Watershed Protection Development Review

Potential Impacts of Hays County WCID No. 1 Proposed Wastewater Discharge on the Algae Communities of Bear Creek and Barton Springs.

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A direct wastewater discharge has been proposed to Bear Creek in the contributing zone of the Barton Springs portion of the Edwards Aquifer from the Hays County Water Control and Improvement District No. 1 (HCWCID1) WWTP serving the Belterra and surrounding developments in Hays County. This discharge will change the flow regime and nutrient loads to both Bear Creek and Barton Springs (Slade 2006, Miertschin and Obenour 2006, COA 2006). Field and lab experiments were conducted to assess the potential alteration of the existing algal community density and structure of both Bear Creek and Barton Springs due to increased nutrient loading if the discharge permit is approved. The question of what is an acceptable level of nutrient addition to these aquatic systems to avoid nuisance algae impacts was also investigated. Periphytometers (Matlock et al 1998) were deployed in the field and sestonic algae growth bioassays (Kiesling et al 2001) were conducted in the lab in July 2006 to determine the limiting nutrient controlling periphytic and sestonic algae growth. Additional sestonic algae growth bioassays were conducted to establish a dose-response relationship for the limiting nutrient to estimate critical nutrient threshold concentrations. A light/dark bottle test (SM 10200J , APHA 1995) was conducted to provide current sestonic algae production rates for use in modeling. Results of this experimentation support the hypothesis that these water bodies are highly oligotrophic and that any nutrient additions of the type proposed by the Belterra wastewater discharge permit will significantly increase algal productivity and likely change their current trophic status.

Introduction

The Belterra development in Hays County, Texas, is currently constructing new single-family residential units and the Hays County Water Control and Improvement District No. 1 serving this area has submitted an application to the Texas Commission on Environmental Quality (TCEQ) for a direct wastewater discharge to Bear Creek, in the contributing zone of the Barton Springs portion of the Edwards Aquifer. If approved, the proposed plant could discharge a monthly average of 800,000 gallons/day of treated effluent with average effluent limitations of 5 mg/L BOD, 5 mg/L TSS, 2 mg/L NH_3 and 1 mg/L P.

City of Austin staff conducted three experiments in July and August 2006 to assess the potential impacts on algal communities in the discharge route. Study sites included two sites on Bear Creek and one in Barton Springs Pool (Table 1), which is the main discharge point of this segment of the Edwards Aquifer. The Davis Pond study site is an impoundment approximately 3 kilometers from the proposed discharge point on Bear Creek. It is approximately 1.6 square kilometers in area and has an average depth of 2 meters. The Bear Creek Pass study site is a natural perennial pool that is adjacent to United States Geological Survey (USGS) gage number 08158810. It is approximately 250 meters long with an average width of 4 meters and an average depth of 1 meter.

Table 1. Study site locations.

Name	Description	Lat/Long
Barton Springs	Perennial spring-fed impounded swimming pool in Austin, Texas	30º15'48"W 97º46'16"N
Bear Creek downstream of Bear Creek Pass	A perennial impoundment with a surface area of approximately 1.6 acres	30º09'19"W 97º56'24"N
Bear Creek at Davis Pond	An intermittently flowing in-channel pool	30º10'41"W 97º58'11''N

Periphytometers were deployed at each site for two weeks. Multiple sestonic algae growth potential bioassays were conducted using ambient water samples at the USGS Austin laboratory. A light/dark bottle test was used to estimate primary productivity in the water column.

The combined experiments had 4 primary objectives:

- Determine the limiting nutrient currently restricting the growth of periphytic and sestonic algae.
- Estimate the current trophic status.
- Estimate current primary productivity.
- Assess the impact of increases in the concentration of the limiting nutrient on sestonic algae and the threshold for predicted nuisance algae growth as a recommended limit to discharge

The proposed Belterra discharge will significantly alter the flow regime of Bear Creek, as wastewater effluent will dominate the total flow for half of every year based on historic USGS flow data (Slade 2006) and dominate the phosphorus loads to both Bear Creek (Slade 2006) and Barton Springs (COA 2006). Potential impacts to both Bear Creek and Barton Springs Pool algal communities include increase in standing crop biomass, alteration of maximum nutrient sufficient algal growth rates, increase in the frequency and duration of algae blooms, changes in community composition, and shift in the community structure from dominance of periphytic algae to dominance of sestonic algae.

Alterations in the existing trophic status of Bear Creek and Barton Springs Pool could alter diurnal DO patterns and reduce water column clarity, negatively impacting aquatic life and the endangered Barton Springs salamander, and affect contact recreation use of both water bodies. Additionally, Barton Springs Pool discharges into Town Lake, a drinking water source of the City of Austin, which is already subject to algae blooms during critical changes in flow regimes that result in anoxic DO conditions at bottom depths (COA 2004).

Perennial pools in Bear Creek and throughout the Central Texas area are the only refugia for aquatic life in intermittent streams, as expected in the semi-arid climate. The large ratio of pool volume to creek flow rates in isolated pools like the Davis Pond and Bear Creek Pass study sites make this type of pool highly vulnerable to eutrophication and DO deficits. Increased nutrient loading from point or non-point sources in critical periods of low or zero flow will result in instream nutrient concentrations that approach actual source load levels.

Due to the potential impacts listed above, the specification of nutrient limits in the Belterra may be an appropriate action by TCEQ to prevent significant degradation of the receiving water uses downstream. Although permit limits in Texas have seldom been below 1.0 mg/L, permit limits in Virginia and Australia have been as low as 0.1 mg/L total phosphorous (Kraume 2005). Current proven technology such as applied at the Durham, Oregon, AWWTF can reach treatment limits in the range of 0.07 mg/L total phosphorous (Stephens 2003). Also, if the water quality based treatment limits represent an economic impact to the development; HCWCID No. 1 may be advised to reconsider land application as an alternative to discharge. Previous development plan submittals to U.S. Fish and Wildlife Service for the Belterra Subdivision have been for land application up to 600,000 gpd (Seawell 2002).

State assessments of Bear Creek have included a comprehensive intensive survey effort in 1986 including water quality, flow regime, and benthic macroinvertebrate sampling (Davis 1986). This effort identified Bear Creek as having extremely low phosphorous levels, and low assimilative capacity for wastewater discharge. The report noted a previous wastewater discharge permit for development above FM 1826 that was denied by the Texas Water Commission in 1986 due to concerns over pollution of the Edwards Aquifer. A conclusion of the study was that "even minor inputs could promote excessive plant growth and result in use impairment" (Davis 1986).

Previous work in the area of nutrient limitation of Colorado River tributary streams was conducted by Southwest Texas State University (currently Texas State University (TSU)) (Short 1988). This study used passive nutrient diffusing substrates and a flow-through tile system in the determination of limiting nutrient and recommended nutrient discharge concentrations. Results of this study, which included three sites on Bear Creek, included a mixed limitation on nitrogen and phosphorous both longitudinally and seasonally in the creek. Also Bear creek was classified with other Hill Country streams having low total phosphorous concentrations (< 20 µg/L) with little filamentous algae growth. The final conclusion of this study was that for sites that are phosphorus-limited such as Bear Creek at Bear Creek Pass, "the threshold concentration for stimulation of algae growth was shown to be between 50 and 100 µg/L". Overall, the study concluded that “Hill Country streams are not significantly impacted at the present time but do show the potential for water quality degradation in terms of excessive algal growth if nutrient levels are increased. Thus the releasing of wastewater effluents into these streams carries a significant probability of causing undesirable water quality conditions and should be avoided if existing conditions are to be maintained”.

Methods

This work was completed during July and August of 2006 and had four components to it. Ambient nutrient and chlorophyll-a water chemistry, a light/dark bottle test, periphytometers, and algae growth potential bioassays (Table 4).

