JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION
Vol. 44, No. 5 AMERICAN WATER RESOURCES ASSOCIATION October 2008

NUTRIENT BIOASSAYS OF GROWTH PARAMETERS FOR ALGAE IN THE NORTH BOSQUE RIVER OF CENTRAL TEXAS[1]

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ABSTRACT: Nutrient dose-response bioassays were conducted using water from three sites along the North Bosque River. These bioassays provided support data for refinement of the Soil and Water Assessment Tool (SWAT) model used in the development of two phosphorus TMDLs for the North Bosque River. Test organisms were native phytoplanktonic algae and stock cultured *Pseudokirchneriella subcapitata* (Korshikov) Hindak. Growth was measured daily by *in vivo* fluorescence. Algal growth parameters for maximum growth (μ_{max}) and half-saturation constants for nitrogen (K_N) or phosphorus (K_P) were determined by fitting maximum growth rates associated with each dose level to a Monod growth rate function. Growth parameters of native algae were compared between locations and to growth parameters of *P. subcapitata* and literature values. No significant differences in half-saturation constants were indicated within nutrient treatment for site or algal type. Geometric mean K_N was 32 μg/l and for K_P 7 μg/l. A significant difference was detected in maximum growth rates between algae types but not between sites or nutrient treatments. Mean μ_{max} was 1.5/day for native algae and 1.2/day for stock algae. These results indicate that watershed-specific maximum growth rates may need to be considered when modeling algal growth dynamics with regard to nutrients.

(KEY TERMS: algae; bioassay; nutrients; rivers/streams; eutrophication; *Pseudokirchneriella subcapitata*; simulation.)

Millican, Jimmy S., Jeffrey A. Back, and Anne M.S. McFarland, 2008. Nutrient Bioassays of Growth Parameters for Algae in the North Bosque River of Central Texas. *Journal of the American Water Resources Association* (JAWRA) 44(5):1219-1230. DOI: 10.1111/j.1752-1688.2008.00219.x

INTRODUCTION

In 2001, two total maximum daily loads (TMDLs) for soluble reactive phosphorus were adopted for classified segments along the North Bosque River in response to water quality concerns regarding excessive nutrients and algae (TNRCC, 2001). These TMDLs were approved by the U. S. Environmental Protection Agency (USEPA), but public concerns have lead to a reevaluation. The Soil and Water Assessment Tool (SWAT) was used to support the development of these TMDLs (Santhi *et al.*, 2001, 2002), and as part of the TMDL reevaluation, new information regarding site-specific nutrient growth parameters for algae will be incorporated into SWAT for simulating the North Bosque River (Hauck *et al.*, 2003).

[1]Paper No. JAWRA-07-0066-P of the *Journal of the American Water Resources Association* (JAWRA). Received May 26, 2007; accepted February 6, 2008.

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Parameter fitting is important in deterministic models, such as SWAT, because poorly fitted rate constants can lead to faulty results even when the general relationship is correctly defined (Bowie *et al.*, 1985). Averages are often used in modeling, but site-specific values are preferred for better parameter estimates. Within SWAT, the biomass concentration of phytoplanktonic algae is related to growth rate parameters μ_{max} – the maximum specific algal growth rate (per day), K_N – the Michaelis-Menton half-saturation constant for nitrogen (N), and K_P – the Michaelis-Menton half-saturation constant for phosphorus (P) as described by a Michaelis-Mention function (Neitsch *et al.*, 2002). The Michaelis-Menton function is often referred to as a Monod function when applied to biological growth (see Tilman, 1982) and has the following simple form:

$$\mu = \frac{\mu_{max*} S}{K_S + S}, \quad (1)$$

where μ equals the growth rate as a function of the maximum growth rate (μ_{max}), the external nutrient concentration (S), and the half-saturation constant of the nutrient of interest (K_S), in this case either N or P. Other models, such as the Droop model that relies on intracellular concentrations of nutrients, may be better predictors of algal growth, but the Monod model is often preferred because it directly relates algal growth to an environmental parameter (Sommer, 1991).

The Monod model is widely accepted for describing algal growth dynamics, but a wide range of parameter values for μ_{max} and S have been produced that vary greatly by algal species (e.g., Bowie *et al.*, 1985; Sommer, 1991; Sterner and Grover, 1998). Dose-response bioassays are used to estimate μ_{max}, K_N, and K_P by evaluating the growth rate of algae under varying concentrations (both limiting and nonlimiting) of the nutrient of interest while maintaining all other nutrients as nonlimiting (Miller *et al.*, 1978). *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) is often used for algal growth experiments for comparative purposes between water bodies. Within a given stream system, the aggregate growth response of the native algal community may be more important than that of individual species. The use of native algae in dose-response bioassays provides a more realistic growth response that also allows for shifts in species abundance and dominance with manipulation of the nutrient environment (López and Dávalos-Lind, 1998).

While a number of studies on the North Bosque River have examined the *in situ* growth dynamics for periphyton in response to increasing nutrient concentrations (Matlock *et al.*, 1998, 1999; McFarland *et al.*, 2004), only limited efforts have been made to look at phytoplankton growth. Growth responses of phytoplankton have been evaluated indirectly through relating in-stream chlorophyll-α to nutrient concentrations, but this evaluation considers an aggregation of data over time and across stream locations, creating considerable scatter (McFarland *et al.*, 2000; Kiesling *et al.*, 2001). This scatter likely represents the response of different algal types within chlorophyll-α samples taken at various locations along the river representing a range of nutrient conditions.

