1 (Fig. 3).

Colony morphology of plated bacteria indicated that diversity remained approximately the same throughout the study and that the addition of "beneficial" bacteria did little to change bacterial assemblage. If anything, diversity was slightly higher prior to supplementation.

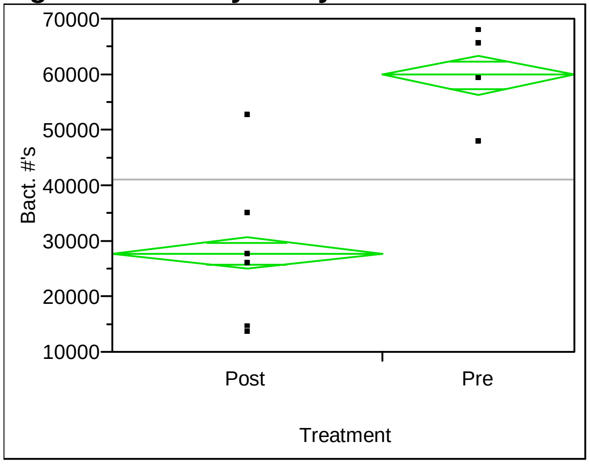
Pellets of the beneficial bacterial inoculum supplied by the manufacturer were extremely difficult to resuspend.. There were 3-4 colony types represented within the pellets with an average number of approximately 18,000,000 cells/g pellet.

Colony morphology of the plated sample provided by the manufacturer was not observed in subsequent environmental samples collected from the lake. Whether bacteria being supplemented into the lake were consumed by heterotrophic algae or resident bacteria, or were simply not viable at all, are definite possibilities. It is also possible that once mixed with resident bacteria found in the lake, the supplemented bacteria numbers simply became so diluted as to be un-detectable. Regardless of the cause, the bacterial assemblage supplied to us by the manufacturer was largely absent from environmental samples collected from the lake.

There was a positive correlation (r^2 =0.8) between water temperature and bacterial counts indicating that species within the lake, whether part of the natural or supplemented assemblage, are temperature-sensitive (Fig. 4). This could partially explain why bacterial numbers decreased over time as water temperature also decreased over the course of this study (Fig. 6). The supplementation of "beneficial" bacteria, even immediately following initial supplementation when water temperatures were still relatively warm, did nothing to increase overall bacterial numbers within the lake. It is impossible to determine from this study if the supplemented bacteria competed with the naturally-occurring assemblage resulting in a suppression of overall numbers, or if the supplemented bacteria survived at all and the decline in numbers is the natural result of temporal variance.

The manufacturer states that *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus licheniformis*, *Pseudomonas fluorescens*, and *Pseudomonas putidia* species are contained within their product. All of these species are extraordinarily common and ubiquitous in the environment and it's very likely they all existed at varying levels within the lake already. Numbers of bacteria in samples taken from Reid Park actually decreased during the post-treatment period. There was a close correlation between water temperature and numbers of bacteria in samples collected from Reid Park Lake. This is not uncommon as many bacteria are temperature-sensitive. Even early in the post-treatment period when temperatures were very similar, numbers of bacteria were significantly less than what existed pre-treatment (Fig. 5).

Figure 1. Oneway Analysis of Bacterial #'s By Treatment



Means and Std Deviations

Level	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
Post	27983.8	13423.2	1664.9	24658	31310
Pre	60016.3	7896.3	1164.2	57671	62361

t Test

Difference	-32032	t Ratio	-15.7669
Upper CL Dif	-28005	Prob > |t|	0.0000
Lower CL Dif	-36060	Prob > t	1.0000
Confidence	0.95	Prob < t	0.0000

Figure. 2. Bacterial Numbers by Date.

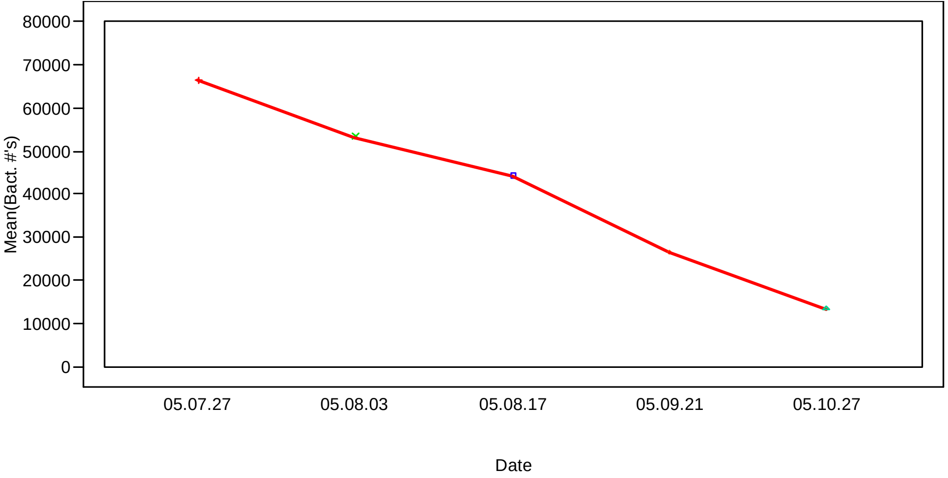
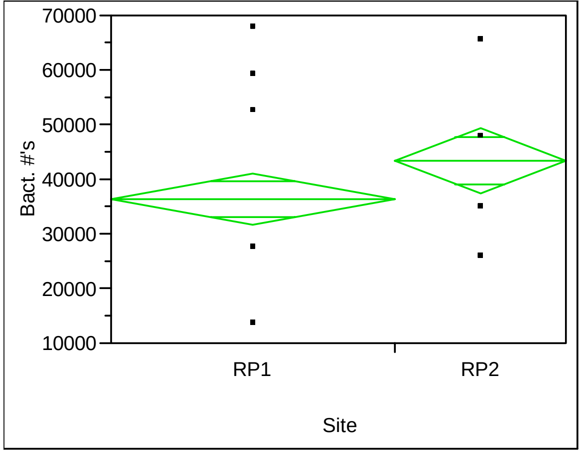