Ambient Nutrients and Phytoplankton Chlorophyll-a

Three ambient nutrient samples were collected at each site during the deployment of the periphytometer (July 6 through July 24) to estimate total nitrogen and phosphorus in both the dissolved and total fractions. Additionally, ambient chlorophyll-a samples were collected from the water column approximately six inches below the water surface of each site. The ambient nutrients and chlorophyll-a samples were analyzed at the Lower Colorado River Authority (LCRA) lab in Austin according to EPA-approved analysis methods (Table 5).

Table 4. Summary of collection dates by experiment.

Event	Barton Springs	Bear Creek Pass	Davis Pond
Periphytometer deployment	06-Jul-2006	07-Jul-2006	07-Jul-2006
Periphytometer retrieval	20-Jul-2006	21-Jul-2006	21-Jul-2006
Light/Dark Bottle Test	13-Jul-2006	14-Jul-2006	14-Jul-2006
Nutrient sample collection #1	06-Jul-2006	07-Jul-2006	07-Jul-2006
Nutrient sample collection #2	13-Jul-2006	13-Jul-2006	13-Jul-2006
Nutrient sample collection #3	24-Jul-2006	24-Jul-2006	24-Jul-2006
AGP (limiting nutrient)	13-Jul-2006	13-Jul-2006	13-Jul-2006
AGP (dose-response #1)	24-Jul-2006	24-Jul-2006	24-Jul-2006
AGP (dose-response #2)	08-Aug-2006	08-Aug-2006	08-Aug-2006

Table 5. Parameters and analysis methods for ambient samples.

Parameter	Analysis Method	PQL*
Nitrate+Nitrite as N	EPA 353.2	0.01 mg/L
Total Kjeldahl Nitrogen as N	EPA 351.2	0.02 mg/L
Ammonia as N	EPA 350.1	0.02 mg/L
Phosphorus as P	EPA 365.4	0.02 mg/L
Orthophosphorus as P	EPA 300	0.01 mg/L
Chlorophyll-a	EPA 446	1.2 µg/L

*PQL=Practical Quantitation Limit

Light/Dark Bottle Test

The light/dark bottle test, as described by Standard Methods number 10200J (APHA 1995), was used to estimate photosynthesis and respiration in the water column by measuring in-situ flux of oxygen. Based on the assumption that one atom of carbon is assimilated for each molecule of oxygen released, the productivity of the system can be calculated.

Oxygen was measured in Wheaton 300mL clear and darkened BOD bottles using a HACH luminescent dissolved oxygen probe. Four replicates of each treatment (light, dark) were used. Bottles were filled simultaneously by immersion approximately 6 inches below the water surface, and DO was measured initially in one set of the clear bottles. Bottles were incubated at ambient light and temperature for 5 hours from 10:00 to 15:00 at each study site using attached floats (Figure 1). After 5 hours, DO was measured in the remaining set of clear bottles and the dark bottles. Sampling was conducted on hot, sunny days in mid-July 2006 in conjunction with the mid-point of the periphytometer deployment. Results from the replicates for each treatment were averaged.

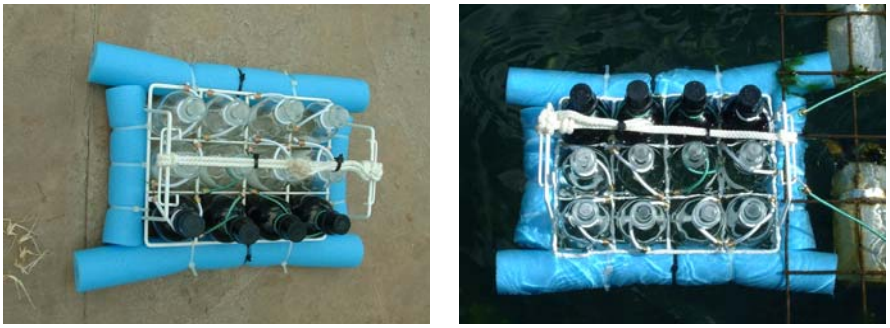

Figure 1. Example of light/dark bottle equipment (left) and deployment adjacent to the periphytometer at Barton Springs Pool (right).

Photosynthesis and respiration were estimated by:

Net photosynthesis	= light bottle DO – initial DO
Respiration	= initial DO – dark bottle DO
Gross photosynthesis	= light bottle DO – dark bottle DO

Net production was estimated by the formula:

Net production (mg C fixed/m^3) = Net Photosynthesis * 12/32 * 1000L/m^3 * PQ

where PQ is the photosynthetic quotient which varies between 1 and 2 depending on whether nitrate or ammonia is available as the nitrogen source. PQ values for study sites (Table 2) were roughly approximated using the molar ratio of organic carbon to nitrate (Davies and Williams 1984, Bell and Kuparinen 1984).

Table 2. Photosynthetic quotient (PQ) values used for study sites.

Site	**PQ (moles O_2 released / moles C fixed)**
Barton Springs	1.42
Bear Creek Pass	1.25
Davis Pond	1.25

Periphytometers

Periphytometers, a passive nutrient diffusion mechanism originally described by Matlock et al (1998), were used to determine the limiting nutrient restricting the growth of periphytic algae. Each periphytometer was deployed with 40 bottles consisting of 4 treatments with 10 replicates per treatment (Table 3). For each 1L narrow-mouth HDPE bottle, a 4.7 cm diameter nylon membrane with a 0.45 μm pore size (Whatman 7404-004) was placed on the mouth bottle, a 3.7 cm diameter glass fiber filter (Whatman 934-AH) was placed on the nylon membrane, and the filters held in place by a screw cap with a hole 2.5 cm in diameter. Non-shading fiberglass window screen was placed over the cap and secured with zip ties to prevent grazing by fish and macroinvertebrates. Each bottle was attached to the rack using a metal hose clamp in a grid format (Figure 2).

Table 3. Periphytometer treatments.

Treatment	Description
Control (C)	1L type III de-ionized (DI) water only
Nitrogen (N)	1L type III DI water + 1mL $NaNO_3$ at 15.3g/L
Phosphorus (P)	1L type III DI water + 1mL of $Na_2HPO_4 \cdot 7H_2O$ at 30 g/L
Nitrogen + Phosphorus (NP)	1L type III DI water + 1mL $NaNO_3$ at 15.3g/L + 1mL of $Na_2HPO_4 \cdot 7H_2O$ at 30 g/L

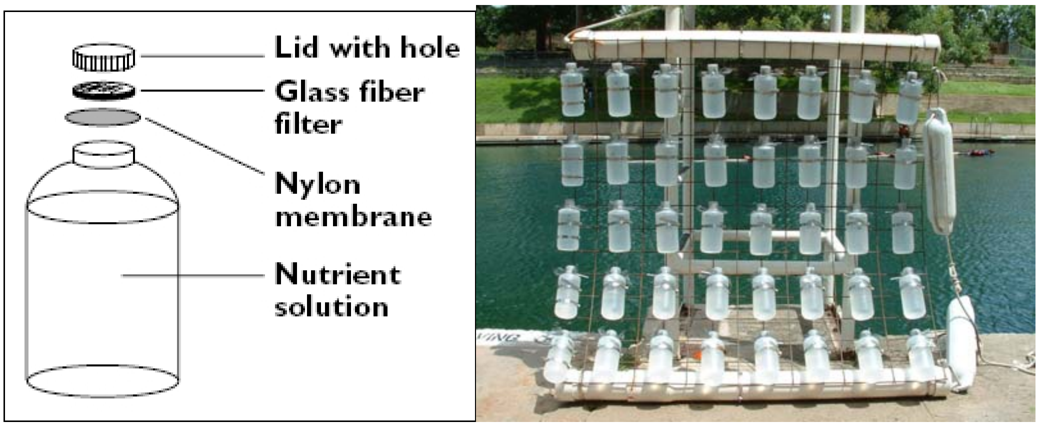

Figure 2. Periphytometer bottle set-up (left) and assembled periphytometer grid prior to deployment at Barton Springs Pool (right)

Treatments were distributed throughout the periphytometer racks using a randomized block design. Racks were deployed for 2 weeks in July 2006. Total and dissolved nutrient samples and ambient chlorophyll-a samples were collected at deployment, after one week and at retrieval. Upon retrieval at each site, 6 of the 10 filters from each treatment were collected, placed into individually labeled glass screw-top vials and delivered to an EPA certified lab (LCRA) for analysis by photometric methods (EPA method 446). The remaining 4 filters from each treatment were frozen for later analysis if necessary. Analysis results were reported by the lab as if 500mL of sample had been filtered. To calculate production rates, the mass of chlorophyll-a was calculated by multiplying reported values in µg/L by 0.5L and dividing by the exposed area of the filters (2.5cm diameter).