Although P was identified as the nutrient most limiting algal growth for the TMDLs, N or N and P limitation occurs in phytoplankton (Kiesling *et al.*, 2001) and periphyton (McFarland *et al.*, 2004) at locations along the North Bosque River. In freshwaters, P generally limits algal growth, but N-dependence, although less studied, is an important secondary limiting nutrient (Elser *et al.*, 1990). Because these TMDLs focus specifically on the North Bosque River, it was important to determine growth parameters for the community of native algae within the river rather than single species. The objective of this study was to determine growth response parameters for native algae to N and P at three locations along the North Bosque River. These parameters were compared to determine if they differed by location on the river and to parameters measured for a stock culture of *P. subcapitata*. To mimic river conditions more closely, ambient river water was used in this study.

SITE DESCRIPTIONS

Three sites were selected representing a variety of conditions along the North Bosque River (Figure 1). All three sites are also designated monitoring sites by the Texas Commission on Environmental Quality (TCEQ). The upper most site, BO070 (TCEQ site 11961), was located on the North Bosque River in the city park of Hico, Texas. The drainage area above BO070 covers about 93,100 ha. The second site, BO080 (TCEQ site 11960), represented mid-river conditions and was located on the North Bosque River at the crossing of Farm to Market (FM) 216 in Iredell, Texas. Site BO080 is about 25 river kilometers downstream of site BO070 and has a drainage area of about 146,000 ha. The most downstream site was BO090 (TCEQ site 11956) located on the North Bosque River in Clifton, Texas at the crossing of FM 219. Site BO090 is about 56 km downstream of site BO080 and has a drainage area of about 253,000 ha. Phosphorus concentrations generally decrease from

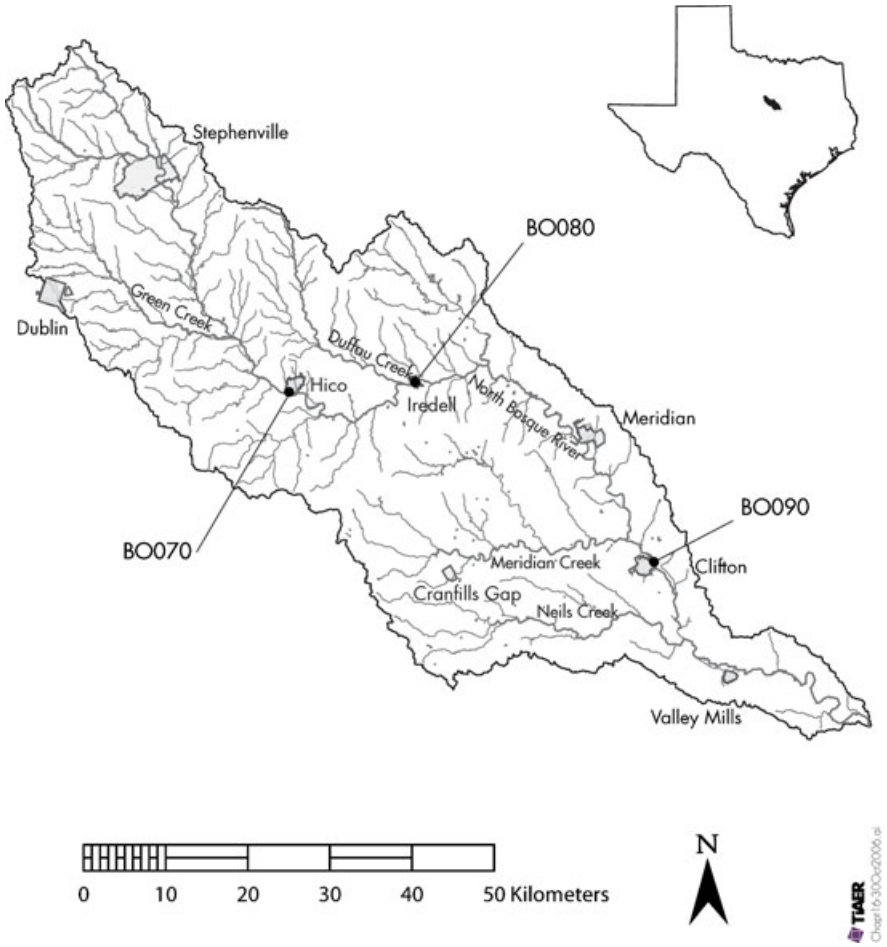

FIGURE 1. Location of Algal Bioassay Sampling Sites Along the North Bosque River.

upstream to downstream with the highest P concentrations occurring at BO070 of the three sites selected (Adams *et al.*, 2005).

METHODS

Seasonal nutrient dose-response bioassays were conducted using water from the three stream sites on eight occasions between February 2004 and July 2005 largely following SM8111B for the growth of algae using chlorophyll-α fluorescence (APHA, 1999). The method was adapted to obtain growth response data for native algae as outlined in López and Dávalos-Lind (1998) with a stock culture of the alga *P. subcapitata* included with each bioassay experiment. The algal assay method was also modified to use test tubes rather than flasks for culturing algae as outlined in Dávalos *et al.* (1989). A further modification involved the use of dilution water at sites with high background nutrient concentrations. High background nutrient concentrations, if not diluted, had the potential of providing nutrient saturated conditions, which would negate the ability to determine expected low K_N and K_P concentrations. Using dilution water when high ambient nutrient concentrations were present was also analogous to determining the mitigating effects of lowering the nutrient concentration (N or P) on algal growth rates of native algae at these high nutrient sites. Dilution water was comprised of ambient water from sites with lower nutrient concentrations following United States Environmental Protection Agency (USEPA) procedures (USEPA, 2002). Ambient water was used as the dilution water to reduce changes in parameters, such as pH and specific conductance, which could affect algal growth rates. Historical instream measurements indicated fairly similar pH values for all three stream sites (median values 8.1-8.4), although a decrease in specific conductance was noted from upstream (median 602 μmhos/cm) to downstream sites (median 434 μmhos/cm) for grab samples collected between 2000 and 2004 (Adams *et al.*, 2005). Sonde measurements of dissolved oxygen, pH, specific

conductance and water temperature were taken in-stream near the time when ambient samples were collected to evaluate differences in these parameters prior to using site's stream water as dilution water.