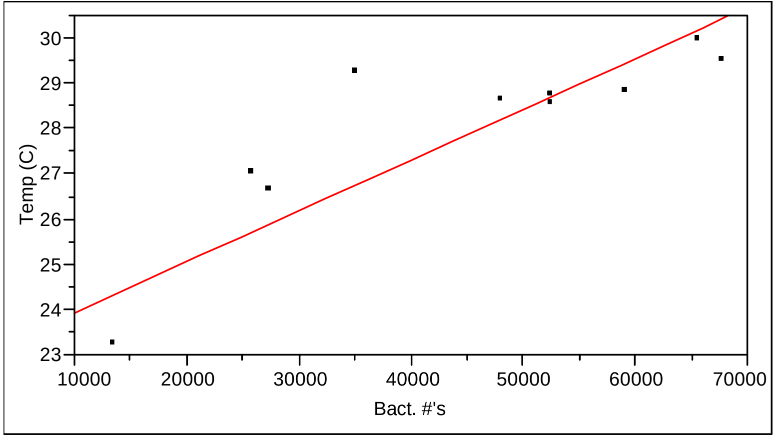
Figure 3. Oneway Analysis of Bacterial. #'s By Site



Means and Std Deviations

Level	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
RP1	36436.0	22513.2	2599.6	31256	41616
RP2	43494.4	15114.7	2253.2	38953	48035

Figure 4. Bivariate Fit of Temp (C) By Bacterial #'s



Linear Fit

Temp (C) = 22.83187 + 0.0001121 Bact. #'s

Summary of Fit

Rsquare	0.812931
RSquare Adj	0.811346
Root Mean Square Error	1.095617
Mean of Response	27.21408

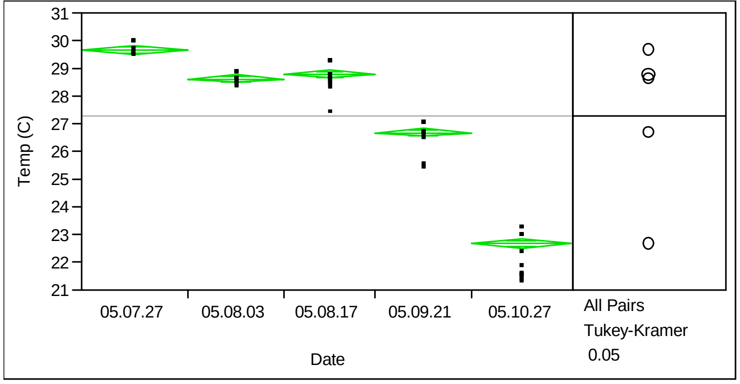
Analysis of Variance

Source	Sum of Squares	Mean Square	F Ratio
Model	615.53329	615.533	512.7836
Error	141.64441	1.200	Prob > F
C. Total	757.17770		<.0001

Parameter Estimates

Term		Estimate	Std Error	t Ratio	Prob>|t|
Intercept		22.83187	0.217838	104.81	<.0001
Bact. #'s		0.0001121	0.000005	22.64	<.0001

Figure 5. Water Temperature by Date.



Rsquare	0.973342
Adj Rsquare	0.972617
Root Mean Square Error	0.427446
Mean of Response	27.31382

Means for Oneway Anova

Level	Mean	Std Error	Lower 95%	Upper 95%
05.07.27	29.6921	0.07441	29.545	29.839
05.08.03	28.6637	0.07804	28.509	28.818
05.08.17	28.8293	0.08078	28.670	28.989
05.09.21	26.7170	0.07804	26.563	26.871
05.10.27	22.6845	0.07677	22.533	22.836

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
2.76193	0.05

Abs(Dif)-LSD	05.07.27	05.08.17	05.08.03	05.09.21	05.10.27
05.07.27	-0.2906	0.5595	0.7306	2.6773	6.7123
05.08.17	0.5595	-0.3155	-0.1446	1.8021	5.8370
05.08.03	0.7306	-0.1446	-0.3048	1.6418	5.6768
05.09.21	2.6773	1.8021	1.6418	-0.3048	3.7301
05.10.27	6.7123	5.8370	5.6768	3.7301	-0.2999

Positive values show pairs of means that are significantly different.

