COMPARISON OF FOUR CLONES OF THE ICHTHYOTOXIC FLAGELLATE *PRYMNESIUM*

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ABSTRACT

Since the mid 1980s blooms of the ichthyotoxic flagellate *Prymnesium parvum* have resulted in recurrent fish kills in Texas lakes, rivers, and reservoirs. South Carolina experienced a bloom of *P. parvum* in a brackish golf course pond in summer 2001. No dead fish were reported since the pond had no resident fish. The following year at Artesian Aquafarms in N.C., all hybrid striped bass perished to blooms of *P. parvum*. In the present study, clonal cultures from each of these blooms were grown in laboratory studies to determine response variation with nutrient-stressed (N-limited, P-limited) and replete cells for growth, hemolytic activity, and ichthyotoxicity. A congener, *P. calathiferum* originally isolated from a New Zealand bloom, was used for comparison. Of the *P. parvum* clones, the TX clone overall grew slower (0.21-0.31 $div \cdot d^{-1}$), had lower hemolytic activity (40-7164 units), but had the highest ichthyotoxicity (1 hr to kill fish in P-limited, 3 hrs in Replete and N-limited). This clone was the most sensitive to nutrient stress and conditioning was reduced to 1 week. In contrast, overall growth and hemolytic activity were greater in the NC (0.21-0.56 $div \cdot d^{-1}$, 77-21399 units) and SC clones (0.20-0.70 $div \cdot d^{-1}$, 45-20795 units) with lower ichthyotoxicity for both ($\geq$4 hrs in N-&P-limited). *Prymnesium calathiferum* showed substantially lower hemolytic activity (8-779 units), but grew faster (0.30-0.73 $div \cdot d^{-1}$) than the *P. parvum* clones. Nitrogen-deficient cultures were similar to or more hemolytic than P-deficient cultures for *P. parvum*, but the P-deficient cultures were the most ichthyotoxic. Under nutrient-replete conditions, *P. calathiferum* was the most ichthyotoxic of the clones with fish mortality occurring in one hour as compared to three hours for the TX clone. Toxicity in *P. parvum* is a complex interaction of hemolytic and ichthyotoxic components.

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INTRODUCTION

Prymnesium parvum N. Carter (1937), a toxic phytoplankton usually <10 µm in size, was first discovered in the 1920s in a brackish tide pool on the Isle of Wight, England (Carter 1937). Its distinguishing features include two chloroplasts, two flagella (12-15 µm) and a single stiff haptonema (3-5 µm) (Green *et al*. 1982). Species identification involves viewing its body scales using electron microscopy since all species of the genus *Prymnesium* have an outer covering of organic scales. *Prymnesium parvum* has two layers of scales with those on the inner layer having wide inflexed rims, while those of the outer layer have narrow rims (Green *et al*. 1982). The scales of its distal face have a pattern of concentric circles, while those of its proximal face have a pattern of radial ridges (Manton and Leedale 1963). Scale morphology (as well as studies on physiology, toxicity, and genetics) led to the conclusion that a once separately identified species (*P. patelliferum*) is only a different life stage of *P. parvum* (Larsen and Medlin 1997, Larsen and Edvardsen 1998, Larsen 1999).

The devastating fish kills caused by *P. parvum* led to detailed studies of its blooms. Carp mortalities associated with *P. parvum* blooms occurred in Israel in 1945 (Reich and Aschner 1947, Yariv and Hestrin 1961) and in China since 1963 (Guo *et al*. 1996). In November-December 1969, the Yuzhnyy Fish Farms in Ukraine experienced fish-killing blooms (Krasnoshchek and. Abramovich 1971). That same year, the Norfolk Thurne Broads, United Kingdom, began experiencing kills of perch, roach, bream, and eels (Holdway *et al*. 1978, Wortley and Phillips 1987) during similar blooms. Annual blooms of *P. parvum* from 1989–1996 in the Sandsfjord, Norway damaged fisheries of Atlantic salmon and rainbow trout (Kaartvedt *et al*. 1991, Larsen and Edvardsen 1998). In Morocco (Sabour *et al*. 2000), *P. parvum* blooms killed carp, barbells, eels, sunpoles, gambusia, shrimp, bivalves, and other invertebrates during 1998-1999. Lake Koronia experienced the first reported *P. parvum* bloom in Greece in August-September 2004. The blooms, ranging in densities from 120-1450 $x10^6$ cells/L in waters having N:P ratios of 10:1, resulted in the death of thousands of birds (30 species, including the endangered *Pelecanus crispus*) and hundreds of fish (Moustaka-Gouni *et al.* 2004).

In the United States, *Prymnesium* blooms were first reported during the mid-1980s in Texas (Figure 1). Affected waters in Texas include Baylor Lake, Brazos River, Buffalo Springs Lake, California Creek, Colorado City Lake, Colorado River, E.V. Spence Reservoir, Lake Diversion, Lake Granbury, Lake Kemp, Lake Sweetwater, Lake Whitney, Lubbock Lake, Moss Creek Lake, Paint Creek, Pecos River, Possum Kingdom Lake/Reservoir, and Red Bluff Reservoir (James and de la Cruz 1989, Rhoades and Hubbs 1992, Texas Parks and Wildlife Department website). More recently, South Carolina experienced a bloom on May 22, 2001 at a brackish golf course pond on Kiawah Island (Lewitus *et al*. 2003). There were no fish kills reported because there were no fish in the pond. Artesian Aquafarms in Elizabeth City, North Carolina experienced *P. parvum* blooms in 2002, which started in March and persisted through October. This aquaculture facility grows hybrid striped bass. When the water source was changed from fresh to brackish to optimize fish growth, *Prymnesium* blooms occurred, causing large fish kills. *Prymnesium parvum* was also observed during a two-year study of the New River, NC where unexplained fish mortalities were commonly reported since 1980, though fish mortality directly linked to *P. parvum* was not established (Tomas *et al*. 2004). Other areas in the U.S. with *P. parvum* blooms have included Colorado, New Mexico, Arizona, and Florida (Websites for Texas Parks and Wildlife Department, Colorado Department of Natural Resources, Arizona Game and Fish Department).

Figure 1. Distribution of *P. parvum* blooms in the United States.

Yariv first isolated the toxin of *P. parvum* in 1958 (Yariv and Hestrin 1961). Properties of prymnesins include a ninety-carbon skeleton with a methyl group as the only carbon branching, five ether rings, possessing a hydrophilic end and a hydrophobic end, and functional groups including conjugated double and triple bonds, chlorine atoms, an amino group and glycosidic residues (Morohashi *et al*. 2001) (Figure 2). Presently, there are two known structures of prymnesins with four known effects - ichthyotoxicity, hemolysis, cytolysis, and neurotoxicity (Igarashi *et al*. 1998). These effects appear to be related to the ability of the prymnesins to change the permeability of cell membranes (Johansson and Granéli 1999).

The ichthyotoxic component affects fish, tadpoles, and invertebrates by disrupting the permeability of the gills (Yariv and Hestrin 1961, James and de la Cruz 1989). Gill damage can occur after only ten to fifteen minutes of exposure. Uptake of the toxin is considered to occur in two stages, 1) a reversible damage to the gills results in a loss of selective permeability and 2) the fish become more sensitive to other toxins due to breakdown in the gill membranes (Ulitzur and Shilo 1966). Cofactors are required to activate the ichthyotoxin including monovalent and divalent cations such as calcium and magnesium (Parnas *et al*. 1962, Parnas 1963). Using streptomycin, neomycin, and spermine have also increased ichthyotoxicity in laboratory experiments (Yariv and Hestrin 1961, Ulitzur and Shilo 1964).

The hemolytic portion of prymnesin was found to contain at least six compounds with two similar glycolipids as the dominating compound of the mixture (Meldahl *et al*. 1994). A specific receptor site on the surface of the blood cells is suggested for the hemolysin, and an aggregation of toxin molecules may be involved (Igarashi *et al*. 1998). The hemolysins become inactivated with high pH, monovalent cations, and possibly by some lipids (Padilla 1970, Ulitzur and Shilo 1970, Igarashi *et al*. 1998).

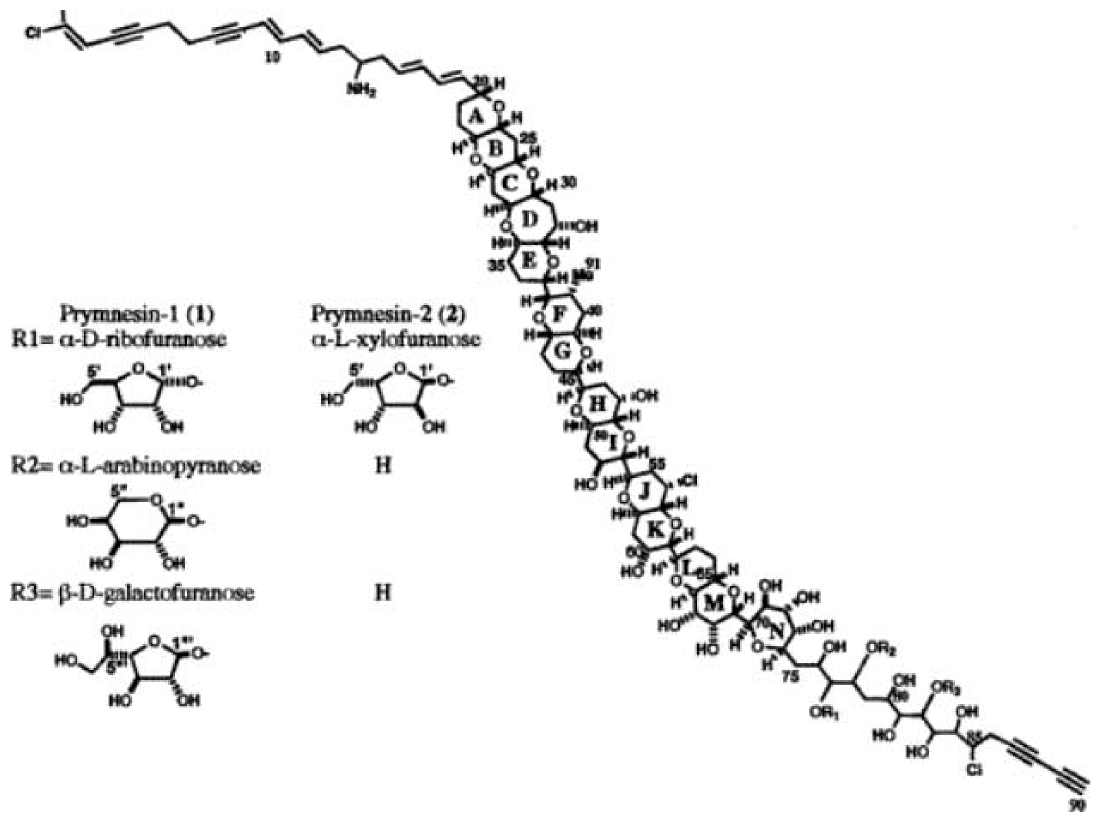

Figure 2. Structure of prymnesin. (from Igarashi *et al*. 1999)

Cytolytic abilities are also reported for the prymnesins. Cells swell due to the free flux of ions (sodium and potassium), amino acids, and nucleotides. As with the ichthyotoxic effect, two stages are involved – water uptake and swelling, followed by cell lysis (Dafni and Giberman 1972).

Neurotoxic effects of prymnesins caused respiratory failure in frogs, mice and cats in laboratory experiments (Parnas 1963). This toxin attacks the central nervous system by blocking postsynaptic membranes at the neuromuscular junction as well as contracting smooth muscle (Parnas and Abbott 1965, Meldahl *et al*. 1994).

One factor implicated in toxin production is nutrient limitation. Shilo (1967) found low levels of phosphorus in the medium with highly toxic *P. parvum* cells. Dafni *et al*. (1972) proposed that phosphate limitation might lead to increased toxin production by disrupting membrane phospholipids, which would act to excrete the toxin. Similarly, blooms in the United Kingdom (Holdway and Watson 1978, Holdway *et al*. 1978, Wortley and Phillips 1987), Norway (Aure and Rey 1992), and Morocco (Sabour *et al*. 2000) occurred in areas with low phosphate concentrations. Johansson and Granéli (1999) studied hemolytic activity of *P. parvum* under both nitrogen and phosphorus limitation and found hemolytic activity to increase under nutrient limitation compared to non-limiting conditions. From these results, they suggested that cellular physiological stress might cause increased toxin production. They argued that this is somewhat perplexing in that the linkage between nutrient limitation and toxicity may not be direct, since nitrogen and phosphorus are not main components of the toxin. In studies for allelopathy, defined as "the release of chemical substances by individuals of a population that have an effect on the individuals of another population" (Hulot and Huisman 2004), toxin production by nutrient-stressed *P. parvum* cultures was associated with inhibiting various phytoplankton (*Thalassiosira weissflogii*, *Prorocentrum minimum*, *Rhodomonas cf. baltica*) and decreased survival rates of the ciliate *Euplotes affinis* (Granéli and Johansson 2003a, 2003b).

While several laboratory studies suggest phosphorus limitation increases toxin production and release, the study of Johansson and Granéli (1999) emphasizes that either nitrogen or phosphorus limitation may lead to increased toxicity. Adding to this confusion are the reports that natural blooms are associated with phosphorus limitation. The recent blooms in the United States have occurred in areas of high eutrophication, but there was some variation among the bloom sites. Two blooms occurred in brackish man-made facilities (NC and SC), while the other occurred in alkaline natural lakes (TX). The blooms in Texas and North Carolina caused fish kills, while those in South Carolina did not due to a lack of fish in the waterbody.

The purpose of this study was to test the relationship between nutrient concentration, growth phase, and toxicity in clones of *P. parvum* from the United States blooms (North Carolina, South Carolina, and Texas) and compare them to a clone of *P. calathiferum* Chang and Ryan (1985), a toxic species from New Zealand.

The research objectives were:

- to determine toxicity as measured by hemolytic activity of the four clones of *Prymnesium*.
- to examine toxicity to *Gambusia holbrooki* of the four clones of *Prymnesium.*
- to examine the effects of nutrient concentration (nitrogen and phosphorus) on hemolytic activity.
- to examine how growth phase (lag, log, stationary) affects hemolytic activity.

The null hypotheses were that:

- the four clones do not differ in their hemolytic activity and are not capable of killing fish.

- neither nutrient concentration nor growth phase affect the hemolytic activity of the four clones.

MATERIALS AND METHODS

Culturing

The Tomas lab (UNCW Center for Marine Science) has clonal cultures of *P. parvum* established from blooms in the United States. One of the fourteen clones from the North Carolina bloom, picked at random, was used in this study (CMS TAC PP7). *Prymnesium parvum* clones from South Carolina (CMS TAC PP22) and Texas (CMS TAC PP18) were also used. Another *Prymnesium* species from New Zealand (*P. calathiferum* (CCMP Strain CHANG1) was used to compare with *P. parvum* from the United States.

Clonal cultures were grown with f/2 media (Guillard and Ryther 1962) at salinities of 4 for *P. parvum* and 36 for *P. calathiferum*. To make the media of different salinities, full strength seawater (at salinity of 38-39), collected 20-50 miles offshore, was filtered through a 47 mm Whatman GF/F glass microfibre filter, and diluted with pyrogen-free deionized water equivalent to Milli-Q ultrapure water. Salinity was checked with a refractometer. Water of the appropriate salinity was then sterilized by autoclaving at 121° C for 15 minutes in Teflon bottles. Sterile nutrients were added aseptically once the salinities were cooled to room temperature. The cultures were maintained in Erlenmeyer (150 mL) and Fernbach (1.5 L) flasks and kept in a walk-in growth chamber of constant temperature (22 ± 1° C) with a 16: 8 hour light:dark photoperiod under cool fluorescent 40-Watt light. For the experiments, only cultures grown in Fernbach flasks were used, with transfers done every 2-3 weeks.

Nutrient Concentration Studies

For the nutrient studies, f/2 media was used to make nutrient-replete, nitrogen-limited, and phosphorus-limited conditions. The nitrogen-limited media was modified to N:P = 4:1, using 16 μM nitrate and 4 μM phosphate. The phosphorus-limited media was modified to N:P = 80:1, using 80 μM nitrate and 1 μM phosphate. Approximately 30 mL of culture grown in nutrient-replete media were transferred into 150 mL of nutrient-deficient media in Erlenmeyer flasks. After one week of growth, the cultures were transferred again to nutrient-deficient media. After another week of growth, the cultures were inoculated into Fernbach flasks and the experiments began. The Texas *P. parvum* clone (for nitrogen and phosphorus limitation) and *P. calathiferum* (for only nitrogen limitation) did not exhibit growth after inoculation into the Fernbach flasks after two weeks of nutrient deprivation. To allow studies of these cultures, they were conditioned for only one week in nutrient-deficient media before inoculation into Fernbach flasks.

Growth Studies

Prymnesium cultures were counted daily using a Beckman Coulter Multisizer 2E Particle Counter with an aperture of 100 μm to generate growth curves as well as to determine the number of cells used in each hemolysis assay. For counting, full strength seawater was diluted with pyrogen-free deionized water to obtain the correct salinity. This water was then filtered through two filters, 47 mm Whatman GF/F glass microfibre filter and a 0.2 μm GTTP Millipore Isopore Membrane Filter, to minimize particle interference. Cultures were diluted with their respective salinities of 4 for *P. parvum* and 36 for *P. calathiferum* to optimize the counter capabilities. Very dense cultures gave a higher percentage of coincidence, defined as a greater chance that more than one organism was passing through the orifice at the same time. The percentage of coincidence was kept below 5% by diluting the samples before counting. Four replicate counts were taken daily from one sample of each culture. Each sample was continuously stirred and 500 µL of each culture was counted. To obtain the number of cells in one mL, the raw count was multiplied by 2 and corrected for the dilution factor. The means of the four replicate counts were used to generate growth curves using SigmaPlot 2001. Using the growth curves, log phase was determined by eye. The mean cells/mL were converted to Log_2 and plotted against the days in log phase. A linear regression was performed on the cell densities in log phase using SigmaPlot 2001. The slope of the line gave the growth rate k.

$$k=\log_2 (N_1/N_0)/(t_1-t_0)$$ (Guillard 1973).

Erythrocyte Lysis Assay

To test hemolytic activity, the erythrocyte lysis assay (ELA, Eschbach *et al*. 2001) was performed. Outdated human red blood cells, obtained from and screened for pathogens by the American Red Cross, were used for this assay due to easier availability and convenience. Other sources, such as fish blood, were not readily available and in insufficient quantities to guarantee a reliable and stable supply. The blood was stored in a 4º C refrigerator and kept on ice while used in the assay. At the time of analysis, the test blood was placed into a 15mL centrifuge tube to which approximately 10 mL of cold ELA buffer were added (Table 1).

The buffer was kept cold so as not to lyse blood cells due to heat shock. The centrifuge tube of blood and buffer was inverted five times and spun at 300 rpm at 4º C for five minutes with a Hermle (Model #Z383K) refrigerated centrifuge. The supernatant was discarded and the cells were resuspended with 10 mL of cold buffer. This washing procedure was repeated until the supernatant was clear (approximately 3-6 times). After the final centrifugation, the

Table 1. ELA Buffer for Hemolysis Assay*

Reagent	Concentration (mM)	Molarity (g/mol)	Grams for 2L
NaCl	150	58.44	17.54
KCl	3.2	74.56	0.4772
$MgSO_4$	1.25	246.48	0.6162
$CaCl_2$	3.75	110.99	0.8234
Trizma pre-set crystals, pH 7.0	12.2	154.8	3.772

* pH adjusted to 7.4 at 4º C.

supernatant was removed and a volume of cold buffer to match the volume of blood was added. A 1:40 through 1:60 dilution of erythrocytes with buffer was made to give an optimal concentration of erythrocytes. This was done to maximize the capability of the microplate reader, giving a full positive control optical density reading of 3.00 OD.

Prymnesium cultures were selected on days representing lag, log, and stationary phases of growth. Fifty mL aliquots of the whole culture were centrifuged at 3200 rpm for 15 minutes at 4º C. The first 50 mL aliquot centrifuged had the supernatant poured off and used in the assay. Due to the small nature of *Prymnesium* cells, obtaining a visible pellet usually took many rounds of centrifuging 50 mL aliquots of culture, removing the majority of the supernatant, and adding more culture to centrifuge. The amount of culture added to obtain a visible pellet was recorded to use in later calculations to correct for the different volumes centrifuged. When a visible pellet of a known cell number was obtained, 3 mL of cold buffer was added and the mixture was sonicated continuously for 30 seconds at an amplitude of 45 on a 20 kHz Ultrasonic Processor (Model #GE 130 PB, Sonics and Materials, Inc.). Cold buffer was used to yield 1:2 and 1:10 dilutions of the supernatant and 1:10 and 1:100 dilutions for the sonicated pellet. The 100% concentrations of the supernatant and pellet were also used in the assay. Throughout the assay, all culture samples were kept on ice.

Using a Transferpette-8 pipette, 125 µl each of erythrocytes and the *P. parvum* dilutions were placed into a Costar 96-well microtitre plate with V-shaped bottoms. Throughout the experiment, the plates were kept on ice. Negative controls consisted of erythrocytes incubated with buffer only; positive controls were erythrocytes incubated with saponin (Sigma S4521) which lysed all erythrocytes. The saponin reagent was made by dissolving 0.008 g of saponin with 50 mL of pyrogen-free DIW to give a concentration of 20 µg/125µL/well. Once made, the saponin was placed into cryovials, kept in a -80º C freezer, and then thawed before use in the assay. Both the negative and positive controls consisted of eight replicate wells and the culture samples consisted of 4-8 replicates. The plates were sealed and incubated at 4º C for 24 hours. After incubation, the plates were centrifuged at 1250 rpm for 10 minutes at 4º C and 250 µl aliquots of the supernatant were then transferred to a 96-well Costar microtitre plate with flat bottoms. The optical densities of the samples (representing released hemoglobin) were read with on a Bio-Tek Powerwave X microplate reader at 415 nm equipped with the K.C. Junior program. The mean of the negative control replicates was subtracted from the rest of the values to correct for any hemolysis by the buffer. Each corrected value was divided by the mean of the saponin replicates and multiplied by 100 to obtain a percentage of lysis. The mean of the replicates were calculated for each culture concentration. This gave percent hemolysis by the supernatant and the pellet.

Toxicity was then normalized on a per cell basis to compare the same number of cells in each clone. As mentioned before, centrifuging the exact number of cells in each culture was not possible due to different cell densities and problems obtaining a pellet. The Coulter count (in cells/mL) was multiplied by the volume of sample centrifuged (50 mL for the supernatant, usually a larger volume for the pellet) as well as the volume of cells in each well (always 125 µL). This number (N) was then divided into the percent hemolysis (PH) value, giving toxicity on a per cell basis. The number was then multiplied by a constant ($1x10^7$) to obtain an easier to read value. This gave normalized hemolysis by the supernatant and pellet.

N = (# cells/mL from Coulter counter) * (Volume centrifuged) * (0.125 mL)

(PH/N) * ($1x10^7$) = Normalized Lysis Value

Toxicity per growth phase (lag, log, stationary) was examined in all four clones. Three replicate trials were performed for each nutrient treatment.

Fish Bioassay

The toxicity of the four clones was also examined with a fish bioassay. Assays using the eastern mosquitofish *Gambusia holbrooki* are commonly used to determine the presence of *Prymnesium* toxicity. For this study, results from the bioassay were compared with those from a hemolysis assay. The procedure outlined by Shilo and Aschner (1953) was used. *Gambusia* were captured in local brackish ponds and maintained in an aquarium at the UNCW Center for Marine Science (IACUC 01-008).

Before the fish bioassay, the cultures were counted every few days with a Coulter Counter to verify that they were in stationary phase. Stationary phase was chosen because the cultures would be the most dense and easier to work with (i.e. centrifuging 40 mL of culture would result in a visible pellet). An aliquot of 200 mL of each clone (NC, SC, TX, Pcal) were placed into sterile beakers. Aliquots of 40 mL of each culture were centrifuged at 3200 rpm for 15 minutes at 4°C. The supernatant was then separated from the pellet. Both fractions, to be used later in hemolysis assays, were placed in a
-80° C freezer.

The fish bioassay took place in 25 Pyrex 50-mL beakers.

- 1 beaker – negative control
- 2 beakers each – NC [100%]; NC [50%]; NC [10%]
- 2 beakers each – SC [100%]; SC [50%]; SC [10%]
- 2 beakers each – TX [100%]; TX [50%]; TX [10%]
- 2 beakers each – Pcal [100%]; Pcal [50%]; Pcal [10%]

The cultures were diluted with their respective sterile media. The 100% concentration consisted of 40 mL of culture. The 50% and 10% concentrations were made by diluting the culture with media. The negative control consisted of 40 mL of aquarium water. One mL of the cofactor DADPA (0.003 M DADPA (3,3' – diaminodipropylamine, Sigma I 7006) was added to every beaker, including the negative control, to make the fish more sensitive to the toxin (Ulitzur and Shilo 1966). Timing began after one *Gambusia* was added to all the beakers. The assay was monitored closely for up to six hours. Observations were recorded every fifteen minutes for the first two hours and every thirty minutes thereafter. Symptoms of toxicity that were looked for included erratic movements, loss of equilibrium, release of blood into the medium, and subsurface bleeding at the snout, gills, and pectoral fins. Death was determined by immobility of the body and gills. At the end of the experiment, dead fish were discarded. The survivors were removed from the experimental beakers and then released into a pond far from the normal collection site.

Statistical Analysis

Growth rates during log phase were examined by linear regression analysis with SigmaPlot 2001, after Log2-transforming the data. Hemolytic activity differences were examined using two-way and three-way analysis of variance (ANOVA) and Tukey HSD with SigmaStat and JMP, after Log10-transforming the hemolytic activity data. A significant level of $\alpha=0.05$ was used in all tests.

RESULTS

Growth: Nutrient-Replete Cultures

The initial cell densities varied significantly (P<0.001) for every trial and clone (Table 2). All four clones exhibited a very short lag phase in all three trials (Figure 3). Throughout the trials, the *P. parvum* clones had similar log phase lengths, but differed in terminal densities. For Trial 1, the *P. parvum* clones reached similar (0.477<P<0.979) terminal cell densities. The NC clone reached the highest terminal density (P<0.001), while the similar (P=0.326) SC and TX clones reached slightly lower terminal densities in Trial 2. For Trial 3, the NC reached a significantly (P<0.001) higher terminal density than the SC clone, while the TX clone had a smaller (P<0.001) terminal density than both the NC and SC clones. *Prymnesium calathiferum* had a shorter log phase and reached a smaller terminal density (P<0.001) for Trials 1 and 2 compared with the *P. parvum* clones. In Trial 3, *P. calathiferum* exhibited no true stationary phase, but rather went from log phase to declining phase.

The three *P. parvum* clones exhibited similar growth rates during log phase for all three trials in the nutrient-replete treatment (Table 3, Figures 4-6). The growth rates for *P. calathiferum* were not significantly different (0.052<P<0.079) from those for the three *P. parvum* clones.

Growth: Nitrogen-Deficient Cultures

The initial cell densities varied significantly (P<0.001) for every trial and clone (Table 2). All four clones exhibited a very short lag phase in all trials. In Trial 1, the three *P. parvum*

Table 2. Initial cell densities of three geographically-distinct clones of *Prymnesium parvum** and *P. calathiferum*.

Nutrient Treatment	Trial	NC	SC	TX	Pcal
Replete					
	1	20058	12469	22621	21298
	2	17815	10855	15775	3645
	3	9255	11830	14330	11790
N-deficient					
	1	938	1252	7555	5505
	2	2535	924	13845	3700
	3	4660	3140	3905	3675
P-deficient					
	1	5760	5212	3755	4780
	2	8185	10470	15550	10415
	3	1360	2135	7525	2910

* NC=North Carolina; SC=South Carolina; TX=Texas

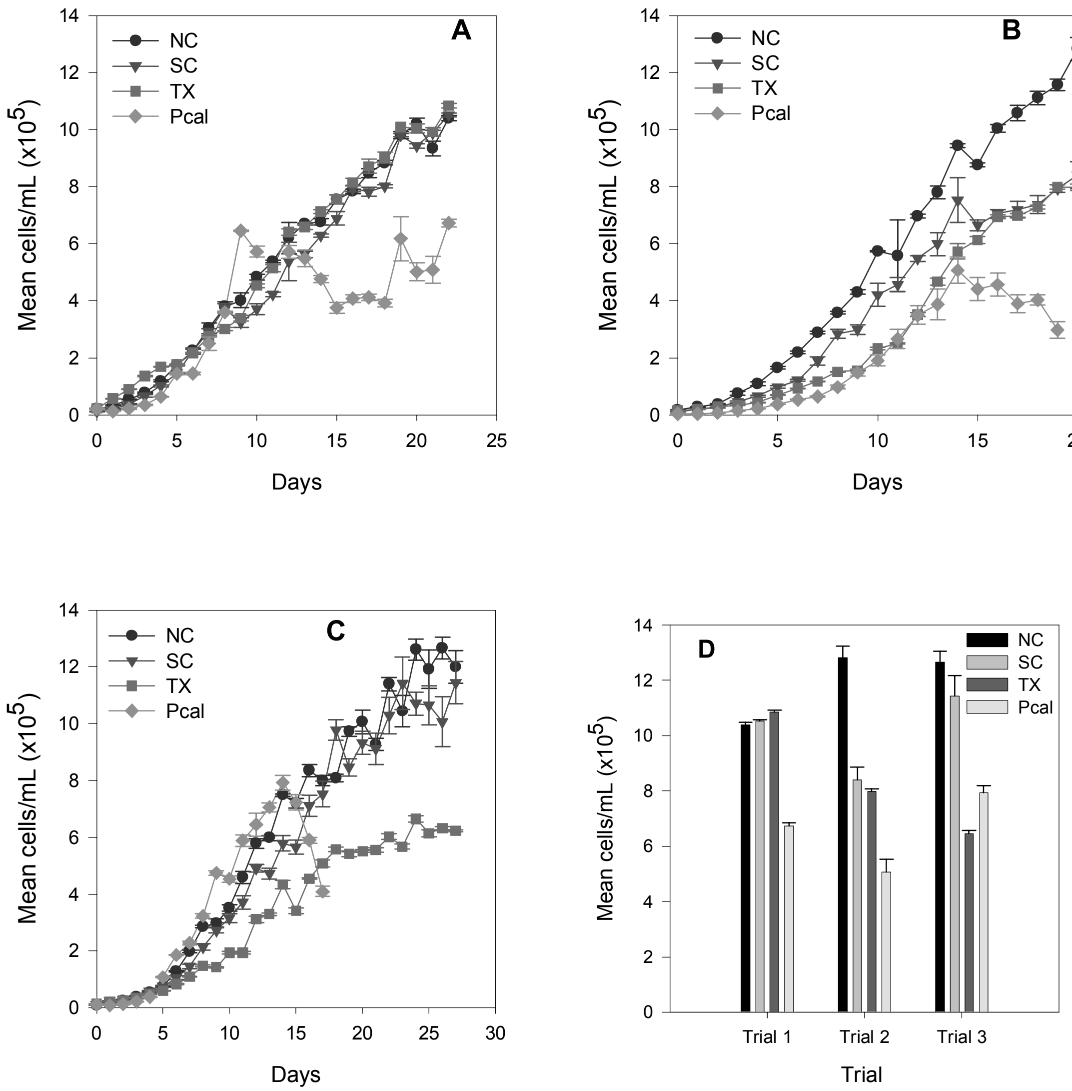

Figure 3. Growth of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* under nutrient-replete conditions. Error bars represent one standard deviation about the mean of four daily counts on a Coulter Counter. (A) Trial 1. (B) Trial 2. (C) Trial 3. (D) Terminal densities of the clones for each trial.

Table 3. Growth rates (k) of three geographically-distinct clones of *Prymnesium parvum** and *P. calathiferum*. Parentheses indicate days in log phase.