The overall algal bioassay procedure had four general steps: (1) collection of ambient water and native algae, (2) filtration of ambient water and concentration of native algae, (3) preparation of dose-response treatment dilutions and concentrations including nutrient analysis of ambient water, and (4) incubation and measurement of algal growth.

Collection of Ambient Water and Native Algae

Samples were collected at each site on eight dates: February 2, May 11, June 21, August 4, and November 9, 2004 and February 8, April 26, and July 12, 2005. Enough river water was collected to fill a minimum of 12 one-liter plastic bottles. The river water was field-filtered through a 63-μm sieve to remove zooplankton. An additional sample of river water was collected and returned to the lab for analysis of nitrite plus nitrate N (NO_2-N + NO_3-N) and soluble reactive P measured as orthophosphate-P (PO_4-P) to determine background concentrations of soluble N and P at each site. NO_2-N + NO_3-N was analyzed using USEPA method 353.2 via colorimetric cadmium reduction using an autoanalyzer (USEPA, 1983). PO_4-P was analyzed colorimetrically using a spectrophotometer following EPA method 365.2 (USEPA, 1983). Although ammonia (NH_3-N) is also a form of soluble N available for algal growth, previous water quality analyses at these sites indicated that NO_2-N + NO_3-N on average comprises over 80 percent of the soluble N present and that NH_3-N concentrations generally occur below the laboratory method detection limit (see Adams *et al.*, 2005). It is noted that preferential uptake of ammonium over nitrite and nitrate by algae occurs (e.g., Dortch, 1990) and emphasis on NH_3-N would be important if it were the dominant N species as found for example in the Port Adelaide River estuary (Ault *et al.*, 2000). Because NO_2-N + NO_3-N was the dominant form of soluble N at these sampling sites, the analysis of NH_3-N was not considered in the treatment calculations to allow a more timely turn around from the laboratory of the soluble nutrient analysis.

Filtration of Ambient Water and Concentration of Native Algae

Eight liters of river water from each site was vacuum filtered through a 47-mm diameter nylon membrane filter (0.45 μm pore size). The vacuum pressure was not directly monitored, but care was taken to filter the algae under gentle pressure to minimize cell damage. About 10-15 hours were needed to filter the 24 l of water and nearly the same pressure was used for each filtration. Algae were harvested from the filter by gently scraping algae with a sterile stainless steel spatula into a beaker. To ensure that a sufficient amount of native algae had been harvested, 2 ml of the inoculum was pipetted into a test tube containing 28 ml of reverse osmosis water. If a minimum fluorescence of 0.200 fluorescence units (fu) was not attained, additional river water was filtered and native algae collected until the desired fluorescence response was achieved. This procedure for native algae was implemented after the first bioassay experiment in February 2004, when it was found that low algae concentrations were most likely responsible for very low fluorescence readings and erratic growth rate patterns observed for site BO090 treatments. Fluorescence readings for N and P treatments of native algae for the February 2004 trial for BO090 were well below 0.2 fu for several days and showed a growth pattern that undulated from day to day rather than following the expected exponential growth pattern.

Preparation of Dose-Response Treatment Dilutions and Concentrations

Ambient nutrient concentrations for each site for PO_4-P and NO_2-N+NO_3-N were used to determine dilutions and concentrations needed to reach target dose concentrations for N and P treatments. A series of six doses for the N treatment were prepared that typically ranged from 50 to 2000-μg/l N (Table 1). Phosphorus treatments consisted of seven separate doses that typically ranged from 10 to 250-μg/l P. The highest target concentrations for N and P were considered in excess to meet maximum algal growth assuming no other limiting factors. Specific target concentrations were adjusted between experiments to help obtain the best growth fit response given the space limitations within the incubator. Ambient water from each site spiked with a concentration of P, N, or both P and N equal to the highest target concentration was used to either concentrate or dilute nutrients to achieve target dose concentrations. Sodium nitrate for N and sodium phosphate dibasic heptahydrate for P were used to produce the nutrient spiked ambient water.

All treatments were implemented in 30-ml Pyrex™ test tubes. Each treatment test tube contained 28 ml of ambient water, which had the limited nutrient (N or P) at a specific dose and the other nutrient (non-limited) provided at the highest target concentration, and 2 ml

TABLE 1. Target Dose Concentrations by Assay Date for N and P.

	Target Dose Concentrations by Assay Date (μg/l)							
Nutrient	February 2004	May 2004	June 2004	August 2004	November 2004	February 2005	April 2005	July 2005
Nitrogen	0	0	0	0	0	0	0	0
	50	50	-	-	-	50	50	50
	100	100	100	100	100	100	100	100
	200	200	200	200	200	200	200	200
	300	300	300	300	300	-	-	-
	500	500	500	500	500	500	500	500
	1000	1000	1000	1000	1000	1000	1000	1000
	-	-	2000	2000	-	2000	2000	2000
	-	-	-	-	2500	-	-	-
Phosphorus	0	0	0	0	0	0	0	0
	10	10	10	10	10	10	10	10
	15	15	15	15	15	15	15	15
	30	30	30	30	30	30	30	30
	50	50	50	50	50	50	50	50
	-	-	75	75	75	75	75	75
	100	100	100	100	100	100	100	100
	250	250	250	250	-	250	250	250
	500	500	-	-	500	-	-	-

of non-limited nutrient spiked algal inoculum. If the ambient concentration of a nutrient was lower than the target dose concentration, the N and P spiked ambient water was added in the appropriate amount to reach the target dose concentration. If the ambient concentration was higher than the target dose concentration, then dilutions were accomplished by adding ambient water spiked with the non-limited nutrient from the site with the lowest concentration of the nutrient being varied or by the addition of reverse osmosis water spiked with the non-limited nutrient. Reverse osmosis water spiked with the non-limited nutrient was used for dilution of ambient water only when no other site had an ambient nutrient concentration low enough to effectively dilute the treatment to desired dose concentrations. Ambient water was primarily used as dilution water to minimize changes in background parameters, such as pH or specific conductance, which may alter algal growth based on instream measurements when ambient water was collected. Treatments were not evaluated after dilution for changes in these parameters. Regardless of whether a treatment required dilution or concentration of nutrients, the non-limited nutrient was always provided at the highest target concentration, which was considered to be in excess for maximum algal growth assuming no other limiting factors.