Level					Mean
05.07.27	A				29.692121
05.08.17		B			28.829286
05.08.03		B			28.663667
05.09.21			C		26.717000
05.10.27				D	22.684516

Levels not connected by same letter are significantly different

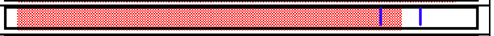
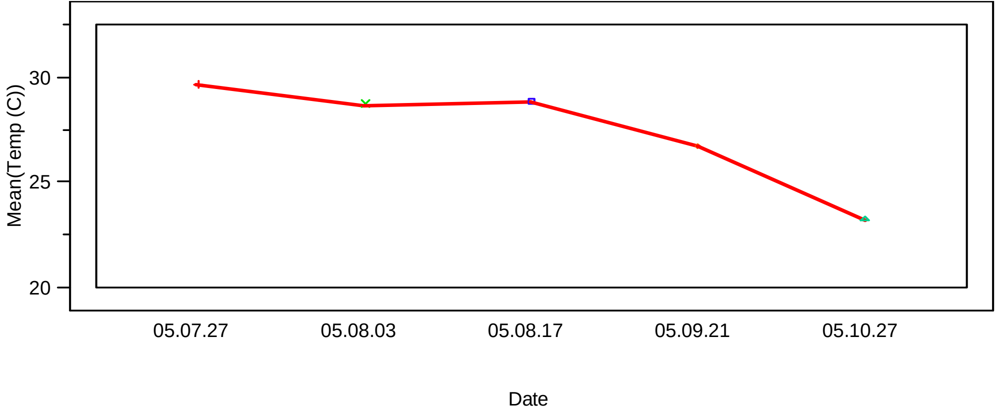
Level	- Level	Difference	Lower CL	Upper CL	Difference
05.07.27	05.10.27	7.007605	6.71232	7.302894	
05.08.17	05.10.27	6.144770	5.83698	6.452564	
05.08.03	05.10.27	5.979151	5.67679	6.281506	
05.09.21	05.10.27	4.032484	3.73013	4.334840	
05.07.27	05.09.21	2.975121	2.67731	3.272937	
05.08.17	05.09.21	2.112286	1.80207	2.422505	
05.08.03	05.09.21	1.946667	1.64184	2.251491	
05.07.27	05.08.03	1.028455	0.73064	1.326270	
05.07.27	05.08.17	0.862835	0.55950	1.166171	
05.08.17	05.08.03	0.165619	-0.14460	0.475839	

Figure 6. Water Temperature by Date



The algal assemblage responded temporally like other urban lakes in the Tucson area receiving reclaimed water. During the height of summer, chlorophytes (“green algae”) and chrysophtes (“diatoms”) dominate followed by cyanobacteria later in the summer and early fall with an overall increase in biomass until shortened photo-period and decreasing temperatures late in the fall once again suppress algal numbers (Fig. 7). We did not monitor long enough to observe a decrease in algal biomass and this may occur as late as December in Tucson depending upon climate. Overall algal biomass was much higher during the post- rather than the pre-treatment as measured by both algae counts (Fig. 7) and levels of chlorophyll *a* (Fig. 8).

If the goal of introducing “beneficial” bacteria is to decrease algal biomass, it was not observed during this study. If the management rationale of this treatment type is through competition with algae (including cyanobacteria) for nutrients, than this rationale is not supported as algae, cyanobacteria, and the bacteria supplied by the manufacturer are common and ubiquitous to all lakes. If competition were to occur to any significant degree as to exclude the growth of one over the other, it likely would occur naturally.

Figure 7. Number of Algal Units/mL by Division and Date

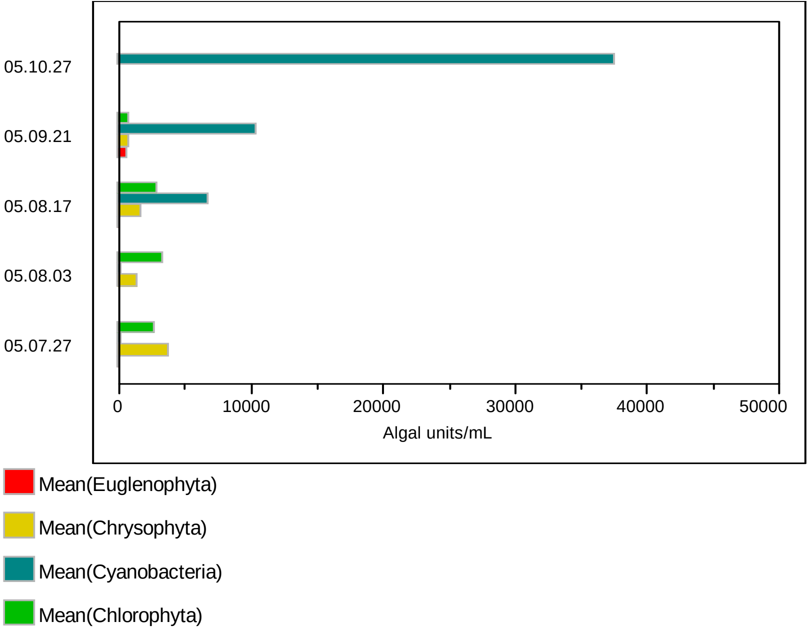
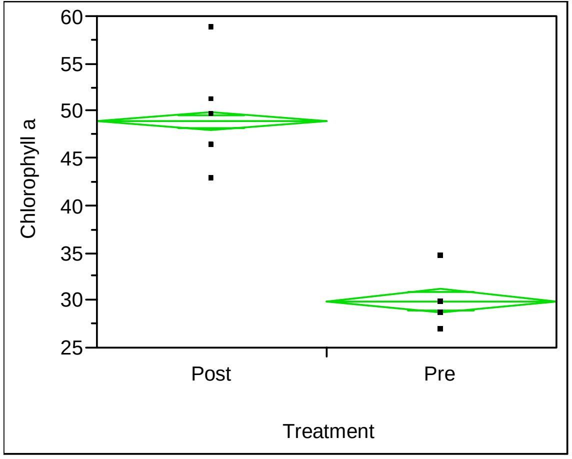