Periphytometers were deployed in unshaded areas, chained to the shore and anchored with concrete weights to keep them stationary (Figure 3).

Figure 3. Site photos (clockwise from top left: Barton Springs, Bear Creek Pass, Davis Pond).

Periphytometer results were used to estimate current trophic status using Lotic Ecosystem Trophic Status Index (LETSI) values calculated as the ratio of control to nutrient saturated (NP treatment) production (Matlock et al 1999, Kiesling et al 2001). LETSI values represent the ratio of baseline primary productivity to maximum primary productivity. Values near 1 indicate the system is near maximum primary productivity.

Sestonic Algae Growth Potential Bioassay

Characterization of the productivity of water column algae was also assessed using an algae growth potential bioassay (AGP) modified from Standard Method 8112 (APHA 1995, Kiesling et al 2001), conducted in two phases. For each phase, the City of Austin collected 5 liters of water in 1 liter plastic containers from each site, preserved the bottles in a small amount of ice and delivered them to the USGS lab in Austin within several hours of collection.

First, the limiting nutrient restricting water column algae growth was determined using ambient water samples from each site inoculated with nutrients at the same concentrations as the periphytometer stock solutions in four treatments with six replicates per treatment: a control with no nutrient addition, high nitrogen, high phosphorus, and a combined high nitrogen and high phosphorus. Exponential growth models were fitted to the measured data to obtain maximum exponential growth rates (r) for each site (MacFarland et al 2001, Kiesling et al 2001). The USGS incubated the samples in borosilicate glass vials at ambient temperature and full light conditions in an environmental growth chamber. Samples were incubated over a period of 5 to 6 days and chlorophyll-a was measured by in-vivo fluorescence (Lorenzen 1966) once per day.

Second, a dose-response relationship for the identified limiting nutrient was estimated along a nutrient enrichment gradient. Samples were inoculated with varying concentrations of the limiting nutrient, and in-vivo fluorescence was measured once per day for approximately 5 days. Four replicates were used per treatment. Phytoplankton growth rates were plotted versus external nutrient concentrations and a Monod function (Monod 1950, Kiesling et al 2001) was fit to the data according to:

$$\mu = \mu_{max} * S / (K_s + S)$$

where μ is the growth rate, μ_{max} is the maximum nutrient saturated growth rate, S is the external nutrient concentration and K_s is the half-saturation constant for growth representing the most rapid portion of biomass increase or potentially a bloom condition.

Statistical Analysis

Statistical analyses were performed using SAS version 9.1, with a critical value for significance (α) of 0.05 unless specified.

Differences between the median values of the periphytometer treatments and blocks were assessed by the non-parametric Kruskal-Wallis test. Comparisons between the median values of the periphytometer treatments as well as the nutrients and chlorophyll-a were assessed by the non-parametric Wilcoxon rank-sum test. ANOVA was used to determine the phytoplankton limiting nutrient at each site.

Coefficients were estimated iteratively for non-linear functions using SAS PROC MODEL. For the AGP studies, the Monod function and starting values for K_s and μ_{max} were explicitly specified (starting K_s=0.001, starting μ_{max}=minimum *r* for any treatment).

Results and Discussion

Ambient Nutrients and Chlorophyll-A

Ambient nutrient and phytoplankton chlorophyll-a samples at all three sites indicate that while Barton Springs maintains lower water column chlorophyll-a concentrations (all values below detection limit at Barton Springs), both total and dissolved nitrogen and dissolved phosphorus are significantly higher in Barton Springs than the Bear Creek sites (Table 6, non-detects taken at detection limit for summation). There was no significant difference between sites for total phosphorus (Wilcoxon rank-sum two-sided prob > |z| = 0.2914). There was no significant difference in total or dissolved nutrient concentrations between the two Bear Creek sites.

Chlorophyll-a values in Barton Springs Pool are typically non-detect (85% of values by method EPA 446, or all data since July 2001, are less than detection limit), and the long-term average chlorophyll-a using all data (by Kaplan-Meier estimation) is 0.394 µg/L (std error = 0.0296). The maximum detected chlorophyll-a value in Barton Springs Pool was 2.23 µg/L (City of Austin Field Sampling Database, 2006). Chlorophyll-a concentrations in Barton Springs are most likely depressed by the high pool turnover rates, which range from 2.1 to 19.2 times per day (Plummer and Associates 2000) depending on spring discharge.

Table 6. Summary of total and dissolved nutrients, chlorophyll-a and physical parameters from ambient samples (mg/L unless specified). Non-detects taken at detection limit for summation.

Event	Diss. N	Total N	Diss. P	Total P	Chl-A (µg/L)
Barton Springs					
#1	1.572	1.482	0.018	0.014	<1.17
#2	1.422	1.442	0.029	0.009	<0.935
#3	1.452	1.792	0.016	0.036	<0.468
Bear Creek Pass					
#1	0.158	0.238	0.007	0.007	3.14
#2	0.058	0.138	0.007	0.007	2.84
#3	0.127	0.206	0.007	0.014	7.28
Davis Pond					
#1	0.183	0.341	0.007	0.01	3.78
#2	0.192	0.204	0.009	0.007	2.05
#3	0.15	0.176	0.007	0.027	2.99

Ambient nutrient samples collected during this study were generally not significantly different from historical non-storm values (City of Austin Field Sampling Database, USGS and the City of Austin) where matching parameters existed and comparisons could be made statistically (Table 7). No historical water quality data was available from the Davis Pond site.

Table 7. Comparison of ambient samples collected during this study (n=3) to available non-storm historical data. Only the shaded constituents were significantly different from background.

Parameter	N	Period of Record	Test*	Prob
Barton Springs				
Ammonia, dissolved	50	1990-2005	Peto	$Pr>\chi^2 = 0.7759$
Ammonia, total	347	1978-2006	Peto	$Pr>\chi^2$ = **
Nitrate, dissolved	72	1990-2005	WRS	$Pr>\|z\| = 0.0279$
Nitrate, total	346	1978-2006	WRS	$Pr>\|z\| = 0.0175$
OP, dissolved	142	1990-2005	Peto	$Pr>\chi^2 = 0.6526$
OP, total	177	1995-2006	Peto	$Pr>\chi^2 = 0.7603$
P, dissolved	87	1990-2005	Peto	$Pr>\chi^2 = 0.8380$
P, total	333	1969-2005	Peto	$Pr>\chi^2 = 0.4147$
Bear Creek Pass				
Nitrate, dissolved	12	1993-1997	Peto	$Pr>\chi^2 = 0.2195$
Nitrate, total	32	1978-1992	Peto	$Pr>\chi^2 = 0.7497$
OP, dissolved	12	1993-1997	Peto	$Pr>\chi^2$ = **
OP, total	9	1991-2004	Peto	$Pr>\chi^2$ = **
Ammonia, dissolved	12	1993-1997	Peto	$Pr>\chi^2 = 0.6642$
Ammonia, total	37	1978-2004	Peto	$Pr>\chi^2 = 0.7439$
P, total	34	1978-2004	Peto	$Pr>\chi^2 = 0.6778$
P, dissolved	12	1993-1997	Peto	$Pr>\chi^2 = 0.1664$

*WRS = Wilcoxon rank-sum test; Peto = modified Peto and Peto test using SAS PROC NPAR1WAY to account for non-detect values
**All current values below detection limit.