Native algae treatments for each site were replicated five times. A separate set of treatments were inoculated with the cultured algae *P. subcapitata* and were prepared in the same manner except that each treatment was not replicated because of limited space within the incubator. Controls containing 28 ml of reverse osmosis water and 2 ml of native algae or *P. subcapitata* inoculum spiked with the non-limited nutrient (N or P) were prepared for each site. Foam plugs, which allowed for air diffusion yet prevented spillage, were used as caps for each test tube.

Incubation and Measurement of Algal Growth

For incubation, the test tubes were placed on clear Plexiglass® slant racks. The incubator was set to the current light/dark cycle as indicated on the U.S. Naval Observatory website (http://aa.usno.navy.mil/data/docs/RS_OneDay.php) for Stephenville, Texas and the mean of the ambient water temperatures recorded on the day of sampling for the three sites (Table 2). Light intensity levels were above light saturation for photosynthesis.

Raw fluorescence readings were taken daily for each treatment using a model 10-AU-005-CE (Turner Designs) fluorometer equipped with a blue mercury

TABLE 2. Incubator Settings for Algae Bioassay Experiments.

Sampling Date	Light (h)	Dark (h)	Temperature (°C)
February 2, 2004	10.95	13.05	7.3
May 11, 2004	13.83	10.17	23.6
June 21, 2004	14.16	9.84	29.3
August 4, 2004	13.77	10.23	30.9
November 9, 2004	10.79	13.21	16.6
February 8, 2005	10.92	13.08	10.8
April 26, 2005	13.59	10.41	19.3
July 12, 2005	14.08	9.92	29.3

vapor lamp, a 436-nm excitation filter, a 680-nm emission filter, and one neutral density square reference filter. Prior to placing test tubes in the fluorometer, algae were evenly distributed within the test tube by using a mini-vortexer. Daily readings were taken until algal growth either stabilized or started to decline as indicated by changes in the fluorescence readings.

DATA ANALYSIS

To determine the maximum specific growth rate for each N or P dose (μd_{max}) of native algae or *P. subcapitata*, the largest specific growth rate (μd) for each replicate within a treatment was determined and averaged across replicates to obtain μd_{max}. Before evaluating the data for μd, the raw fluorescence values of replicates for each dose were graphed on a natural log scale and plotted over time to view the data for potential outliers.

To determine if results from a replicate were an "outlier" with regard to their impact on estimating the maximum growth rate, data were visually assessed by overlaying results from all five replicates for a given treatment. If the trajectory or slope of florescence readings for a given replicate was obviously different than the other replicates, the replicate was flagged as a potential outlier. Data results and lab notes were then evaluated to see if there were any unusual observations noted with the replicate, and basic statistics for the maximum slope were calculated with and without the outlier observation between replicates. If there was not a clear reason based on lab notes to remove a replicate, best professional judgment was used to determine if the flagged replicate inordinately influenced the calculated slope for removal as an outlier. About 3% of project replicates were removed as outliers prior to calculating the largest specific growth rate for each treatment.

After removing outliers, if necessary, dates when largest μd occurred for each replicate were determined by visually evaluating each plot for when the steepest slope occurred. On a natural log scale, the steepest slope closely approximates exponential growth of the algae and generally occurred between day 1 and day 5. The two or three points defined in a straight line representing the steepest slope were used to calculate the largest μd for a dose-replicate as follows:

$$\mu d = \frac{\mathrm{Ln}(F_2/F_1)}{t_2 - t_1}, \tag{2}$$

where μd is the specific growth rate (per day) for a given replicate, t_1 is the first day selected, t_2 is the second day selected, F_1 is the fluorescence on t_1, and F_2 is the fluorescence on t_2. These slopes representing the largest μd for each replicate were averaged across replicates to represent the maximum growth (μd_{max}) for each dose.

The μd_{max} associated with each dose for a treatment was plotted (e.g., Figure 2), and the SAS PROC NLIN procedure was used to fit initial parameters for the Monod growth model (SAS, 2000). For the example shown in Figure 2, μd_{max} represents μ, $\mu_{max(unadj)}$ represents μ_{max}, and the N dose concentrations represent S from Equation (1). Of note, μ_{max} is represented as an unadjusted value ($\mu_{max(unadj)}$), because for use in SWAT, μ is adjusted for a standard photoperiod and temperature.

Estimated μ_{max} values were adjusted for photoperiod and water temperature prior to comparison with literature values using equations obtained from the SWAT theoretical documentation (Neitsch *et al.*, 2002).

Photoperiod adjustments were performed as follows:

$$\mu_{max(day\_adj)} = \mu_{max(unadj)*}(24/T_{DL}), \tag{3}$$

where $\mu_{max(day\_adj)}$ is adjusted for daily photoperiod and T_{DL} is the total photoperiod or day length.