Figure 8. Oneway Analysis of Chlorophyll *a* By Treatment



Means and Std Deviations

Level	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
Post	48.9423	5.01817	0.58335	47.780	50.105
Pre	29.9693	2.87326	0.42364	29.116	30.823

t Test

Post-Pre
Assuming unequal variances

Difference	18.9729	t Ratio	26.31661
Std Err Dif	0.7209	DF	117.353
Upper CL Dif	20.4007	Prob > |t|	0.0000
Lower CL Dif	17.5452	Prob > t	0.0000
Confidence	0.95	Prob < t	1.0000

Nutrient levels were typical of a hyper-eutrophic lake and orders of magnitude higher than what it would take to be “limiting” to algal growth in any way. There was little difference in nutrient levels over time (Fig. 9). This may be due to the constant influx of nutrient-rich reclaimed water and relatively low residence time of water within the lake due to its use as irrigation water within Reid Park.

Oxidized forms of nitrogen, nitrate and nitrite, were indeed lower during the post-treatment period. Ammonia was also slightly lower; however, levels of total nitrogen were slightly higher during the post-treatment period. This is likely due to incorporation of nitrogen into algal biomass thereby making the organic N pool (not measured in this study) higher during this period. The waxing and waning of inorganic and organic forms of nutrients are common in hyper-eutrophic systems as some nutrients are incorporated into biomass during high growth phases and leached from this same biomass back into the water during periods of senescence.

Total P was slightly lower during the post-treatment period but still several orders of magnitude greater than what it would take to deem a lake as eutrophic. Total organic carbon was slightly higher during the post-treatment period and, like total N, is likely due to increased incorporation into algal biomass.

There was no difference in nutrient levels between sites RP1 and RP2 (Fig. 10).

Figure 9. Nutrient Levels by Pre- and Post-Treatment

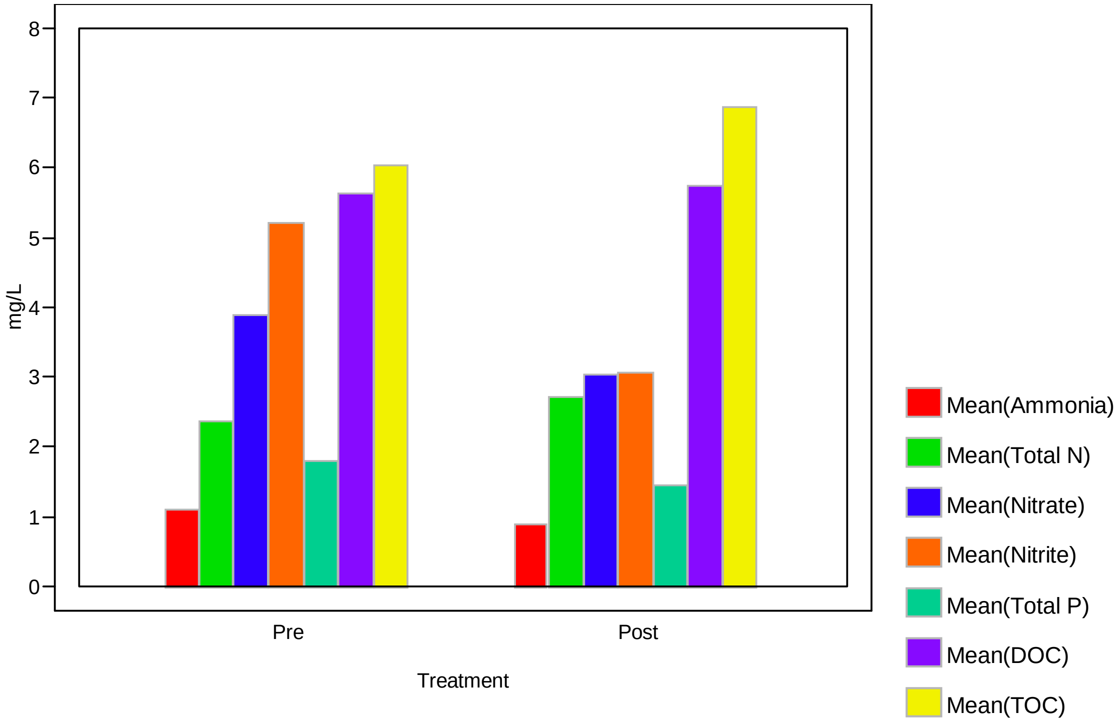
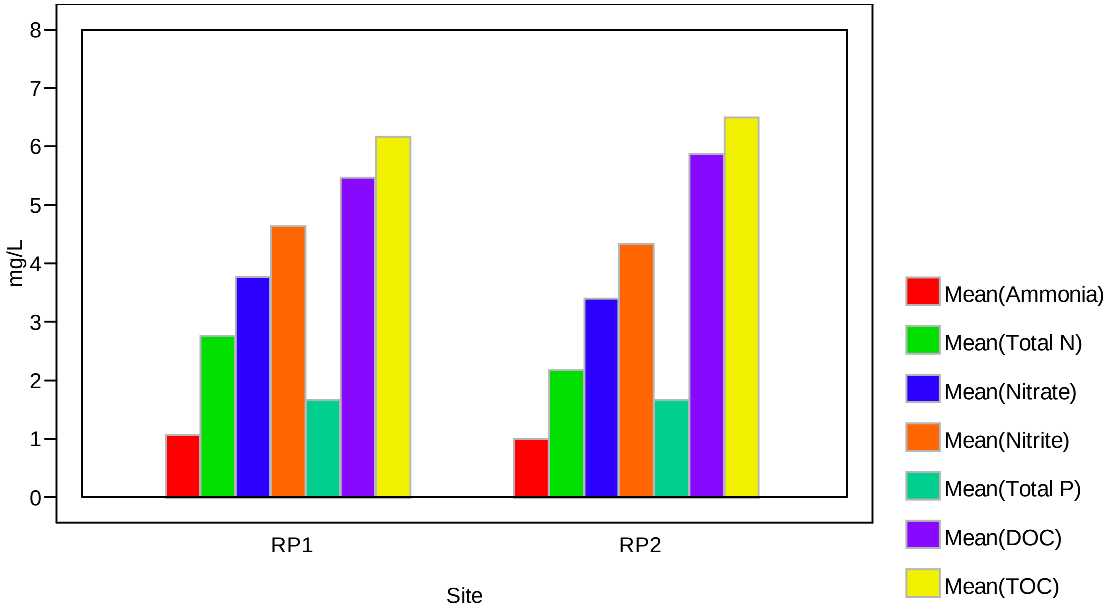