Nitrate (total and dissolved) in Barton Springs was significantly greater during the study period by Wilcoxon rank-sum than historical data (average total nitrate during the study period was approximately equal to the 95th percentile of the historical data), most likely due to flow effects. Barton Springs nitrate is inversely related to Barton Springs mean daily discharge (COA 2005), and flow was extremely low during the study period. However, total nitrate concentrations have been greater than one standard deviation of the long-term mean since August 2005 according to routine monitoring data (Figure 4), and total nitrate during the study period is not significantly different from total nitrate from January 2005 to June 2006 (Wilcoxon rank-sum pr>|z| = 0.2089). Although nitrate concentrations during the study period were elevated, they do not represent a single, short-term spike.

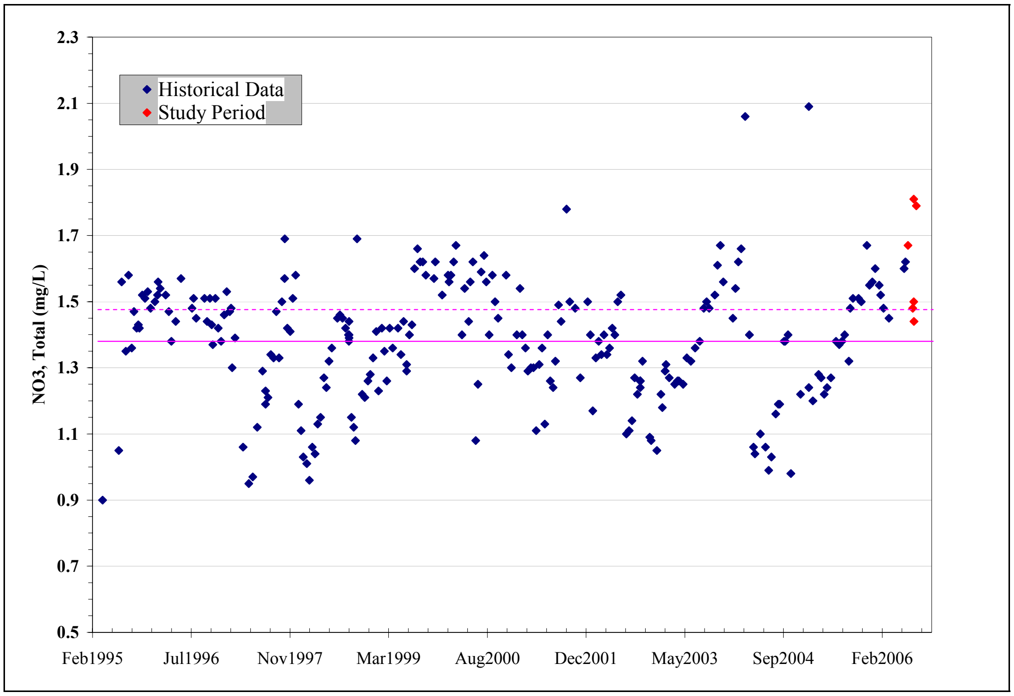

Figure 4. Total nitrate in Barton Springs before and during the study period (displayed since 1995 when City of Austin intensive monitoring began) with long-term mean nitrate (solid line) and one standard deviation of the mean (dashed line).

During July 2006, Barton Springs was in a period of extreme low flow with an average daily discharge of 26 ft^3/s (Figure 5). Bear Creek flows continued to recede during the study period, and although the USGS gage registered zero flow after 20 July 2006, both sites on Bear Creek maintained pooled water sufficient to completely cover the periphytometer. There were no significant rainfall events during the periphytometer deployments.

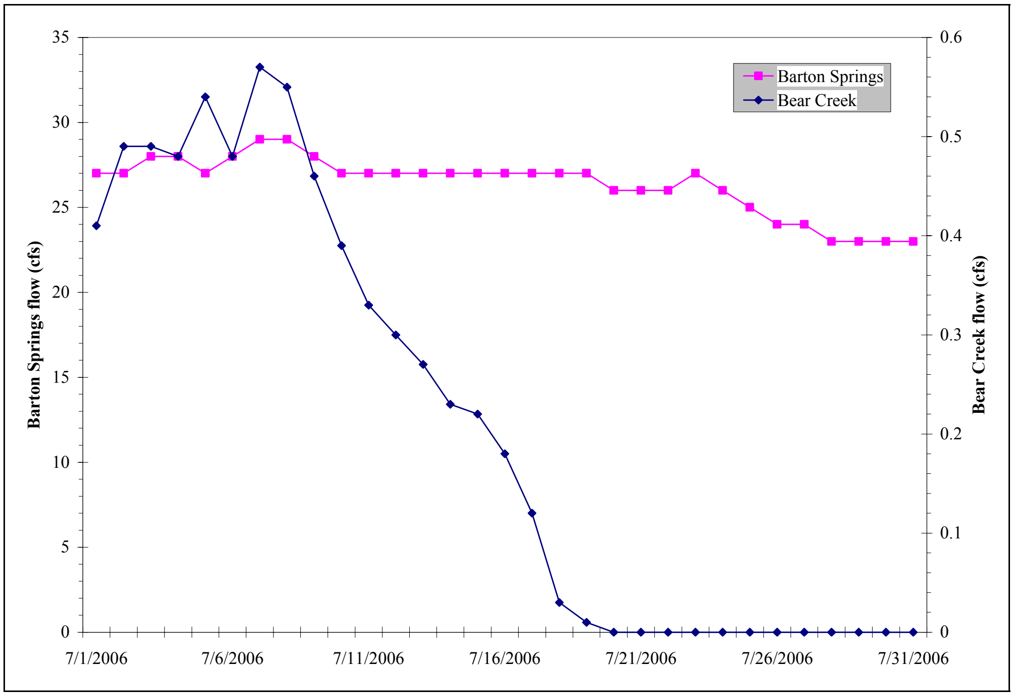

Figure 5. USGS mean daily discharge data from Barton Springs (08155500) and Bear Creek Pass (08158810) from 01-July-2006 to 31-July-2006. Average daily discharge from Barton Springs based on USGS gage data (1978-2006) is 64 ft^3/s.

Comparison of the molar ratio of nitrogen to phosphorus can be used to determine the limiting nutrient based on ecological stoichiometry. The expected nitrogen to phosphorus atomic ratio in most phytoplankton without nutrient limitation is 16:1 (Redfield 1958), although this ratio may vary by taxa (Rhee and Gotham 1980). Studies of river phytoplankton have estimated that Redfield ratios above 20 suggest phosphorus limitation (Schanz and Juon 1983). Atomic ratios from ambient samples suggest that the majority of samples were phosphorus limited, although one sample from Bear Creek Pass and one sample from Davis Pond on two separate sampling events indicated the potential for either phosphorus or nitrogen limitation (Table 8).

Table 8. Atomic Redfield ratios from ambient nutrient samples.

	N:P (Dissolved)	N:P (Total)
Barton Springs		
Deployment	193	234
After 1 week	108	354
After 2 weeks	201	110
Bear Creek Pass		
Deployment	50	75
After 1 week	18	44
After 2 weeks	40	33
Davis Pond		
Deployment	58	75
After 1 week	47	64
After 2 weeks	47	14

Light/Dark Bottle Test
Results from the light/dark bottle test (Table 9) indicate that both Bear Creek sites yield positive net photosynthesis, while respiration at Barton Springs Pool is greater than net photosynthesis (Table 10).

Table 9. Mean DO (mg/L) and standard deviation from light/dark bottle test conducted in July 2006.

Treatment	**Mean**	**StdDev**	**Min**	**Median**	**Max**
Barton Springs					
Initial	6.84	0.26	6.54	6.83	7.14
Final-Light	6.17	0.13	6.00	6.20	6.30
Final-Dark	6.53	0.10	6.38	6.57	6.60
Bear Creek at Davis Pond					
Initial	10.08	0.10	9.98	10.07	10.21
Final-Light	10.31	0.07	10.27	10.28	10.41
Final-Dark	10.01	0.05	9.94	10.02	10.04
Bear Creek at Bear Creek Pass					
Initial	6.81	0.08	6.74	6.80	6.91
Final-Light	7.11	0.02	7.09	7.11	7.14
Final-Dark	6.80	0.11	6.66	6.81	6.91

Table 10. Estimated photosynthesis and respiration (mg/L DO) from light/dark bottle test and ambient chlorophyll-a in July 2006.