Temperature adjustments were made on $\mu_{max(day\_adj)}$ using the following equation to obtain the adjusted μ_{max} assuming a base temperature of 20°C:

$$\mu_{max} = \left[\frac{\mu_{max(day\_adj)}}{1.047^{(T-20)}}\right], \tag{4}$$

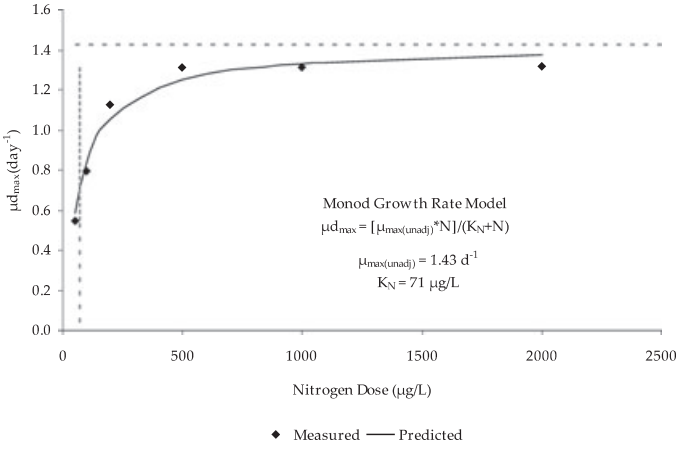

FIGURE 2. Example Plot Showing Use of the Monod Function to Estimate Dose-Response Growth Rate Parameters. The maximum specific growth rate (μd_{max}) was measured for individual dose treatments. The horizontal dashed line represents the estimate of $\mu_{max(unadj)}$ (1.43/day) unadjusted for temperature and photoperiod and the vertical dashed line represents the estimate of half-saturation constant, K_N (71 $\mu g/l$).

where T is incubator temperature used for each bioassay experiment based on the mean ambient water temperature.

Estimated growth rate parameters μ_{max}, K_N, and K_P were compared between sites and algae types (native or stock culture) using analysis of variance procedures. The SAS UNIVARIATE procedure was used to confirm that the dose-response data were normally distributed based on site and nutrient type (SAS, 2000). The Hartley's test was also applied to evaluate for equal variances (Ott, 1984). To be normally distributed the half-saturation constants were transformed to their natural log value. The analysis of variance test was implemented using the SAS GLM procedure. A two-way ANOVA of site and algal type was conducted by nutrient that included evaluation of an interaction factor between site and algal type. Significance was indicated at $\alpha = 0.05$. The interaction factor between site and algal was not significant, so significance by site and algal type were considered separately, assuming the full model was significant. For μ_{max}, a further evaluation was done after the initial two-way ANOVA to evaluate if differences occurred between N and P treatments. If a significant difference ($p < 0.05$) existed for μ_{max} and K values between sites, then the Tukey's honestly significant difference test was implemented to determine which sites were different from one another.

RESULTS AND DISCUSSION

As noted in the methods section, the fluorescence readings for February 2004 for native algae at site BO090 were very low and showed erratic patterns indicating a lack of exponential growth, which did not occur with the *P. subcapitata* treatments. Because exponential growth did not occur, the February 2004 BO090 native algal treatments could not be included in the growth parameter analyses. Corrective action to avoid this problem was taken by implementing procedures establishing minimum native algae fluorescence values for all other experiment dates (see Methods).

Ambient River Water Concentrations

Ambient nutrient concentrations for PO_4-P were consistently highest at site BO070 and ranged from 64 to 340 $\mu g/l$ (Table 3). At BO080, PO_4-P concentrations ranged from 1 to 54 $\mu g/l$, while at BO090, PO_4-P concentrations ranged from 3 to 11 $\mu g/l$. The NO_2-N + NO_3-N concentrations showed a less consistent pattern across sites. The highest and lowest concentrations occurred at BO080 with values ranging from 9 to 2080 $\mu g/l$. Concentrations of NO_2-N + NO_3-N at BO070 ranged from 20 to 683 $\mu g/l$, while concentrations at BO090 ranged from 30 to 502 $\mu g/l$ (Table 3).

Half-Saturation Constant Values

The N half-saturation constants (K_N) were similar between native algae and *P. subcapitata* cultures, ranging from 200 $\mu g/l$ to <10 $\mu g/l$ (Figure 3). By sampling date, the K_N for native algae showed less variation than for *P. subcapitata* algae between sites, although temporal patterns in K_N showed some similarities for the two algal types. Slightly higher K_N values were observed in May 2004 at all three sites and again in April 2005 at BO070 (Figure 3a). For *P. subcapitata*, slightly elevated K_N values were indicated in June 2004 and April 2005 for sites BO070 and BO090 and for BO080 in August 2004 (Figure 3b).

Phosphorus half-saturation constants (K_P) ranged from 20 to 1 $\mu g/l$ and were about an order of magnitude less than K_N values (Figure 4). Generally, higher K_P concentrations occurred in the spring and summer than in the winter. The highest K_P for native algae

TABLE 3. Ambient River Water Concentrations for Soluble N and P Used to Set Treatment Concentrations.

Sampling Date	Site	NO_2-N + NO_3-N ($\mu g/l$)	PO_4-P ($\mu g/l$)
February 2, 2004	BO070	20	340
	BO080	9	3
	BO090	112	3
May 11, 2004	BO070	151	143
	BO080	14	54
	BO090	30	6
June 21, 2004	BO070	389	285
	BO080	12	95
	BO090	58	11
August 4, 2004	BO070	205	199
	BO080	2080	1
	BO090	122	5
November 9, 2004	BO070	145	252
	BO080	228	21
	BO090	343	9
February 8, 2005	BO070	112	74
	BO080	919	8
	BO090	502	4
April 26, 2005	BO070	683	85
	BO080	300	8
	BO090	328	4
July 12, 2005	BO070	85	64
	BO080	14	4
	BO090	299	4