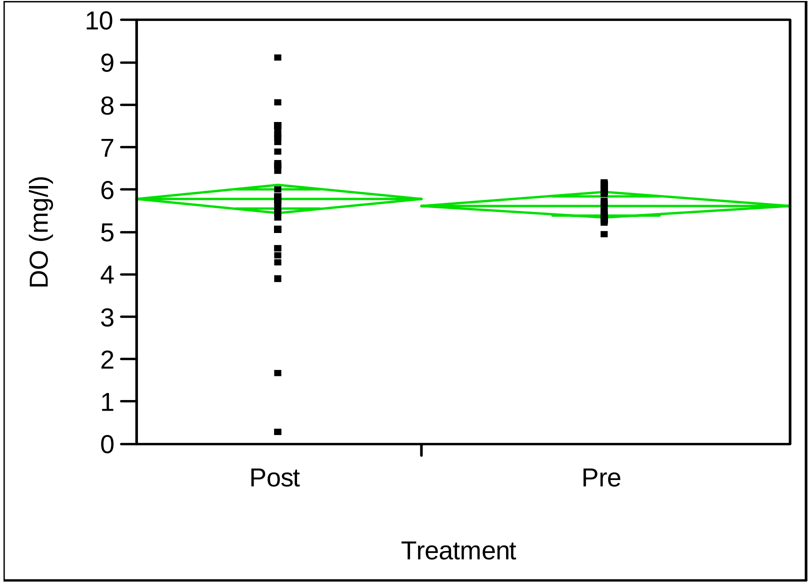
Figure 10. Nutrient Levels by Site



While there was no difference in mean levels of dissolved oxygen between the pre- and post-treatment periods, spikes and depressions were more dynamic during the post-treatment period (Fig. 11). Again, this is not uncommon for a hyper-eutrophic lake during late summer and early fall as increases in DO near the surface are common due to increased algal biomass and photosynthetic rate while depletion of DO near the bottom occurs due to the death and decomposition of the same algae near the bottom. Dealing with the spikes and sags of DO in eutrophic lakes is always problematic and it's preferred, from a managerial standpoint, to have DO levels in a relatively steady-state rather than to experience extremes in highs or lows.

Oxygen sinks within the lake, as measured by both BOD and COD, were greater during the post-treatment period (Fig. 12). Since bacterial numbers were actually lower during the post-treatment period, these increases are likely due to increased algal biomass.

Figure 11. Dissolved Oxygen Levels by Treatment Period.

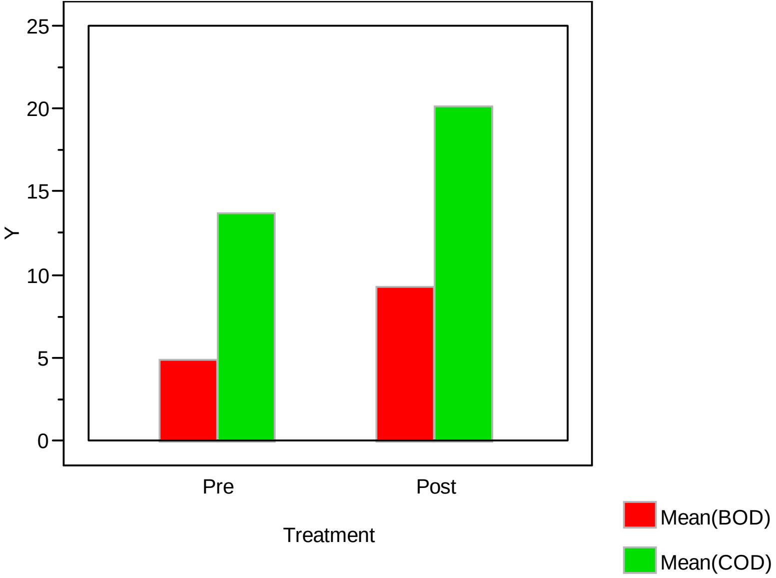


Means and Std Deviations

Level	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
Post	5.80551	1.77736	0.25391	5.2950	6.3160
Pre	5.65286	0.38259	0.04820	5.5565	5.7492

Difference	0.15265	t Ratio	0.590662
Std Err Dif	0.25844	DF	51.47032
Upper CL Dif	0.67139	Prob > |t|	0.5573
Lower CL Dif	-0.36608	Prob > t	0.2787
Confidence	0.95	Prob < t	0.7213

Figure 12. Levels of BOD and COD by Treatment Period.