Site	**Net Photosynthesis**	**Respiration**	**Gross Photosynthesis**	**Ambient chlorophyll-a (µg/L)**
Barton Spring	-0.66	0.31	-0.36	<0.935
Davis Pond	0.23	0.08	0.30	2.05
Bear Creek Pass	0.30	0.01	0.31	2.84

UV inhibition may have resulted in the anomalous negative net photosynthesis values at Barton Springs as samples were incubated near the surface and the water is exceptionally clear. A second light/dark bottle test was conducted at Barton Springs Pool on 17 August 2006 by the same methods although two racks were deployed simultaneously: one floated at the surface as done previously and one on the bottom of the pool in approximately 1.7 meters of water. During this deployment, measured DO values were significantly lower for all three treatments (initial, final-light, final-dark) than the July test (Table 11), and again there was a negative net photosynthesis (Table 12) confirming the July test results. As there was no statistically significant difference for the final-light bottle DO values between the surface and bottom depths (Wilcoxon rank-sum pr>|z| = 0.1939), it is presumed that UV inhibition did not adversely affect the July test.

Table 11. Mean DO (mg/L) from light/dark bottle test with two depths at Barton Springs Pool site in August 2006.

Treatment	Mean	StdDev	Min	Median	Max
Surface					
Initial	5.30	0.09	5.20	5.30	5.40
Final-Light	5.25	0.06	5.17	5.26	5.31
Final-Dark	5.21	0.03	5.18	5.22	5.24
Pool Bottom (1.7m)					
Initial	5.19	0.03	5.16	5.2	5.22
Final-Light	5.16	0.08	5.1	5.13	5.27
Final-Dark	5.11	0.09	5	5.11	5.2

Table 12. Estimated photosynthesis and respiration (mg/L DO) from light/dark bottle test in Barton Springs at surface and depth in August 2006.

Depth	Net Photosynthesis	Respiration	Gross Photosynthesis
Surface	-0.04	0.09	0.05
Bottom	-0.05	0.09	0.03

Despite lower measured initial DO values during the August light/dark bottle test dates, estimated gross photosynthesis was greater than during the July date. Spring discharge values were approximately 30% lower in August (20 ft^3/s). No additional chlorophyll-a sample was collected due to time constraints. Gross and net phytoplankton production rates were calculated for each study site (Table 13).

Table 13. Gross and net production (mg C m^{-3} h^{-1}) for Barton Springs (August 2006) and Bear Creek (July 2006) light-dark bottle tests.

Site	Net Production	Gross Production
Barton Springs Surface	*	5.32
Barton Springs Bottom	*	3.73
Davis Pond	21.09	32.22
Bear Creek Pass	27.89	33.28

*negative net production.

Periphytometer

On retrieval, periphytometer bottles at Barton Springs were almost completely covered by attached blue-green and filamentous algae (Figure 6), and bottles from both Bear Creek sites were essentially clean of periphyton. Mean chlorophyll-a for the control samples at Barton Springs are two times greater than the most enriched treatment in Bear Creek (Table 14).

Figure 6. Example of difference between Barton Springs (left) and Bear Creek (Bear Creek Pass, right) in periphytometer bottles on retrieval.

Table 14. Mean chlorophyll-a in μg/cm^2 from periphytometer filters after 14 day deployment. One standard deviation of the mean in parenthesis.

Treatment	Barton Springs	Bear Creek Pass	Davis
C	5.98 (2.83)	0.23 (0.03)	0.18 (0.06)
N	5.63 (2.41)	0.19 (0.03)	0.43 (0.13)
NP	6.06 (1.99)	2.36 (0.83)	2.16 (0.42)
P	6.18 (2.53)	0.41 (0.13)	0.44 (0.13)

Comparison of the mean chlorophyll-a by blocks yields no significant difference at either of the two Bear Creek sites, indicating no spatial bias to the randomized placement of the blocks on the periphytometer (Table 15). Although the Barton Springs Pool periphytomer did yield a difference between blocks, the location of the blocks with the higher mean chlorophyll-a did not correspond with an increase in the potential availability of light, to proximity of the algae-covered pool walls (colonization effect) or to any other apparent variable that could explain this effect.

Table 15. Kruskal-Wallis test of chlorophyll-a by block.

Site	DF	χ^2	Pr > χ^2
Barton Springs Pool	5	14.91	0.0108
Bear Creek Pass	5	0.55	0.9902
Davis Pond	5	1.27	0.9376

Mean chlorophyll-a from the filters at Barton Springs Pool showed no significant difference among all treatments (Table 16), and Wilcoxon rank-sum tests indicates no statistically significant difference between any two individual treatments at Barton Springs Pool. ANOVA with a blocking factor to account for variability due to the spatial placement of the blocks also yields no statistically significant difference between treatments at Barton Springs (df=3, F=0.10, Pr>F=0.9569).

Because of the significant block effect and lack of power to detect differences between treatments, the remaining 4 frozen filters for each treatment from the Barton Springs periphytometer were submitted to the laboratory for analysis. After analyzing the 4 remaining filters for each treatment for Barton Springs, the statistically significant block effect remained (Kruskal-Wallis χ^2=26.49, df=9, Pr>χ^2=0.0017). Again, there was no clear spatial pattern in block mean chlorophyll-a from the periphytometer. With the inclusion of the additional filters, there was still no statistically significant difference between treatments in Barton Springs (Kruskal-Wallis χ^2=1.39, df=3, Pr>χ^2=0.7070), with or without the inclusion of a blocking factor.

Table 16. Kruskal-Wallis test of chlorophyll-a by treatment.

Site	DF	χ^2	Pr > χ^2
Barton Springs Pool	3	0.287	0.9625
Bear Creek Pass	3	19.82	0.0002
Davis Pond	3	19.10	0.0003

Both Bear Creek sites did yield a significant treatment effect (Figure 7). Multiple comparison analysis indicates that the NP treatment was significantly higher than all other treatments at both Bear Creek sites indicating a co-limitation of nitrogen and phosphorous (Table 17). Barton Springs periphyton production rates were much higher than Bear Creek production rates (Figure 7).

Table 17. Multiple comparison analysis of periphytometer filters Wilcoxon two-sample test (prob > |z|).

Comparison	Barton Springs	Bear Creek Pass	Davis Pond
N vs. C	1.0000	0.1282	0.0049
P vs. C	0.8102	0.0082	0.0081
N vs. P	0.6889	0.0051	0.9361
N vs. NP	0.6889	0.0051	0.0050
P vs. NP	1.0000	0.0051	0.0050
NP vs. all others	0.8155	0.0004	0.0004