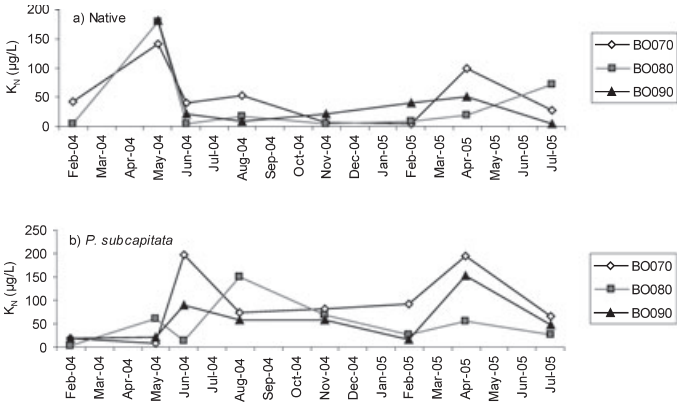

FIGURE 3. Estimated Half-Saturation Constants (K_N) for N Dose-Response Algal Bioassay Experiments by Sampling Date for (a) Native and (b) *P. subcapitata* Algae.

occurred in June 2004 and July 2005 for site BO070, August 2004 and April 2005 for site BO080, and June 2004 and April 2005 for site BO090 (Figure 4a). The highest K_P concentrations for *P. subcapitata* occurred in June 2004 and July 2005 at site BO070, June 2004 and April 2005 for site BO080, and August 2004 and April 2005 at site BO090 (Figure 4b).

Seasonal variation in K values was anticipated, because dominant algal species will vary with temperature. A more intensive study over multiple seasons would be needed to clearly evaluate potential seasonal fluctuations. Because the SWAT model currently uses a constant value for K_N and K_P and does not consider seasonal differences, K_N and K_P concentrations were compared across sampling dates to determine if there were significant differences between sites and between the native and *P. subcapitata* algal species (Table 4).

Geometric mean K_N values from both the native algae and *P. subcapitata* inoculated N treatments were well within the range suggested by Neitsch

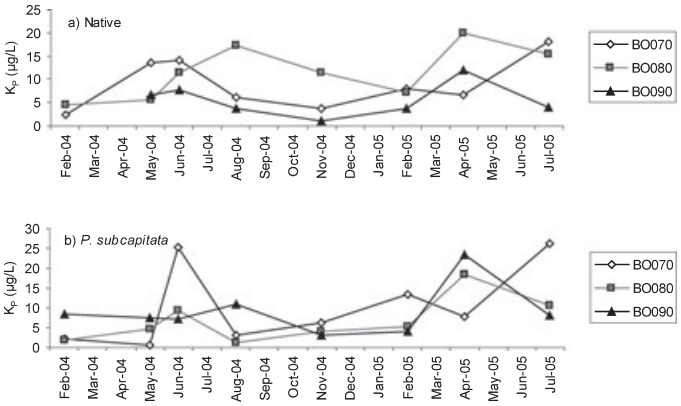

FIGURE 4. Estimated Half-Saturation Constants (K_P) for P Dose-Response Algal Bioassay Experiments by Sampling Date for (a) Native and (b) *P. subcapitata*.

TABLE 4. Geometric Mean, Minimum, and Maximum Half-Saturation Estimates for Native Algae and *P. subcapitata* for Individual Sampling Sites and All Sites Combined.

		Native Algae		*P. subcapitata*	
Site	Statistic	K_N (µg/l)	K_P (µg/l)	K_N (µg/l)	K_P (µg/l)
All sites	Geometric mean	22	7	44	6
	Minimum	3	1	4	1
	Maximum	180	20	197	26
BO070	Geometric mean	31	7	61	6
	Minimum	4	2	7	1
	Maximum	142	18	197	26
BO080	Geometric mean	15	10	33	5
	Minimum	3	5	4	1
	Maximum	178	20	150	19
BO090	Geometric mean	24	5	44	8
	Minimum	4	1	16	3
	Maximum	180	12	153	23

et al. (2002) of 10-300 µg/l as potential parameter values for SWAT (Table 4). The mean of the combined K_N for all three sites for native algae was approximately 50% less than K_N for *P. subcapitata*. By site, geometric mean K_N values from native algae N treatments were about 50% less for BO070 and BO080, and 45% less for BO090 when compared to *P. subcapitata* N treatments. It is possible that because the cultured algae were maintained under optimal nutrient conditions prior to the bioassay that luxury uptake of nutrients occurred. If so, this luxury uptake would have given the cultured algae an advantage over the native algae under nutrient limiting conditions. Minimum K_N values obtained from N treatments for both algae types were below the range given in the SWAT manual for all sites except for site BO090 *P. subcapitata* inoculated treatments.

Geometric mean K_P concentrations for P dose-response treatments were similar between both algae types (Table 4), and all were within the range given in the SWAT guidance of 0.001-0.05 mg/l (Neitsch *et al.*, 2002). The K_P concentrations obtained from all sites for native algae P treatments ranged from 1 to 20 µg/l with a mean of 7 µg/l. Phosphorus treatments inoculated with *P. subcapitata* had K_P values that ranged from 1 to 26 µg/l with a mean of 6 µg/l. Geometric mean K_P values for native algae at sites BO080 and BO090 were about 50% lower than K_P values for *P. subcapitata*. Unlike sites BO080 and BO090, the geometric mean K_P value at BO070 was slightly larger for native algae than *P. subcapitata* treatments.

Although a wide variability occurred in K_N and K_P values, statistically no significant differences ($p > 0.05$) were indicated for K_N or K_P concentrations between sites or algae types when compared across sampling dates. While it is generally accepted that

μ_{max} will increase with increasing temperatures, the relationship of K values with temperature is less clear, but this relationship is included in some physical models (e.g., Aksnes and Egge, 1991). K_N indicated no significant relationships with temperature, while K_P indicated marginal positive correlations for sites BO070 ($r = 0.66$, $p = 0.0767$, $n = 8$) and BO080 ($r = 0.57$, $p = 0.1401$, $n = 8$). Sterner and Grover (1998) found a significant positive relationship of K_N with temperature, but also found that inclusion of a temperature dependence for K_N only marginally improved their ability to predict algal growth rates.