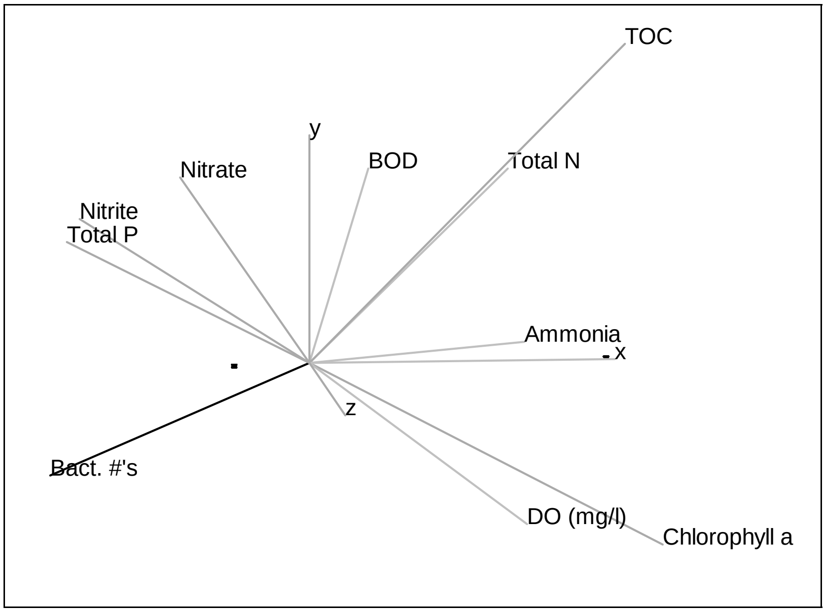
In order to examine correlations between response variables and both algal biomass (as measured by chlorophyll *a*) and bacterial numbers, we used principal components analysis (PCA) (Fig. 13). Principal components analysis is a classical statistical method also called a Karhounen-Louve or Hotteling transformation. PCA uses linear transformations and matrix algebra to choose a new coordinate system for the data cloud so that the centroid is set to zero and the first principal component axis goes through the maximum amount of variation with the second axis exactly orthogonal to the first. This sets the framework for the remaining principal components axes. In essence, PCA reduces dimensionality of a data set so that correlations among several variables can be examined simultaneously. We used a 3-dimensional representation of the data cloud (Gabriel bi-plot) so that the PCA axes could be visualized. The relative distances between each axis are eigenvectors and an eigenvector report is given below the Gabriel bi-plot. The method in which the bi-plot represents the 3-dimensional correlations is that the axes that are closest to one another have some degree of positive correlation while those in opposite quadrants are inversely proportional.

There appeared to be an inverse relation between chlorophyll *a* and bacterial numbers. Since bacterial numbers were lowest during the period when algae levels were peaking, we can only conclude either that algae levels somehow suppressed bacterial growth or that there was no treatment effect at all and the lake behaved as it normally would have in lieu of any treatment.

There was a strong positive correlation between chlorophyll *a* and levels of dissolved oxygen. This is not surprising due to increased photosynthetic rate. There was a relatively weak positive correlation between chlorophyll *a* and ammonia and a strongly inverse correlation between chlorophyll *a* and oxidized forms of nitrogen such as nitrate and nitrite. This is typical as it requires far less energy for algae to incorporate reduced and/or organic forms of nitrogen compared to more oxidized forms. There was an inverse relationship between chlorophyll *a* and total P. This relationship is probably an artifact of there being, essentially, no limitation of total P for the growth of algae.

Bacterial numbers appear to have an inverse correlation to levels of both total N and TOC meaning that as levels of both total N and TOC were elevated, bacterial numbers declined. This inverse relationship makes some sense due to heterotrophic bacteria's' consumption and degradation of organic carbon. We should be reminded, however, that bacterial numbers were significantly higher during the pre-treatment period so this inverse relationship with TOC does not imply that the treatment played any role in reducing levels of TOC.

Figure 13. PCA of Chlorophyll *a*, Bacterial Numbers, and Response Variables



Principal Components

Eigenvalue	6.0935	2.0351	1.2330	0.5491	0.0894	0.0000	0.0000	0.0000	-0.0000	-0.0000
Percent	60.9349	20.3506	12.3297	5.4912	0.8936	0.0000	0.0000	0.0000	-0.0000	-0.0000
Cum Percent	60.9349	81.2856	93.6153	99.1064	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000
Eigenvectors										
Bact. #'s	-0.37694	-0.04671	-0.17243	-0.33441	0.59580	-0.13591	-0.35191	0.26163	0.29909	0.24285
Chlorophyll a	0.39667	0.08072	0.06227	-0.17641	0.26093	-0.18104	0.37193	-0.16217	-0.23438	0.69333
DO (mg/l)	0.29073	0.22091	0.52780	0.27711	-0.00388	-0.29050	-0.00433	0.38191	0.52942	0.02729
Ammonia	0.29483	-0.36967	-0.33542	0.30883	0.11244	0.02857	0.02107	0.67839	-0.30718	-0.00826
Total N	0.30730	-0.39372	0.00152	-0.44071	0.16559	0.27261	0.42456	-0.01778	0.40865	-0.32715
Nitrate	-0.18492	-0.41698	0.51830	0.41089	0.39411	0.36513	-0.04383	-0.20562	-0.11005	0.09758
Nitrite	-0.30356	0.25248	0.42594	-0.39228	-0.07979	0.26300	0.24859	0.48846	-0.36267	-0.03678
Total P	-0.37415	0.03073	-0.27924	0.28031	-0.25459	0.32526	0.42623	0.11505	0.39970	0.42100
BOD	0.11531	0.62677	-0.21743	0.25703	0.52850	0.28399	0.17429	-0.05403	-0.02736	-0.29415
TOC	0.39378	0.13939	0.00259	-0.15267	-0.17617	0.63270	-0.53042	0.04291	0.09912	0.27899