Nutrient Treatment	Trial	NC	SC	TX	Pcal
Replete					
	1	0.27 (5-10)	0.48 (4-8)	0.26 (5-12)	0.61 (5-19)
	2	0.27 (5-14)	0.30 (6-14)	0.31 (9-16)	0.42 (7-14)
	3	0.21 (9-17)	0.20 (8-18)	0.24 (5-18)	0.30 (5-14)
N-deficient					
	1	0.38 (4-9)	0.45 (2-6)	0.23 (0-3)	0.58 (1-4)
	2	0.22 (2-10)	0.33 (4-8)	0.21 (0-3)	0.46 (1-4)
	3	0.32 (2-5)	0.43 (2-5)	0.30 (1-3)	0.66 (1-3)
P-deficient					
	1	0.44 (5-10)	0.51 (5-10)	0.24 (2-7)	0.73 (3-8)
	2	0.54 (2-7)	0.49 (2-7)	0.22 (1-7)	0.69 (2-6)
	3	0.56 (9-15)	0.70 (4-13)	0.23 (1-5)	0.46 (6-11)

* NC=North Carolina; SC=South Carolina; TX=Texas

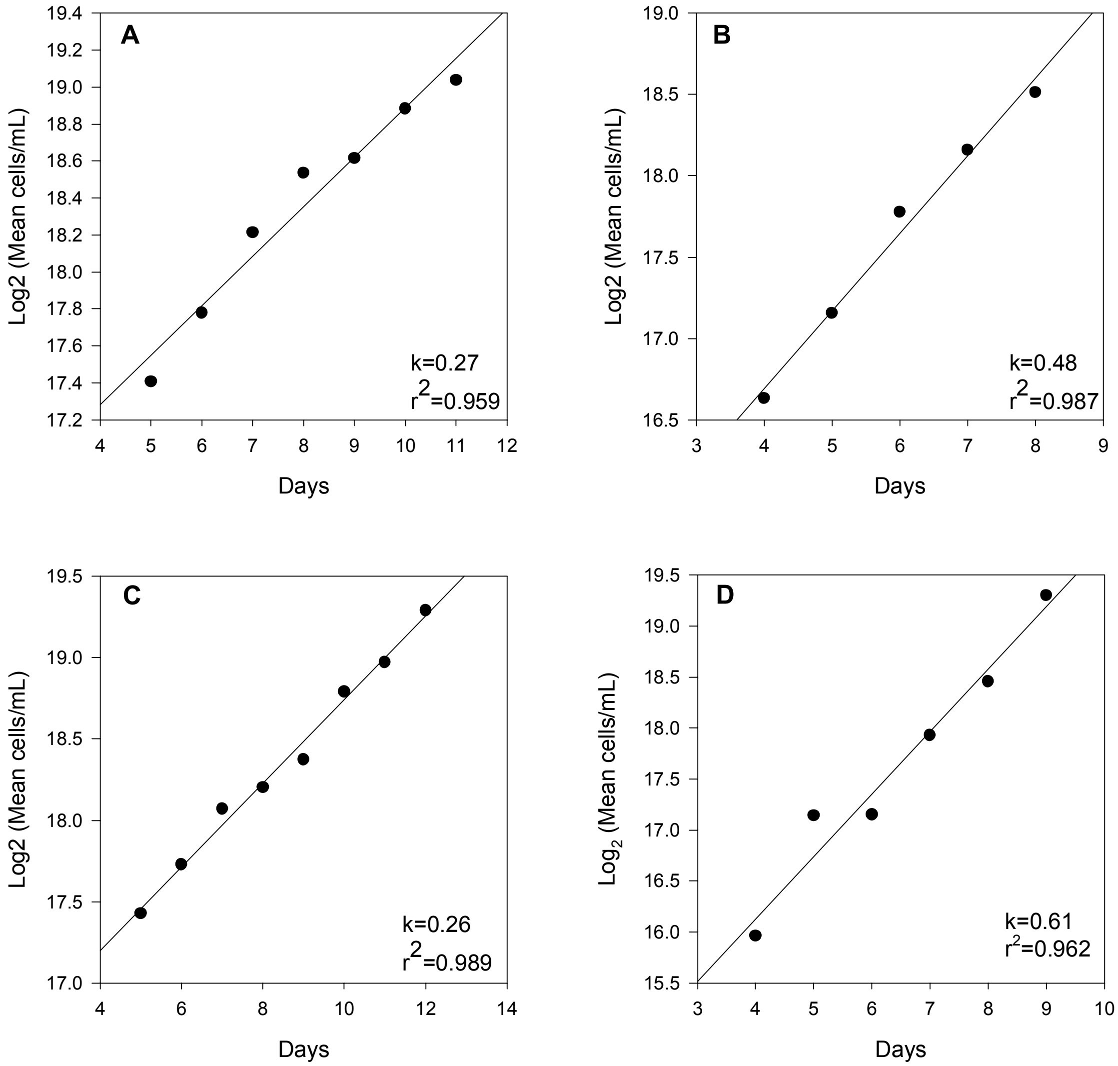

Figure 4. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under nutrient-replete conditions for Trial 1. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

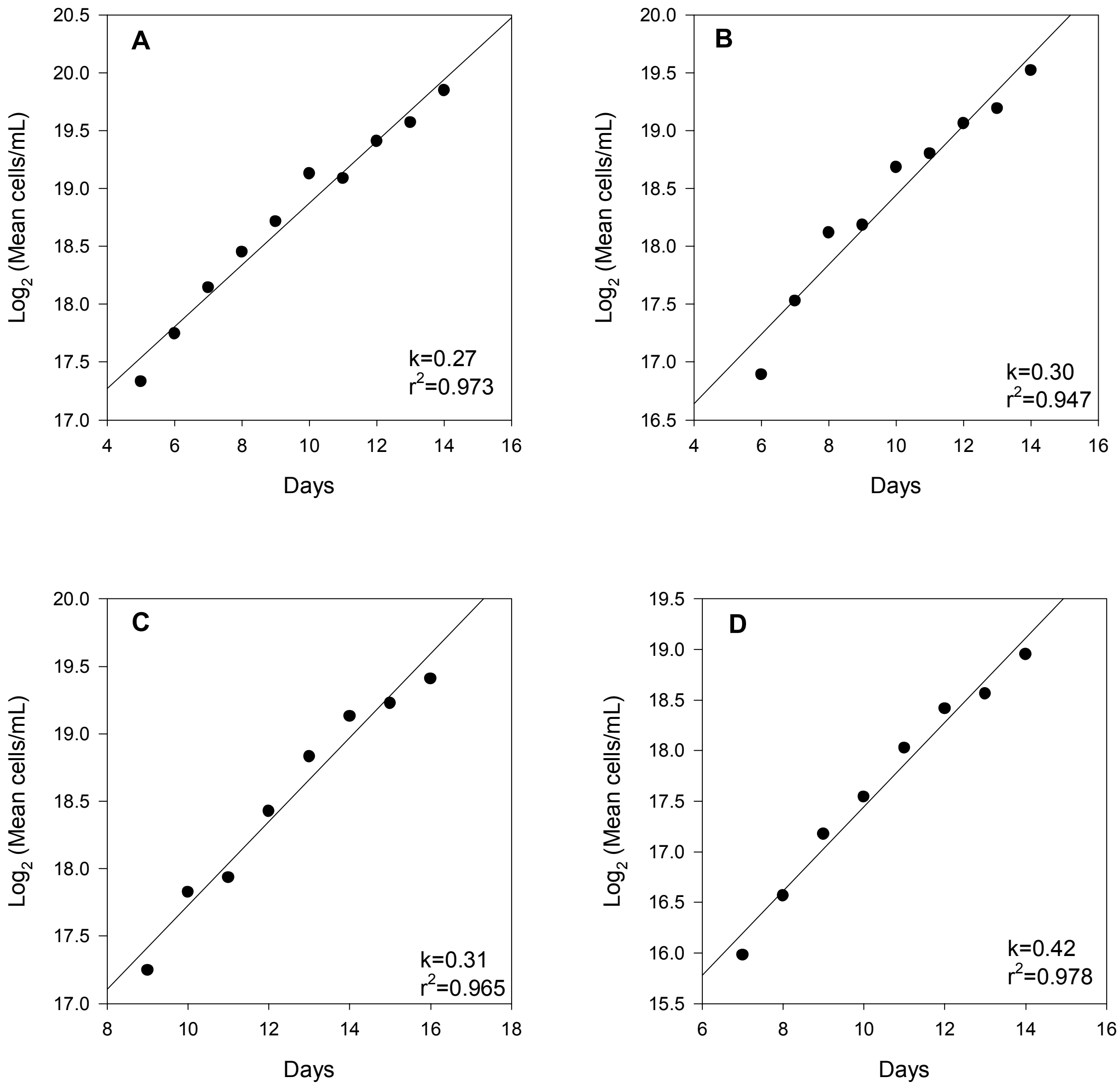

Figure 5. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under nutrient-replete conditions for Trial 2. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

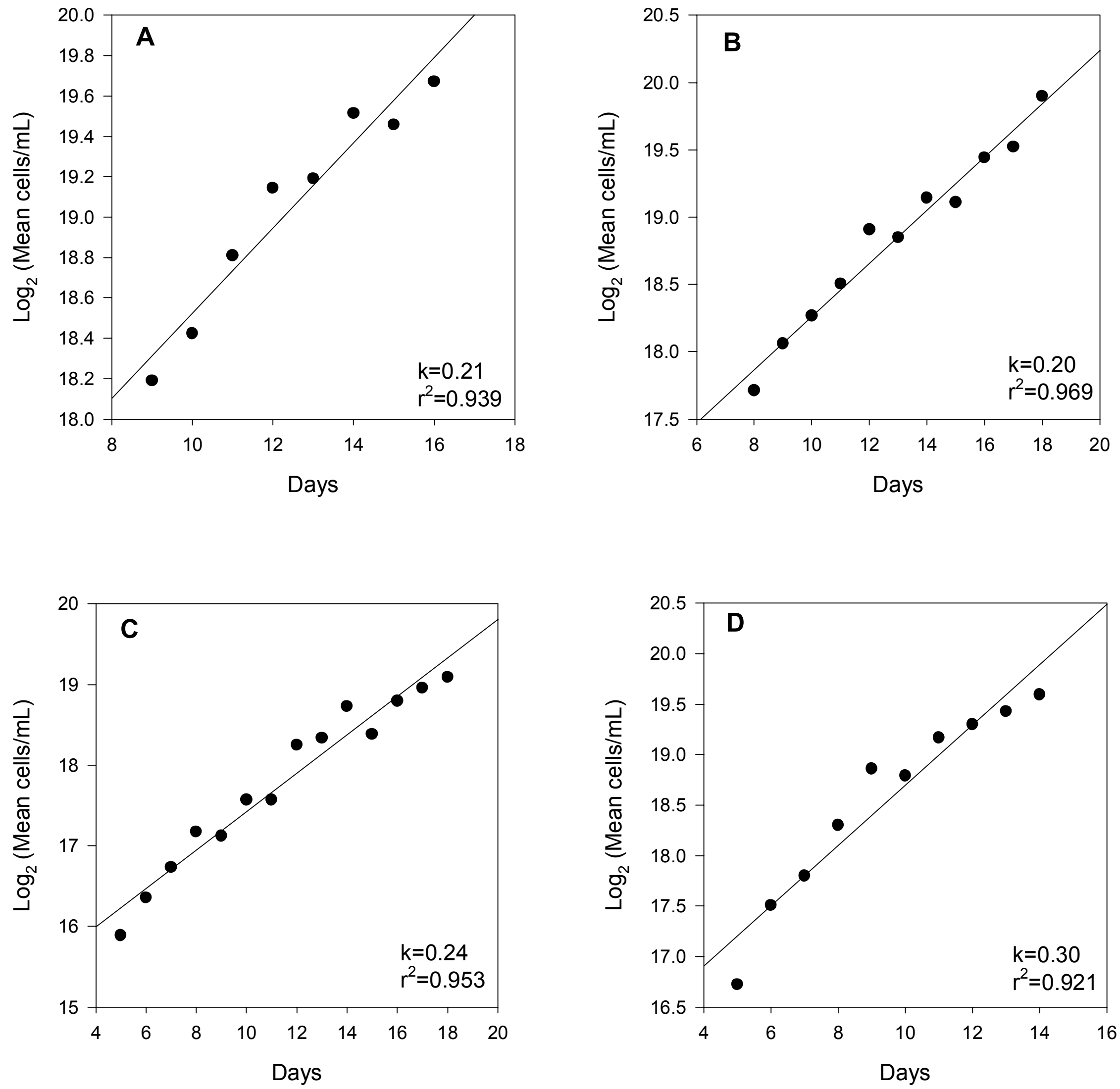

Figure 6. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under nutrient-replete conditions for Trial 3. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

clones all reached similar terminal cell densities of approximately $1.2\text{-}1.3 \times 10^5$ cells/mL, though the TX clone had a shorter log phase than the other clones (Figure 7). *Prymnesium calathiferum* reached a higher terminal density ($P<0.001$) than the *P. parvum* clones. In Trial 2, the SC clone had a slightly shorter log phase, but reached a statistically similar ($P=0.720$) terminal density to the NC clone. The TX clone differed from both the NC and SC clones by having a shorter log phase and a larger ($P<0.001$) terminal density. *Prymnesium calathiferum* also had a very short log phase, but reached the smallest terminal density ($P<0.001$) of the four clones. In Trial 3, NC and SC clones grew to similar terminal densities ($P=0.998$), while the terminal density of the TX clone was significantly lower ($P<0.001$) than those of the other *P. parvum* clones, but similar ($P=0.754$) to that of *P. calathiferum*.

The SC clone had a high growth rate for the *P. parvum* clones for all three trials, but was only significantly different from the TX clone ($P=0.016$) and not the NC clone ($P=0.113$) (Table 3, Figures 8-10). In Trial 1, the NC and SC clones had similar growth rates, while the TX clone had a slightly lower growth rate. In Trials 2 and 3, the NC and TX had similar ($P=0.113$) growth rates. *Prymnesium calathiferum* had the significantly ($P\leq0.013$) highest growth rate of the four clones for all three trials.

Growth: Phosphorus-Deficient Cultures

The initial cell densities varied significantly ($P<0.001$) for every trial and clone (Table 2). All four clones had short lag and log phases. Lag phase was longer in Trial 3. The SC clone had significantly higher ($P<0.001$) terminal densities than the NC clone for all three trials (Figure 11). The TX clone reached the smallest terminal density ($P<0.001$) of the four clones for all

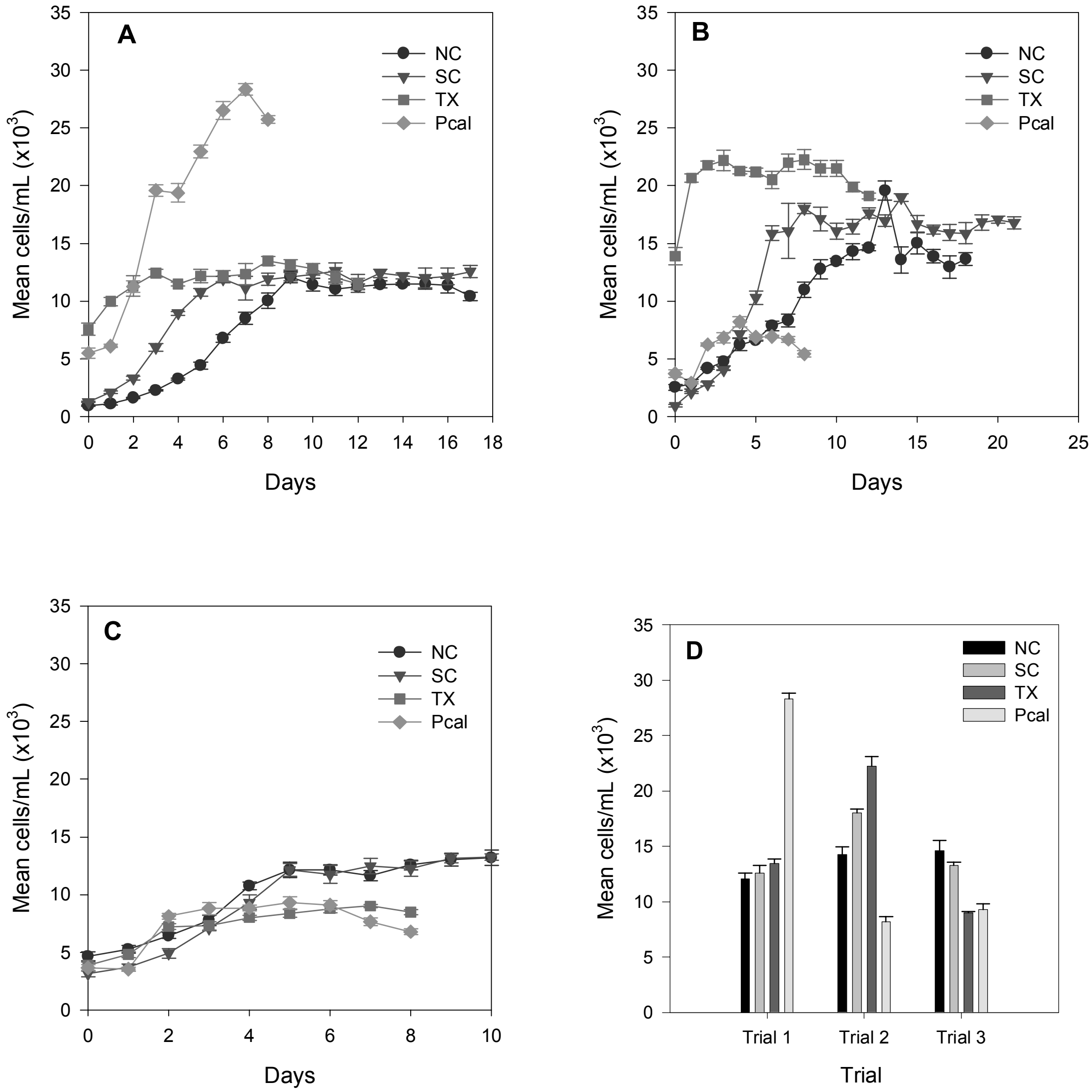

Figure 7. Growth of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* under N-deficient conditions. Error bars represent one standard deviation about the mean from four daily counts on a Coulter Counter. (A) Trial 1. (B) Trial 2. (C) Trial 3. (D) Terminal densities of the clones for each trial.

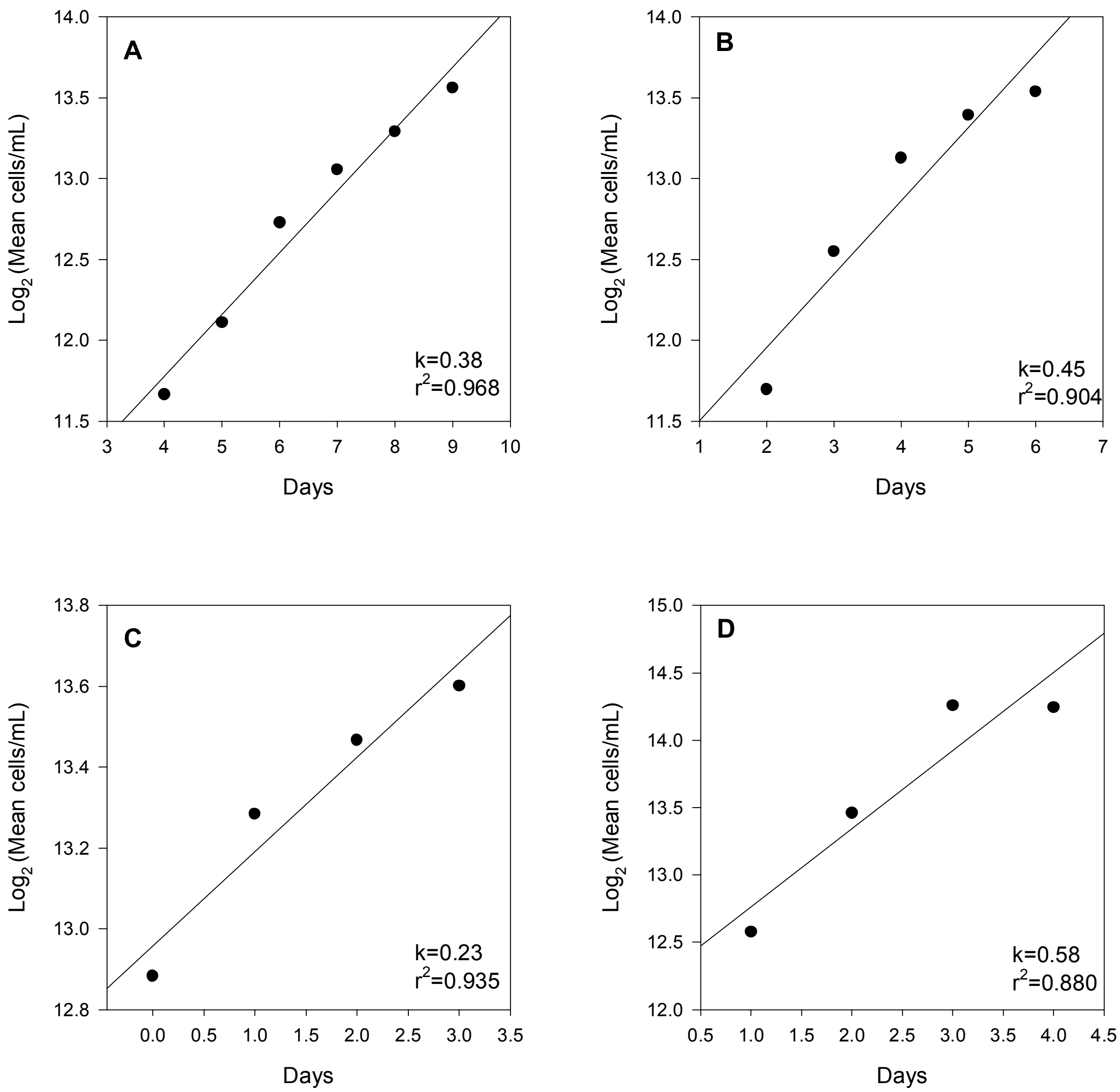

Figure 8. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under N-deficient conditions for Trial 1. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

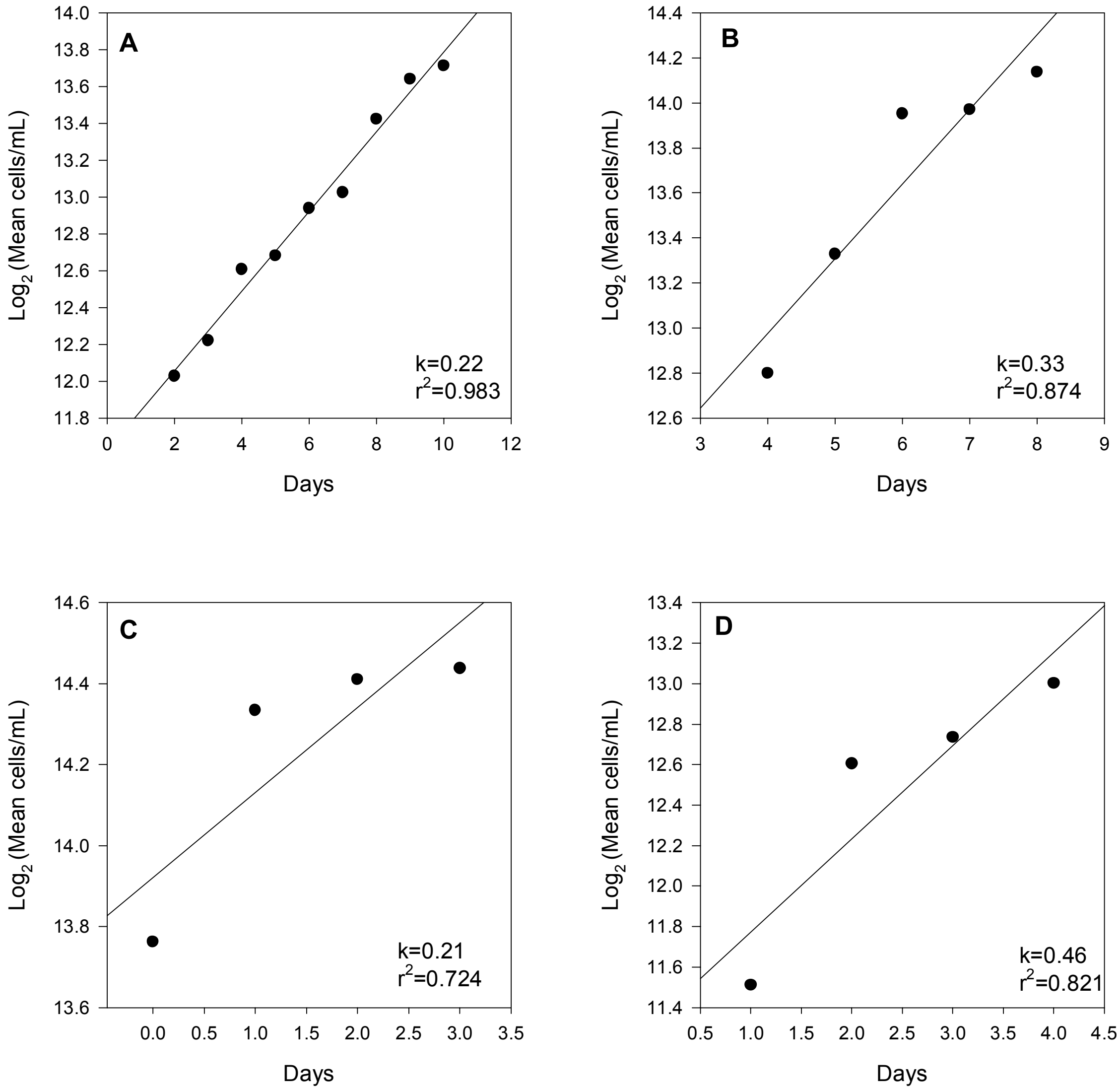

Figure 9. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under N-deficient conditions for Trial 2. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

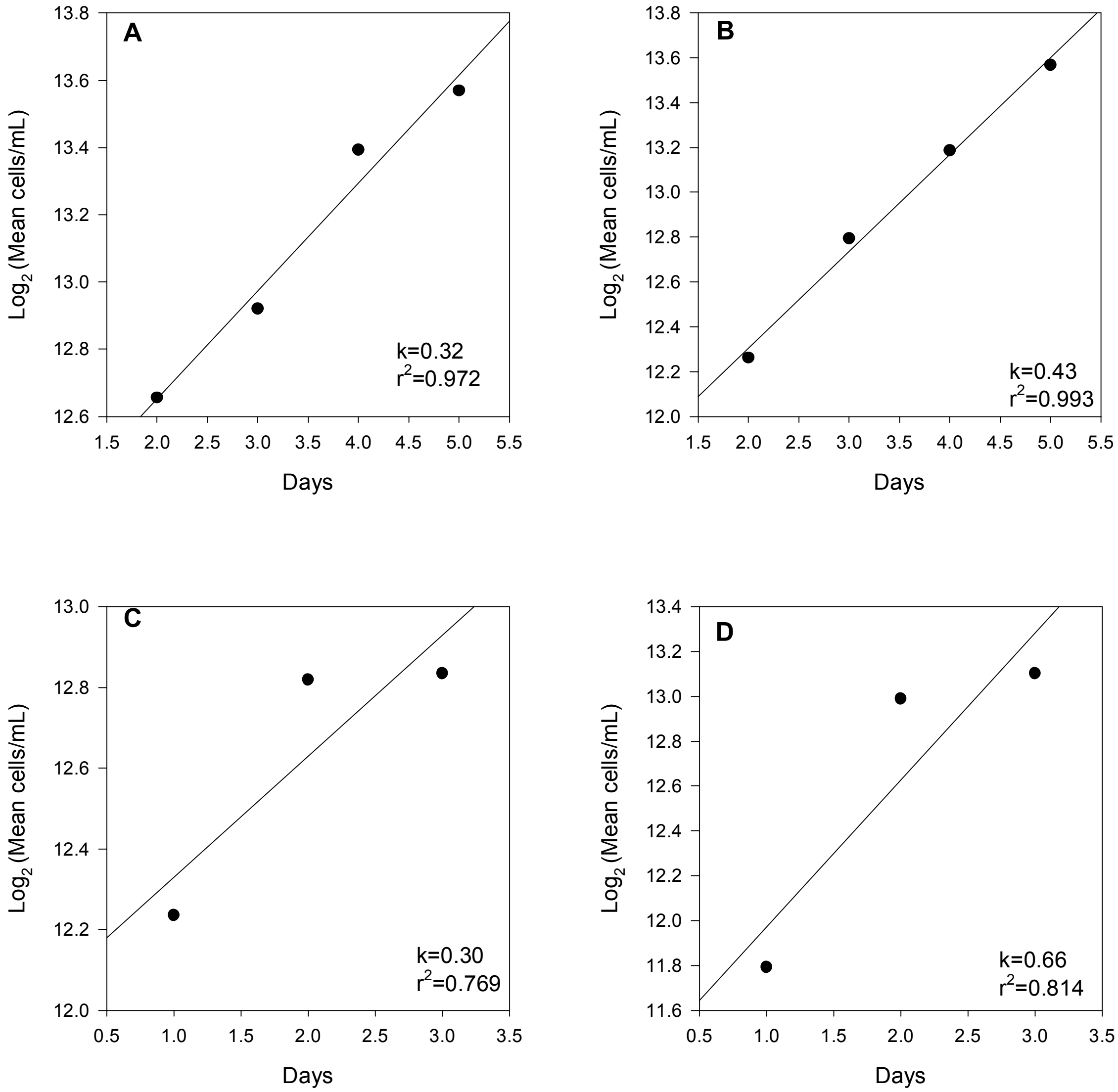

Figure 10. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under N-deficient conditions for Trial 3. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

three trials. *Prymnesium calathiferum* reached a terminal density higher than the TX clone, but less than the NC and SC clones for all three trials ($P<0.001$).

For Trials 1 and 2, the NC and SC clones had similar growth rates. For Trial 3, the SC clone had a much higher growth rate than the other three clones. The growth rate of the TX clone was much lower than the other clones for all trials, but was only significantly different from the SC clone ($P=0.042$) and *P. calathiferum* ($P=0.021$). *Prymnesium calathiferum* had the highest growth rate of the four clones for Trials 1 and 2 (Table 3, Figures 12-14), but the growth rates of the NC, SC, and *P. calathiferum* clones were not significantly different ($P\geq0.638$).

Overall, when averaging the trials and comparing nutrient treatments, all clones reached their maximum growth under nutrient-replete conditions (Figure 15). Nutrient-deficient conditions produced limited growth. The P-deficient conditions produced larger terminal densities than the N-deficient conditions for all four clones. *Prymnesium calathiferum* had the significantly ($P\leq0.019$) highest growth rate throughout the nutrient treatments (Table 3). The NC and SC clones had their highest growth rates in the nutrient-deficient treatments, particularly in the P-deficient treatment, which was significantly different ($P\leq0.035$) from the nutrient-replete treatment. The TX and *P. calathiferum* clones had similar growth rates throughout the nutrient treatments.

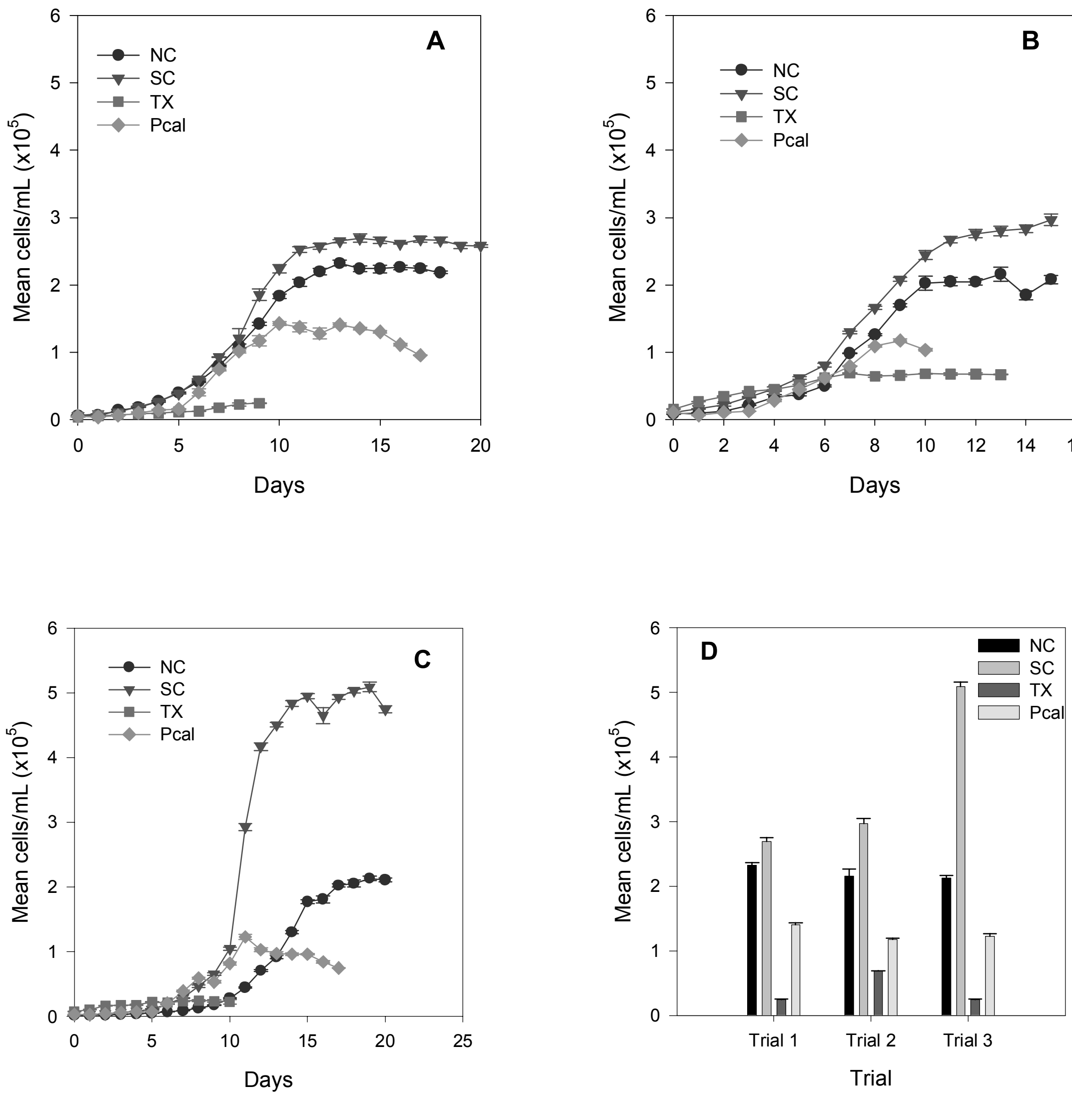

Figure 11. Growth of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* under P-deficient conditions. Error bars represent one standard deviation about the mean from four daily counts on a Coulter Counter. (A) Trial 1. (B) Trial 2. (C) Trial 3. (D) Terminal densities of the clones for each trial.