Maximum Growth Rates

Variation in maximum growth rates was anticipated because of variations in dark light cycles in the incubator associated with day length and ambient water temperature. Higher maximum growth rates were expected with longer photoperiods and higher temperatures and were adjusted for using Equations (3 and 4) prior to conducting statistical comparisons. Maximum growth rates prior to adjustment [$\mu_{max(unadj)}$] generally showed the highest values in August 2004 and the lowest values in February 2004 and 2005 because of extremes in temperature and photoperiod (Figure 5). Adjusted μ_{max} values showed relatively little variation between sites or algal types (Figure 6), although between dates, there appeared to be a slight drop in the adjusted μ_{max} values in June 2004.

Maximum growth rates (μ_{max}) for native algae ranged from 0.84 to 2.49/day for both N and P treatments (Table 5). Native algae produced similar mean μ_{max} results of 1.48/day for N treatments and 1.51/day for P treatments. Overall μ_{max} results for *P. subcapitata* inoculated nutrient treatments were generally lower than those obtained for native algae. Across sites and nutrient treatment, μ_{max} results for *P. subcapitata* ranged from 0.62 to 1.81/day. Similar to native algae, overall mean μ_{max} values for *P. subcapitata* showed little variation between nutrient treatments with values of 1.20/day for N and 1.26 day for P treatments. Mean μ_{max} results for both algae and nutrient types decreased slightly from upstream (BO070) to downstream (BO090) sites.

A relatively wide range of μ_{max} values from literature and model documentation are referenced for both total phytoplankton and green algae in the USEPA manual, *Rates, Constants, and Kinetics Surface Water Quality Modeling* (Bowie *et al.*, 1985). The μ_{max} values for native algae obtained from this study were within the range of literature source values given by Jorgensen (1979) that proposed a typical range for total phytoplankton of 0.58-3.0/day at 20°C. The native algae μ_{max} values for this study

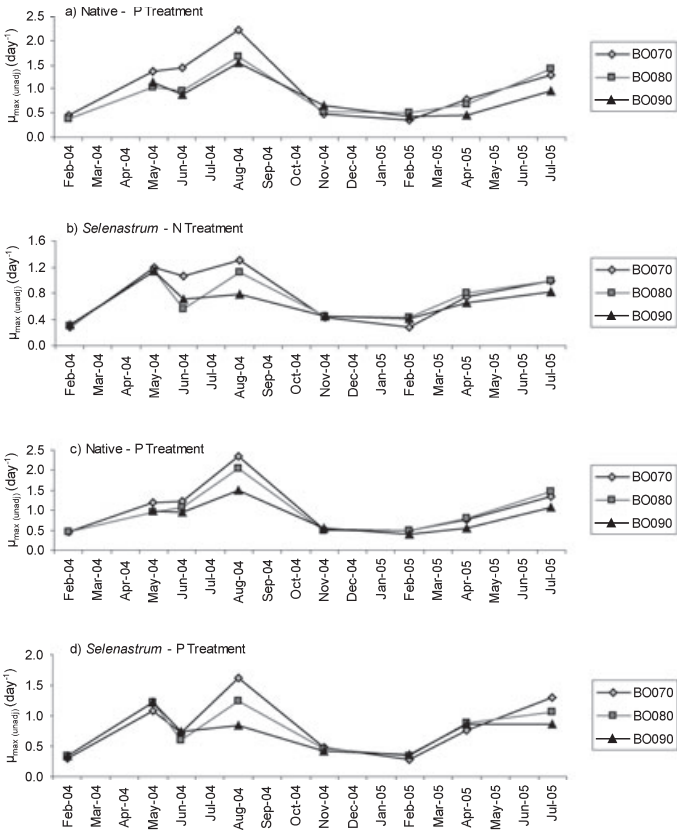

FIGURE 5. Maximum Growth Rates Unadjusted for Water Temperature and Photoperiod [$\mu_{max(unadj)}$] for (a) Native Algae N Treatments, (b) *P. subcapitata* Algae N Treatments, (c) Native Algae P Treatments and (d) *P. subcapitata* Algae P Treatments by Sampling Date.

were also within the range of model documentation values of 0.20-8.0/day at 20°C from Grenney and Kraszewski (1981) and Baca and Arnett (1976) as cited in Bowie *et al.* (1985).

The range of μ_{max} values cited in the USEPA modeling manual (Bowie *et al.*, 1985) for green algae were generally higher than those obtained for the *P. subcapitata* inoculated treatments from this study. Typical μ_{max} values given by Collins and Wlosinski (1983) of 0.7-2.1/day at 20°C would encompass the range of μ_{max} values obtained for *P. subcapitata* from this study with the exception of the minimum values for site BO080 of 0.62 and 0.66 day for N and P treatments, respectively. The mean μ_{max} values obtained for *P. subcapitata* treatments for all sites were within the above-referenced values.

In comparing μ_{max} values, the analysis of variance procedure indicated no significant interaction between site and algae or differences between sites or nutrient treatments within an algal type. Significant differences were indicated for μ_{max} between the native and *P. subcapitata* algae. The native algae had a significantly higher μ_{max} than the *P. subcapita-*