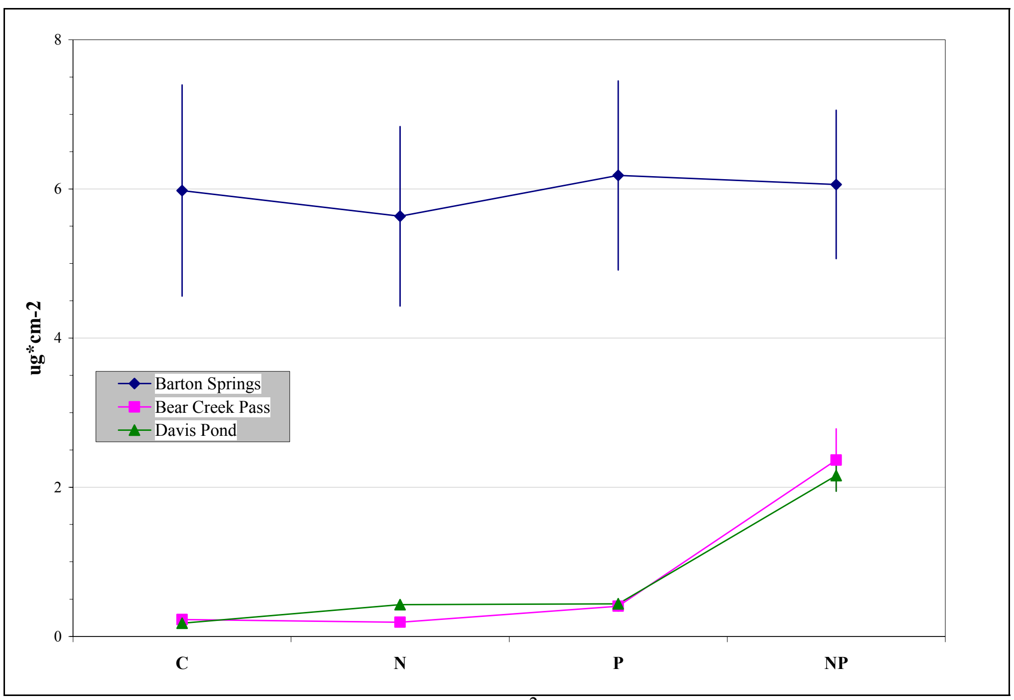

Figure 7. Mean chlorophyll-a production (µg chl-a/cm^2) from periphytometers by treatment and site (vertical bars show one standard deviation of the mean).

Examination of LETSI values from periphytometer data indicate the substantial difference in current trophic status between Bear Creek and Barton Springs periphyton (Figure 8). Although ambient phosphorus varies between sites, the difference is small in magnitude and site means are not significantly different (Pr>χ^2=0.7370). Despite relatively low ambient total phosphorus in Barton Springs Pool, Barton Springs periphytometers yield LETSI values very near 1, indicating that Barton Springs Pool periphyton are operating near maximum potential productivity. LETSI values near 1 were also encountered in a study of the Bosque River, Texas (Kiesling et al 2001) at nutrient-enriched sites with ambient total phosphate concentrations greater than 0.2 mg/L (as P). A Michaelis-Menten function fit to the Bosque River LETSI values indicate predicted LETSI values of 0.50 (or current periphyton production is 50% of maximum potential periphyton production) at phosphate concentrations of 0.04 mg/L (as P).

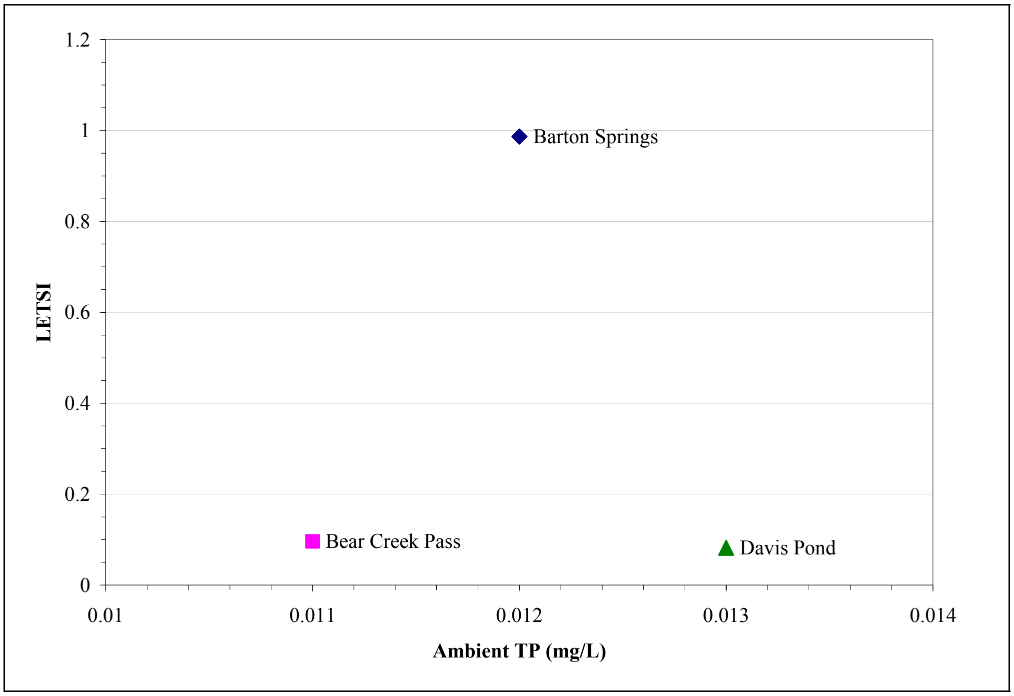

Figure 8. LETSI (ratio of average control production to average NP production) from periphytometer data versus average ambient total phosphorus concentration.

Sestonic Algae Growth Potential Bioassay

A significant treatment effect was obtained for each of the 3 study sites, documenting that sestonic algae were limited by at least one nutrient (Figure 9, table 18). Based on maximum exponential growth rates determined from AGP bioassay results, Bear Creek phytoplankton are currently co-limited by both nitrogen and phosphorus. Barton Springs phytoplankton are currently limited by phosphorus (Table 19).

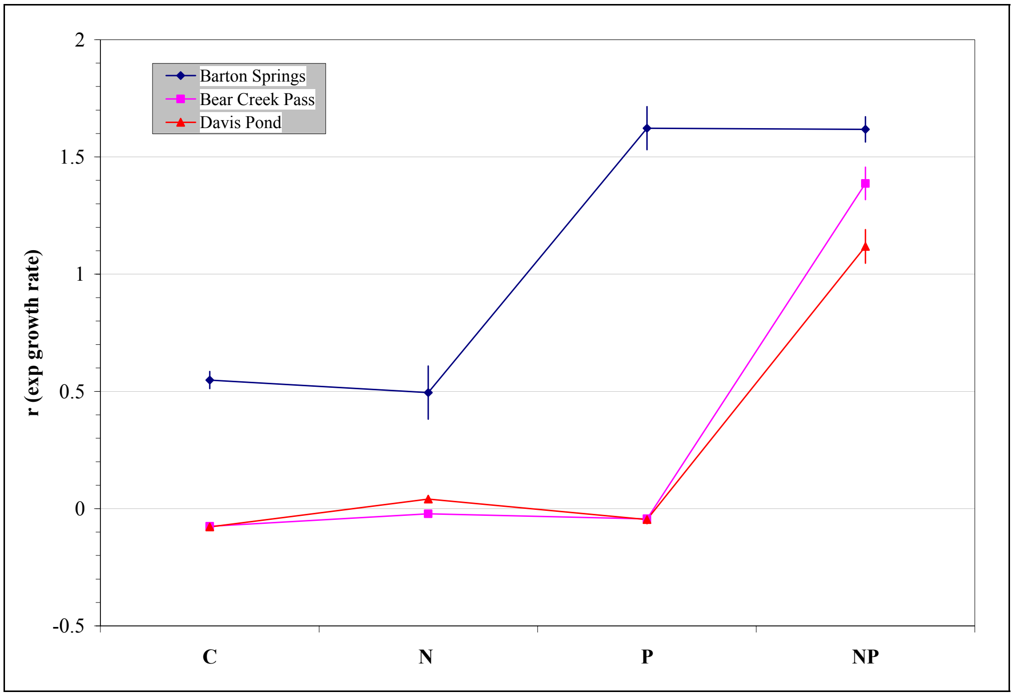

Figure 9. Summary of exponential growth rates from phytoplankton bioassay. Error bars indicate one standard deviation of the mean.

Table 18. Kruskal-Wallis test of AGP maximum growth rates by treatment.

Site	DF	χ^2	Pr > χ^2
Barton Springs Pool	3	17.36	0.0006
Bear Creek Pass	3	17.34	0.0006
Davis Pond	3	19.49	0.0002

Table 19. Multiple comparison analysis of AGP maximum growth rates by Wilcoxon two-sample test (Pr > |z|).