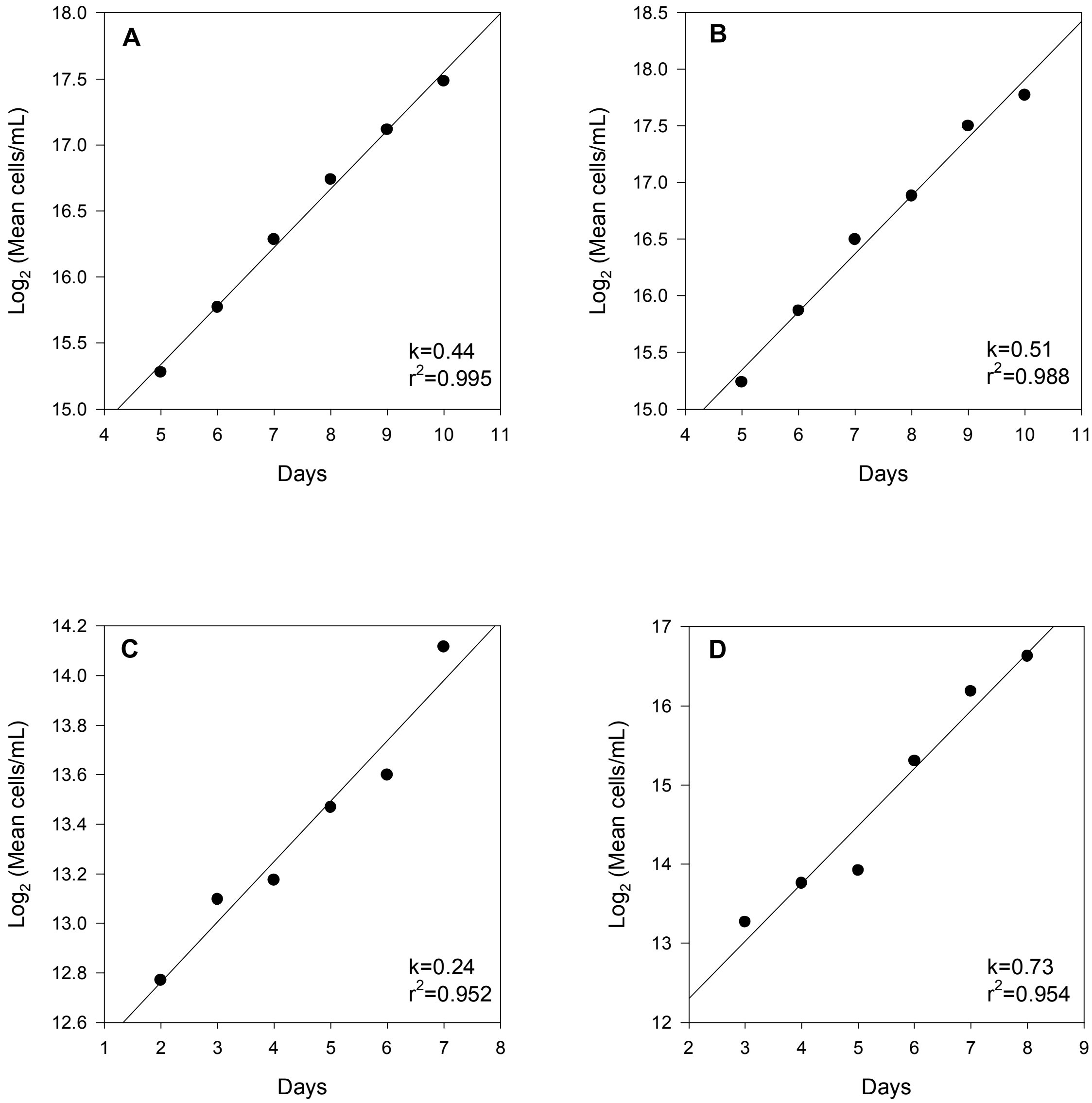

Figure 12. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under P-deficient conditions for Trial 1. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

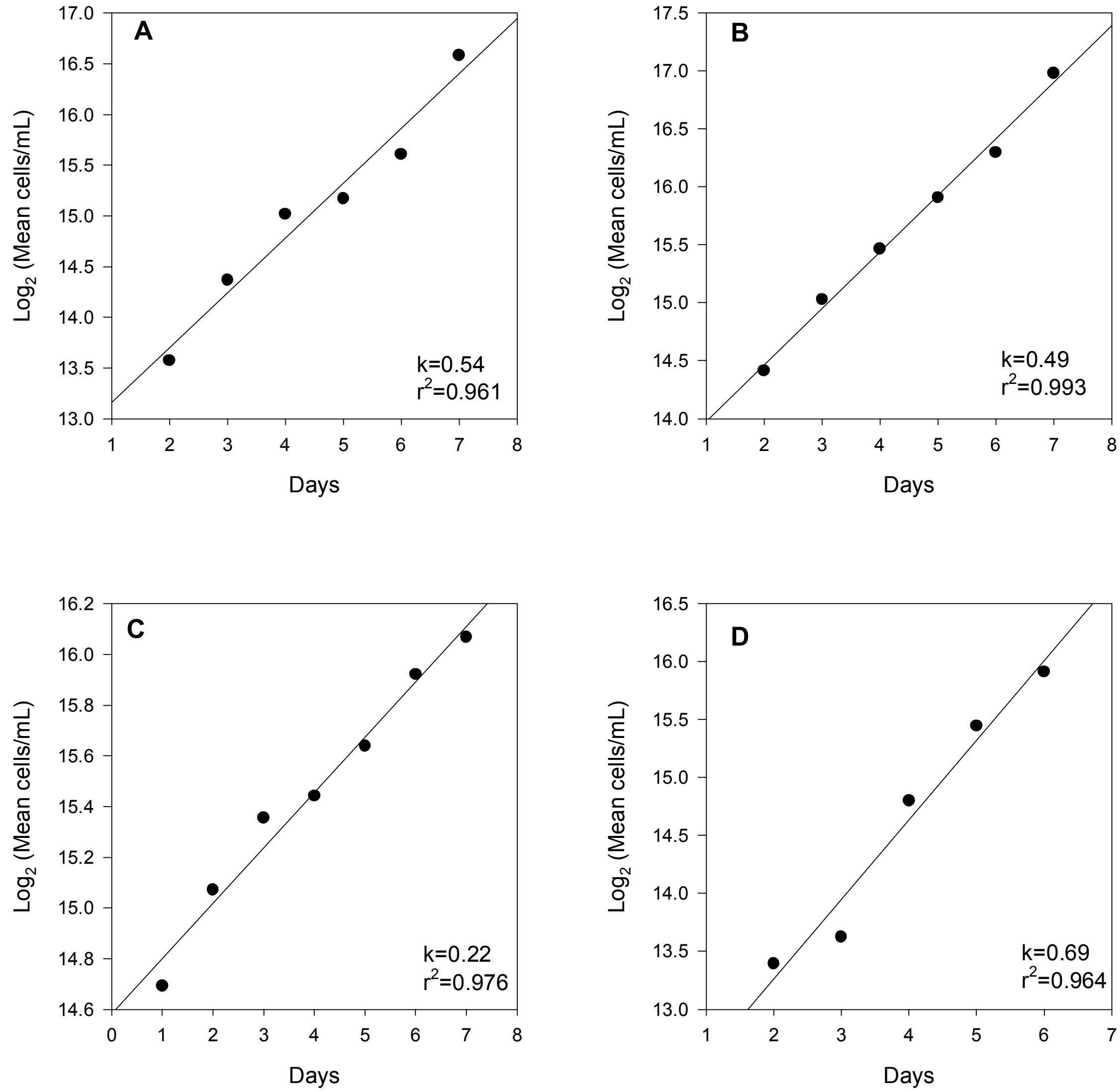

Figure 13. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under P-deficient conditions for Trial 2. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

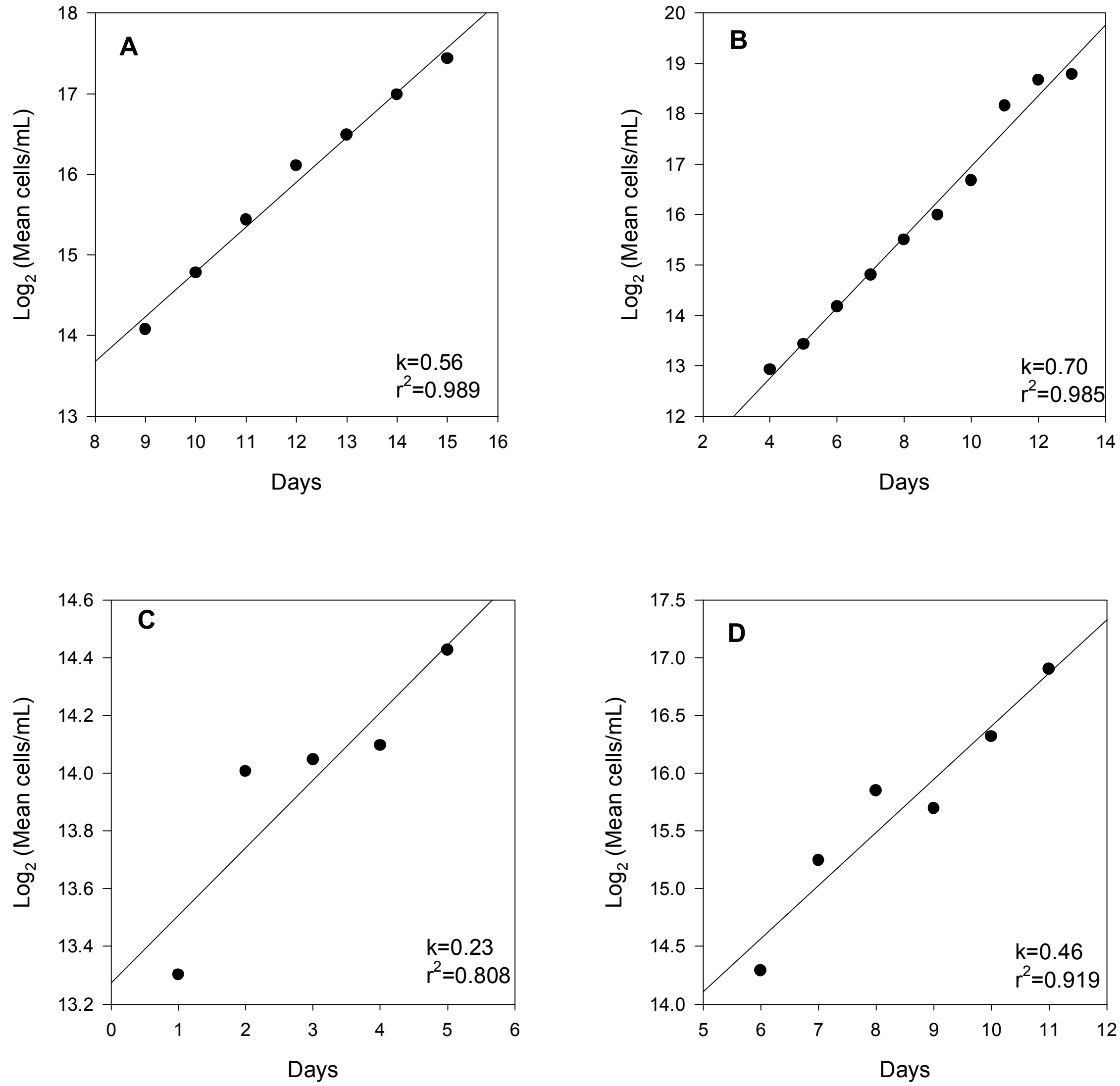

Figure 14. Regression analysis for log phase for three geographically-distinct clones of *Prymnesium parvum* and *P. calathiferum* under P-deficient conditions for Trial 3. (A) North Carolina (B) South Carolina (C) Texas (D) *P. calathiferum*

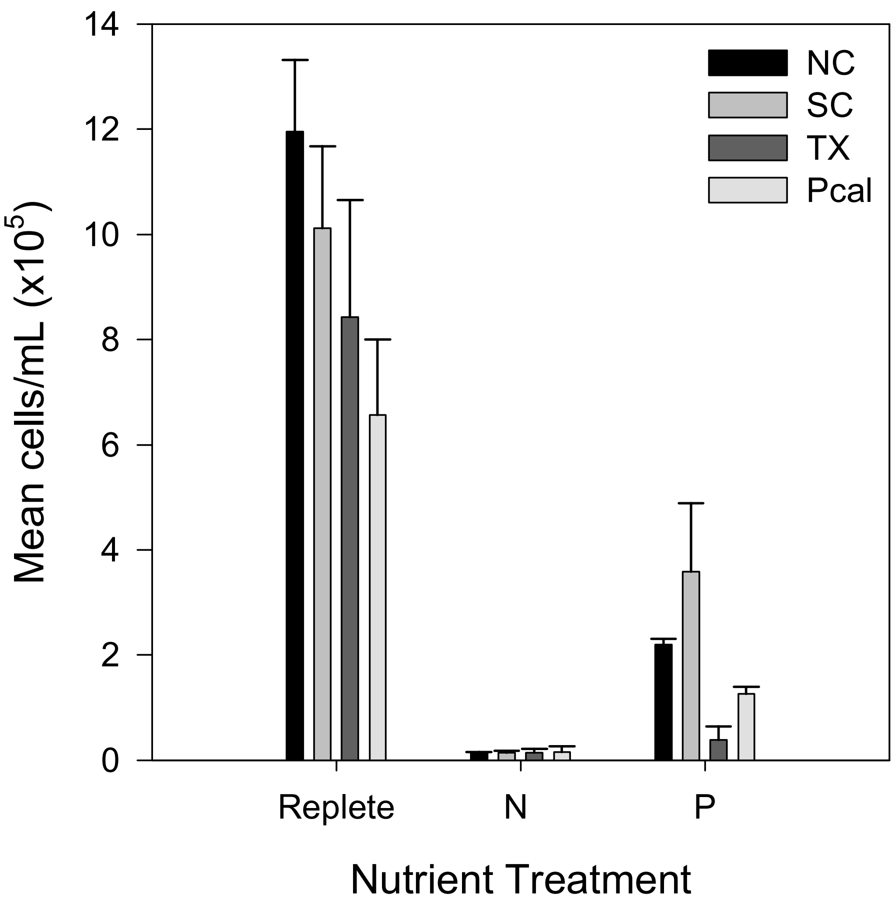

Figure 15. Mean terminal densities of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean of three trials.

Hemolytic Activity: Nutrient-Replete Cultures

There were no significant differences in mean hemolytic activity among the three *P. parvum* clones for either the supernatant ($P>0.300$) or the pellet ($P>0.600$) for any of the growth phases (Figures 16 and 17). For the supernatant, the NC and SC clones were significantly more hemolytic ($P<0.040$) than *P. calathiferum* in lag and stationary phases, but the TX clone (lag, $P=0.077$; stationary, $P=0.065$) was not statistically different from *P. calathiferum*. In log phase, all three *P. parvum* clones were significantly more hemolytic ($P\leq 0.004$) than *P. calathiferum*. For the pellet, the three *P. parvum* clones were more hemolytic ($P<0.040$) than *P. calathiferum* in lag and log phases. However, in stationary phases, there were no differences in mean hemolytic activity ($0.999<P<0.5$) among the four clones.

Lag phase was the most hemolytic ($P<0.030$) phase for the supernatant in all four clones. Lag phase was also the most hemolytic ($P\leq 0.007$) for the pellet for the NC and SC clones. For the TX clone, lag phase was the most hemolytic for the pellet, but was only statistically different from stationary phase ($P=0.003$) and not log phase ($P=0.059$). For *P. calathiferum*, there were no differences in hemolytic activity ($P>0.200$) among the growth phases for the pellet. There was no difference in hemolytic activity between the supernatant and the pellet for the NC, SC, and *P. calathiferum* clones. For the TX clone, the pellet was significantly more hemolytic ($P<0.001$) than the supernatant.

Hemolytic Activity: Nitrogen-Deficient Cultures

There were clonal differences in mean hemolytic activity for the supernatant (Figure 18A). The NC and SC clones were similar in hemolytic activity, but more

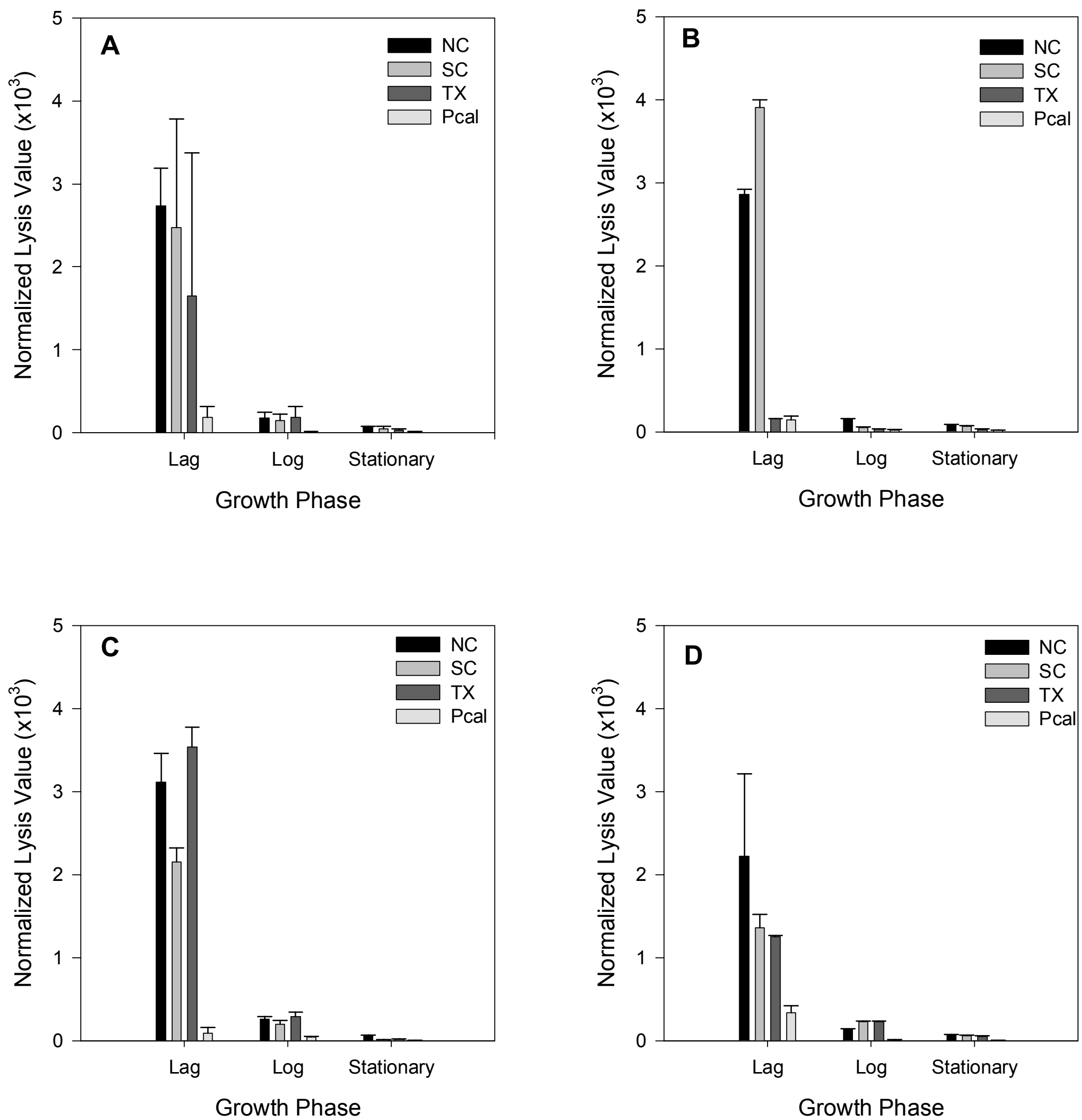

Figure 16. Hemolytic activity by the supernatant of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown under nutrient-replete conditions. Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

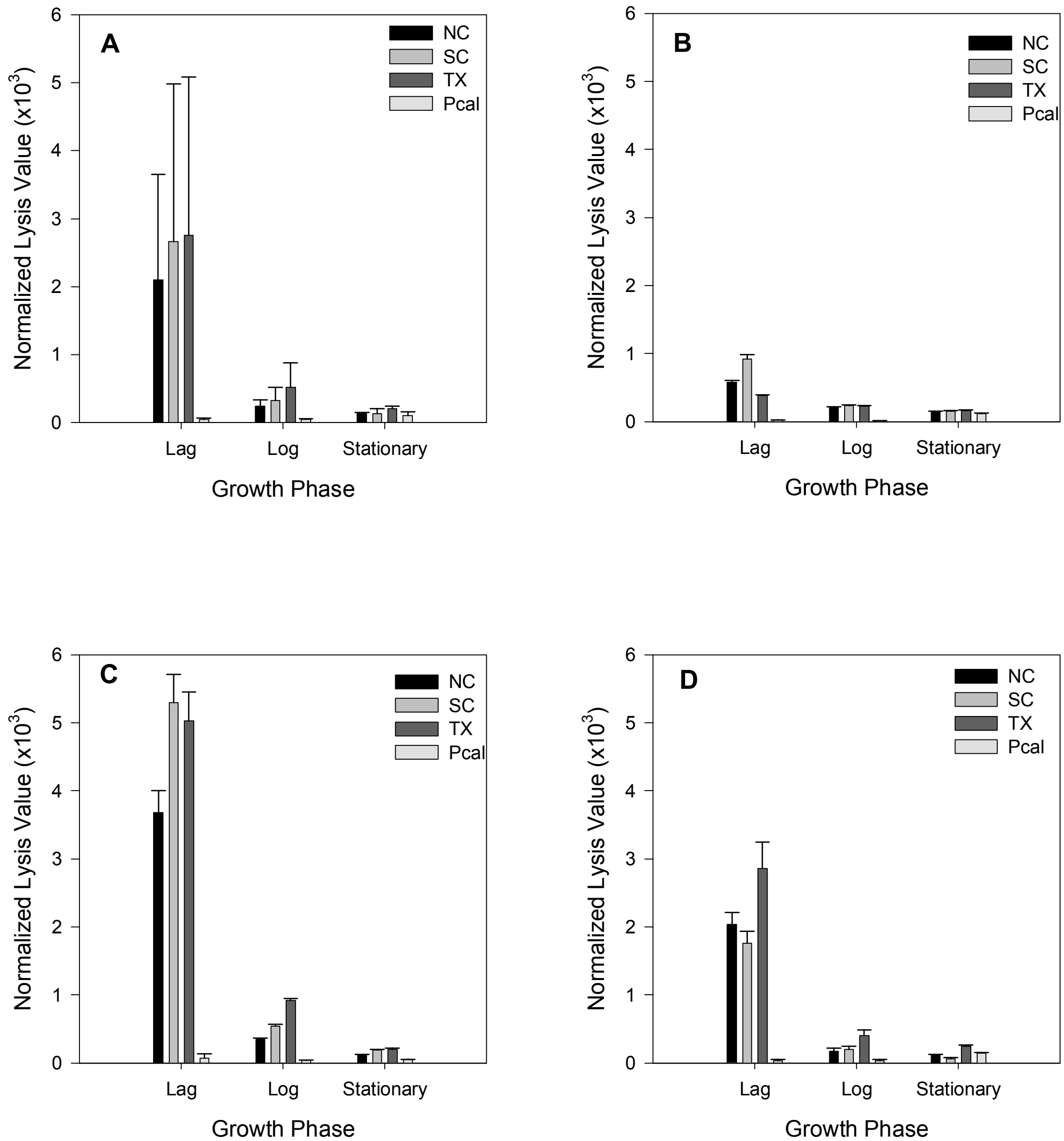

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Figure 17. Hemolytic activity by the pellet of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown under nutrient-replete conditions. Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3

hemolytic (P<0.001) than both the TX and *P. calathiferum* clones in all growth phases. This trend was supported by all three of the individual trials (Figure 18B-D). Overall, the TX clone was more hemolytic (P<0.001) than *P. calathiferum*, but differences in growth phase were seen. In lag phase, there was no significant difference (P=0.743) between the TX and *P. calathiferum* clones, but the TX clone was more hemolytic (P≤0.003) than *P. calathiferum* in log and stationary phases. There were no significant differences in hemolytic activity (P>0.100) among the growth phases for any of the *P. parvum* clones. For *P. calathiferum*, lag phase was more hemolytic than log (P=0.016) and stationary (P=0.010) phases.

For the pellet, there were no significant differences (P>0.400) in mean hemolytic activity among the three *P. parvum* clones (Figure 19A). In addition, all *P. parvum* clones were significantly more hemolytic (P<0.001) than *P. calathiferum* for all growth phases. For the NC and SC clones, lag phase was more hemolytic (P<0.050) than stationary phase. For the TX clone, there was no difference in hemolytic activity among the growth phases. For *P. calathiferum*, lag phase was the most hemolytic (P≤0.037). The supernatant was the most hemolytic for the NC, SC, and *P. calathiferum* clones (P≤0.011). For the TX clone, the pellet was the most hemolytic (P<0.001).

Hemolytic Activity: Phosphorus-Deficient Cultures

For the supernatant, clonal differences in mean hemolytic activity were seen in the *P. parvum* clones for individual growth phases (Figure 20A). The NC clone was significantly more hemolytic (P=0.016) than the TX clone during lag phase, while the TX clone was significantly more hemolytic (P=0.022) than the SC clone during stationary

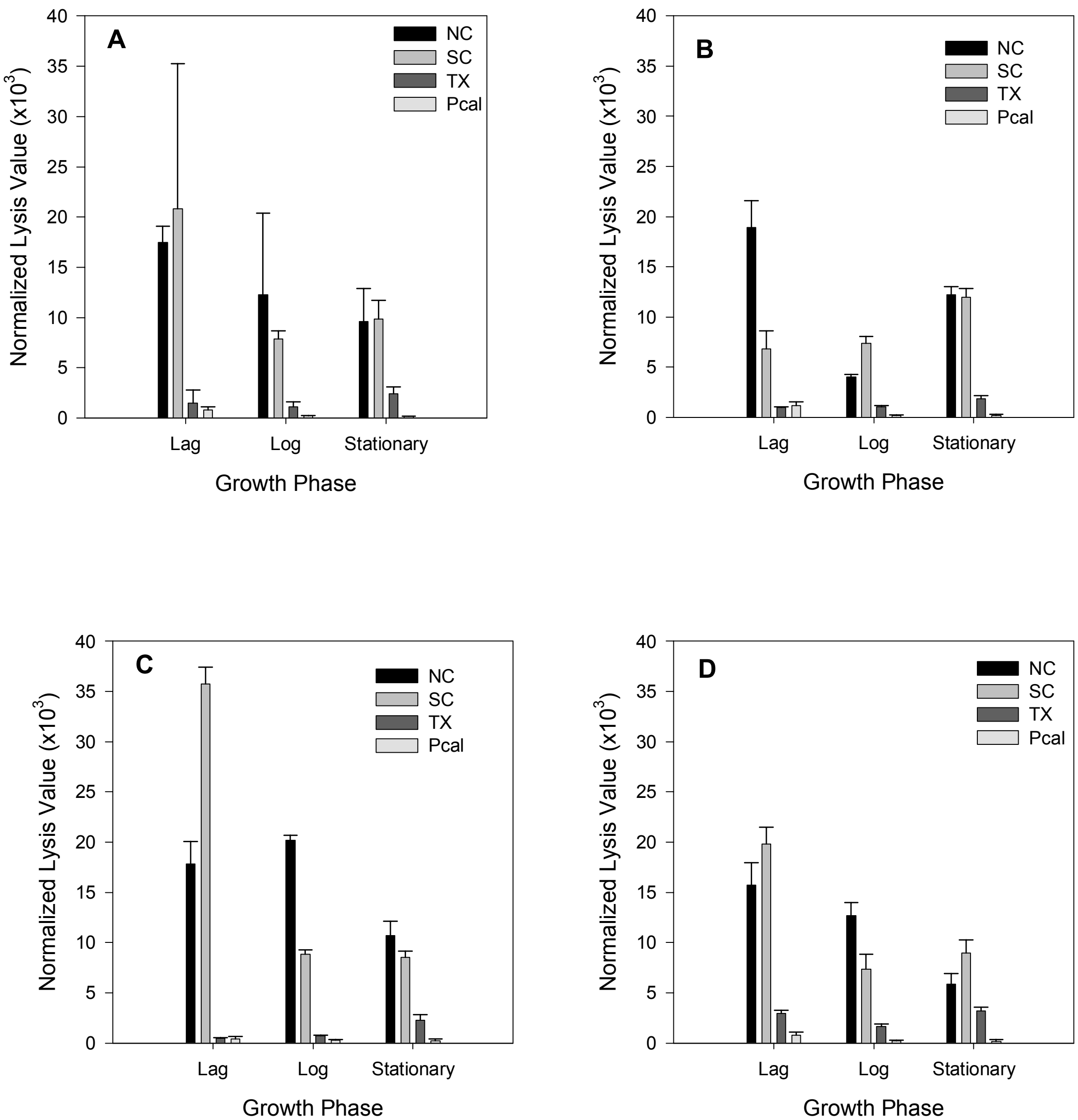

Figure 18. Hemolytic activity by the supernatant of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown under N-deficient conditions. Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

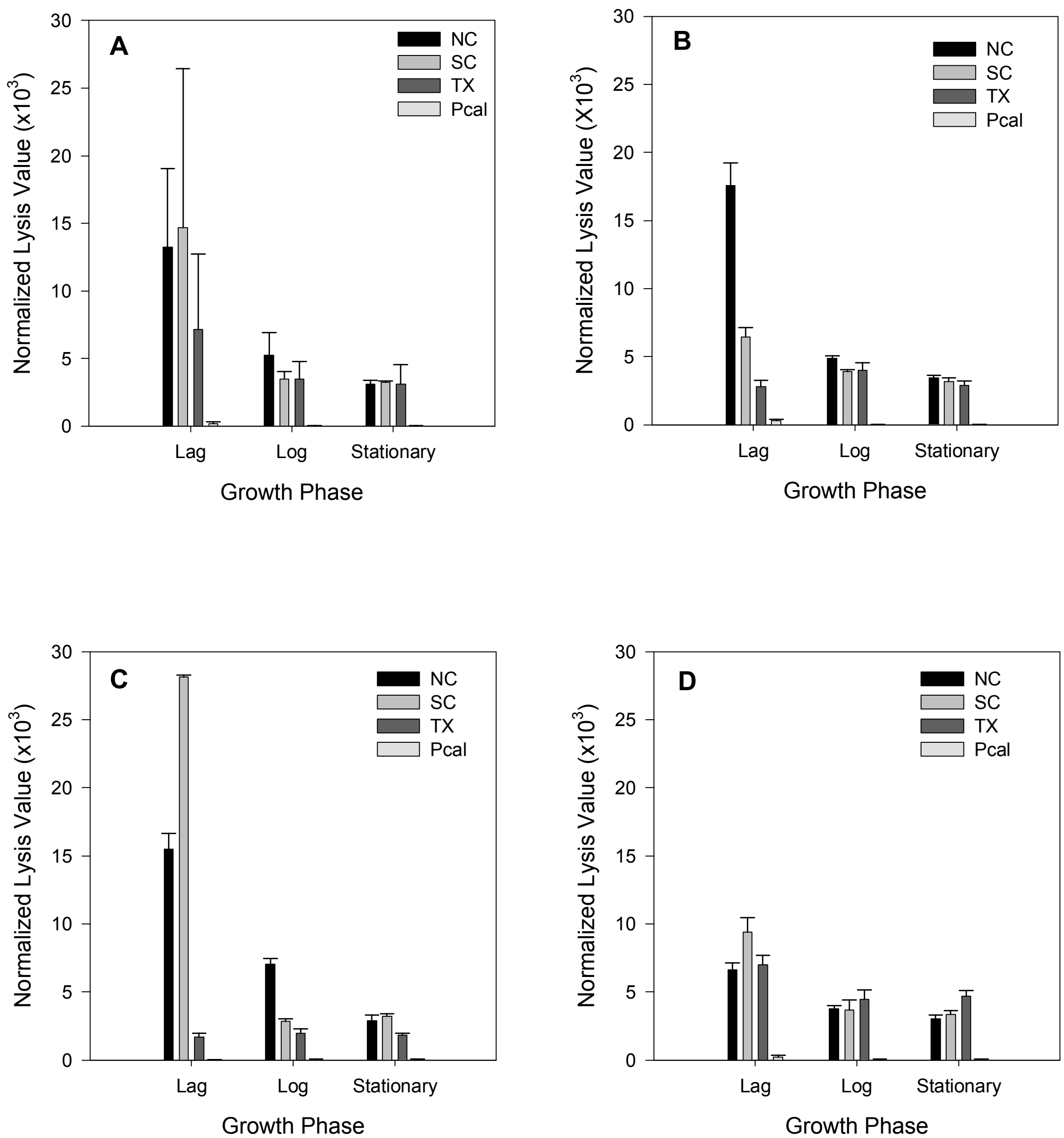

Figure 19. Hemolytic activity by the pellet of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown under N-deficient conditions. Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

phase. These two differences were supported by all three of the individual trials (Figure 20B-D). The NC and SC clones were significantly more hemolytic ($P<0.001$) than *P. calathiferum* for all growth phases. Overall, the TX clone was also more hemolytic ($P<0.001$) than *P. calathiferum*, but differences were seen in growth phases. The TX clone was only significantly more hemolytic ($P<0.001$) than *P. calathiferum* for log and stationary phases, but there was no significant difference ($P=0.059$) during lag phase. Lag phase was the most hemolytic phase ($P\leq0.022$) for the NC, SC, and *P. calathiferum* clones, but all phases of the TX clone had similar hemolytic activity ($P\geq0.755$).