Summary and Discussion

There was no discernible effect or benefit of supplementing the bacterial population in Reid Park Lake. It is unclear as to why bacteria counts decreased during the post-treatment period. This makes some sense with the last sampling in late October when water temperatures were beginning to cool but makes less sense with the samplings in mid-August and late-September when water temperatures should have played no role in suppressing bacterial biomass.

The ecologic interplay of bacteria and algae (in this case including cyanobacteria) in aquatic ecosystems is still poorly understood. Oligotrophic systems do not generally have a higher biomass of true bacteria that “out-compete” algae whereas eutrophic systems generally have a high biomass of algae but whether it out-competes bacteria for resources is unknown. Certainly, in Reid Park Lake, nutrients are nowhere near limiting for algal or bacterial growth yet we observed that as bacterial numbers decreased post-treatment, algae numbers skyrocketed.

There are at least 2 possible scenarios why we observed algal numbers increase and bacterial numbers decrease post-treatment:

1) The treatment did nothing, at least nothing that could be quantified.

2) The treatment actually supplied an energy source for the growth of heterotrophic algae.

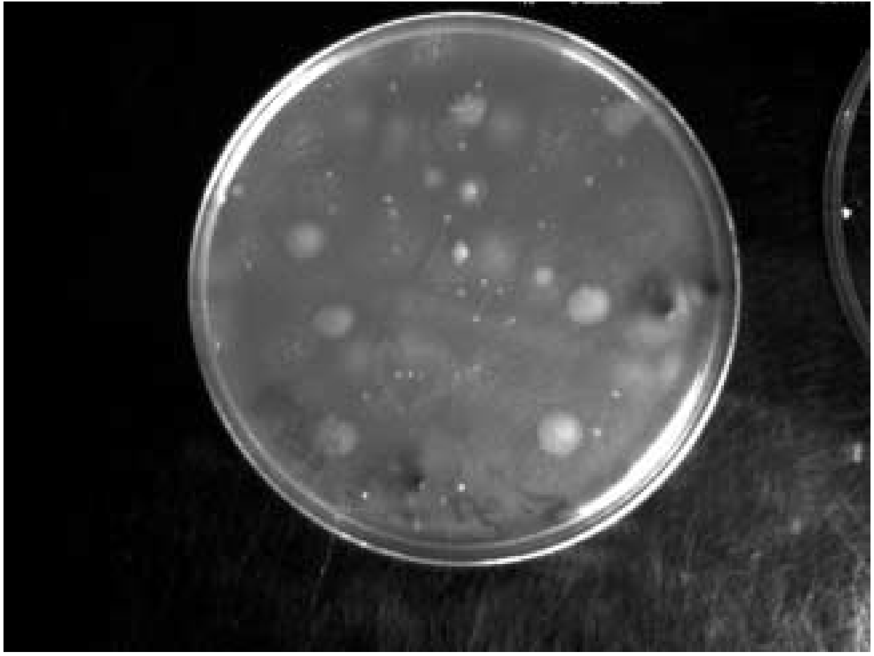
We did see a conversion away from strongly autotrophic species (i.e. green algae) to a more heterotrophic assemblage (i.e. cyanobacteria) and predation by these heterotrophic algae on bacteria is well-known. Whether or not this occurred in Reid Park Lake is not quantifiable in this study but is still a possibility.

In either scenario listed above, the treatment did not appear to decrease either numbers of algae or levels of nutrients and the lake responded temporally as any hyper-eutrophic urban lake would have with or without the supplementation of “beneficial” bacteria.