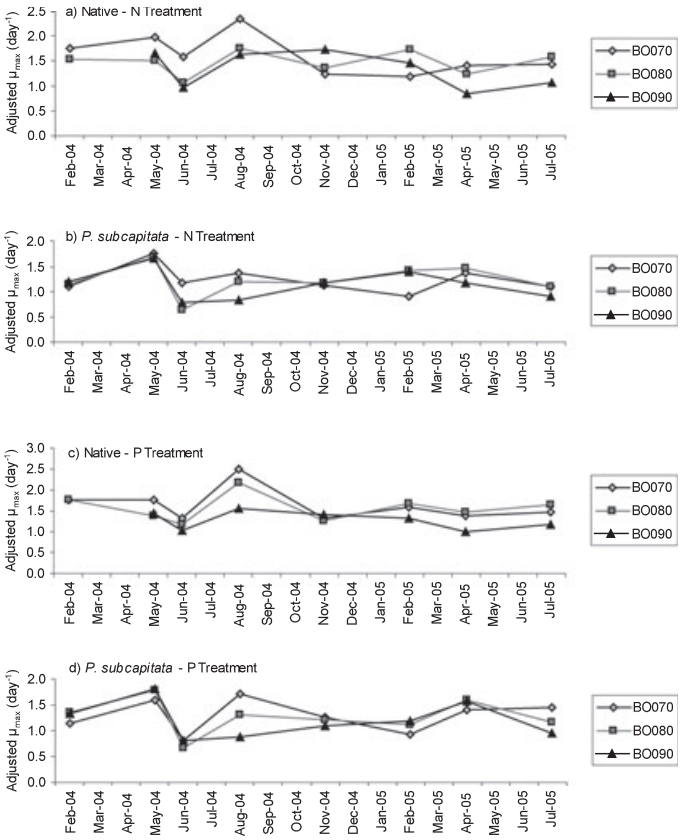

FIGURE 6. Maximum Growth Rates (μ_{max}) Adjusted for Water Temperature and Photoperiod for (a) Native Algae N Treatments, (b) *P. subcapitata* Algae N Treatments, (c) Native Algae P Treatments and (d) *P. subcapitata* Algae P Treatments by Sampling Date.

TABLE 5. Mean, Minimum, and Maximum Growth Rate (μ_{max}) Estimates for Native Algae for Individual Sampling Sites and All Sites Combined.

		Native Algae (μ_{max}/day)		***P. subcapitata* (μ_{max}/day)**	
Site	**Statistic**	**P Treatment**	**N Treatment**	**P Treatment**	**N Treatment**
All sites	Mean	1.51	1.48	1.26	1.20
	Minimum	1.00	0.84	0.66	0.62
	Maximum	2.49	2.34	1.81	1.75
BO070	Mean	1.64	1.62	1.29	1.24
	Minimum	1.32	1.18	0.80	0.91
	Maximum	2.49	2.34	1.71	1.75
BO080	Mean	1.57	1.47	1.28	1.22
	Minimum	1.17	1.07	0.66	0.62
	Maximum	2.16	1.76	1.80	1.68
BO090	Mean	1.28	1.34	1.20	1.14
	Minimum	1.00	0.84	0.81	0.79
	Maximum	1.57	1.73	1.81	1.66

ta algae. The structure of the native algae community *vs*. the single alga in the *P. subcapitata* treatment may have allowed for greater maximum growth rates for the native algae through more efficient assimilation of nutrients (Piehler *et al.*, 2002). Because native algae represent a community rather than a single alga, different algal taxa may dominate at different nutrient concentrations. These results indicate that watershed-specific maximum growth rate parameters may need to be considered when modeling algal growth dynamics with regard to nutrients.

Estimates of μ_{max} were more reliable than estimates of K_N or K_P. This reliability is due in part to the inherent difficulties in preparing dose treatments representative of the low concentrations generally associated with K_N and K_P (Grover, 1989). For K_N, even though NH_3-N concentrations in the ambient water were considered a minor contribution compared to NO_2-N + NO_3-N, further work should consider concentrations of both for low concentration bioassays in defining K_N. In addition, internal storage of nutrients, particularly for the cultured algae, may complicate low dose bioassays allowing growth when external nutrients alone would be limiting (Droop, 1973). The Monod model relates growth only to external concentrations. Although accounting for internal concentrations would allow a more realistic representation of growth dynamics, the difficulty in reliably doing this limits the use of internal algal concentrations as a parameter in most widely used water quality models.

While the parameter estimates for μ_{max}, K_N, and K_P derived from this study could be yet further refined, the derived values provide a better estimate of parameter values to be used in the SWAT model for the native algae in the North Bosque River than the broad range of literature values. This study also clearly indicates for μ_{max} that native algae rather than "test" specimens, such as *P. subcapitata,* should be used for evaluating growth parameters.

SUMMARY

Half-saturation constant mean values from native algae N treatments were considerably lower than those obtained from *P. subcapitata* treatments; however, mean values for both algae types fell within the range of typical values given in the SWAT user's manual. Mean K values for P treatments were typically higher for native algae treatments with the exception of site BO090 when compared to *P. subcapitata* treatments. For both types of algae, mean K_P values were lower than mean K_N values because of the lower amounts of P required for algae growth. Analysis of variance results indicated no significant difference in K values within each nutrient treatment

between either sites or algae types. The high temporal variability in the data may have reduced the ability to detect differences between sites or algae types, but evaluation of temporal variability was beyond the scope of this study.

These growth parameters provide a more realistic parameterization of the SWAT model for studies on the TMDL within the North Bosque River. Similarities appeared to exist in μ_{max} values obtained between sites within algal type (native or cultured). When comparing different algal types, a significant difference was detected. Larger mean μ_{max} values were indicated for the native algae than the *P. subcapitata*. Although not statistically different, mean μ_{max} values for native algae appeared to decrease from upstream to downstream for both nutrient treatments. Differences in ambient nutrient concentrations along the North Bosque River may account for the slight site differences noted in μ_{max} for the native algae, because algae that exist in areas with high-nutrient concentrations tend to have higher μ_{max} values (Dodds, 2002). Because statistically significant differences were not indicated between sites or between nutrient treatments, a single μ_{max} value may be appropriate for SWAT simulations of native algal growth.

ACKNOWLEDGMENTS

Support for this research was provided through the Texas Commission on Environmental Quality, Work Order 582-6-70859-04 as part of the project, Monitoring to Support North Bosque River Model Refinement. We take full responsibility for the information presented herein, but would like to recognize the initial guidance and advice given us by Dr. Owen Lind and Dr. Laura Dávalos-Lind of the Department of Biology at Baylor University in Waco, Texas on procedures for conducting algal bioassay experiments.

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