For the pellet, there were no differences in mean hemolytic activity among the *P. parvum* clones for lag and log phases, but the TX clone was statistically more hemolytic than the NC ($P=0.027$) and SC ($P=0.008$) clones in stationary phase (Figure 21A). This difference was supported in all three of the individual trials (Figure 21B-D). Overall, the *P. parvum* clones were more hemolytic ($P<0.001$) than *P. calathiferum*, but this was not seen in the individual growth phases for the NC and SC clones. *Prymnesium calathiferum* was significantly less hemolytic than the NC and SC clones only for lag ($P<0.001$) and log ($P\leq0.022$) phases, but there was no difference among the three clones in stationary phase. For the NC and SC clones, lag phase was the most hemolytic phase ($P\leq0.012$). For the TX and *P. calathiferum* clones, there were no significant differences among growth phases. There was no difference among the supernatant and pellet for the four clones ($0.194\leq P\leq0.395$).

Hemolytic Activity: Effect of Nutrient Concentration

For the supernatant of the NC clone, the nutrient-replete culture was significantly less hemolytic ($P<0.001$) than the nutrient-deficient cultures for all growth phases (Figure

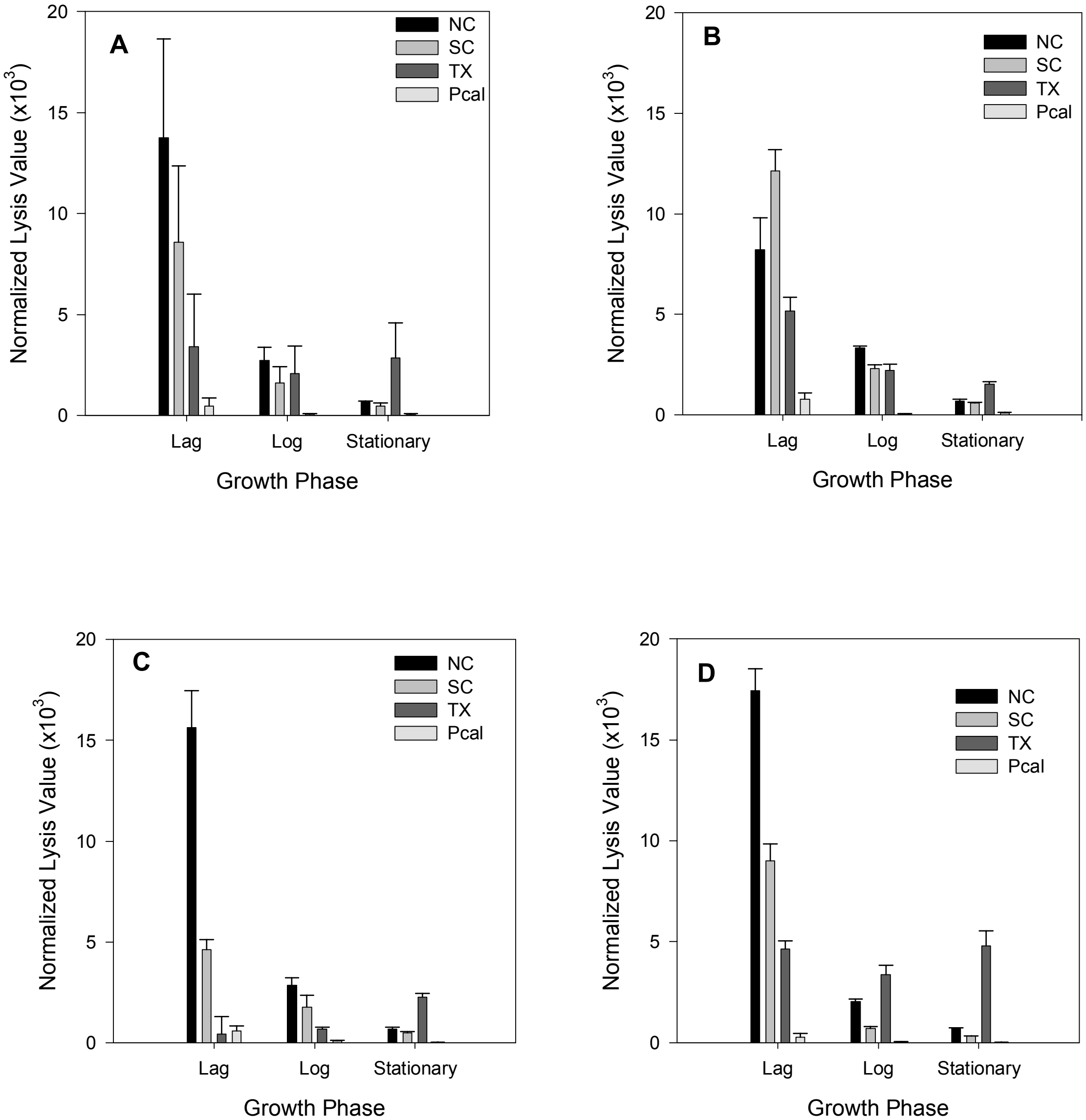

Figure 20. Hemolytic activity by the supernatant of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown under P-deficient conditions. Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

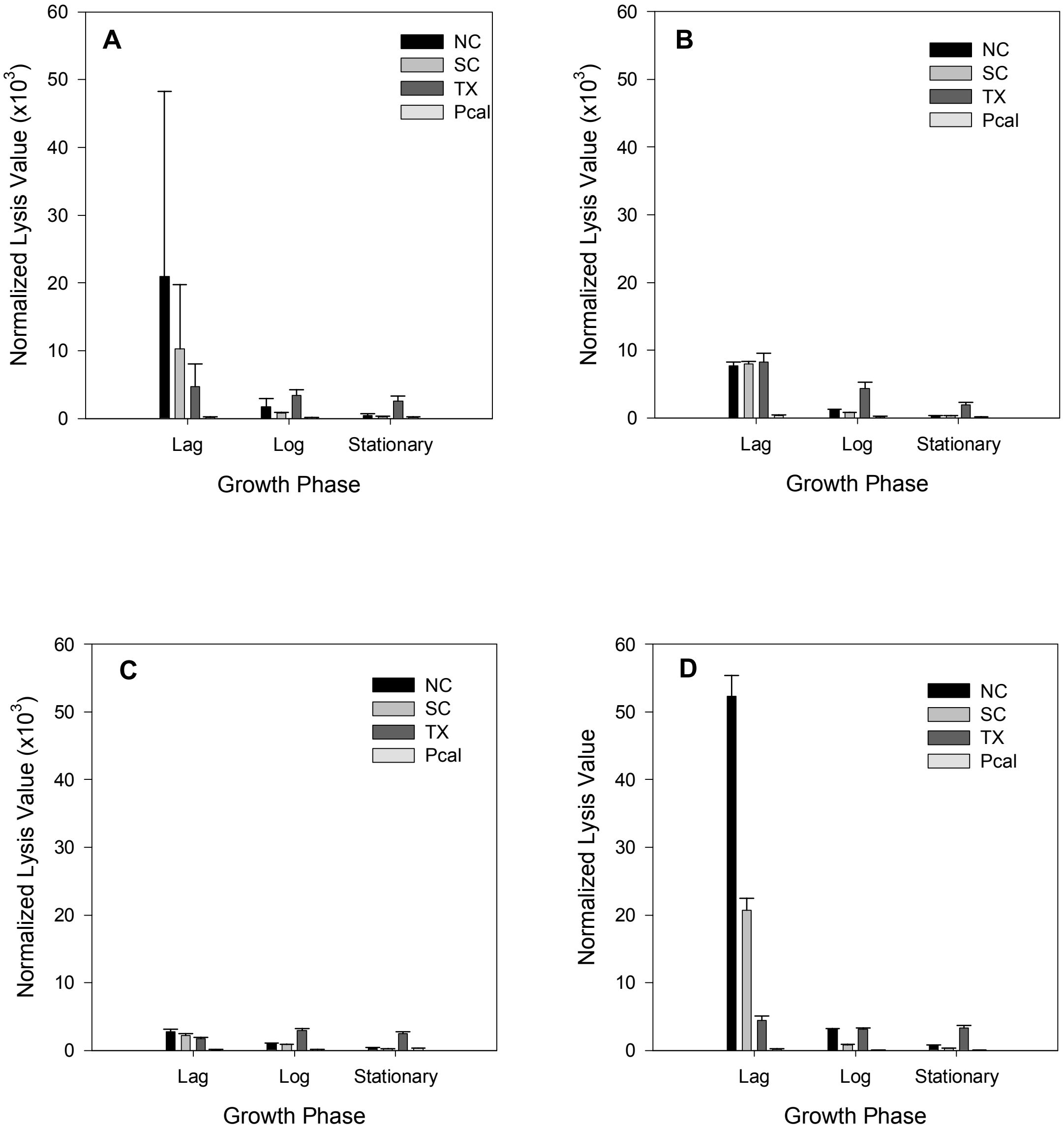

Figure 21. Hemolytic activity by the pellet of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown under P-deficient conditions. Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

22A). In lag phase, there was no significant difference in hemolytic activity for the nutrient-deficient cultures ($P=0.622$). This was supported in two of the three individual trials (Figure 22B-D). In the first trial, however, the N-deficient culture was significantly more hemolytic ($P<0.001$) than the P-deficient culture. In log and stationary phases, the N-deficient culture was more hemolytic ($P\leq0.001$) than the P-deficient culture.

For the pellet of the NC clone, the nutrient-replete culture was significantly less hemolytic than the nutrient-deficient cultures ($P\leq0.012$) for both lag and log phases (Figure 23A). In stationary phase, however, the nutrient-replete culture was less hemolytic ($P<0.001$) than the N-deficient culture, but there was no difference ($P=0.132$) between the nutrient-replete and P-deficient cultures. The N-deficient culture was only significant more hemolytic than the P-deficient culture in stationary phase ($P=0.006$) and not in log phase ($P=0.121$). For Trials 1 and 2, the N-deficient culture was more hemolytic than the P-deficient culture for all growth phases (Figure 23B-C). However, in Trial 3, the P-deficient culture had a much higher normalized hemolysis value in lag phase than in the other trials and was more hemolytic than the N-deficient culture (Figure 23D).

For the supernatant of the SC clone, the nutrient-replete culture was significantly less hemolytic than the nutrient-deficient cultures ($P\leq0.05$) in all growth phases (Figure 24A). In lag phase, there was no difference between the nutrient-deficient cultures. The P-deficient culture was the most hemolytic for Trial 1 (Figure 24B), but the N-deficient culture was the most hemolytic Trials 2 and 3 in lag phase (Figure 24C-D). However, the N-deficient culture was significantly more hemolytic ($P\leq0.008$) than the P-deficient culture in log and stationary phases.

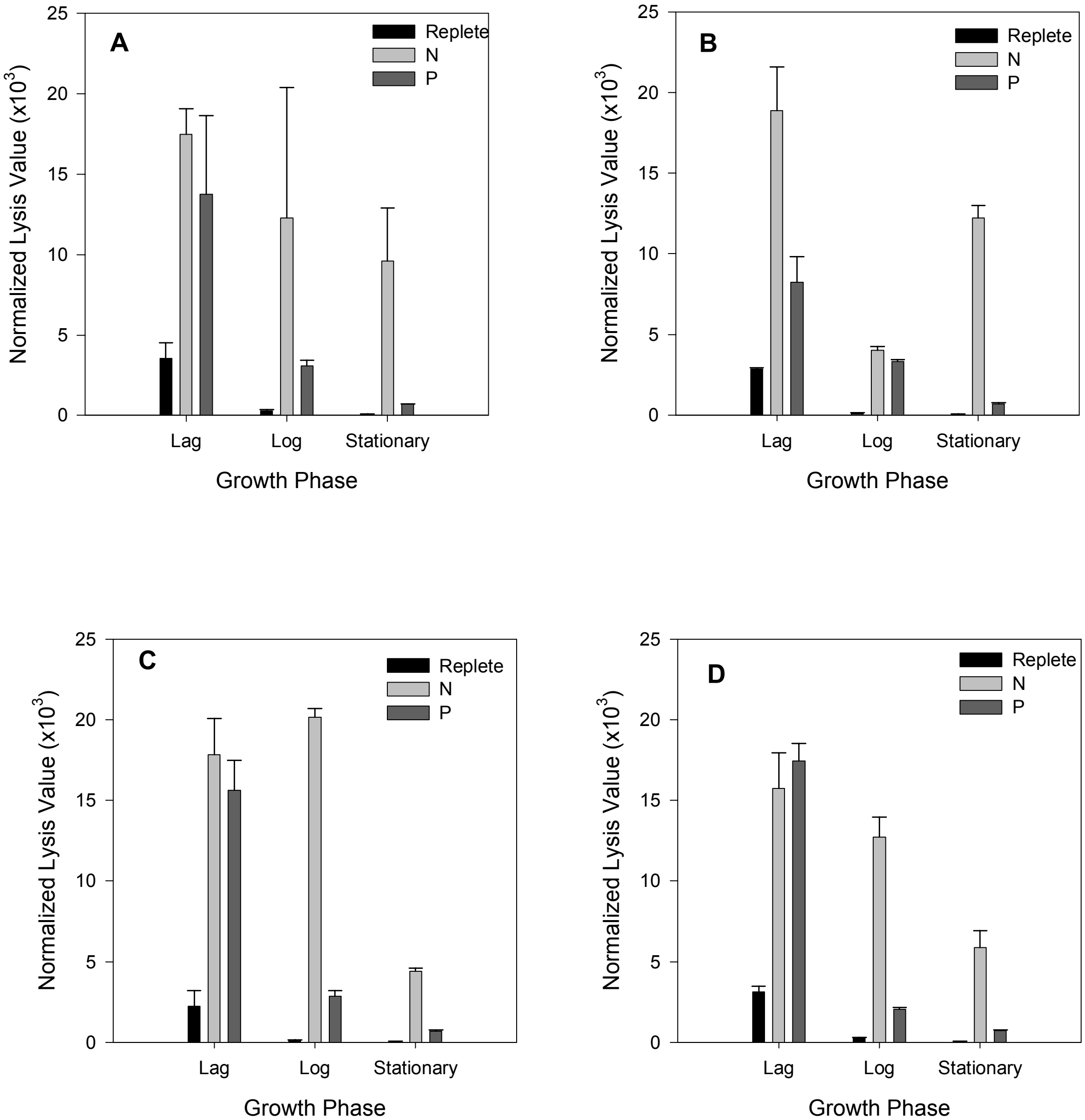

Figure 22. Hemolytic activity by the supernatant of the North Carolina *Prymnesium parvum* clone grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

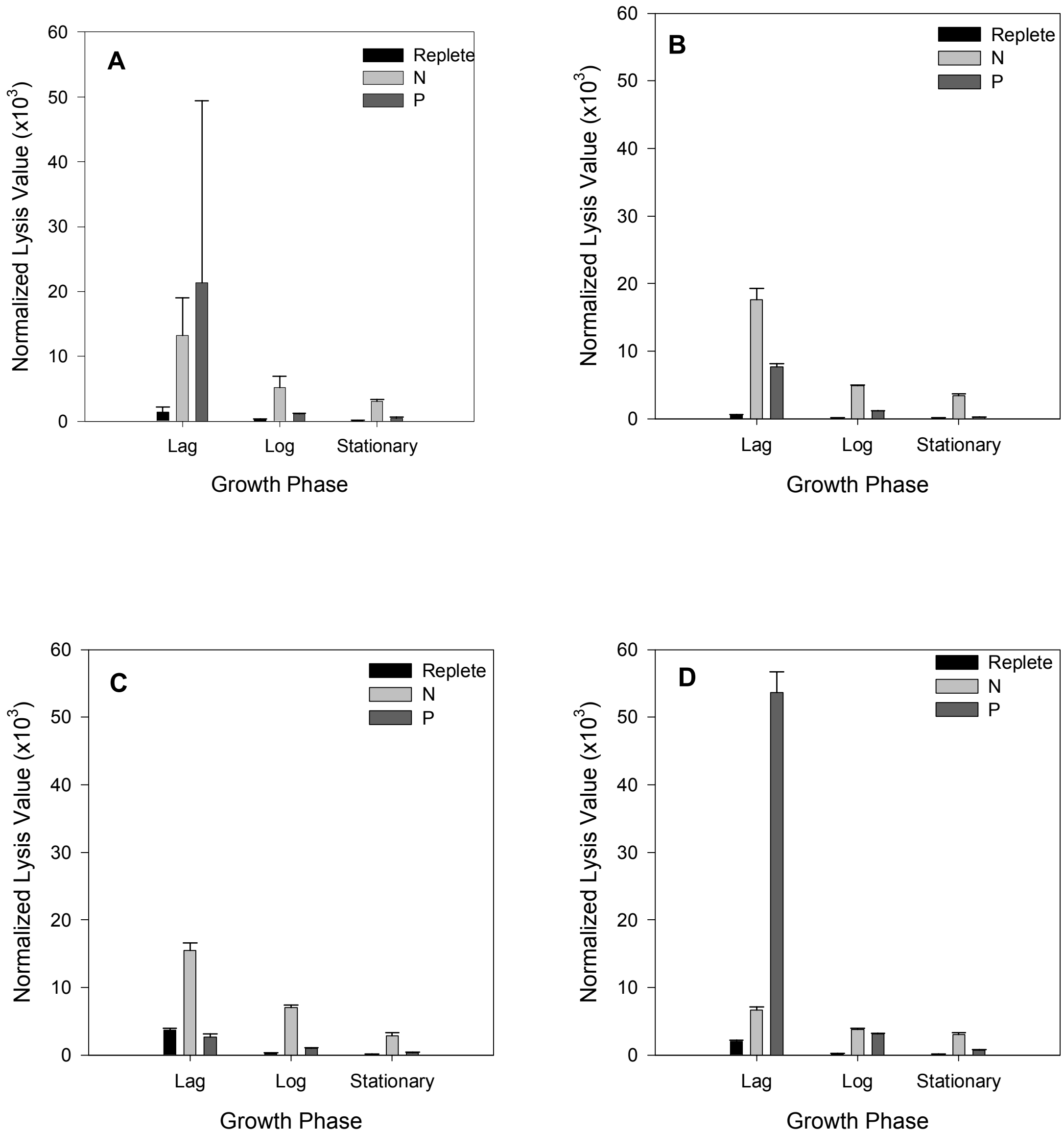

Figure 23. Hemolytic activity by the pellet of the North Carolina *Prymnesium parvum* clone grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

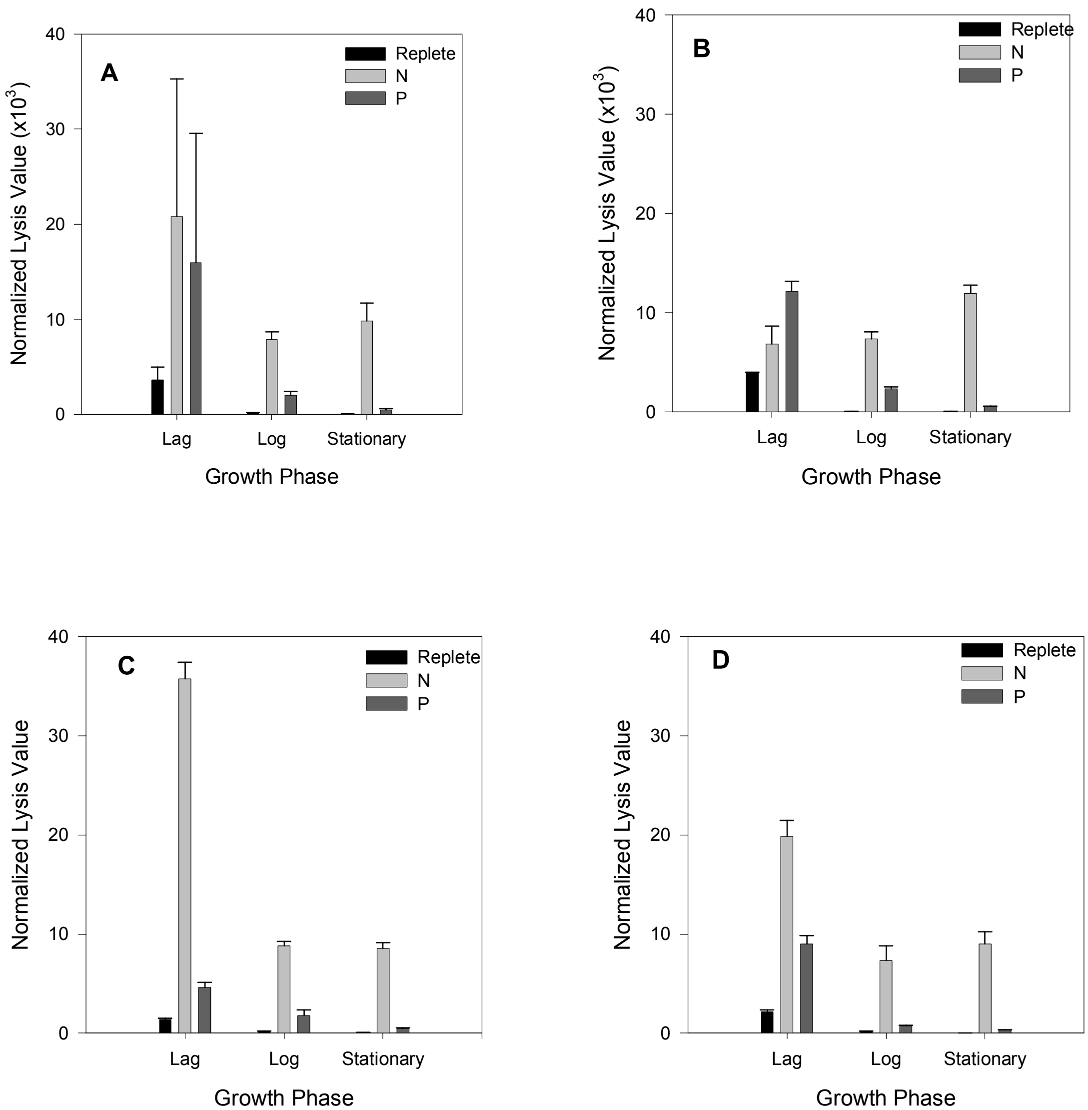

Figure 24. Hemolytic activity by the supernatant of the South Carolina *Prymnesium parvum* clone grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

For the pellet of the SC clone, the nutrient-replete culture was significantly less hemolytic ($P=0.010$) than the nutrient-deficient cultures in lag phase (Figure 25A). There was no overall difference in hemolytic activity between the nutrient-deficient cultures in lag phase. The P-deficient culture was the most hemolytic in two trials (Figure 25B, 25D), while the N-deficient culture was the most hemolytic in one trial (Figure 25C). In log and stationary phases, the N-deficient culture was more hemolytic than the other cultures ($P<0.040$), but the P-deficient and nutrient-replete cultures were not significantly different ($P>0.130$).

For the TX clone, the nutrient-replete culture was significantly less hemolytic than the nutrient-deficient cultures for only log and stationary phases for hemolysis by both the supernatant ($P\leq0.041$) and pellet ($P<0.080$) (Figures 26 and 27). There were no significant differences between the nutrient-deficient cultures for any of the growth phases.

For the supernatant of *P. calathiferum*, the nutrient-replete culture was less hemolytic ($P=0.027$) than the N-deficient culture in lag phase (Figure 28). There was no significant difference between the nutrient-replete and P-deficient cultures in lag phase. The nutrient-replete culture was significantly less hemolytic than the both nutrient-deficient cultures in log and stationary phases ($P\leq0.010$). The N-deficient culture was more hemolytic than the P-deficient culture in log ($P=0.069$) and stationary ($P=0.007$) phases, but there was no difference between the two nutrient-deficient cultures in lag phase. For the pellet of *P. calathiferum*, there were no effects of nutrient concentration on mean hemolytic activity (Figure 29).

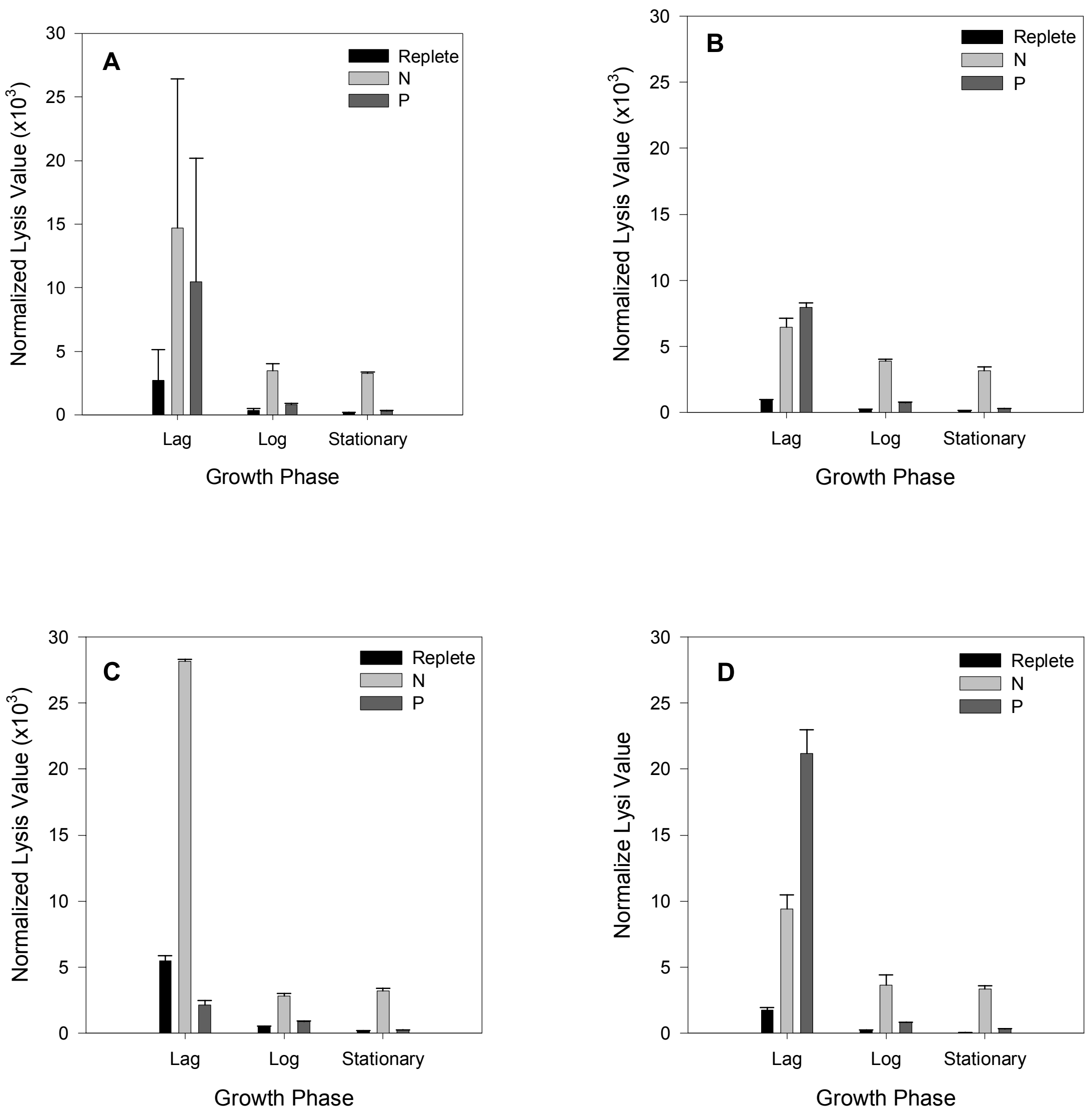

Figure 25. Hemolytic activity by the pellet of the South Carolina *Prymnesium parvum* clone grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

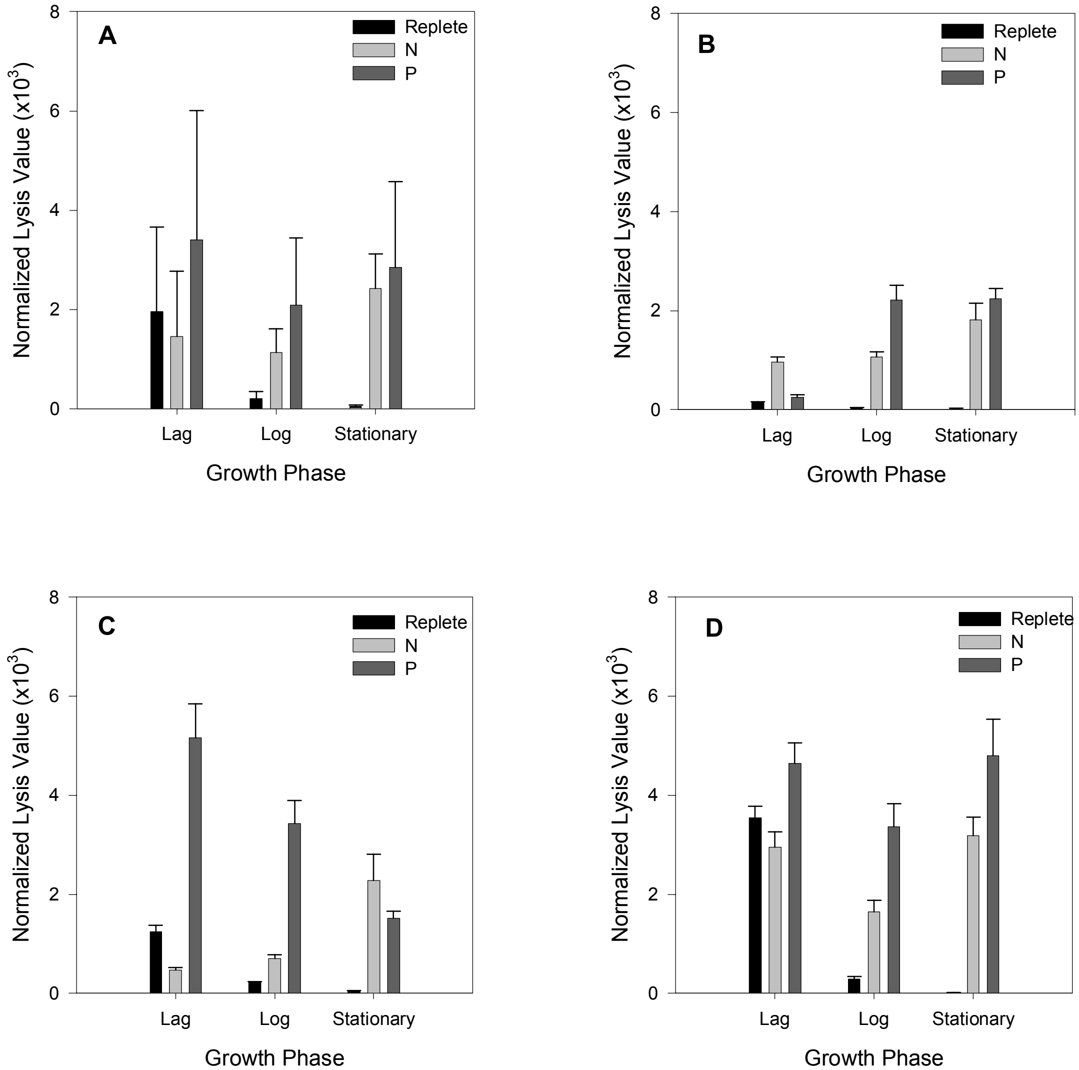

Figure 26. Hemolytic activity by the supernatant of the Texas *Prymnesium parvum* clone grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