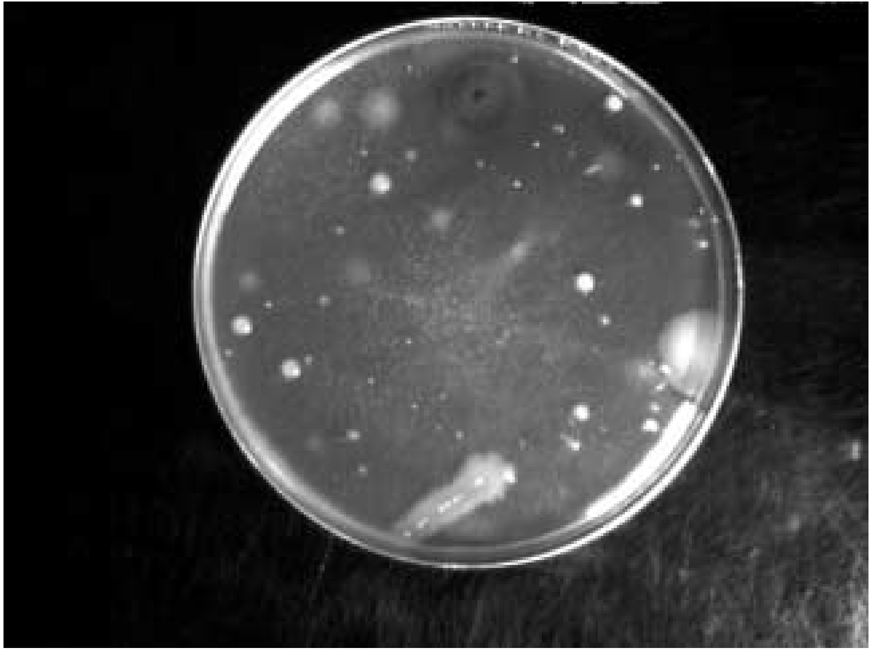
Appendix

Bacterial colonies from pellet supplied by the manufacturer

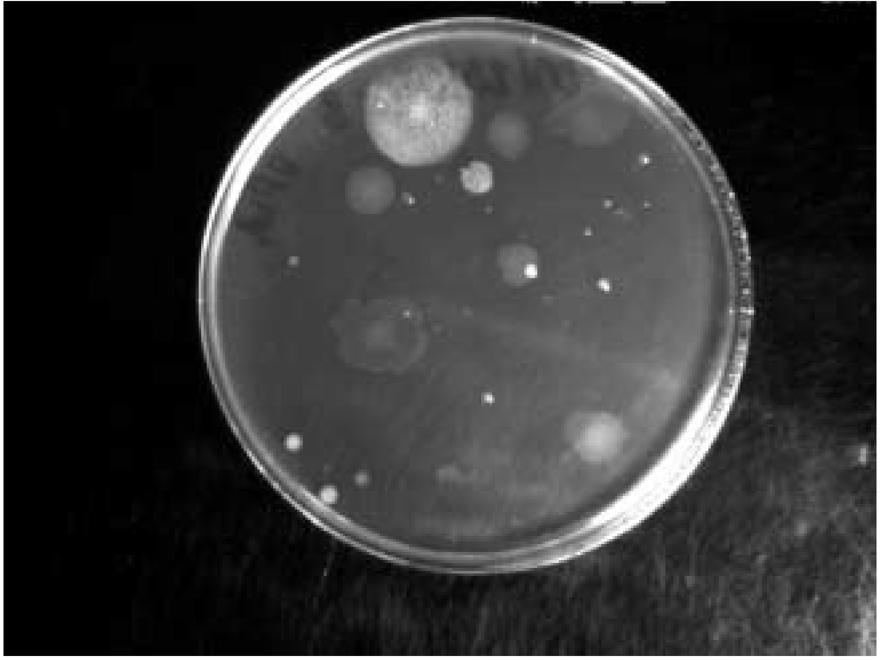
Pre-treatment bacterial colonies from the 07/27/05 sampling



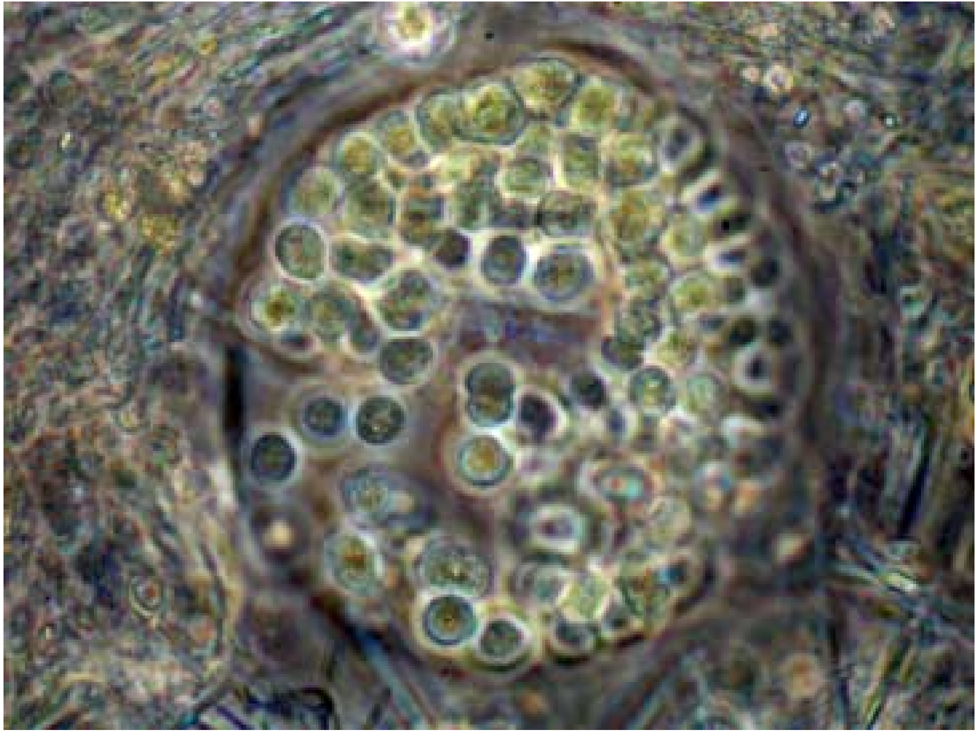
Post-treatment bacterial colonies from the 08/17/05 sampling



Post-treatment bacterial colonies from the 09/21/05 sampling



Colony of *Gomphosphaeria sp*. (a species of cyanobacteria) collected during the 09/21/05 sampling



Colony of *Microcystis sp.* (a species of cyanobacteria) collected during the 08/17/05 sampling