Comparison	Barton Springs	Bear Creek Pass	Davis Pond
N vs. C	0.6889	0.0131	0.0081
P vs. C	0.0051	0.0782	0.1003
N vs. P	0.0051	0.2298	0.0051
N vs. NP	0.0051	0.0051	0.0051
P vs. NP	0.8102	0.0051	0.0051
NP vs. C	0.3643	0.0051	0.0005
NP vs. all other treatments combined	0.0124	0.0004	0.0004

Comparison of Barton Springs Pool and Bear Creek growth rates from sestonic algae indicate that while Bear Creek maintains higher ambient chlorophyll-a concentrations than Barton Springs, Barton Springs yielded a higher maximum nutrient sufficient growth rate. The lower ambient chlorophyll-a concentrations in Barton Springs Pool are most likely a function of the high turnover rate of the pool (Plummer and Associates 2000), while the higher maximum growth rates at Barton Springs are most likely a function of the higher ambient nutrient concentrations.

Using a modified LETSI to compare the ratio of control growth rates to maximum growth rates (from AGP data) indicates that Barton Springs phytoplankton are also currently growing at a higher proportion of the maximum primary productivity than the Bear Creek sites (Table 20).

Table 20. Modified LETSI (ratio of control growth rate to nutrient sufficient growth rate) for AGP data.

Site	LETSI (Modified)
Barton Springs	0.339
Bear Creek Pass	0.054
Davis Pond	0.070

As the Bear Creek sites indicated co-limitation for phytoplankton, a combined nitrogen and phosphorus (NP) positive control treatment was added to the original study design of the AGP dose-response test for the Bear Creek sites. No positive growth response was obtained over time from the AGP in response to the phosphorus-only nutrient additions for the Davis Pond site, although the Davis Pond phytoplankton did respond strongly to the positive NP control (Figure 10).

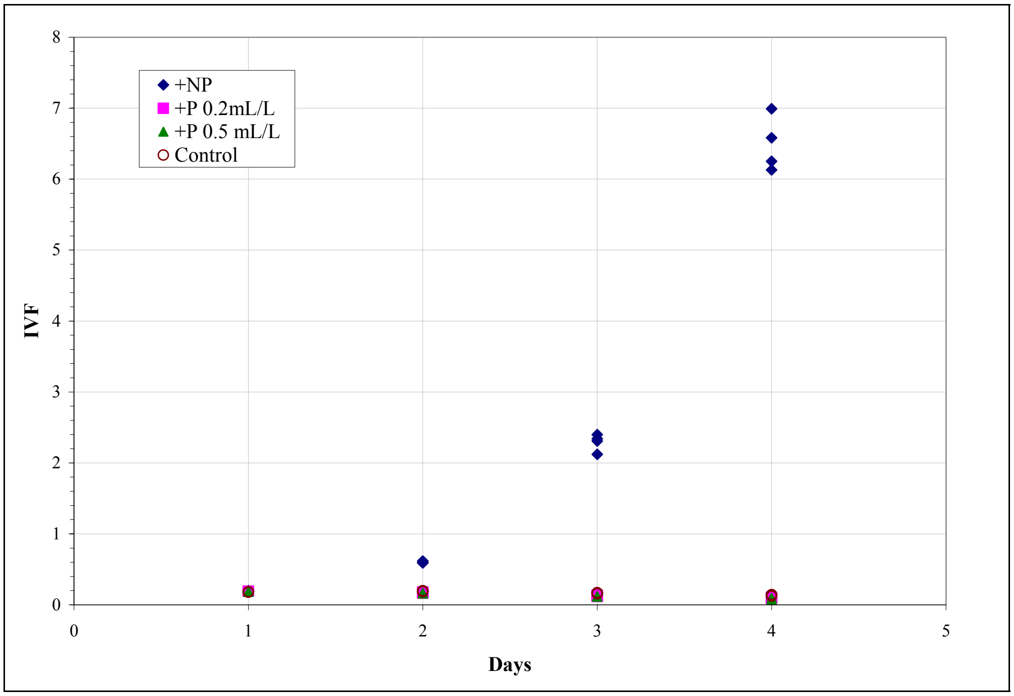

Figure 10. In-vivo fluorescence (IVF) for Davis Pond AGP test with phosphorus-only and nitrogen+phosphorus addition over time.

A slight positive response was obtained through day three for the Bear Creek Pass site dose-response AGP to the phosphorus-only additions, although the strong positive response to the nitrogen and phosphorus positive control underscores the co-limitation by nitrogen and phosphorus of Bear Creek Pass phytoplankton (Figure 11). IVF most likely decreased in the phosphorus-only additions for Bear Creek Pass after day three due to exhaustion of available nitrogen. Ambient total nitrogen was slightly greater (+0.03 mg/L) at the Bear Creek Pass site than the Davis Pond site at the time of sample collection.

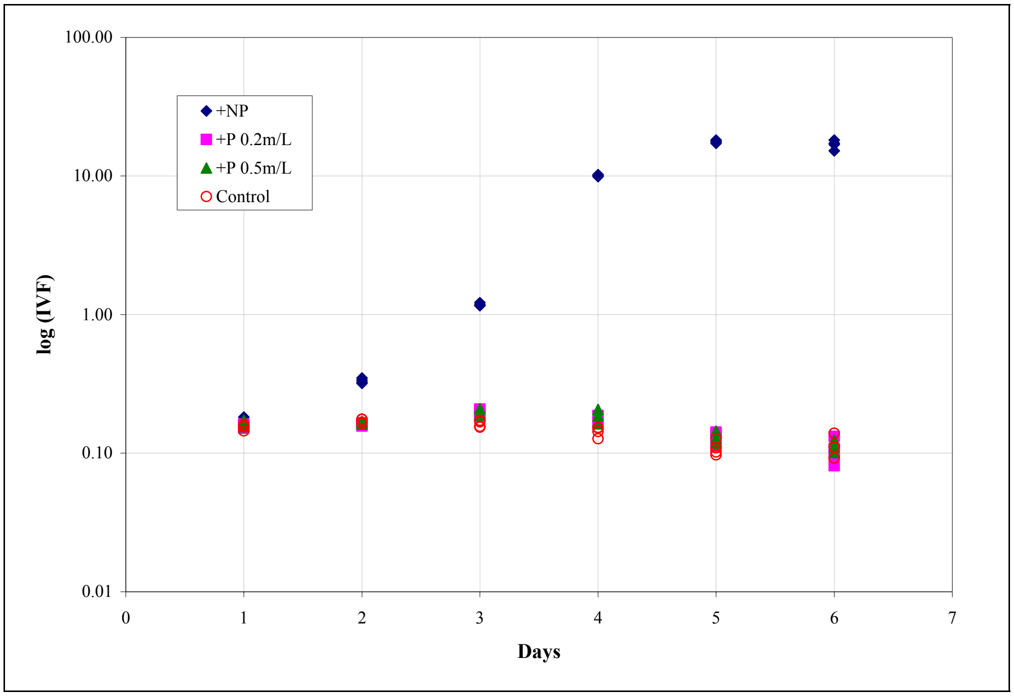

Figure 11. In-vivo fluorescence (log-scale) for Bear Creek Pass AGP test with phosphorus-only and nitrogen+phosphorus addition over time.

Barton Springs dose-response AGP to phosphorus-only additions yielded a strong positive response relative to the no nutrient addition control (Figure 12). Exponential curves fit to Barton Springs dose-response IVF data over time yielded predicted growth rates with high r^2 values (>95%) for all treatments. The lowest phosphorus dose concentration from the dose-response AGP at Barton Springs was 0.01 mg/L, higher than some measured ambient total phosphorus concentration at Barton Springs. To better define the hyperbolic portion of the Monod function and more effectively bracket the lower end of ambient phosphorus concentrations expected in Barton Springs, two additional low phosphorus doses (0.0093 mg/L, 0.019 mg/L) were tested in an additional AGP dose-response test at Barton Springs using ambient water collected on August 8, 2006. Dose-response data from both sample events were combined to estimate the Monod function.