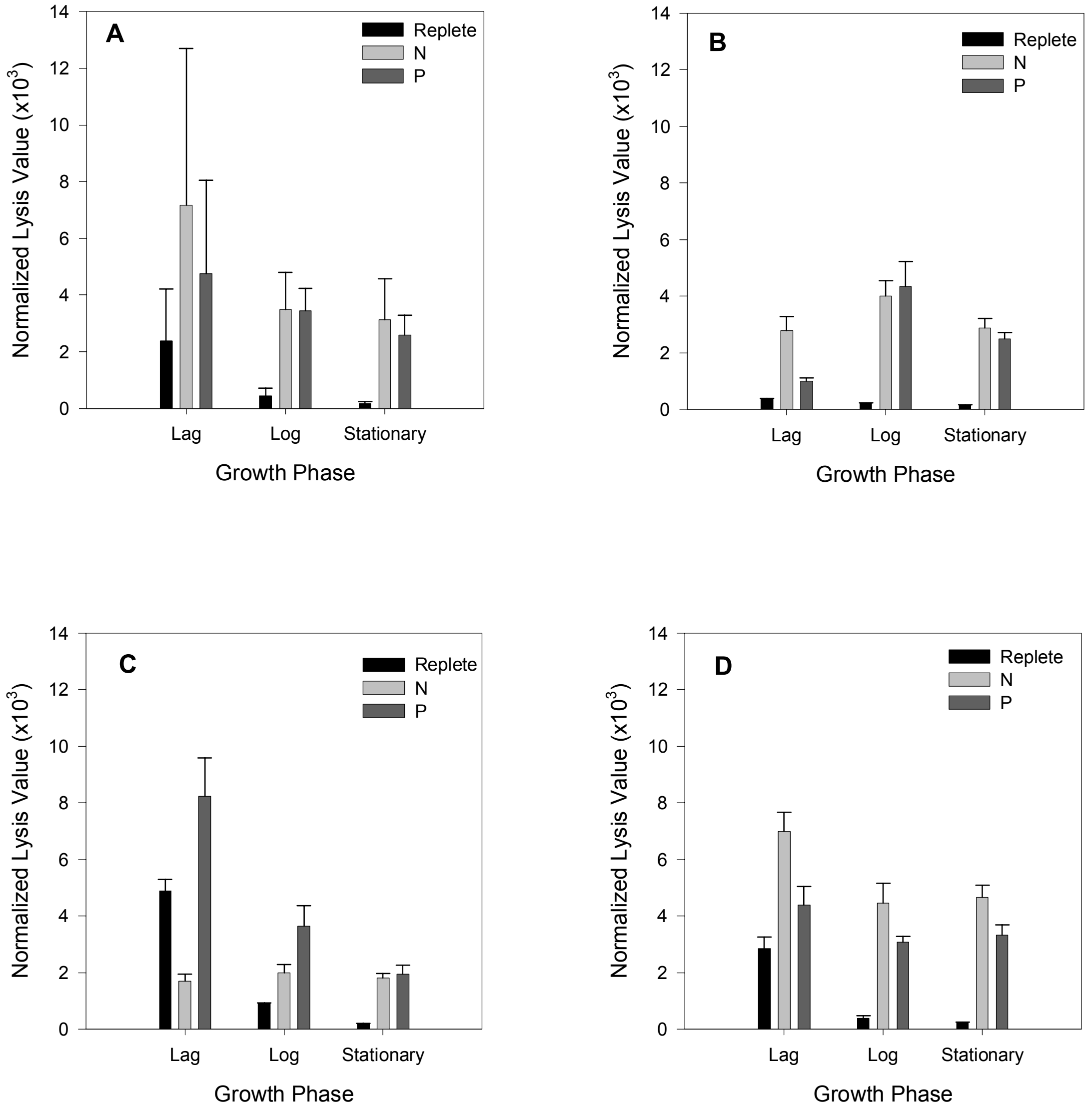

Figure 27. Hemolytic activity by the pellet of the Texas *Prymnesium parvum* clone grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

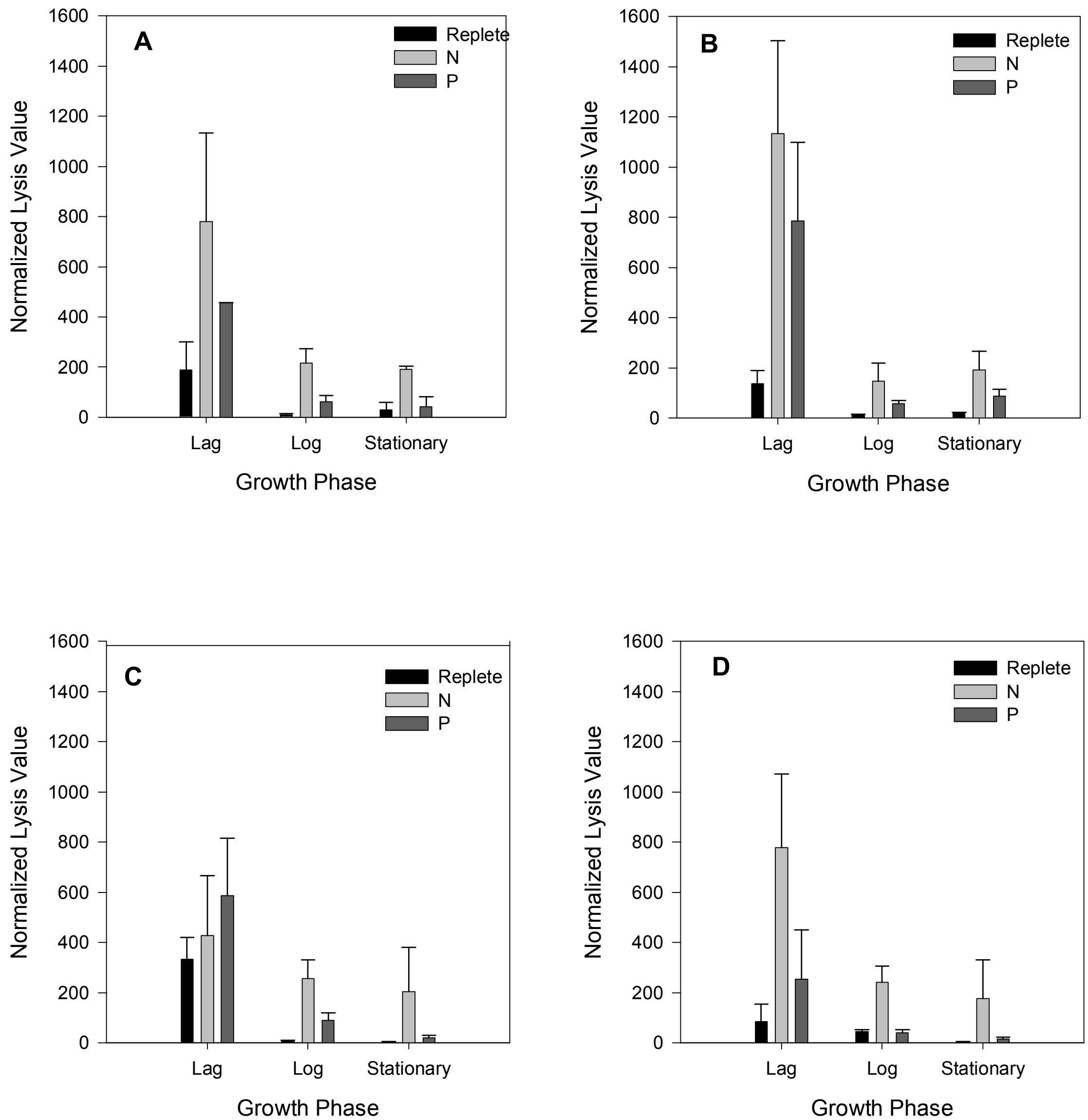

Figure 28. Hemolytic activity by the supernatant of *Prymnesium calathiferum* grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

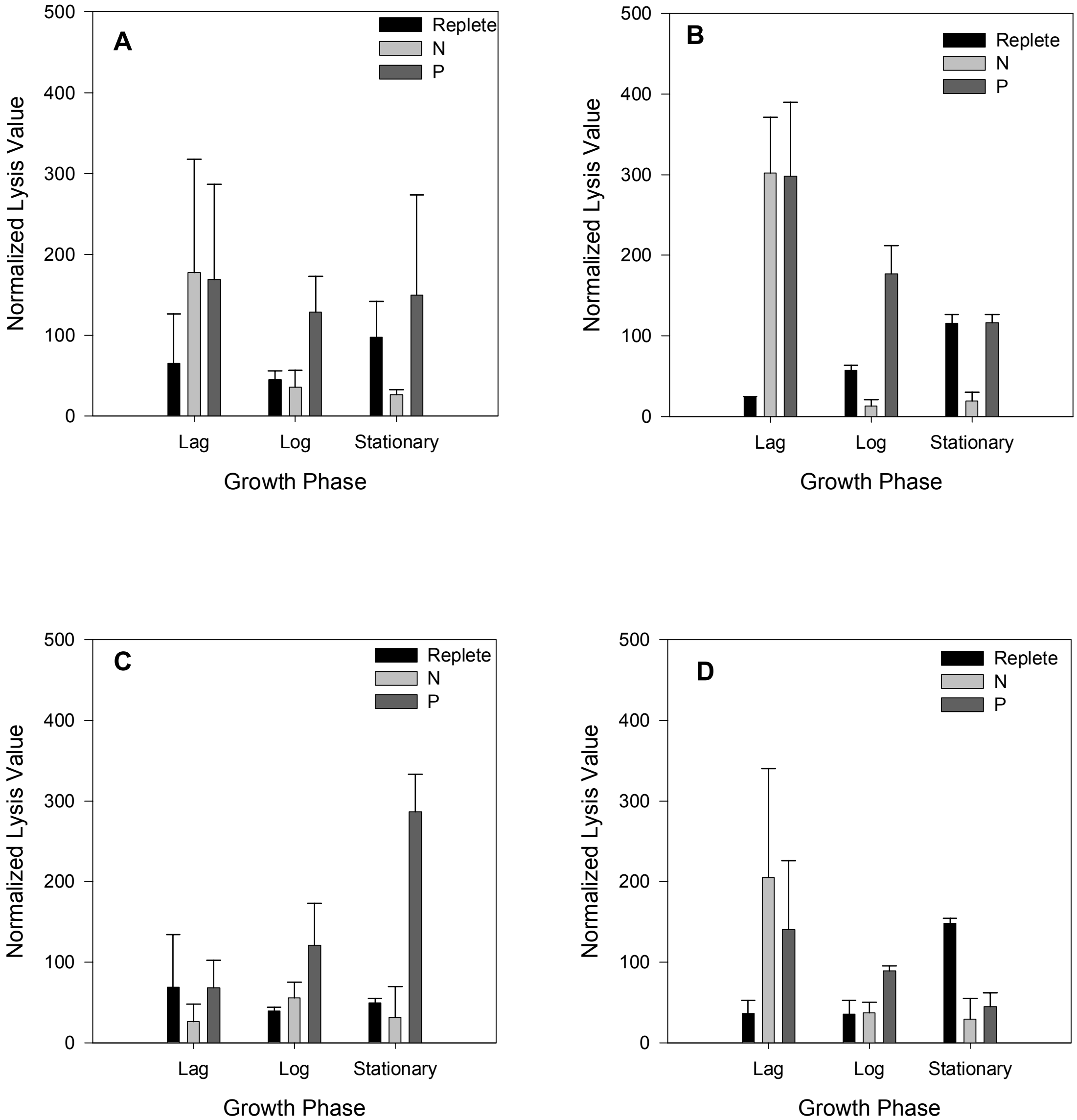

Figure 29. Hemolytic activity by the pellet of *Prymnesium calathiferum* grown under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean. (A) Means of the three trials. (B) Trial 1. (C) Trial 2. (D) Trial 3.

Ichthyotoxicity

The fish in the control beakers survived in each trial and did not show signs of ichthyotoxicity. None of the test fish displayed visible symptoms of the hemolytic part of the toxin. There was no visible bleeding from the snout or the gills, and there was no release of blood into the surrounding medium.

The nutrient-replete *P. calathiferum* culture was more ichthyotoxic than the *P. parvum* cultures. Two fish were killed in one hour in the 100% concentration of *P. calathiferum*, while only one fish was killed in three hours in the 100% concentration of the TX clone (Table 4). Fish were not killed in any of the concentrations for the other *P. parvum* clones. *Prymnesium calathiferum* was also the least dense and least hemolytic of the four clones for this nutrient treatment (Figures 30-31).

The P-deficient *P. parvum* cultures were the most ichthyotoxic of the nutrient treatments. For the NC clone, fish were killed at all concentrations of the P-deficient culture. Only one fish was killed with the N-deficient NC culture and no fish were killed with the nutrient-replete NC culture. For the SC clone, only the P-deficient culture caused fish kills. For the TX clone, all six fish were killed at all culture concentrations of the P-deficient cultures. Three fish were killed with the N-deficient TX culture and one was killed in the nutrient-replete TX culture.

The TX clone was the most ichthyotoxic, most hemolytic, and least dense of the *P. parvum* clones for all nutrient treatments (Table 4, Figures 30-31). The TX clone killed fish in all nutrient treatments, while the SC clone only killed fish under P-deficient conditions and the NC clone only killed under nutrient-deficient conditions. In addition, it took less time to kill fish exposed to the TX clone compared with the NC and SC clones. It took only one hour to kill fish

Table 4. Time (in hours) required to kill two fish in each concentration for three geographically-distinct clones of *Prymnesium parvum** and *P. calathiferum*.

Nutrient Treatment	Concentration	NC	SC	TX	Pcal
Replete					
	10%	—	—	—	—
	50%	—	—	—	—
	100%	—	—	3**	1.5
N-deficient					
	10%	—	—	—	—
	50%	—	—	3**	—
	100%	4**	—	3	—
P-deficient					
	10%	6**	—	2	—
	50%	5.5	—	1	5.5**
	100%	4	5.5	1	—

* NC=North Carolina; SC=South Carolina; TX=Texas
**=only 1 fish killed; — = no fish killed

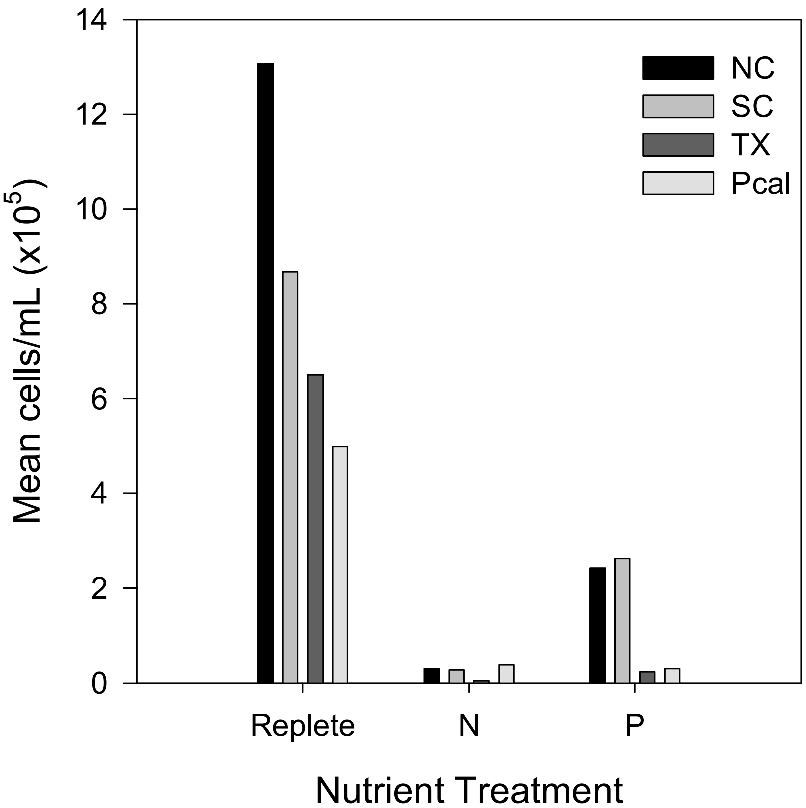

Figure 30. Cell densities for the fish bioassays of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* under different nutrient treatments (Replete, N-Deficient, P-Deficient).

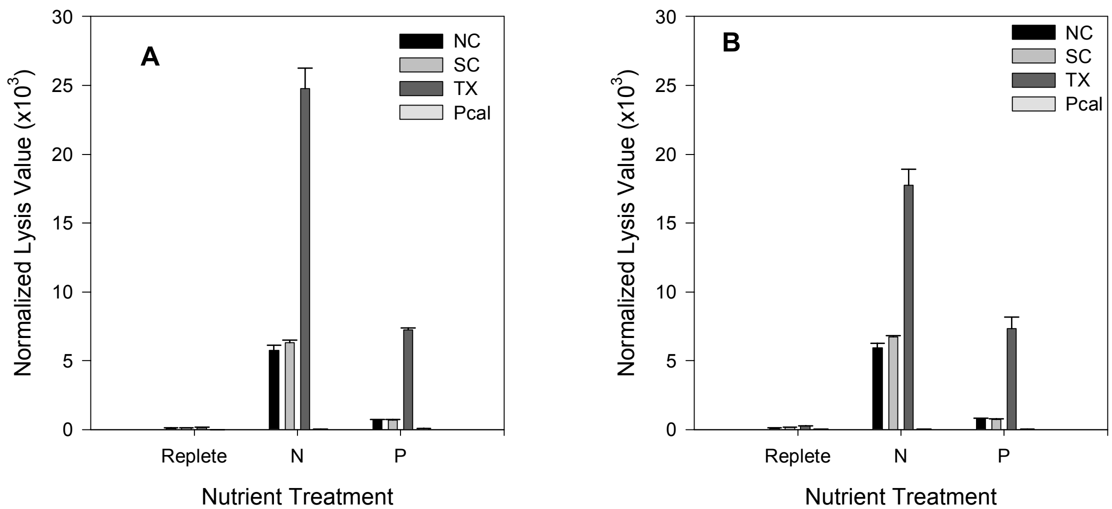

Figure 31. Hemolytic activity for the fish bioassays of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* under different nutrient treatments (Replete, N-Deficient, P-Deficient). Error bars represent one standard deviation about the mean (n=8). (A) Hemolytic activity by the supernatant. (B) Hemolytic activity by the pellet.

with the TX clone, while it took over three hours for the NC and SC clones to kill fish. For the 100% concentration of the phosphorus-deficient cultures, it took the NC clone 4 hours to kill fish, while TX killed fish in 2.5 hours.

All *P. parvum* clones were more hemolytic in nitrogen-deficient conditions, yet more ichthyotoxic under phosphorus-deficient conditions (Table 4, Figures 30-31).

DISCUSSION

Growth

The *P. parvum* clones had similar growth rates for the nutrient-replete treatments, but had differences when grown in nutrient-deficient media. All four clones experienced smaller terminal densities in the nutrient-deficient treatments, indicating that nutrient limitation caused limited growth. The TX clone, however, had lower growth rates than the SC clone for the N-deficient treatment and both the NC and SC clones in the P-deficient treatment. As mentioned previously, the TX clone showed no growth after two weeks of pre-conditioning in nutrient-deficient media. Even with only one week of pre-conditioning, growth was severely limited with smaller terminal densities.

Hemolytic Activity

There were more similarities than differences in hemolytic activity among the *P. parvum* clones. In the nutrient-replete treatment, there were no differences among the clones. In the N-deficient treatment, clonal differences occurred in the supernatant only with the NC and SC clones having similar hemolytic activity and the TX clone being less hemolytic. For the P-deficient treatment, clonal differences were seen only in the individual growth phases. For the supernatant, the TX clone was less hemolytic than the NC clone in lag phase, but more hemolytic than the SC clone in stationary phase. For the pellet, the TX clone was more hemolytic than the NC and SC clones in stationary phase.

Overall, the NC and SC clones were more similar to each other than to the TX clone. The NC and SC clones had similar hemolytic activity, but each deviated from the TX clone on various occasions.

Hemolytic Activity: Effect of Nutrient Concentration

The effect of nutrient concentration on hemolytic activity varied with clone. For all *P. parvum* clones, the N-deficient cultures were more hemolytic than the nutrient-replete cultures.

For the NC and SC clones, both nutrient-deficient cultures were more hemolytic than the nutrient-replete cultures for the supernatant, while the P-deficient cultures were similar to or more hemolytic than the nutrient-replete cultures for the pellet. This result agrees with the study of Hagstrom and Granéli (2005), which found hemolytic activity in nutrient-sufficient and P-deficient cultures to be similar. Also for the NC and SC clones, the N-deficient cultures were similar to or more hemolytic than the P-deficient cultures. This finding agrees with Johansson and Granéli (1999), who found N-deficient cultures to be more hemolytic on a per cell basis than P-deficient cultures, though their finding was not statistically significant.

For the TX clone, both nutrient-deficient cultures were more hemolytic than the nutrient-replete cultures, and there was no difference in hemolytic activity between the nutrient-deficient cultures. This was similar to the finding by Johansson and Granéli (1999), which looked at total hemolytic activity.

Based on the findings of this study, nitrogen deficiency caused greater hemolytic activity than nitrogen sufficiency for *P. parvum*. Phosphorus deficiency (compared with phosphorus sufficiency) caused greater hemolytic activity in the supernatant and had greater or similar hemolytic activity in the pellet depending on clone and growth phase. Nitrogen deficiency caused similar or greater hemolytic activity than phosphorus deficiency depending on clone and growth phase.

Ichthyotoxicity

For the fish bioassay, the TX clone was the most ichthyotoxic of the *P. parvum* clones. The TX clone was the most stressed by nutrient deficiencies in terms of growth, and while this did not correlate well with hemolytic activity, more nutrient-stressed in this case correlated with being more ichthyotoxic. The TX clone was the most hemolytic on a per cell basis.

If a water sample containing *P. parvum* was hemolytic, there might be a higher chance of having a fish kill. More studies are needed, however. Previous reports found no relationship between hemolytic activity and ichthyotoxicity (Simonsen and Moestrup 1997). Kim and Padilla (1977) found three prymnesin fractions that were hemolytic and only one fraction that was both ichthyotoxic and hemolytic. Both the hemolysis assay and fish bioassay relate information about the toxicity of *Prymnesium*, but it remains to be seen whether the two toxin properties are related.

The nutrient-replete *P. parvum* cultures showed minimal ichthyotoxicity. Previous studies found *P. parvum* to be ichthyotoxic even under nutrient-replete conditions (Yariv and Hestrin 1961, Reich and Parnas 1962, Ulitzur and Shilo 1964, Kim and Padilla 1977). Inactivation of ichthyotoxicity under laboratory settings can occur with constant illumination, heating, high pH, and high salinity (Reich and Parnas 1962, Parnas *et al.* 1962, Parnas 1963, Ulitzur and Shilo 1964, Shilo 1981). None of these factors occurred during the experiment.

Nutrient-deficient cultures were the most ichthyotoxic, particularly the P-deficient cultures. Fish kills have mostly occurred in high nitrogen P-limited systems, although Moustaka-Gouni *et al*. (2004) did report a fish kill in a N-limited waterbody. Since all the clones came from regions associated with P-limited conditions at the time of the bloom (Lewitus *et al*. 2003, Tomas *et al*. 2004), finding that the clones were most ichthyotoxic during P-deficient conditions was consistent with field observations. The N:P ratios in this study were consistent with natural conditions.

Extrapolating this bioassay into natural environments, if a eutrophic waterbody experienced a *P. parvum* bloom, fish kills would likely occur. If a non-eutrophic waterbody experienced a *P. parvum* bloom, fish kills may or may not occur. Since only one trial was done for each treatment, solid conclusions cannot be drawn from the fish bioassays. It should also be noted that the *P. parvum* densities used in this fish bioassay, approximately 10^2 cells/L, were well below documented densities for fish kills. While the number of *P. parvum* cells is never an indicator of whether or not a fish kill will occur, studies have shown that at least 10^4 cells/L are required (Reich and Aschner 1947). Most fish kills occur with blooms between 10^6 and 10^9 cells/L (Edvardsen and Paasche 1998). In this study, the TX was the most ichthyotoxic clone while being the least dense.

In this study, the clones that came from samples with fish kills (NC and TX clones) were the most ichthyotoxic. In this case, it seems that once a bloom has killed fish, it may be more likely to kill fish again in the future. Most areas have recurrent fish kills associated with *P. parvum* blooms. *Prymnesium* may produce resting stages in natural environments. These resting stages that exist in other harmful algal bloom species including *Gymnodinium catenatum*, *Pyrodinium bahamense*, and species of *Alexandrium* are a period of dormancy brought on by adverse environmental conditions (Anderson 1998, Usup and Azanza 1998, Amorim *et al*. 2002). Despite the lack of information on resting stages in *Prymnesium*, this by no means suggests that the cysts do not exist. *Prymnesium* cysts would be very small and difficult to detect, especially in a small population size. The next step in future *Prymnesium* research is to confirm the presence of these cysts and their ability to form motile populations.

What might be an explanation for the differences in the *P. parvum* clones? The basic assumption is that the three clones should behave similarly (similar growth rates, hemolytic activity, and ichthyotoxicity) because they are *P. parvum*. However, the clones did show differences. These differences may be attributed to different environmental conditions, including geographical origin, type of waterbody, and whether fish kills occurred. Another explanation may be the genetic makeup of the different clones. Further studies should examine the genetic differences between the NC, SC, and TX clones. While this study focused on physiological ecology, other studies should compare the toxins from each clone. Six hemolytic compounds have recently been isolated for the NC clone (Wright *et al*. 2005). Further experiments should also compare clones from the same geographic area, but one from a natural waterbody and one from a manmade waterbody.

Another question is whether clones in the same bloom vary in characteristics. This is a fundamental problem in the study of harmful algal blooms – if a bloom occurs, do all the clones respond in the same manner? This study examined geographical differences. One solution to this problem would be to isolate many different clones from the same bloom and test genetic differences. The development of genetic fingerprinting for HAB species, including *P. parvum*, is critical.

Variation in harmful algal bloom species is not rare. Larsen published several studies looking at three geographically-distinct clones of *P. parvum* from Norway, Denmark, and England (Larsen and Medlin 1997, Larsen and Edvardsen 1998, Larsen and Bryant 1998). The studies found that the clones from the same area were more physiologically similar than clones from different areas of the same species. The three *P. parvum* clones did display differences in genetics, growth rate, and toxicity (Larsen and Bryant 1998). Intraspecific variation has also been found in non-*Prymnesium* species. Strom and Bright (2003) found intraspecific variation in organic and inorganic nitrogen requirements for the haptophyte *Emiliana huxleyi*. Doblin *et al*. (2000) found intraspecific variation in selenium requirements for the dinoflagellate *Gymnodinium catenatum*.

Comparison between *P. parvum* and *P. calathiferum*

There were notable differences between the *P. parvum* and *P. calathiferum* cultures. When *P. calathiferum* reached stationary phase, clumps of particles appeared along with an unpleasant odor. Neither characteristic was ever associated with the *P. parvum* clones. *Prymnesium calathiferum* reached stationary phase more rapidly, normally having lower terminal densities, and had the highest growth rates. So, why does *P. calathiferum* grow faster than *P. parvum*? These are the first growth rates on *P. calathiferum* and there are no known published growth rates for comparison. In the short term, *P. calathiferum* may have some competitive advantage in being able to grow faster.

Relative to hemolytic activity, *P. parvum* was highly hemolytic, while *P. calathiferum* showed limited hemolytic activity. *Prymnesium calathiferum* was used as a negative control in this study. Again, these are the first observations on *P. calathiferum*.

However, *P. calathiferum* was reported as being ichthyotoxic. In a previous study, the supernatant from a nutrient-replete *P. calathiferum* culture killed fish in three hours (Chang 1985). In the fish bioassays performed for this study, the nutrient-replete *P. calathiferum* culture killed fish in 1.5 hours while the nutrient-deficient cultures killed no fish. The fish killed at the 50% concentration of the P-deficient culture was probably not due to the culture since the fish at the 100% concentration were not killed. From these results, it is unlikely that fish would be killed in a eutrophic waterbody where a *P. calathiferum* bloom formed.

More work needs to be done on *P. calathiferum*. The initial finding of *P. calathiferum* was from a bloom where it was not even the dominant phytoplankton (Chang and Ryan 1985). From this study, there is a distinct difference in toxicity between *P. parvum* and *P. calathiferum*. Since *P. parvum* produces prymnesin, it is assumed that *P. calathiferum* also produces the same toxin. However, there are no studies confirming that *P. calathiferum* produces prymnesin.

Hemolytic Activity: Supernatant versus Pellet

This study also examined where hemolytic activity was the greatest – in the cells (represented by the pellet) or released into the surrounding medium (represented by the supernatant). Prymnesin is an unusual toxin in that it is extracellular and released into the medium (Shilo and Aschner 1953). This should mean that the extracellular component (the supernatant in this study) should be the most toxic. Studies have found the extracellular component to be highly hemolytic (Simonsen and Moestrup 1997, Fistarol *et al*. 2003).

The results from this study vary according to clone and nutrient treatment. For the P-deficient cultures of the four clones, hemolytic activity in the cells equaled that in the surrounding medium. For the N-deficient cultures, the hemolytic activity in the extracellular component was greater in the NC, SC, and *P. calathiferum* clones, while the hemolytic activity in the cells were greater in the TX clone. For the nutrient-replete cultures, hemolytic activity was greater in the cells than was released for the TX clone, while hemolytic activity was equally distributed between the intracellular and extracellular components of the NC, SC, and *P. calathiferum* clones.

Why is the media more hemolytic than the cells if the toxin is extracellular? Possibly there was no trigger to release toxin. Nitrogen deficiency was the only nutrient treatment to show the supernatant being most hemolytic. One suggestion is that the membranes are less intact and more leaky due to imbalanced metabolism from nitrogen deficiency. Being stressed by limited nutrients is thought to lead to an imbalance in metabolism, such that the toxin is expelled through leaks in the membrane (Dafni *et al*. 1972). The TX clone was the only organism to show the pellet being the most hemolytic. Perhaps membrane leakage is less likely in nitrogen deficiency for the TX clone.

Hemolytic Activity: Growth Phase

Hemolytic activity was also assessed at different growth phases – lag phase, log phase, and stationary phase. As a secondary metabolite, toxins should accumulate when the organism is in active growth and be at their highest levels in stationary phase (Calvo *et al*. 2002).

This study found that there was either no difference among the growth phases or that lag phase was the most hemolytic. Both findings were very inconsistent with previous studies. A few studies on *P. parvum* have found hemolysis to be highest in stationary phase (Shilo and Rosenberger 1960, Igarashi *et al*. 1995, Rosetta *et al*. 2003). Padilla (1970) found that the hemolysin accumulated in log phase. Simonsen and Moestrup (1997) found that hemolytic activity was greatest in log phase, and that lag and stationary phases were similar to each other and less hemolytic. One study that looked at the dinoflagellate *Alexandrium* found that total hemolysis increased with growth, but that hemolysis on a per cell basis decreased from log to stationary phases (Arzul *et al*. 1999).

In addition, finding lag phase to be the most hemolytic or similar to later phases in the supernatant was also inconsistent with previous studies. Simonsen and Moestrup (1997) found hemolytic activity in the medium to be highest in stationary phase, but not present in lag or log phases. Shilo (1967) found that in lag phase, only the intracellular toxin was present. The extracellular toxin was not present until later in the growth cycle.

The best explanation for lag phase being the most hemolytic would be carryover from inoculation. For the nutrient-replete cultures, they were inoculated from cultures in stationary phase where hemolysin production is known to occur – not only accumulating in the cells but also released into the surrounding medium. When cultures are inoculated, they receive the cells and the medium, which have both presumably accumulated the toxin. Lag phase being more hemolytic than stationary phase could be due to toxin release by stressed organisms. Inoculation into a new surrounding would seem to be stressful – if not, lag phase would not exist, and the cells would start multiplying rapidly. Possibly this stress may cause the cells to rupture and release prymnesin. Nonetheless, it would be impossible for hemolysin production to occur in lag phase. Enzyme function is thought to be low in lag phase. Most enzymes are inactivated during stationary phase, and during lag phase, the organisms are replenishing their nutrient supply in order to re-activate the enzymes and rapidly reproduce (Fogg and Thake 1987).

Summary

As this study found, intraspecific variation in growth rate, hemolytic activity, and ichthyotoxicity did occur in geographically-distinct strains of *P. parvum*. The next step would be to examine genetic differences. The question as to whether nitrogen or phosphorus limitation greater affects toxicity in *P. parvum* still does not have a firm answer. Experiments using different forms of nitrogen and using different concentrations of nitrate and phosphate should be done to look at the effects on toxicity, particularly hemolytic activity.

Observations of low hemolytic activity and ichthyotoxicity limited to nutrient-replete conditions suggest *P. calathiferum* should pose a minimal problem. However, observations of high hemolytic activity and ichthyotoxicity support *P. parvum* being a major problem. The question exists why has the United States recently started experiencing *P. parvum* blooms? Has it always been there and just not noticed? Alternatively, has it been introduced from other areas? The larger question is how do you get rid of the *P. parvum* blooms? Two mitigation and control problems exist – just the bloom and the toxicity of the bloom. Eliminating the organism all together poses many problems – in a manmade waterbody, such as the NC and SC ponds, elimination would be an easier problem than in a natural system such as TX. There are many management methods, including ammonium sulfate, copper sulfate, potassium permanganate, hydrogen peroxide, and clay. Each has its set of problems (Barkoh and Fries 2005, Hagstrom and Graneli 2005).

This study suggests that eutrophication may increase the ichthyotoxicity and possibly the hemolytic activity of *P. parvum* blooms. Waterbodies affected by *P. parvum* should be monitored for nitrate, ammonia, and phosphate concentrations. When the waters become severely N- or P-limited, decreasing the nutrient source may be beneficial to decrease the potential of fish kills.

CONCLUSIONS

- Intraspecific variation in growth rate, hemolytic activity, and ichthyotoxicity occurred in the three clones of *P. parvum* used in this study.
- *Prymnesium parvum* and *P. calathiferum* showed discernible differences in growth rate, hemolytic activity, and ichthyotoxicity.
- Nitrogen deficiency caused greater hemolytic activity than nitrogen replete conditions for *P. parvum*.
- Phosphorus deficiency (compared with phosphorus replete conditions) caused greater hemolytic activity in the supernatant and had greater or similar hemolytic activity in the pellet depending on clone and growth phase.
- Nitrogen deficiency caused similar or greater hemolytic activity than phosphorus deficiency depending on clone and growth phase.
- *Prymnesium calathiferum* cultures grown under nutrient-replete conditions were more ichthyotoxic than those under nutrient-deficient conditions.
- Phosphorus deficiency caused the greatest ichthyotoxicity in the *P. parvum* clones.
- There was no difference in hemolytic activity between the supernatant and the pellet in the phosphorus-deficient treatment for the four clones. For the nutrient-replete treatment,

there was no difference between the supernatant and pellet for the NC, SC, and *P. calathiferum* clones.

- For the nitrogen-deficient treatment, the supernatant was the most hemolytic for the NC, SC, and *P. calathiferum* clones.
- For the nutrient-replete and nitrogen-deficient treatments, the pellet was the most hemolytic for the TX clone.
- There was either no difference in hemolytic activity among the growth phases or lag phase was the most hemolytic.

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APPENDICES

Appendix A. Daily Counts of Cultures.

North Carolina *P. parvum* clone – Trial 1 of Nutrient-Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	968	19360		12	7646	611680	
	976	19520			8173	653840	
	1121	22420	20510		7323	585840	626900
	1037	20740			8203	656240	
1	1781	35620		13	8342	667360	
	1729	34580			8383	670640	
	1618	32360	33830		8421	673680	669580
	1638	32760			8333	666640	
2	2717	54340		14	8544	683520	
	2729	54580			8392	671360	
	2685	53700	54470		8284	662720	676160
	2763	55260			8588	687040	
3	4296	85920		15	9376	750080	
	4128	82560			9523	761840	
	4031	80620	76984		9687	774960	756080
	4028	80560			9218	737440	
4	5768	115360		16	9790	783200	
	6093	121860			9690	775200	
	5834	116680	118715		9896	791680	783460
	6048	120960			9797	783760	
5	8692	173840		17	10357	828560	
	8769	175380	173725		10510	840800	
	8603	172060			10798	863840	847600
	8681	173620			10715	857200	
6	11508	230160		18	11035	882800	
	11321	226420			11079	886320	
	10867	217340	224930		11153	892240	883800
	11290	225800			10923	873840	
7	4042	323360		19	12075	966000	
	3887	310960			12426	994080	
	3749	299920	303660		12266	981280	981300
	3505	280400			12298	983840	
8	5026	402080		20	12929	1034320	
	4581	366480			12478	998240	
	4745	379600	379640		12976	1038080	1020080
	4630	370400			12621	1009680	
9	5364	429120		21	11277	902160	
	4608	368640			11936	954880	
	5174	413920	401520		11510	920800	933780
	4930	394400			11966	957280	
10	6153	492240		22	13149	1051920	
	6004	480320			12916	1033280	
	5881	470480	483100		12908	1032640	
	6117	489360			13034	1042720	1040140
11	6719	537520					
	6767	541360					
	6630	530400					
	6758	540640	537480				

South Carolina *P. parvum* clone – Trial 1 of Nutrient-Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	622	12440		12	6589	527120	
	572	11440			5579	446320	
	672	13440			6864	549120	
	627	12540	12465		6025	482000	501140
1	1057	21120		13	6844	547520	
	995	19880			7091	567280	
	899	17980			7069	565520	
	1097	21920	20225		7162	572960	563320
2	1787	35740		14	7776	622080	
	1755	35100			7843	627440	
	1813	36260			7866	629280	
	2018	40360	36865		7946	635680	628620
3	3074	61480		15	8437	674960	
	3543	70860			8343	667440	
	3145	62900			8622	689760	
	3499	69980	66305		9008	720640	688200
4	4972	99440		16	9942	795360	
	4977	99540			9872	789760	
	5092	101840			9758	780640	
	5266	105320	101535		10096	807680	793360
5	7224	144480		17	9584	766720	
	7228	144560			9673	773840	
	7326	146520			9814	785120	
	7421	148420	145995		10044	803520	782300
6	7224	144480		18	9996	799680	
	7228	144560			10030	802400	
	7326	146520			9940	795200	
	7421	148420	224460		10090	807200	801120
7	10480	209600		19	12323	985840	
	11806	236120			12301	984080	
	11109	222180			12198	975840	
	11497	229940	292540		12185	974800	980140
8	3499	279920		20	11712	936960	
	3742	299360			11685	934800	
	3636	290880			11902	952160	
			373973		11854	948320	943060
9	4178	334240		21	12402	992160	
	3805	304400			12507	1000560	
	3914	313120			12249	979920	
	4271	341680	323360		12256	980480	988280
10	4373	349840		22	13197	1055760	
	4715	377200			13070	1045600	
	4809	384720			13230	1058400	
			370587		13098	1047840	1051900
11	5217	417360					
	5314	425120					
	5198	415840					
	5384	430720	422260				

Texas *P. parvum* clone – Trial 1 of Nutrient-Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	1124	22480		12	7909	632720	
	1137	22740			8220	657600	
	1173	23460			7942	635360	
	1090	21800	22620		7974	637920	640900
1	2757	55140		13	8348	667840	
	2831	56600			8124	649920	
	2849	56980			8163	653040	
	2948	58960	56920		8328	666240	659260
2	4523	90460		14	8843	707440	
	4427	88540			8903	712240	
	4568	91360			8868	709440	
	4479	89580	89985		9011	720880	712500
3	6804	136080		15	9487	758960	
	6889	137780			9469	757520	
	6690	133800			9218	737440	
	6993	139860	136880		9647	771760	756420
4	8460	169200		16	10007	800560	
	8357	167140			10310	824800	
	8475	169500			10101	808080	
	8466	169320	168790		10351	828080	815380
5	8778	175560		17	10625	850000	
	8980	179600			10679	854320	
	8673	173460			10970	877600	
	8837	176740	176340		11291	903280	871300
6	10758	215160		18	11100	888000	
	10795	215900			11427	914160	
	10991	219820			11059	884720	
	10898	217960	217210		11527	922160	902260
7	3396	271680		19	12614	1009120	
	3455	276400					
	3466	277280					
	3435	274800	275040				1009120
8	3827	306160		20	12344	987520	
	3686	294880			12477	998160	
	3814	305120			12567	1005360	
	3755	300400	301640		12819	1025520	1004140
9	4124	329920		21	12322	985760	
	4358	348640			12192	975360	
	4359	348720			12554	1004320	
	4133	330640	339480		12551	1004080	992380
10	5661	452880		22	13590	1087200	
	5776	462080			13407	1072560	
	5588	447040			13617	1089360	
	5637	450960	453240		13602	1088160	1084320
11	6383	510640					
	6501	520080					
	6385	510800					
	6631	530480	518000				

Prymnesium calathiferum – Trial 1 of Nutrient-Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	1037	20740		12	6858	548640	571800
	1110	22220			7077	566160	
	872	17440	19830		7532	602560	
	946	18920			7123	569840	
1	989	19780		13	7142	571360	
	593	11860			6517	521360	
	603	12060	14280		6443	515440	548784
	671	13420			6966	557280	
2	1126	22520			7231	578480	
	1058	21160		14	5820	465600	
	1267	25340			5862	468960	
	1034	20680	22696		5979	478320	476180
	1189	23780			6148	491840	
3	1772	35440		15	4417	353360	
	1651	33020			4752	380160	
	1804	36080	34970		4627	370160	
	1767	35340			4981	398480	548784
4	3196	63920		16	4956	396480	
	3124	62480			5085	406800	
	3282	65640	63780		5019	401520	407960
	3154	63080			5338	427040	
5	7323	146460		17	5048	403840	
	7057	141140			5152	412160	
	7311	146220			5067	405360	
	6934	138680	144460		5345	427600	412240
	7490	149800			4860	388800	
6	7322	146440		18	4751	380080	
	7121	142420			4877	390160	
	7551	151020	145495		5144	411520	392640
	7105	142100			6836	546880	
7	2702	216160		19	6921	553680	
	3237	258960			8559	684720	
	3356	268480	249520		8564	685120	617600
	3181	254480			5943	475440	
8	4536	362880		20	5931	474480	
	4455	356400			6468	517440	
	4468	357440	359420		6727	538160	501380
	4512	360960			5757	460560	
9	8105	648400		21	6067	485360	
	8081	646480			6475	518000	
	8059	644720	645620		7111	568880	508200
	8036	642880			8450	676000	
10	7031	562480		22	8398	671840	
	6876	550080			8584	686720	
	7282	582560			8187	654960	
	7421	593680	572200				672380
11	9827	786160					
	9941	795280					
	10170	813600	800840				
	10353	828240					

North Carolina *P. parvum* clone – Trial 2 of Nutrient Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	877	17540		12	8641	691280	
	871	17420			8730	698400	
	922	18440	17815		8656	692480	
	893	17860			8818	705440	696900
1	1360	27200		13	9662	772960	
	1418	28360			9835	786800	
	1461	29220	28145		9417	753360	
	1390	27800			10085	806800	779980
2	1904	38080		14	11749	939920	
	1863	37260			11730	938400	
	1892	37840	38585		11819	945520	
	2058	41160			11932	954560	944600
3	3373	67460		15	10931	874480	
	3582	71640			10868	869440	
	3921	78420	75005		10963	877040	
	4125	82500			11064	885120	876520
4	4980	99600		16	12473	997840	
	5341	106820			12334	986720	
	5706	114120	108865		12678	1014240	
	5746	114920			12712	1016960	1003940
5	8166	163320		17	12974	1037920	
	7951	159020			13284	1062720	
	8438	168760	164995		13695	1095600	
	8444	168880			12958	1036640	1058220
6	11238	224760		18	13551	1084080	
	10665	213300			13750	1100000	
	10985	219700	219835		14199	1135920	
	11079	221580			14089	1127120	1111780
7	14602	292040		19	14314	1145120	
	14198	283960			14292	1143360	
	14429	288580	289560		14446	1155680	
	14683	293660			14827	1186160	1157580
8	17924	358480		20	15642	1251360	
	17626	352520			15733	1258640	
	17977	359540	358265		15985	1278800	
	18126	362520			16778	1342240	1282760
9	21635	432700					
	20941	418820					
	21819	436380	430620				
	21729	434580					
10	7168	573440					
	7197	575760					
	7184	574720					
	7151	572080	574000				
11	4608	368640					
	7637	610960					
	7728	618240					
	7905	632400	557560				

South Carolina *P. parvum* clone – Trial 2 of Nutrient Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	517	10340		12	6760	540800	
	528	10560			6812	544960	
	569	11380			6811	544880	548340
	563	11260	10885		7034	562720	
1	947	18940		13	6904	552320	
	919	18380			7460	596800	
	1011	20220			7463	597040	599300
	976	19520	19265		8138	651040	
2	1312	26240		14	8542	683360	
	1209	24180			9538	763040	
	1408	28160			8841	707280	753180
	1547	30940	27380		10738	859040	
3	2204	44080		15	8076	646080	
	2145	42900			8132	650560	
	2307	46140			8470	677600	664840
	2477	49540	45665		8564	685120	
4	3052	61040		16	8753	700240	
	3165	63300			8598	687840	
	3376	67520			8911	712880	705720
	3544	70880	65685		9024	721920	
5	4822	96440		17	8755	700400	
	4672	93440			8825	706000	
	4889	97780			8896	711680	720140
	5131	102620	97570		9531	762480	
6	5939	118780		18	9136	730880	
	6072	121440			8805	704400	
	5966	119320			9189	735120	737820
	6355	127100	121660		9761	780880	
7	9056	181120		19	9811	784880	
	8962	179240			9762	780960	
	9283	185660			9939	795120	793400
	10511	210220	189060		10158	812640	
8	13685	273700		20	9990	799200	
	13564	271280			10389	831120	
	14347	286940			10311	824880	
	15330	306600	284630		11337	906960	840540
9	14955	299100					
	14870	297400					
	13895	277900					
	15956	319120	298380				
10	4681	374480					
	5209	416720					
	5265	421200					
	5925	474000	421600				
11	5409	432720					
	5514	441120					
	5821	465680					
	6109	488720	457060				

Texas *P. parvum* clone – Trial 2 of Nutrient Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	799	15980		12	4394	351520	
	777	15540			4429	354320	
	814	16280	15775		4281	342480	352080
	765	15300			4500	360000	
1	962	19240		13	5647	451760	
	949	18980			5781	462480	
	986	19720	19505		5874	469920	466520
	1004	20080			6024	481920	
2	1498	29960		14	6893	551440	
	1430	28600			7430	594400	
	1468	29360	29430		6853	548240	573080
	1490	29800			7478	598240	
3	1710	34200		15	7623	609840	
	1635	32700			7510	600800	
	1734	34680	33915		7654	612320	613080
	1704	34080			7867	629360	
4	2147	42940		16	8812	704960	
	2156	43120			8674	693920	
	2211	44220	43490		8637	690960	694860
	2184	43680			8620	689600	
5	3530	70600		17	8644	691520	
	3519	70380			8826	706080	
	3485	69700	70160		8631	690480	697520
	3498	69960			8775	702000	
6	4619	92380		18	9094	727520	
	4665	93300			9008	720640	
	4717	94340	93480		9186	734880	729140
	4695	93900			9169	733520	
7	5842	116840		19	9999	799920	
	5913	118260			9864	789120	
	5895	117900	117530		9962	796960	799120
	5856	117120			10131	810480	
8	7502	150040		20	9781	782480	
	7588	151760			10001	800080	
	7598	151960	150990		10052	804160	
	7510	150200			10072	805760	798120
9	7628	152560					
	7780	155600					
	7818	156360	155340				
	7842	156840					
10	2841	227280					
	2877	230160					
	2969	237520					
	2925	234000	232240				
11	3115	249200					
	3111	248880					
	3163	253040					
	3140	251200	250580				

Prymnesium calathiferum – Trial 2 of Nutrient Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	182	3640		12	3870	309600	
	190	3800			4289	343120	
	192	3840	3675		4496	359680	349740
	171	3420			4832	386560	
1	182	3640		13	4194	335520	
	181	3620			4467	357360	
	185	3700	3685		4954	396320	387280
	189	3780			5749	459920	
2	323	6460		14	5770	461600	
	310	6200			5956	476480	
	348	6960	6635		6633	530640	507420
	346	6920			7012	560960	
3	707	14140		15	4864	389120	
	674	13480			5432	434560	
	742	14840	14500		5738	459040	441320
	777	15540			6032	482560	
4	1077	21540		16	5130	410400	
	1070	21400			5551	444080	
	1060	21200	21765		5814	465120	456600
	1146	22920			6335	506800	
5	1861	37220		17	4371	349680	
	1877	37540			4801	384080	
	1837	36740	37305		5088	407040	390800
	1886	37720			5280	422400	
6	2602	52040		18	4822	385760	
	2637	52740			4982	398560	
	2730	54600	53365		5031	402480	403440
	2704	54080			5337	426960	
7	3156	63120		19	3257	260560	
	3163	63260			3635	290800	298020
	3275	65500	64620		3952	316160	
	3330	66600			4057	324560	
8	4497	89940		20	3870	309600	
	4693	93860			4289	343120	
	4924	98480	97105		4496	359680	
	5307	106140			4832	386560	349740
9	6626	132520					
	7348	146960					
	7504	150080	147860				
	8094	161880					
10	2105	168400					
	2295	183600					
	2499	199920					
	2656	212480	191100				
11	2797	223760					
	3239	259120					
	3492	279360					
	3806	304480	266680				

North Carolina *P. parvum* clone – Trial 3 of Nutrient Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	468	9360		14	7506	600480	
	437	8740			7374	589920	
	467	9340	9255		7405	592400	
	479	9580			7625	610000	748920
1	855	17100		15	9285	742800	
	870	17400			9374	749920	
	864	17280	17310		9407	752560	720100
	873	17460			9380	750400	
2	1225	24500		16	8818	705440	
	1315	26300			8901	712080	
	1365	27300	25970		8999	719920	835200
	1289	25780			9287	742960	
3	1947	38940		17	10231	818480	
	1895	37900			10257	820560	
	1981	39620	38745		10477	838160	800020
	1926	38520			10795	863600	
4	2537	50740		18	9827	786160	
	2589	51780			9833	786640	
	2750	55000	53955		10016	801280	808680
	2915	58300			10325	826000	
5	3634	72680		19	9996	799680	
	3599	71980			10034	802720	
	3842	76840	75045		10045	803600	972120
	3934	78680			10359	828720	
6	6215	124300		20	11867	949360	
	6530	130600			12098	967840	
	6405	128100	128090		12281	982480	1006000
	6468	129360			12360	988800	
7	9700	194000		21	12178	974240	
	9826	196520			12166	973280	
	10049	200980	198575		12697	1015760	926740
	10140	202800			13259	1060720	
8	14283	285660		22	11233	898640	
	14364	287280			11520	921600	
	14113	282260	286670		11770	941600	1139140
	14574	291480			11814	945120	
9	15047	300940		23	14013	1121040	
	14023	280460			13960	1116800	
	15753	315060	298795		14423	1153840	1044040
	14936	298720			14561	1164880	
10	17875	357500		24	15310	1224800	
	16855	337100			15424	1233920	
	18059	361180			16077	1286160	1260560
	17540	350800	351645		16217	1297360	
11	21668	433360		25	13993	1119440	
	22720	454400			14518	1161440	1190960
	23198	463960			15083	1206640	
	24284	485680	459350		15954	1276320	
12	7044	563520		26	15417	1233360	1266080
	7205	576400			15698	1255840	
	7170	573600			15681	1254480	
	7532	602560	579020		16508	1320640	
13	7044	563520		27	14413	1153040	
	7205	576400			14601	1168080	
	7170	573600	598200		14930	1194400	
	7532	602560			16024	1281920	720100

South Carolina *P. parvum* clone – Trial 3 of Nutrient Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	554	11080		14	6078	486240	
	547	10940			5954	476320	
	599	11980	11830		6238	499040	578840
	666	13320			6292	503360	
1	1109	22180		15	5669	453520	
	1222	24440			5781	462480	
	1204	24080	24225		5907	472560	565500
	1310	26200			6244	499520	
2	1405	28100		16	6877	550160	
	1331	26620			7105	568400	
	1534	30680	28555		7296	583680	711720
	1441	28820			7664	613120	
3	1889	37780		17	6906	552480	
	1961	39220			6915	553200	
	2103	42060	40945		6913	553040	752020
	2236	44720			7541	603280	
4	2689	53780		18	8493	679440	
	2911	58220			8763	701040	
	2895	57900	57585		8764	701120	977500
	3022	60440			9566	765280	
5	3608	72160		19	8900	712000	
	3804	76080			9073	725840	
	3763	75260	75765		9451	756080	845760
	3978	79560			10177	814160	
6	4896	97920		20	11722	937760	
	5148	102960			11999	959920	
	5313	106260	105280		12393	991440	932940
	5699	113980			12761	1020880	
7	6835	136700		21	10332	826560	
	7206	144120			10424	833920	
	7463	149260	146285		10384	830720	911380
	7753	155060			11148	891840	
8	10714	214280		22	11099	887920	
	10600	212000			11568	925440	
	10170	203400	214590		11679	934320	1028520
	11434	228680			12301	984080	
9	13754	275080		23	10675	854000	
	13043	260860			11046	883680	
	13947	278940	273040		11619	929520	1142120
	13864	277280			12229	978320	
10	15543	310860		24	12027	962160	
	14760	295200			12347	987760	
	16585	331700	315595		13279	1062320	1071020
	16231	324620			13773	1101840	
11	17560	351200		25	14519	1161520	
	18174	363480			14624	1169920	
	18300	366000			12620	1009600	1064860
	20267	405340	23464.91		15343	1227440	
12	6078	486240		26	12869	1029520	
	5954	476320			13072	1045760	
	6238	499040	491240		13630	1090400	1007100
	6292	503360			13980	1118400	
13	5669	453520		27	12376	990080	
	5781	462480			12927	1034160	
	5907	472560			13565	1085200	
	6244	499520	472020		14375	1150000	1143900

Texas *P. parvum* clone – Trial 3 of Nutrient Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	728	14560		14	5314	425120	
	697	13940			5240	419200	
	700	14000	14330		5475	438000	311600
	741	14820			5638	451040	
1	1054	21080		15	4102	328160	
	1016	20320			4277	342160	
	1106	22120	21195		4287	342960	330320
	1063	21260			4424	353920	
2	1299	25980		16	5685	454800	
	1317	26340			5665	453200	
	1318	26360	26210		5722	457760	433340
	1308	26160			5682	454560	
3	1678	33560		17	6224	497920	
	1680	33600			6254	500320	
	1798	35960	34490		6406	512480	341800
	1742	34840			6563	525040	
4	2449	48980		18	6883	550640	
	2405	48100			6890	551200	
	2514	50280	48995		7111	568880	455080
	2431	48620			6993	559440	
5	3044	60880		19	6735	538800	
	2999	59980			6657	532560	
	3015	60300	60685		6789	543120	508940
	3079	61580			6948	555840	
6	4174	83480		20	6828	546240	
	4106	82120			6920	553600	
	4122	82440	83785		6874	549920	557540
	4355	87100			6950	556000	
7	5423	108460		21	6901	552080	
	5457	109140			6821	545680	
	5428	108560	109075		7069	565520	542580
	5507	110140			6991	559280	
8	7568	151360		22	7395	591600	
	7283	145660			7456	596480	
	7474	149480	147730		7665	613200	551440
	7221	144420			7590	607200	
9	7180	143600		23	6938	555040	
	6911	138220			7065	565200	
	7250	145000	142585		7188	575040	555640
	7176	143520			7172	573760	
10	9407	188140		24	8127	650160	
	9832	196640			8251	660080	
	9653	193060	194415		8433	674640	602120
	9991	199820			8418	673440	
11	9407	188140		25	7509	600720	
	9832	196640			7659	612720	
	9653	193060			7693	615440	567260
	9991	199820	194415		7887	630960	
12	3866	309280		26	7918	633440	
	3920	313600			7783	622640	
	3735	298800	311600		7890	631200	664580
	4059	324720			7991	639280	
13	4061	324880		27	7735	618800	
	4139	331120			7769	621520	
	4117	329360			7790	623200	
	4199	335920	330320		7841	627280	614960

Prymnesium calathiferum – Trial 3 of Nutrient Replete Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	614	12280		12	7527	602160	
	614	12280			7749	619920	
	563	11260	11790		8515	681200	645260
	567	11340			8472	677760	
1	434	8680		13	8613	689040	
	464	9280			8708	696640	
	476	9520	9095		8910	712800	705940
	445	8900			9066	725280	
2	635	12700		14	9617	769360	
	718	14360			9658	772640	
	630	12600	13610		10027	802160	792460
	739	14780			10321	825680	
3	1056	21120		15	8550	684000	
	1124	22480			9042	723360	
	1121	22420	22090		9289	743120	722580
	1117	22340			9248	739840	
4	1889	37780		16	7223	577840	
	1928	38560			7359	588720	
	2014	40280	38700		7404	592320	590120
	1909	38180			7520	601600	
5	5264	105280		17	4808	384640	
	5362	107240			5148	411840	
	5421	108420	108090		5353	428240	
	5571	111420			5156	412480	409300
6	9319	186380					
	9236	184720					
	9431	188620	186085				
	9231	184620					
7	11052	221040					
	11329	226580					
	11460	229200	228145				
	11788	235760					
8	15615	312300					
	16210	324200					
	16210	324200	323030				
	16571	331420					
9	23394	467880					
	23616	472320					
	24358	487160	475205				
	23673	473460					
10	5528	442240					
	5622	449760					
	5851	468080					
	5685	454800	453720				
11	7177	574160					
	7214	577120					
	7716	617280					
	7321	585680	588560				

North Carolina *P. parvum* clone – Trial 1 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	122	976		12	578	11560	
	128	1024			554	11080	
	114	912	938		557	11140	11270
	105	840			565	11300	
1	161	1288		13	575	11500	
	135	1080			549	10980	
	126	1008	1116		585	11700	11445
	136	1088			580	11600	
2	197	1576		14	580	11600	
	212	1696			572	11440	
	207	1656	1630		568	11360	11480
	199	1592			576	11520	
3	277	2216		15	604	12080	
	291	2328			549	10980	
	295	2360	2278		581	11620	11485
	276	2208			563	11260	
4	418	3344		16	568	11360	
	404	3232			522	10440	
	410	3280	3244		586	11720	11355
	390	3120			595	11900	
5	591	4728		17	510	10200	
	579	4632			511	10220	10410
	528	4224	4420		513	10260	
	512	4096			548	10960	
6	838	6704					
	828	6624					
	818	6544	6782				
	907	7256					
7	991	7928					
	1107	8856					
	1030	8240	8510				
	1127	9016					
8	1278	10224					
	1315	10520					
	1288	10304	10020				
	1129	9032					
9	619	12380					
	586	11720					
	633	12660	12095				
	581	11620					
10	549	10980					
	584	11680					
	604	12080					
	546	10920	11415				
11	525	10500					
	565	11300					
	534	10680					
	587	11740	11055				

South Carolina *P. parvum* clone – Trial 1 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	161	1288		12	546	10920	
	146	1168			607	12140	
	162	1296	1252		542	10840	11535
	157	1256			612	12240	
1	262	2096		13	613	12260	
	282	2256			611	12220	
	256	2048	2106		608	12160	12400
	253	2024			648	12960	
2	418	3344		14	615	12300	
	396	3168			618	12360	
	402	3216	3314		591	11820	12180
	441	3528			612	12240	
3	766	6128		15	626	12520	
	705	5640			613	12260	
	797	6376	5990		623	12460	11995
	727	5816			537	10740	
4	1112	8896		16	557	11140	
	1095	8760			609	12180	
	1120	8960	8948		625	12500	12135
	1147	9176			636	12720	
5	1294	10352		17	595	11900	
	1372	10976			615	12300	
	1332	10656	10754		649	12980	
	1379	11032			651	13020	12550
6	1472	11776					
	1451	11608					
	1532	12256	11890				
	1490	11920					
7	1279	10232					
	1416	11328					
	1307	10456	11122				
	1559	12472					
8	1423	11384					
	1444	11552					
	1567	12536	11880				
	1506	12048					
9	581	11620					
	623	12460					
	612	12240	12095				
	603	12060					
10	614	12280					
	614	12280					
	619	12380					
	621	12420	12340				
11	594	11880					
	675	13500					
	612	12240					
	640	12800	12605				

Texas *P. parvum* clone – Trial 1 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	413	8260		12	569	11380	
	382	7640			609	12180	
	363	7260	7555		556	11120	
	353	7060			558	11160	11460
1	508	10160					
	491	9820					
	517	10340	9970				
	478	9560					
2	544	10880					
	546	10920					
	541	10820	11320				
	633	12660					
3	602	12040					
	614	12280					
	630	12600	12425				
	639	12780					
4	564	11280					
	568	11360					
	584	11680	11460				
	576	11520					
5	574	11480					
	596	11920					
	621	12420	12160				
	641	12820					
6	613	12260					
	607	12140					
	633	12660	12145				
	576	11520					
7	579	11580					
	650	13000					
	577	11540	12335				
	661	13220					
8	673	13460					
	675	13500					
	698	13960	13480				
	650	13000					
9	648	12960					
	673	13460					
	631	12620	13135				
	675	13500					
10	664	13280					
	652	13040					
	632	12640	12830				
	618	12360					
11	565	11300					
	625	12500					
	624	12480					
	592	11840	12030				

Prymnesium calathiferum – Trial 1 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	299	5980					
	288	5760					
	262	5240	5505				
	252	5040					
1	309	6180					
	307	6140					
	310	6200	6105				
	295	5900					
2	586	11720					
	550	11000					
	583	11660	11260				
	533	10660					
3	983	19660					
	1011	20220					
	974	19480	19590				
	950	19000					
4	934	18680					
	934	18680					
	997	19940	19375				
	1010	20200					
5	1149	22980					
	1118	22360					
	1139	22780	22945				
	1183	23660					
6	1287	25740					
	1360	27200					
	1358	27160	26515				
	1298	25960					
7	1432	28640					
	1386	27720					
	1409	28180	28335				
	1440	28800					
8	1299	25980					
	1303	26060					
	1281	25620					
	1264	25280	25735				

North Carolina *P. parvum* clone – Trial 2 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	144	2880	
	123	2460	
	123	2460	2535
	117	2340	
1	164	3280	
	147	2940	
	125	2500	2805
	125	2500	
2	219	4380	
	215	4300	
	212	4240	4175
	189	3780	
3	266	5320	
	237	4740	
	231	4620	4775
	221	4420	
4	332	6640	
	282	5640	
	318	6360	6240
	316	6320	
5	323	6460	
	323	6460	
	327	6540	6570
	341	6820	
6	407	8140	
	391	7820	
	366	7320	7855
	407	8140	
7	413	8260	
	449	8980	
	394	7880	8335
	411	8220	
8	559	11180	
	584	11680	
	518	10360	10985
	536	10720	
9	681	13620	
	625	12500	
	600	12000	12775
	649	12980	
10	653	13060	
	672	13440	
	679	13580	13435
	683	13660	
11	731	14620	
	669	13380	
	723	14460	
	734	14680	14285
12	732	14640	
	719	14380	
	744	14880	14600
	725	14500	
13	1004	20080	
	922	18440	
	966	19320	19570
	1022	20440	
14	723	14460	
	699	13980	
	615	12300	13565
	676	13520	
15	771	15420	
	700	14000	
	790	15800	15010
	741	14820	
16	680	13600	
	728	14560	
	676	13520	13865
	689	13780	
17	698	13960	
	636	12720	
	604	12080	12955
	653	13060	
18	713	14260	
	682	13640	
	658	13160	
	674	13480	13635