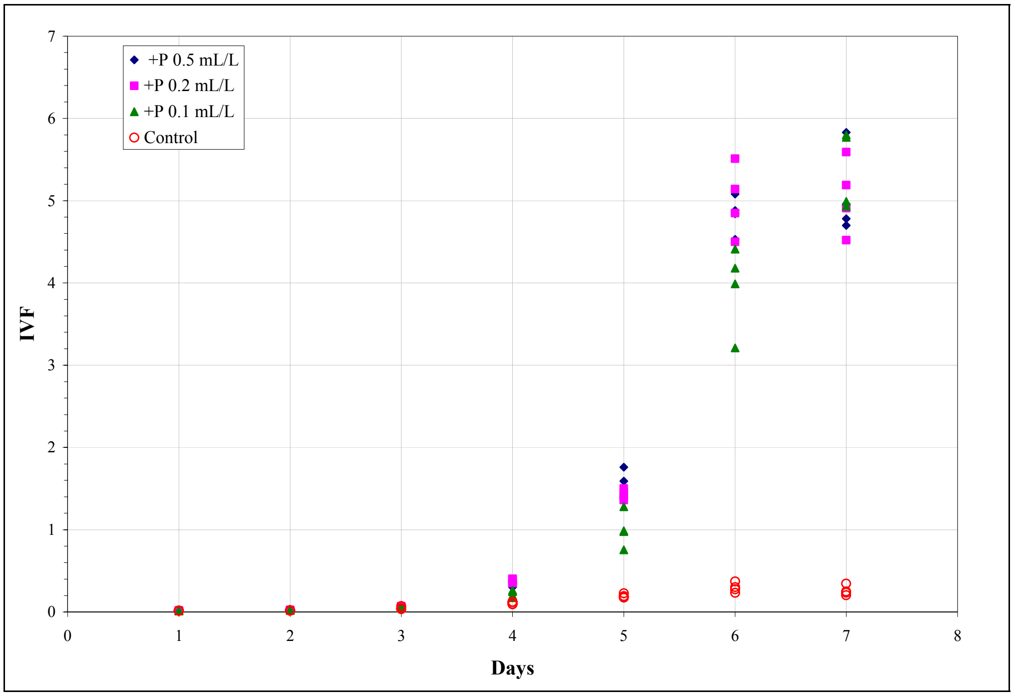

Figure 12. In-vivo fluorescence for Barton Springs AGP test with phosphorus-only addition over time.

The combined AGP dose-response data from Barton Springs followed the expected Monod function pattern (r^2=0.37) in response to the limiting nutrient (phosphorus) additions (Figure 13, Table 21). The low K_s values, relative to ambient total phosphorus concentrations in Barton Springs, highlight the sensitivity of Barton Springs phytoplankton to increases in phosphorus loading. This is also may be applicable to Bear creek conditions once the algae community responds to stimulus from increased nutrient additions.

Table 21. Monod function parameters from Barton Springs AGP for phosphorus (mg/L).

Coefficient	Estimate	Approx. Std Error	Approx. Pr > |t|
μ_{max}	1.20	0.04	<0.0001
K_s	0.004	0.001	0.0016

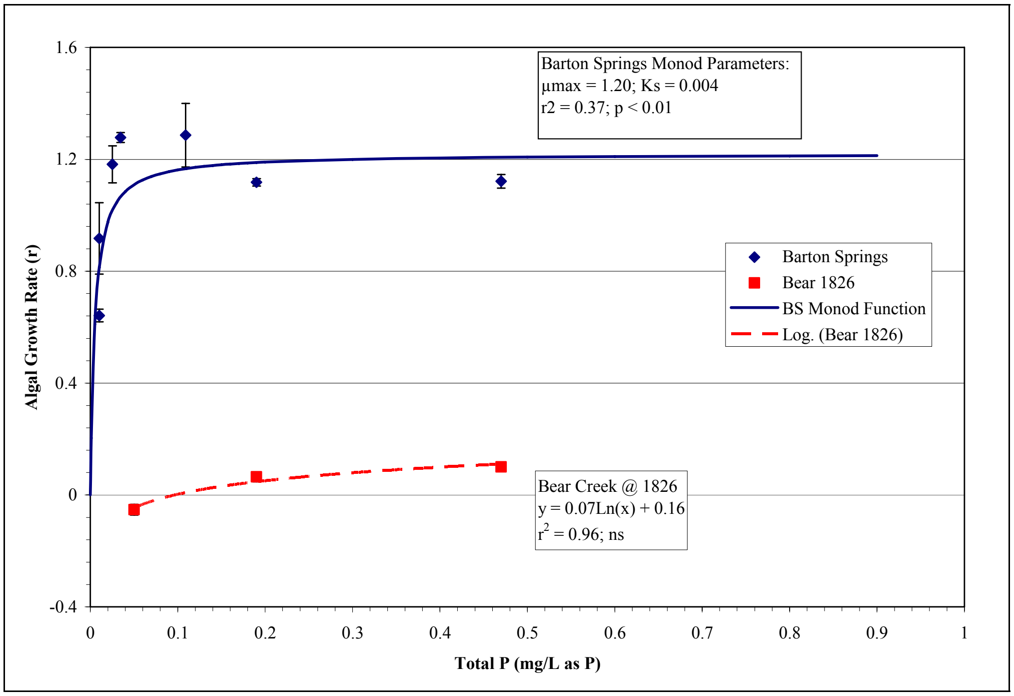

Figure 13. AGP results from dose-response test with phosphorus. No response for Davis Pond phytoplankton to phosphorus-only addition.

Conclusions

The following conclusions are made from the experiments conducted to date in Bear Creek:

- Bear Creek periphytic and sestonic algae are currently in an oligotrophic state, operating at less than 10% of nutrient saturated growth, and are co-limited by both ambient nitrogen and phosphorus concentrations.
- Any combined increase of nitrogen and phosphorus concentrations in Bear Creek will increase periphyton and phytoplankton productivity.
- The proposed discharge, if approved, could significantly alter the trophic status and algal community structure of Bear Creek.

Additional conclusions made from the experiments conducted in Barton Springs pool include:

- Barton Springs Pool periphyton are currently operating at nutrient saturated growth rates.
- Additional nutrient inputs may not increase periphyton growth rates in Barton Springs Pool.
- Periphytic algae dominate the algal community of Barton Springs Pool.
- Barton Springs Pool phytoplankton are currently in an oligotrophic state, and are limited by ambient phosphorus concentrations.
- Any increase in ambient phosphorus concentrations will increase Barton Springs Pool phytoplankton growth rates.
- Although high turnover rates under nominal flow conditions may not result in an increase in sestonic algae biomass in Barton Springs Pool, there would be an increase in the efflux of phytoplankton biomass to Barton Creek below Barton Springs Pool and thus Town Lake.

- Under low flow conditions, or if flows are sufficiently decreased through aquifer pumping, Barton Springs could shift from a periphyton-dominated system to a system dominated by phytoplankton.

Conclusions concerning nutrient limitations for wastewater discharges such as proposed by the HCWCID No. 1 permit are as follows:

- The algae community at Bear Creek sites is anticipated to change as background nutrient concentrations increase. Sestonic algae response curves are anticipated to resemble those determined for Barton Springs when phosphorous is limiting.
- At concentration exceeding the half saturation constant, K_s = 0.004 mg/L total phosphorous, and a surplus of nitrogen, growth of sestonic algae is anticipated to be unacceptably high in Bear Creek. The lowest detection limits available from contract laboratories are also in this concentration range (<0.007 mg/L).
- Any discharge of treated wastewater with phosphorous concentrations above the 0.004 mg/L will result in a corresponding increase in algae growth in the downstream reaches of Bear Creek and potentially increase nuisance conditions at Barton Springs.

The above results are the first using these methods in Austin area streams. Although the results are directly applicable to setting nutrient limits for discharges, more insight into algae community behavior in oligotrophic hill country streams may be obtainable with additional testing. Additional studies are planned to repeat these experiments under different seasonal conditions. In addition, repeating the Bear Creek studies at a saturated level of nitrogen and ultra low range of phosphorous dosage may further define a dose response curve for this nutrient poor stream. Further testing in Barton Springs pool is planned to assist efforts to control nuisance algae conditions inhibiting recreational uses.

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