South Carolina *P. parvum* clone – Trial 2 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	129	1032		12	877	17540	
	118	944			923	18460	
	101	808	924		892	17840	17635
	114	912			835	16700	
1	276	2208		13	862	17240	
	263	2104			814	16280	
	253	2024	2078		851	17020	16955
	247	1976			864	17280	
2	344	2752		14	952	19040	
	327	2616			923	18460	
	370	2960	2830		924	18480	18975
	374	2992			996	19920	
3	494	3952		15	843	16860	
	492	3936			829	16580	
	486	3888	4014		773	15460	16670
	535	4280			889	17780	
4	366	7320		16	776	15520	
	338	6760			786	15720	
	350	7000	7125		814	16280	16185
	371	7420			861	17220	
5	464	9280		17	795	15900	
	521	10420			754	15080	
	478	9560	10290		823	16460	15925
	595	11900			813	16260	
6	785	15700		18	723	14460	
	794	15880			799	15980	
	844	16880	15865		805	16100	15865
	750	15000			846	16920	
7	769	15380		19	823	16460	
	911	18220			860	17200	
	674	13480	16060		794	15880	16830
	858	17160			889	17780	
8	921	18420		20	833	16660	
	878	17560			820	16400	
	899	17980	18020		851	17020	17045
	906	18120			905	18100	
9	848	16960		21	793	15860	
	869	17380			819	16380	
	772	15440	17110		846	16920	
	933	18660			899	17980	16785
10	852	17040					
	789	15780					
	845	16900	16060				
	726	14520					
11	800	16000					
	799	15980					
	854	17080					
	836	16720	16445				

Texas *P. parvum* clone – Trial 2 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	711	14220		12	939	18780	
	738	14760			959	19180	
	659	13180	13895		958	19160	
	671	13420			966	19320	19110
1	1026	20520					
	1030	20600					
	1060	21200	20660				
	1016	20320					
2	1086	21720					
	1105	22100					
	1098	21960	21770				
	1065	21300					
3	1163	23260					
	1121	22420					
	1061	21220	22190				
	1093	21860					
4	1064	21280					
	1046	20920					
	1080	21600	21280				
	1066	21320					
5	1038	20760					
	1050	21000					
	1071	21420	21165				
	1074	21480					
6	1065	21300					
	1045	20900					
	1007	20140	20500				
	983	19660					
7	1051	21020					
	1105	22100					
	1103	22060	22000				
	1141	22820					
8	1062	21240					
	1167	23340					
	1115	22300	22250				
	1106	22120					
9	1083	21660					
	1064	21280					
	1118	22360	21500				
	1035	20700					
10	1083	21660					
	1064	21280					
	1118	22360	21500				
	1035	20700					
11	969	19380					
	994	19880					
	993	19860					
	1019	20380	19875				

Prymnesium calathiferum – Trial 2 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	206	4120					
	184	3680					
	185	3700	3700				
	165	3300					
1	141	2820					
	143	2860					
	147	2940	2920				
	153	3060					
2	308	6160					
	312	6240					
	306	6120	6230				
	320	6400					
3	366	7320					
	328	6560					
	317	6340	6820				
	353	7060					
4	423	8460					
	378	7560					
	425	8500	8205				
	415	8300					
5	333	6660					
	355	7100					
	336	6720	6880				
	352	7040					
6	348	6960					
	351	7020					
	340	6800	6930				
	347	6940					
7	343	6860					
	330	6600					
	345	6900	6685				
	319	6380					
8	262	5240					
	272	5440					
	290	5800					
	261	5220	5425				

North Carolina *P. parvum* clone – Trial 3 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	247	4940					
	249	4980					
	206	4120	4660				
	230	4600					
1	256	5120					
	269	5380					
	285	5700	5285				
	247	4940					
2	323	6460					
	315	6300					
	312	6240	6450				
	340	6800					
3	382	7640					
	419	8380					
	369	7380	7750				
	380	7600					
4	551	11020					
	516	10320					
	545	10900	10755				
	539	10780					
5	645	12900					
	623	12460					
	570	11400	12145				
	591	11820					
6	642	12840					
	596	11920					
	597	11940	12140				
	593	11860					
7	613	12260					
	568	11360					
	581	11620	11635				
	565	11300					
8	642	12840					
	619	12380					
	651	13020	12585				
	605	12100					
9	676	13520					
	674	13480					
	642	12840	13040				
	616	12320					
10	700	14000					
	673	13460					
	645	12900					
	623	12460	13205				

South Carolina *P. parvum* clone – Trial 3 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	160	3200					
	141	2820					
	157	3140	3140				
	170	3400					
1	196	3920					
	179	3580					
	166	3320	3700				
	199	3980					
2	242	4840					
	227	4540					
	236	4720	4905				
	276	5520					
3	341	6820					
	370	7400					
	356	7120	7100				
	353	7060					
4	434	8680					
	451	9020					
	471	9420	9320				
	508	10160					
5	606	12120					
	571	11420					
	615	12300	12140				
	636	12720					
6	554	11080					
	579	11580					
	575	11500	11760				
	644	12880					
7	641	12820					
	620	12400					
	654	13080	12460				
	577	11540					
8	567	11340					
	608	12160					
	642	12840	12255				
	634	12680					
9	657	13140					
	633	12660					
	661	13220	13140				
	677	13540					
10	660	13200					
	670	13400					
	677	13540					
	645	12900	13260				

Texas *P. parvum* clone – Trial 3 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	208	4160					
	190	3800					
	201	4020	3905				
	182	3640					
1	228	4560					
	234	4680					
	246	4920	4820				
	256	5120					
2	364	7280					
	373	7460					
	366	7320	7225				
	342	6840					
3	382	7640					
	341	6820					
	363	7260	7305				
	375	7500					
4	414	8280					
	404	8080					
	388	7760	8005				
	395	7900					
5	438	8760					
	423	8460					
	412	8240	8370				
	401	8020					
6	445	8900					
	426	8520					
	425	8500	8775				
	459	9180					
7	455	9100					
	443	8860					
	450	9000	9015				
	455	9100					
8	411	8220					
	439	8780					
	420	8400					
	425	8500	8475				

Prymnesium calathiferum – Trial 3 of Nitrogen-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	193	3860					
	179	3580					
	194	3880	3675				
	169	3380					
1	174	3480					
	181	3620					
	188	3760	3545				
	166	3320					
2	397	7940					
	410	8200					
	397	7940	8130				
	422	8440					
3	443	8860					
	475	9500					
	420	8400	8795				
	421	8420					
4	424	8480					
	455	9100					
	440	8800	8840				
	449	8980					
5	444	8880					
	451	9020					
	459	9180	9290				
	504	10080					
6	433	8660					
	446	8920					
	460	9200	9095				
	480	9600					
7	386	7720					
	361	7220					
	380	7600	7645				
	402	8040					
8	342	6840					
	321	6420					
	348	6960					
	345	6900	6780				

North Carolina *P. parvum* clone – Trial 1 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	252	5040		12	10774	215480	
	340	6800			10784	215680	
	249	4980	5760		11033	220660	219400
	311	6220			11289	225780	
1	249	4980		13	11335	226700	
	326	6520			11607	232140	
	226	4520	5970		11616	232320	232115
	393	7860			11865	237300	
2	692	13840		14	11082	221640	
	665	13300			10930	218600	
	743	14860	13630		11322	226440	223870
	626	12520			11440	228800	
3	904	18080		15	11074	221480	
	883	17660			11088	221760	
	905	18100	18180		11670	233400	223870
	944	18880			11394	227880	
4	1295	25900		16	11007	220140	
	1308	26160			11164	223280	
	1359	27180	26995		11316	226320	226130
	1437	28740			11375	227500	
5	1911	38220		17	10676	213520	
	1999	39980			10902	218040	
	1957	39140	39765		11073	221460	224310
	2086	41720			10982	219640	
6	2725	54500		18	9292	185840	
	2774	55480			9309	186180	
	2863	57260	55890		9360	187200	
	2816	56320			9495	189900	218165
7	3928	78560					
	4032	80640					
	3951	79020	79865				
	4062	81240					
8	5327	106540					
	5384	107680					
	5486	109720	109380				
	5679	113580					
9	7073	141460					
	7000	140000					
	7104	142080	142080				
	7239	144780					
10	9097	181940					
	9155	183100					
	8996	179920	183165				
	9385	187700					
11	9960	199200					
	10082	201640					
	10060	201200					
	10534	210680	203180				

South Carolina *P. parvum* clone – Trial 1 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	641	5128		12	12604	252080	
	622	4976			12890	257800	
	649	5192	5212		12894	257880	257580
	694	5552			13128	262560	
1	994	7952		13	13077	261540	
	1023	8184			13211	264220	
	1125	9000	8246		13316	266320	264885
	981	7848			13373	267460	
2	594	11880		14	13272	265440	
	566	11320			13226	264520	
	590	11800	11570		13424	268480	269180
	564	11280			13914	278280	
3	992	19840		15	13115	262300	
	917	18340			13183	263660	
	948	18960	19115		13266	265320	266030
	966	19320			13642	272840	
4	1316	26320		16	13000	260000	
	1362	27240			13050	261000	
	1335	26700	26720		13145	262900	261270
	1331	26620			13059	261180	
5	1975	39500		17	13140	262800	
	1917	38340			13282	265640	
	1890	37800	38645		13427	268540	267425
	1947	38940			13636	272720	
6	2934	58680		18	13099	261980	
	3018	60360			13239	264780	
	3058	61160	59895		13336	266720	266350
	2969	59380			13596	271920	
7	4657	93140		19	12732	254640	
	4624	92480			12850	257000	
	4566	91320	92555		12823	256460	258035
	4664	93280			13202	264040	
8	5614	112280		20	12810	256200	
	6443	128860			12821	256420	
	5314	106280	120915		12983	259660	
	6812	136240			12971	259420	257925
9	8697	173940					
	9304	186080					
	9393	187860	185395				
	9685	193700					
10	10830	216600					
	11198	223960					
	11189	223780	224040				
	11591	231820					
11	12466	249320					
	12489	249780					
	12569	251380					
	12964	259280	252440				

Texas *P. parvum* clone – Trial 1 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	173	3460					
	189	3780					
	188	3760	3755				
	201	4020					
1	272	5440					
	285	5700					
	280	5600	5725				
	308	6160					
2	363	7260					
	341	6820					
	336	6720	6985				
	357	7140					
3	449	8980					
	444	8880					
	425	8500	8765				
	435	8700					
4	453	9060					
	490	9800					
	436	8720	9245				
	470	9400					
5	579	11580					
	555	11100					
	539	10780	11335				
	594	11880					
6	640	12800					
	621	12420					
	606	12120	12405				
	614	12280					
7	867	17340					
	921	18420					
	917	18340	17755				
	846	16920					
8	1070	21400					
	1146	22920					
	1010	20200	22380				
	1250	25000					
9	1190	23800					
	1214	24280					
	1263	25260					
	1212	24240	24395				

Prymnesium calathiferum – Trial 1 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	596	4768		12	6715	134300	
	588	4704			6039	120780	
	607	4856	4780		6757	135140	127610
	599	4792			6011	120220	
1	492	3936		13	6850	137000	
	519	4152			7055	141100	
	477	3816	3860		7010	140200	140610
	442	3536			7207	144140	
2	316	6320		14	6666	133320	
	301	6020			6695	133900	
	319	6380	6180		6792	135840	135235
	300	6000			6894	137880	
3	556	11120		15	6400	128000	
	473	9460			6453	129060	
	445	8900	9870		6622	132440	130045
	500	10000			6534	130680	
4	645	12900		16	5490	109800	
	732	14640			5432	108640	
	673	13460	13885		5619	112380	111075
	727	14540			5674	113480	
5	755	15100		17	4756	95120	
	755	15100			4734	94680	
	835	16700	15535		4804	96080	
	762	15240			4751	95020	95225
6	1873	37460					
	2266	45320					
	1698	33960	40390				
	2241	44820					
7	3698	73960					
	3586	71720					
	3733	74660	74420				
	3867	77340					
8	4857	97140					
	5124	102480					
	5155	103100	101300				
	5124	102480					
9	5677	113540					
	5582	111640					
	6430	128600	116860				
	5683	113660					
10	6912	138240					
	7182	143640					
	7195	143900	142695				
	7250	145000					
11	6784	135680					
	7024	140480					
	6401	128020					
	7177	143540	136930				

North Carolina *P. parvum* clone – Trial 2 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	440	8800		12	10040	200800	
	403	8060			10138	202760	
	391	7820	8185		10093	201860	204145
	403	8060			10558	211160	
1	536	10720		13	10098	201960	
	464	9280			10751	215020	
	449	8980	9540		10947	218940	215930
	459	9180			11390	227800	
2	563	11260		14	8992	179840	
	599	11980			8911	178220	
	615	12300	12200		9433	188660	184605
	663	13260			9585	191700	
3	1035	20700		15	10037	200740	
	1031	20620			10270	205400	
	1063	21260	21145		10604	212080	
	1100	22000			10667	213340	207890
4	1451	29020					
	1526	30520					
	1756	35120	33155				
	1898	37960					
5	1791	35820					
	1795	35900					
	1833	36660	36890				
	1959	39180					
6	2422	48440					
	2428	48560					
	2514	50280	49855				
	2607	52140					
7	4896	97920					
	4908	98160					
	4923	98460	98235				
	4920	98400					
8	6247	124940					
	6235	124700					
	6282	125640	125835				
	6403	128060					
9	8327	166540					
	8559	171180					
	8438	168760	169670				
	8610	172200					
10	9546	190920					
	9820	196400					
	10440	208800	202470				
	10688	213760					
11	10010	200200					
	10141	202820					
	10104	202080					
	10690	213800	204725				

South Carolina *P. parvum* clone – Trial 2 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	500	10000		12	13646	272920	
	533	10660			13616	272320	
	518	10360	10470		13688	273760	275980
	543	10860			14246	284920	
1	771	15420		13	13640	272800	
	779	15580			13937	278740	
	810	16200	15955		13941	278820	280170
	831	16620			14516	290320	
2	1049	20980		14	13870	277400	
	1031	20620			14082	281640	
	1077	21540	21835		14113	282260	283125
	1210	24200			14560	291200	
3	1654	33080		15	14556	291120	
	1652	33040			14493	289860	
	1643	32860	33420		14916	298320	
	1735	34700			15381	307620	296730
4	2117	42340					
	2166	43320					
	2297	45940	45245				
	2469	49380					
5	2956	59120					
	3058	61160					
	3100	62000	61510				
	3188	63760					
6	3894	77880					
	3941	78820					
	4088	81760	80605				
	4198	83960					
7	6362	127240					
	6445	128900					
	6495	129900	129410				
	6580	131600					
8	8227	164540					
	8250	165000					
	8272	165440	166200				
	8491	169820					
9	10271	205420					
	10324	206480					
	10407	208140	208125				
	10623	212460					
10	12013	240260					
	11906	238120					
	12239	244780	243970				
	12636	252720					
11	13115	262300					
	13184	263680					
	13453	269060					
	13625	272500	266885				

Texas *P. parvum* clone – Trial 2 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	783	15660		12	3330	66600	
	732	14640			3440	68800	
	801	16020	15550		3370	67400	67700
	794	15880			3463	69260	
1	1325	26500		13	3298	65960	
	1337	26740			3377	67540	
	1300	26000	26480		3338	66760	
	1334	26680			3370	67400	66750
2	1708	34160					
	1718	34360					
	1718	34360	34435				
	1743	34860					
3	2063	41260					
	2055	41100					
	2168	43360	41945				
	2103	42060					
4	2250	45000					
	2213	44260					
	2255	45100	44515				
	2185	43700					
5	2608	52160					
	2521	50420					
	2606	52120	51025				
	2470	49400					
6	3103	62060					
			62060				
7	3432	68640					
	3467	69340					
	3428	68560	68695				
	3412	68240					
8	3168	63360					
	3271	65420					
	3369	67380	64390				
	3266	65320					
9	3175	63500					
	3235	64700					
	3320	66400	65550				
	3380	67600					
10	3375	67500					
	3427	68540					
	3407	68140	68060				
	3419	68380					
11	3235	64700					
	3362	67240					
	3468	69360					
	3434	68680	67495				

Prymnesium calathiferum – Trial 2 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	575	11500					
	504	10080					
	508	10160	10415				
	496	9920					
1	366	7320					
	328	6560					
	339	6780	6685				
	304	6080					
2	508	10160					
	599	11980					
	497	9940	10770				
	550	11000					
3	631	12620					
	632	12640					
	633	12660	12635				
	631	12620					
4	1300	26000					
	1465	29300					
	1494	29880	28520				
	1445	28900					
5	2310	46200					
	2229	44580					
	2187	43740	44665				
	2207	44140					
6	3126	62520					
	3046	60920					
	3122	62440	61675				
	3041	60820					
7	3920	78400					
	4011	80220					
	3967	79340	79385				
	3979	79580					
8	5414	108280					
	5477	109540					
	5341	106820	108845				
	5537	110740					
9	5948	118960					
	5754	115080					
	5793	115860	117215				
	5948	118960					
10	5093	101860					
	5124	102480					
	5292	105840					
	5151	103020	103300				

North Carolina *P. parvum* clone – Trial 3 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	77	1540		12	3472	69440	
	64	1280			3616	72320	
	71	1420	1360		3454	69080	70645
	60	1200			3587	71740	
1	170	1360		13	4473	89460	
	182	1456			4678	93560	
	197	1576	1462		4676	93520	91840
	182	1456			4541	90820	
2	242	1936		14	6558	131160	
	240	1920			6311	126220	
	249	1992	1928		6490	129800	130090
	233	1864			6659	133180	
3	299	2392		15	8918	178360	
	308	2464			8663	173260	
	311	2488	2468		8847	176940	177005
	316	2528			8973	179460	
4	469	3752		16	8840	176800	
	472	3776			9199	183980	
	450	3600	3758		8667	173340	180945
	488	3904			9483	189660	
5	630	5040		17	9881	197620	
	612	4896			10145	202900	
	621	4968	5040		10046	200920	202370
	657	5256			10402	208040	
6	848	6784		18	9948	198960	
	794	6352			10068	201360	
	825	6600	6664		10456	209120	205070
	865	6920			10542	210840	
7	1076	8608		19	10496	209920	
	1063	8504			10296	205920	
	1111	8888	8756		10656	213120	212955
	1128	9024			11143	222860	
8	1601	12808		20	10201	204020	
	1572	12576			10343	206860	
	1590	12720	12786		10530	210600	
	1630	13040			11040	220800	210570
9	2027	16216					
	2228	17824					
	2108	16864	17296				
	2285	18280					
10	3581	28648					
	3394	27152					
	3630	29040	28106				
	3448	27584					
11	2270	45400					
	2225	44500					
	2177	43540					
	2189	43780	44305				

South Carolina *P. parvum* clone – Trial 3 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	110	2200		12	10452	418080	
	101	2020			10240	409600	
	110	2200	2135		10540	421600	416590
	106	2120			10427	417080	
1	350	2800		13	11209	448360	
	323	2584			11059	442360	
	346	2768	2756		11207	448280	450230
	359	2872			11548	461920	
2	428	3424		14	11823	472920	
	443	3544			11866	474640	
	447	3576	3492		12051	482040	483280
	428	3424			12588	503520	
3	719	5752		15	12368	494720	
	707	5656			12198	487920	
	701	5608	5638		12357	494280	494500
	692	5536			12527	501080	
4	1001	8008		16	11238	449520	
	917	7336			11790	471600	
	990	7920	7764		11256	450240	464660
	974	7792			12182	487280	
5	1470	11760		17	12308	492320	
	1317	10536			12297	491880	
	1360	10880	11026		12147	485880	492940
	1366	10928			12542	501680	
6	2305	18440		18	12504	500160	
	2332	18656			12493	499720	
	2334	18672	18494		12341	493640	503220
	2276	18208			12984	519360	
7	3494	27952		19	12605	504200	
	3617	28936			12460	498400	
	3605	28840	28584		12835	513400	508620
	3576	28608			12962	518480	
8	5769	46152		20	11681	467240	
	5530	44240			11552	462080	
	6087	48696	46442		11820	472800	
	5835	46680			12349	493960	474020
9	7981	63848					
	8336	66688					
	7783	62264	65214				
	8507	68056					
10	13199	105592					
	12690	101520					
	13268	106144	104592				
	13139	105112					
11	7480	299200					
	7207	288280					
	7375	295000					
	7179	287160	292410				

Texas *P. parvum* clone – Trial 3 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	340	6800					
	410	8200					
	359	7180	7525				
	396	7920					
1	523	10460					
	500	10000					
	521	10420	10095				
	475	9500					
2	832	16640					
	795	15900					
	866	17320	16460				
	799	15980					
3	824	16480					
	845	16900					
	864	17280	16935				
	854	17080					
4	884	17680					
	905	18100					
	908	18160	17530				
	809	16180					
5	1085	21700					
	1083	21660					
	1156	23120	22045				
	1085	21700					
6	1069	21380					
	1074	21480					
	1029	20580	21185				
	1065	21300					
7	1140	22800					
	1201	24020					
	1122	22440	23295				
	1196	23920					
8	1190	23800					
	1139	22780					
	1296	25920	24425				
	1260	25200					
9	1114	22280					
	1061	21220					
	1294	25880	23705				
	1272	25440					
10	1062	21240					
	1361	27220					
	1015	20300					
	1006	20120	22220				

Prymnesium calathiferum – Trial 3 of Phosphorus-Deficient Treatment

Day	Cells/0.5 mL	Cells/mL	Mean cells/mL	Day	Cells/0.5 mL	Cells/mL	Mean cells/mL
0	154	3080		12	5025	100500	
	132	2640			5008	100160	
	150	3000	2910		5268	105360	102910
	146	2920			5281	105620	
1	107	2140		13	4803	96060	
	139	2780			4751	95020	
	125	2500	2405		5053	101060	97200
	110	2200			4833	96660	
2	182	3640		14	4696	93920	
	195	3900			4767	95340	
	213	4260	3905		4914	98280	96145
	191	3820			4852	97040	
3	267	5340		15	4820	96400	
	292	5840			4752	95040	
	262	5240	5465		4761	95220	96025
	272	5440			4872	97440	
4	314	6280		16	4137	82740	
	309	6180			4175	83500	
	338	6760	6470		4177	83540	84045
	333	6660			4320	86400	
5	368	7360		17	3764	75280	
	341	6820			3695	73900	
	386	7720	7060		3719	74380	
	317	6340			3730	74600	74540
6	990	19800					
	1002	20040					
	998	19960	20050				
	1020	20400					
7	1851	37020					
	1896	37920					
	1989	39780	38850				
	2034	40680					
8	2861	57220					
	3017	60340					
	2966	59320	59040				
	2964	59280					
9	2742	54840					
	2527	50540					
	2714	54280	53105				
	2638	52760					
10	3980	79600					
	4064	81280					
	4287	85740	81860				
	4041	80820					
11	5870	117400					
	6111	122220					
	6263	125260					
	6301	126020	122725				

Appendix B: Erythyrocyte Lysis Assay Data

Replete: Trial Averages

Hemolytic activity of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nutrient replete conditions. Values for means of three individual trials ± one standard deviation.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis (Cell)	% Lysis	Lysis(Cell)
	Lag	70.877 ±33.219	3543.172 ±973.283	48.645 ±36.153	3639.586 ±1371.491	20.116 ±11.471	1958.295 ±1701.731	1.114 ±0.739	187.553 ±133.058
	Log	77.973 ±10.283	259.223 ±111.171	33.886 ±11.621	143.808 ±79.522	30.255 ±11.339	203.530 ±144.659	3.766 ±2.385	8.662 ±5.333
	Stationary	51.658 ±4.143	77.711 ±10.591	27.845 ±17.538	45.837 ±30.097	18.363 ±11.003	40.742 ±30.975	3.907 ±4.034	28.323 ±31.049

Hemolytic activity of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nutrient replete conditions. Values for means of three individual trials ± one standard deviation.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis (Cell)	% Lysis	Lysis(Cell)
	Lag	89.000 ±8.742	1440.031 ±765.165	97.592 ±8.031	2713.572 ±2415.977	91.475 ±20.171	2395.659 ±1824.391	1.054 ±0.514	64.847 ±61.544
	Log	98.496 ±2.214	261.677 ±124.744	97.841 ±4.381	326.539 ±190.61	101.594 ±5.484	456.98 ±263.425	13.522 ±3.972	45.182 ±10.9
	Stationary	91.596 ±3.684	113.368 ±36.977	83.426 ±29.258	126.509 ±80.509	97.253 ±9.442	174.982 ±59.814	38.472 ±9.628	97.390 ±44.456

Replete: Trial 1

Hemolytic activity (n=8 ± one standard deviation) of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and
P. calathiferum grown in nutrient replete conditions. All clones were inoculated on February 7, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis (Cell)	% Lysis	Lysis (Cell)	% Lysis	Lysis (Cell)	% Lysis	Lysis (Cell)
2	Lag	97.46 ±1.849	2862.794 ±54.226	90.041 ±2.018	3907.191 ±87.592	8.73 ±0.32	155.230 ±5.685	1.966 ±0.716	138.626 ±50.452
15	Log	69.763 ±3.763	147.636 ±7.963	22.541 ±2.033	52.408 ±4.727	17.273 ±0.741	36.534 ±1.567	3.416 ±0.387[a]	21.904 ±2.481 [a]
22	Stationary	53.797 ±4.834	82.739 ±7.436	42.817 ±4.815	65.116 ±7.324	19.288 ±2.517	28.456 ±3.714	8.397 ±1.026	19.979 ±2.441

*-n=4; a=Log phase on Day 7

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nutrient replete conditions. All clones were inoculated on February 7, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis (Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
2	Lag	98.179 ±5.615*	576.677 ±32.989*	106.395 ±6.712*	923.373 ±58.259*	108.266 ±1.16*	384.869 ±4.124*	1.645 ±0.099*	23.197 ±1.393*
15	Log	96.610 ±7.160	204.452 ±15.152	100.65 ±5.698	234.010 ±13.248	107.607 ±3.754	227.594 ±7.940	2.545 ±0.239*[a]	16.322 ±1.536*[a]
22	Stationary	95.814 ±5.998	147.360 ±9.226	100.605 ±4.045	152.998 ±6.153	107.926 ±5.542	159.225 ±8.178	48.429 ±4.782	115.221 ±11.379

*-n=4; a=Log phase on Day 7

Replete: Trial 2

Hemolytic activity (n=8 ± one standard deviation) of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nutrient replete conditions. All clones were inoculated on March 14, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis (Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis (Cell)	% Lysis	Lysis (Cell)
2	Lag	53.530 ±23.923	2219.527 ±992.023	23.277 ±2.780	1360.109 ±162.430	22.983 ±2.152	1249.307 ±117.001	1.379 ±0.353	334.794 ±85.737
9	Log	35.520 ±2.988	131.877 ±11.104	33.352 ±2.256	181.877 ±12.302	21.794 ±1.040	224.448 ±10.712	0.707 ±0.304	7.649 ±3.285
20	Stationary	53.952 ±2.907	67.304 ±3.622	32.167 ±1.555	61.240 ±2.959	26.153 ±1.348	52.435 ±2.702	0.530 ±0.324[a]	2.846 ±1.738[a]

a - Stationary phase on Day 19

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nutrient replete conditions. All clones were inoculated on March 14, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis (Cell)	% Lysis	Lysis (Cell)
2	Lag	88.699 ±7.789	3677.734 ±322.965	90.666 ±7.063	5297.750 ±412.731	92.556 ±7.837	5031.126 ±426.052	0.576 ±0.548	69.433 ±66.112
9	Log	93.061 ±5.638	345.746 ±20.949	100.079 ±4.255	545.751 ±23.204	89.473 ±2.035	921.451 ±20.965	3.595 ±0.458	38.895 ±4.959
20	Stationary	96.067 ±3.702	119.841 ±4.618	100.030 ±5.432	190.437 ±10.340	101.628 ±4.455	203.760 ±8.931	9.190 ±0.961[a]	49.332 ±5.159[a]

a - Stationary phase on Day 19

Replete: Trial 3

Hemolytic activity (n=8 ± one standard deviation) of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nutrient replete conditions. All clones were inoculated on April 25, 2005. (n=8)

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	33.637 ±3.801 [a]	3108.704 ±351.310 [a]	32.618 ±2.513 [a]	2154.015 ±165.964 [a]	31.670 ±2.120	3536.292 ±236.691	0.633 ±0.510	85.886 ±69.160
11	Log	74.649 ±7.610 *	260.059 ±26.506*	45.766 ±9.681 *	197.140 ±41.693*	35.262 ±6.140 *	290.246 ±50.527*	6.318 ±1.092 * [b]	44.297 ±7.655* [b]
27	Stationary	46.882 ±3.246	65.542 ±4.330	8.550 ±1.229 [d]	11.156 ±1.604 [d]	6.927 ±0.513	17.795 ±1.317	0.588 ±0.007 [c]	2.298 ±2.744 [c]

* - n=4; a - Lag phase on Day 1; b- Log phase on Day 7, c-Stationary phase on Day 17; d-Stationary phase on Day 29

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nutrient replete conditions. All clones were inoculated on April 25, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis (Cell)	% Lysis	Lysis(Cell)
0	Lag	88.049 ±7.647[a]	2034.317 ±176.708[a]	95.714 ±10.011[a]	1755.746 ±183.672[a]	69.085 ±9.496	2857.046 ±392.690	0.712 ±0.337	35.805 ±16.947
11	Log	100.933 ±25.938	175.813 ±45.173	92.793 ±22.836*	199.855 ±49.175*	96.869 ±21.334	398.672 ±87.788	9.624 ±4.649[b]	35.514 ±17.160[b]
27	Stationary	89.013 ±7.504	118.748 ±10.011	49.664 ±7.372 [d]	36.094 ±4.968[d]	93.848 ±9.674	241.097 ±24.857	37.777 ±0.055 [c]	147.685 ±6.85[c]

* - n=4; a - Lag phase on Day 1; b- Log phase on Day 7, c-Stationary phase on Day 17; d-Stationary phase on Day 29

N-Deficient: Trial Averages

Hemolytic activity of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nitrogen-deficient conditions (N:P=4:1). Values for means of three individual trials ± one standard deviation.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
	Lag	33.662 ±10.573	17487 ±1601.087	28.369 ±9.451	20795.93 ±14476.8	5.259 ±1.680	1456.904 ±1309.345	2.226 ±1.504	779.944 ±352.989
	Log	63.162 ±36.237	12291.53 ±8079.995	57.482 ±28.469	7850.901 ±837.061	8.108 ±1.062	1133.417 ±472.738	1.151 ±0.151	214.897 ±59.021
	Stationary	76.324 ±22.766	9599.747 ±3307.954	84.901 ±9.164	9815.413 ±1873.03	19.587 ±7.686	2423.249 ±690.949	1.504 ±1.355	190.649 ±12.719

Hemolytic activity of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nitrogen-deficient conditions (N:P=4:1). Values for means of three individual trials ± one standard deviation.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
	Lag	92.521 ±7.958	13228.492 ±5806.117	90.382 ±5.191	14670.09 ±11760.71	82.794 ±6.939	7164.427 ±5545.626	1.785 ±1.322	177.615 ±139.822
	Log	91.208 ±1.756	5224.072 ±1682.887	93.794 ±3.869	3469.88 ±561.342	92.024 ±4.961	3484.36 ±1315.627	0.839 ±0.384	35.194 ±21.286
	Stationary	87.965 ±7.376	3107.485 ±283.225	91.067 ±2.425	3235.977 ±111.060	92.008 ±6.426	3123.968 ±1442.671	0.749 ±0.431	26.451 ±6.237

N-Deficient: Trial 1

Hemolytic activity (n=8 ± one standard deviation) of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nitrogen- deficient conditions (N:P=4:1). The NC and SC clones were inoculated on March 14, 2005, TX was inoculated on May 16, 2005, and Pcal was inoculated on May 22, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
3	Lag	26.878 ±3.801	18,882.043 ±2669.789	25.568 ±6.709	6830.685 ±1791.935	4.543 ±0.481 [a]	962.087 ±101.913 [a]	3.899 ±1.271 [a]	1133.365 ±369.273 [a]
7	Log	21.347 ±1.202	4013.765 ±225.960	51.381 ±6.902	7398.104 ±646.164	7.597 ±0.764 [c]	1060.522 ±106.638 [c]	1.037 ±0.509 [b]	147.373 ±72.330 [b]
14	Stationary	87.611 ±5.588	12,211.162 ±778.779	91.055 ±6.382	11,961.745 ±838.374	13.664 ±2.509 [e]	1817.574 ±333.708 [e]	3.068 ±1.221 [d]	190.731 ±75.919 [d]

a – Lag Phase on Day 0; b- Log phase on Day 2; c-Log phase on Day 4; d-Stationary phase on Day 8; e-Stationary phase on Day 11

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nitrogen-deficient conditions (N:P=4:1). The NC and SC clones were inoculated on March 14, 2005, TX was inoculated on May 16, 2005, and Pcal was inoculated on May 22, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
3	Lag	97.582 ±9.243	17,577.219 ±1664.694	94.370 ±9.621	6464.586 ±658.947	75.179 ±13.090 [a]	2793.115 ±486.370 [a]	2.806 ±0.643 [a]	302.081 ±69.203 [a]
7	Log	90.645 ±3.109	4869.523 ±167.000	95.057 ±2.924	3907.284 ±120.173	97.700 ±13.290 [c]	4011.579 ±545.716 [c]	0.405 ±0.223 [b]	13.079 ±7.212 [b]
14	Stationary	93.405 ±6.075	3425.974 ±222.801	90.236 ±7.957	3152.211 ±277.942	86.481 ±10.128 [e]	2875.958 ±336.774 [e]	1.246 ±0.681 [d]	19.363 ±10.577 [d]

a – Lag Phase on Day 0; b- Log phase on Day 2; c-Log phase on Day 4; d-Stationary phase on Day 8; e-Stationary phase on Day 11

N-Deficient: Trial 2

Hemolytic activity (n=8 ± one standard deviation) of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nitrogen- deficient conditions (N:P=4:1). NC was inoculated on April 11, 2005, SC was inoculated on April 1, 2005, TX was inoculated on May 16, 2005, and Pcal was inoculated on May 31, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	28.264 ±3.505	17,840.126 ±2212.11	20.636 ±1.132	35,735.106 ±1960.151	4.056 ±0.476	467.076 ±54.835	0.988 ±0.553	427.387 ±238.959
3	Log	82.760 ±2.172 [b]	20,158.048 ±529.033 [b]	88.506 ±4.589 [c]	8816.828 ±457.214 [c]	9.329 ±1.005 [a]	701.361 ±75.535 [a]	1.094 ±0.318	256.648 ±74.623
8	Stationary	91.241 ±12.035 [e]	10,708.154 ±1412.216[e]	89.280 ±6.774 [f]	8511.660 ±645.708 [f]	28.272 ±6.541 [d]	2276.333 ±526.546 [d]	0.689 ±0.601	203.326 ±177.137

a-Log phase on Day 4; b- Log phase on Day 5; c-Log phase on Day 7; d- Stationary phase on Day 11; e- Stationary phase on Day 18; f-Stationary phase on Day 21

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nitrogen-deficient conditions (N:P=4:1). NC was inoculated on April 11, 2005, SC was inoculated on April 1, 2005, TX was inoculated on May 16, 2005, and Pcal was inoculated on May 31, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	83.348 ±6.186	15,473.113 ±1148.420	84.512 ±4.329	28,143.889 ±1441.445	84.441 ±11.868	1705.773 ±239.761	0.292 ±0.236	26.280 ±21.278
3	Log	89.803 ±5.137 [b]	7055.985 ±403.562 [b]	96.875 ±5.589 [c]	2836.916 ±163.760 [c]	89.855 ±14.102[a]	1986.893 ±311.843 [a]	1.137 ±0.388	55.541 ±18.986
8	Stationary	90.920 ±13.405 [e]	2883.898 ±425.147 [e]	93.799 ±6.175 [f]	3193.766 ±210.211[f]	90.486 ±7.021 [d]	1821.380 ±141.299 [d]	0.464 ±0.571	31.101 ±38.260

a-Log phase on Day 4; b- Log phase on Day 5; c-Log phase on Day 7; d- Stationary phase on Day 11; e- Stationary phase on Day 18; f-Stationary phase on Day 21

N-Deficient: Trial 3

Hemolytic activity (n=8 ± one standard deviation) of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nitrogen-deficient conditions (N:P=4:1). NC and SC were inoculated on May 4, 2005, TX was inoculated on May 22, 2005, and Pcal was inoculated on May 31, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis (Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	45.844 ±6.390	15,738.825 ±2193.979	38.905 ±3.211	19,821.984 ±1636.208	7.179 ±0.755	2941.548 ±309.398	1.790 ±0.670	779.079 ±291.914
3	Log	85.380 ±8.473 [b]	12,702.783 ±1260.536 [b]	32.558 ±6.608*	7337.772 ±1489.092*	7.399 ±1.092 [a]	1638.369 ±241.887 [a]	1.323 ±0.357	240.670 ±64.974
10	Stationary	50.120 ±8.856	5,879.926 ±1039.101	74.370 ±10.606	8972.830 ±1279.726	16.825 ±1.988 [c]	3175.841 ±375.304 [c]	0.754 ±0.652 [d]	177.889 ±153.802 [d]

* - n=4; a-Log phase on Day 2; b-Log phase on Day 4; c-Stationary phase on Day 8

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in nitrogen-deficient conditions (N:P=4:1). NC and SC were inoculated on May 4, 2005, TX was inoculated on May 22, 2005, and Pcal was inoculated on May 31, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	96.634 ±7.112	6635.144 ±488.400	92.264 ±10.293	9401.804 ±1048.959	88.762 ±8.664	6994.394 ±682.639	2.256 ±1.494	204.563 ±135.550
3	Log	93.177 ±6.724 [b]	3746.708 ±270.339 [b]	89.451 ±18.002*	3665.439 ±737.596*	88.516 ±13.879 [a]	4454.609 ±698.533 [a]	0.975 ±0.354	36.962 ±13.417
10	Stationary	79.570 ±7.723	3012.582 ±292.433	89.168 ±6.618	3361.954 ±249.544	99.059 ±9.075 [c]	4674.566 ±428.318 [c]	0.539 ±0.472 [c]	28.889 ±25.889 [c]

* - n=4; a-Log phase on Day 2; b-Log phase on Day 4; c-Stationary phase on Day 8

P-Deficient: Trial Averages

Hemolytic activity of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in phosphorus-deficient conditions (N:P=80:1). Values for means of three individual trials ± one standard deviation.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
	Lag	43.472 ±31.767	13755.502 ±4880.402	29.767 ±9.941	8581.064 ±3782.827	12.659 ±8.903	3406.254 ±2602.884	2.243 ±1.731	457.391 ±407.998
	Log	85.607 ±4.026	3084.742 ±347.592	81.398 ±7.211	2039.98 ±382.495	25.611 ±9.827	2081.198 ±1357.01	1.968 ±0.892	62.139 ±25.679
	Stationary	91.756 ±2.4481	691.985 ±15.783	92.751 ±4.173	462.845 ±139.360	61.078 ±35.632	2849.406 ±1723.339	3.144 ±3.767	40.949 ±41.264

Hemolytic activity of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in phosphorus-deficient conditions (N:P=80:1). Values for means of three individual trials ± one standard deviation.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
	Lag	89.540 ±6.881	21339.202 ±28043.698	72.926 ±14.657	10430.26 ±9753.698	93.006 ±9.359	4758.803 ±3284.013	2.284 ±0.723	168.536 ±117.662
	Log	88.774 ±1.456	1085.792 ±113.142	94.589 ±1.934	809.984 ±79.376	94.769 ±1.411	3456.237 ±782.519	9.712 ±5.746	128.736 ±44.239
	Stationary	94.243 ±5.381	448.771 ±250.089	94.165 ±7.07	298.831 ±48.01	99.335 ±5.971	2595.053 ±697.868	13.084 ±9.523	149.204 ±124.195

P-Deficient: Trial 1

Hemolytic activity (n=8 ± one standard deviation) by the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in phosphorus-deficient conditions (N: P = 80:1). NC was inoculated on April 11, 2005, SC and Pcal were inoculated on April 3, 2005, and TX was inoculated on May 31, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	29.593 ±5.713	8218.893 ±1586.902	39.526 ±3.406	12,133.848 ±1045.656	12.115 ±1.583	5162.640 ±674.380	2.345 ±0.937	784.908 ±313.779
5	Log	82.760 ±2.172	3330.526 ±87.407	86.497 ±6.937[a]	2310.445 ±185.311[a]	18.831 ±2.525	2215.244 ±297.063	2.649 ±0.584[b]	56.967 ±12.545[b]
	Stationary	92.877 ±11.889[e]	681.241 ±87.195[e]	96.034 ±4.491[f]	595.829 ±27.859[f]	23.021 ±2.207[c]	1509.645 ±144.743[c]	7.481 ±2.181[d]	88.516 ±25.810[d]

a- Log phase on Day 6; b – Log phase on Day 7; c- Stationary phase on Day 9; d-Stationary phase on Day 14; e- Stationary Phase on Day 18; f- Stationary phase on Day 20

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in phosphorus-deficient conditions (N: P = 80:1). NC was inoculated on April 11, 2005, SC and Pcal were inoculated on April 3, 2005, and TX was inoculated on May 31, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	94.459 ±5.885	7716.049 ±480.824	87.995 ±4.092	7944.989 ±369.493	92.630 ±15.259	8223.559 ±1354.554	3.025 ±0.931	297.773 ±91.618
5	Log	89.803 ±5.137	1165.795 ±66.677	95.956 ±3.243[a]	753.857 ±25.477[a]	96.266 ±19.211	4355.501 ±869.135	15.589 ±3.156[b]	176.408 ±35.713[b]
	Stationary	98.297 ±14.068[e]	257.499 ±36.849[e]	94.675 ±4.706[f]	309.156 ±15.366[f]	101.162 ±16.604[c]	1951.100 ±320.289[c]	18.664 ±1.556[d]	116.227 ±9.691[d]

a- Log phase on Day 6; b – Log phase on Day 7; c- Stationary phase on Day 9; d-Stationary phase on Day 14; e- Stationary Phase on Day 18; f- Stationary phase on Day 20

P-Deficient: Trial 2

Hemolytic activity (n=8 ± one standard deviation) of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in phosphorus-deficient conditions (N: P = 80:1). All clones were inoculated on May 2, 2005, except TX, which was inoculated on May 17, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	79.816 ±9.483	15,613.867 ±1854.853	30.123 ±3.303	4603.973 ±504.832	4.041 ±0.943	415.873 ±97.056	3.821 ±1.493	586.911 ±229.334
5	Log	88.453 ±11494 [a]	2838.957 ±368.890 [a]	76.299 ±14.844*	1769.515 ±586.286*	21.120 ±2.973	662.139 ±93.234	2.296 ±0.787*	90.011 ±29.955*
15	Stationary	88.948 ±12.007	684.609 ±92.412	88.055 ±12.382	474.825 ±66.767	93.649 ±8.362 [c]	2245.051 ±200.443 [c]	1.263 ±0.672 [b]	19.567 ±10.416 [b]

* - n=4; a-Log phase on Day 6; b- Stationary phase on Day 10; c-Stationary Phase on Day 13

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in phosphorus-deficient conditions (N: P = 80:1). All clones were inoculated on May 2, 2005, except TX, which was inoculated on May 17, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	83.154 ±12.497	2709.547 ±407.155	72.063 ±11.050	2159.623 ±331.103	83.840 ±11.012	1691.755 ±222.176	2.245 ±1.133	67.616 ±34.138
5	Log	87.744 ±9.586 [a]	1005.788 ±109.872 [a]	93.221 ±6.706*	866.111 ±62.303*	93.463 ±9.609	2930.251 ±301.325	9.441 ±4.049*	120.795 ±51.799*
15	Stationary	88.138 ±11.172	357.042 ±45.253	86.853 ±11.294	246.497 ±32.052	104.179 ±9.464 [c]	2497.490 ±226.854 [c]	18.500 ±2.988 [b]	286.560 ±46.281 [b]

* - n=4; a-Log phase on Day 6; b- Stationary phase on Day 10; c-Stationary Phase on Day 13

P-Deficient: Trial 3

Hemolytic activity (n=8 ± one standard deviation) of the supernatant for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P. calathiferum* grown in phosphorus-deficient conditions (N: P = 80:1). NC and SC were inoculated on March 14, 2005, TX was inoculated on June 9, 2005, and Pcal was inoculated on July 13, 2005.

Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	21.006 ±1.309	17,433.747 ±1086.058	19.653 ±1.848	9005.370 ±846.678	21.882 ±1.930 [a]	4640.248 ±410.315 [a]	0.463 ±0.355 [a]	254.445 ±195.218 [a]
9	Log	21.918 ±1.403	2027.403 ±129.803	28.548 ±3.811	700.353 ±93.503	36.881 ±5.100 [b]	3366.210 ±465.487 [b]	0.958 ±0.314 [c]	39.439 ±12.947 [c]
20	Stationary	93.443 ±4.186	710.106 ±31.807	94.165 ±4.803	317.882 ±16.211	66.563 ±10.217 [d]	4793.522 ±735.687 [d]	0.688 ±0.381 [e]	14.763 ±8.178 [e]

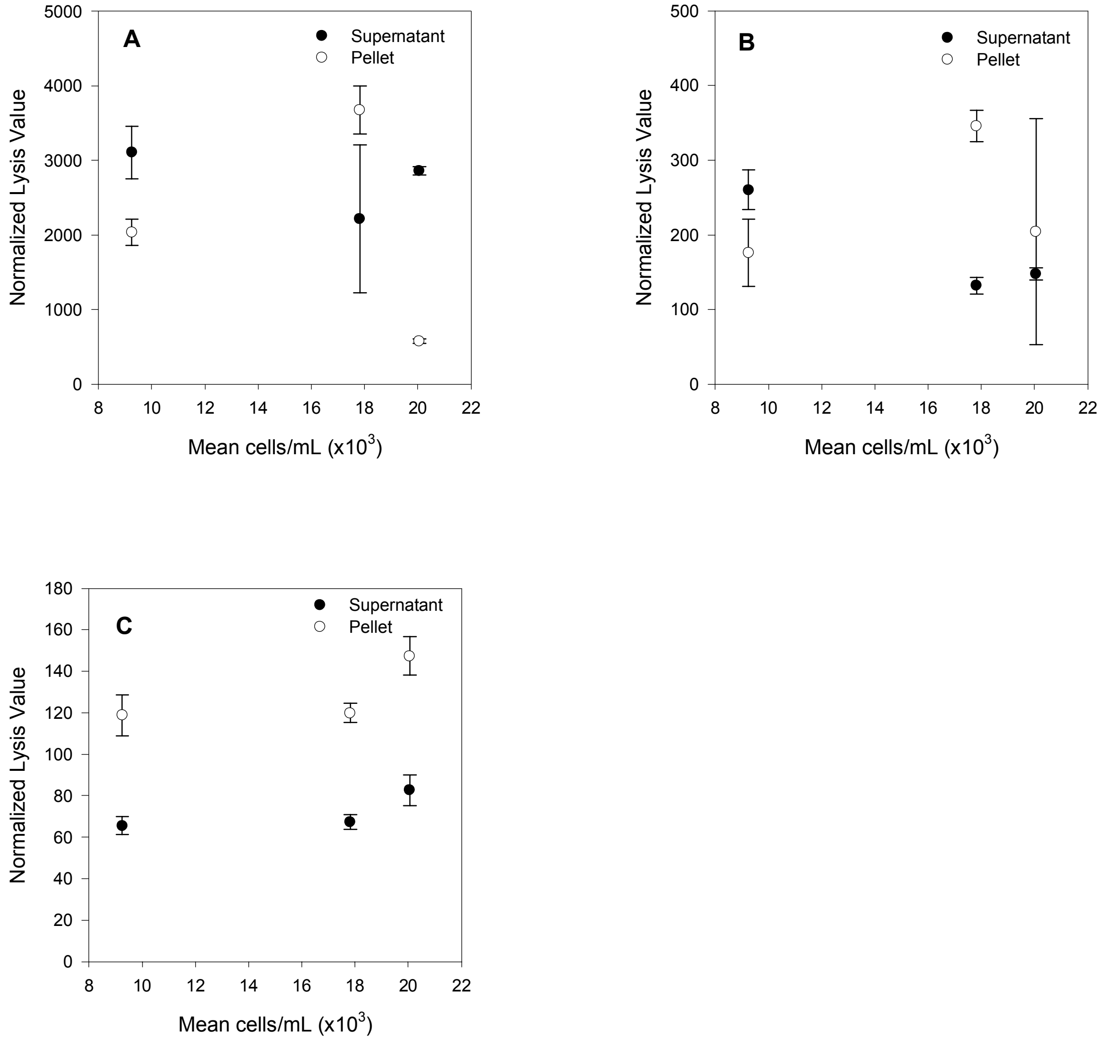
a- Lag phase on Day 2; b- Log phase on Day 4; c-Log phase on Day 7; d- Stationary phase on Day 10; e-Stationary phase on Day 17

Hemolytic activity (n=8 ± one standard deviation) of the pellet for lag, log, and stationary phases of three geographically-distinct clones of *Prymnesium parvum* (NC=North Carolina; SC=South Carolina; TX=Texas) and *P.calathiferum* grown in phosphorus-deficient conditions (N: P = 80:1). NC and SC were inoculated on March 14, 2005, TX was inoculated on June 9, 2005, and Pcal was inoculated on July 13, 2005.

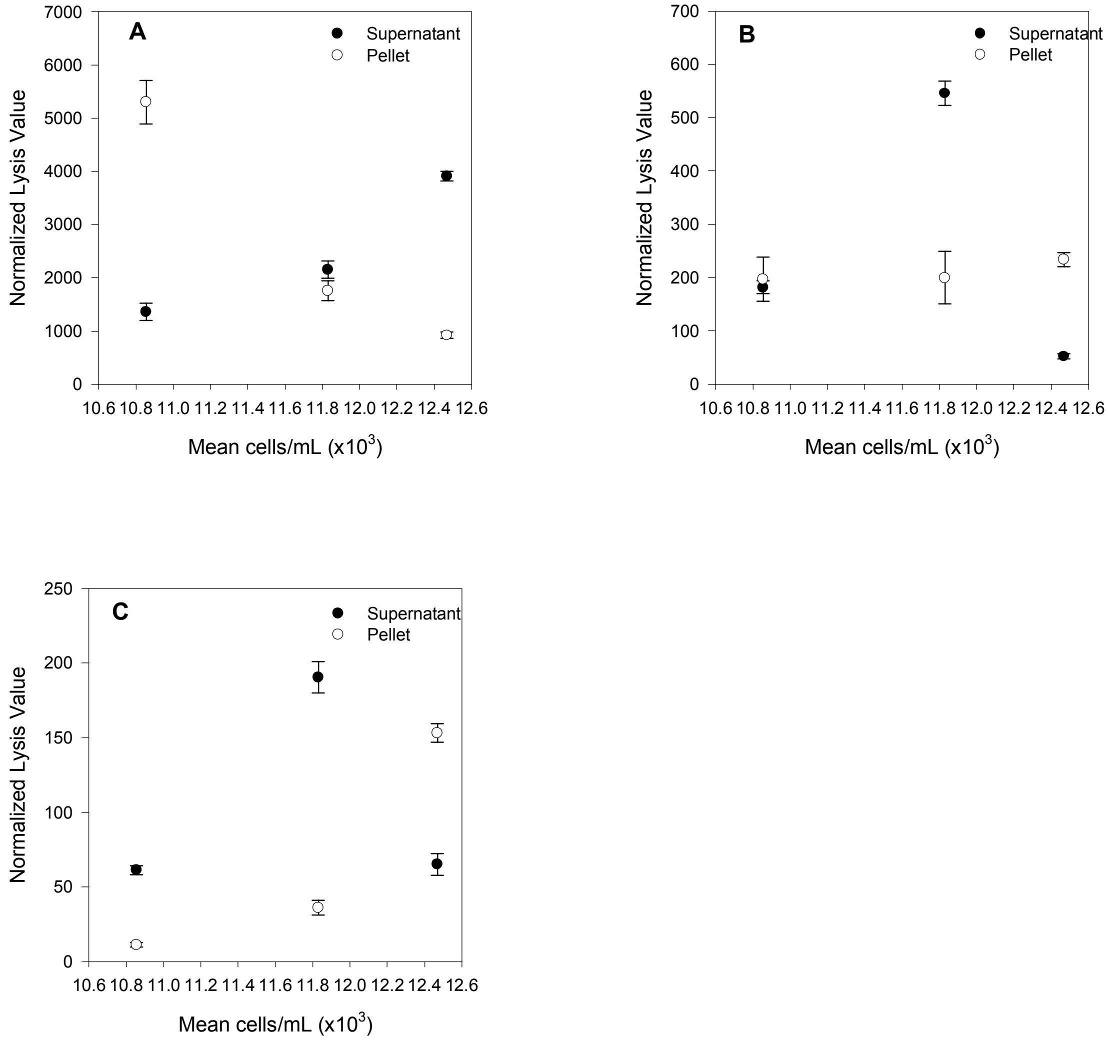
Day	Growth Stage	NC		SC		TX		Pcal	
		% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)	% Lysis	Lysis(Cell)
0	Lag	82.008 ±4.688	52,355.271 ±2992.825	58.719 ±4.962	20,697.243 ±1748.742	102.547 ±15.667 [a]	4361.096 ±666.249 [a]	1.581 ±0.690 [a]	140.219 ±85.173 [a]
9	Log	92.055 ±2.157	3153.710 ±73.894	90.261 ±2.653	820.128 ±24.105	94.578 ±6.279 [b]	3082.960 ±204.665 [b]	4.107 ±0.288 [c]	89.005 ±6.243 [c]
20	Stationary	96.294 ±4.450	731.772 ±33.814	100.966 ±5.223	340.841 ±17.629	92.663 ±9.692 [d]	3336.569 ±348.953 [d]	2.088 ±0.772 [e]	44.826 ±16.565 [e]

a- Lag phase on Day 2; b- Log phase on Day 4; c-Log phase on Day 7; d- Stationary phase on Day 10; e-Stationary phase on Day 17

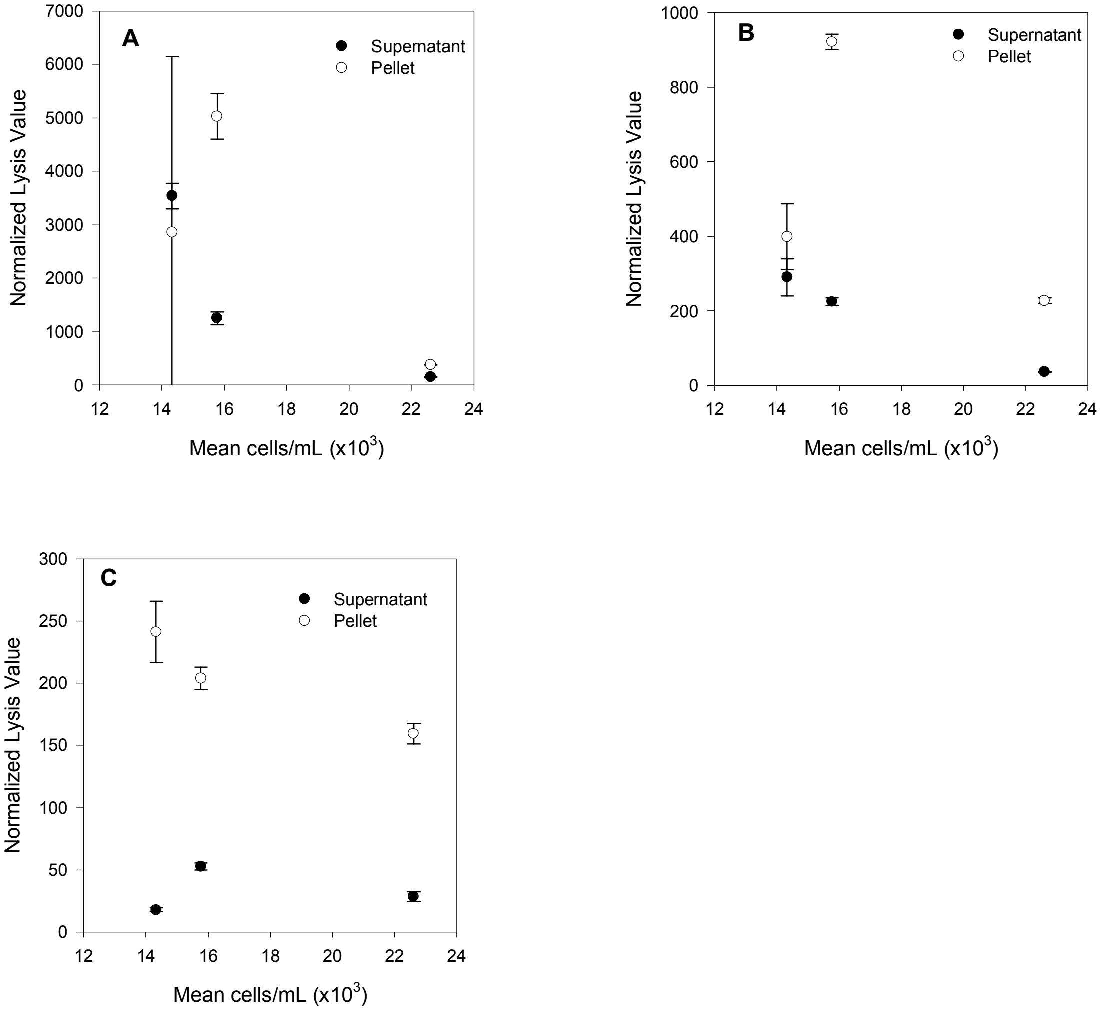
Appendix C: Comparison of Initial Cell Density and Hemolytic Activity



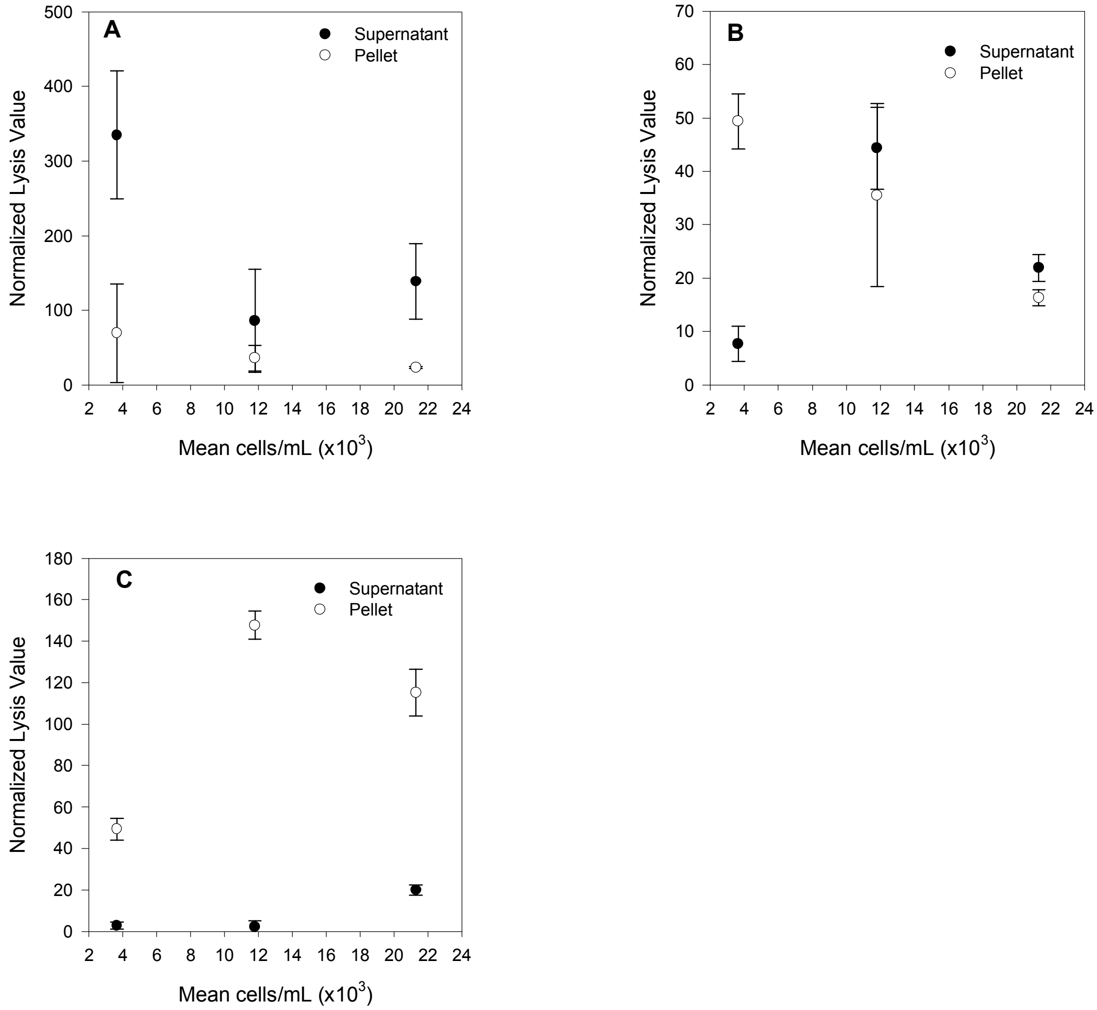
Comparison of initial cell density and hemolytic activity of North Carolina *P. parvum* clone under nutrient-replete conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



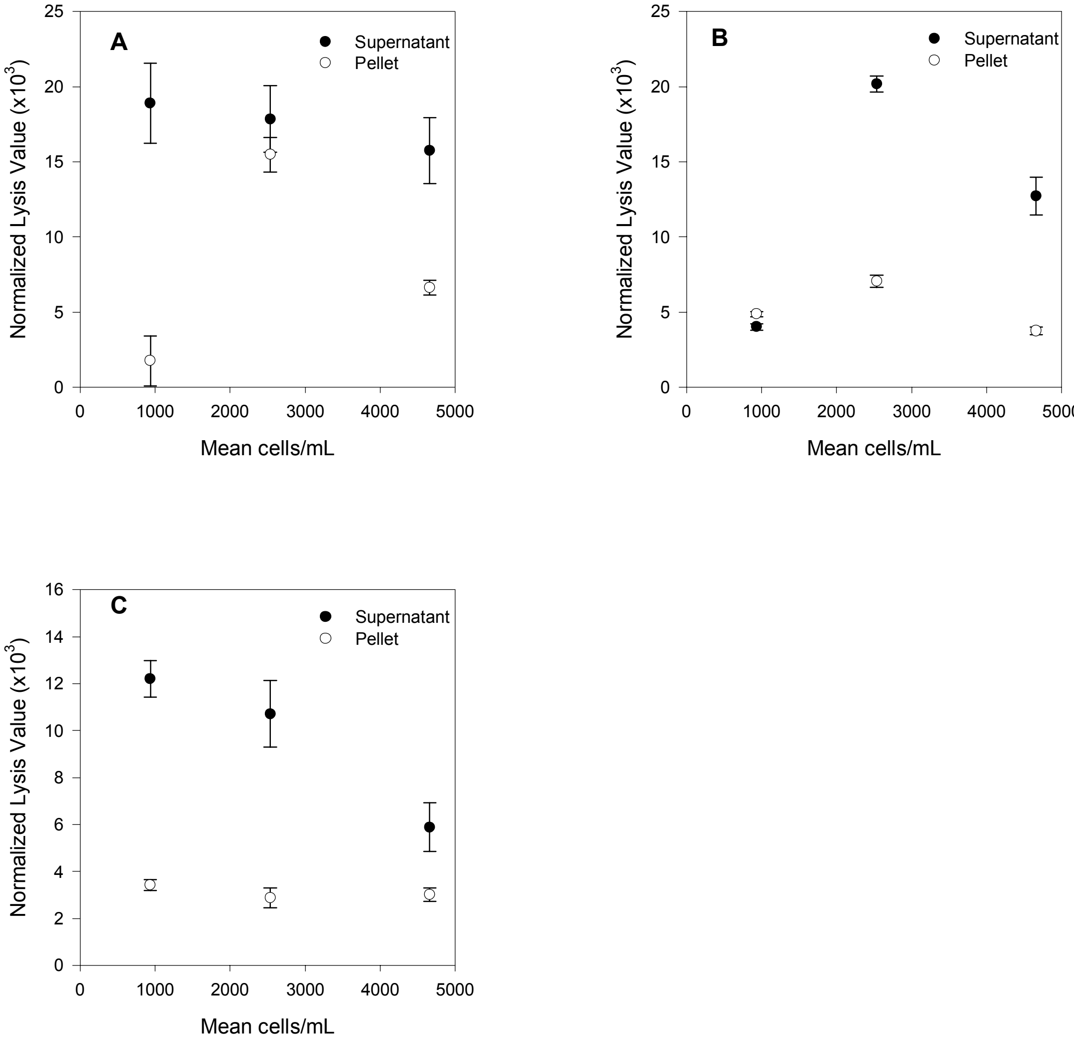
Comparison of initial cell density and hemolytic activity of South Carolina *P. parvum* clone under nutrient-replete conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



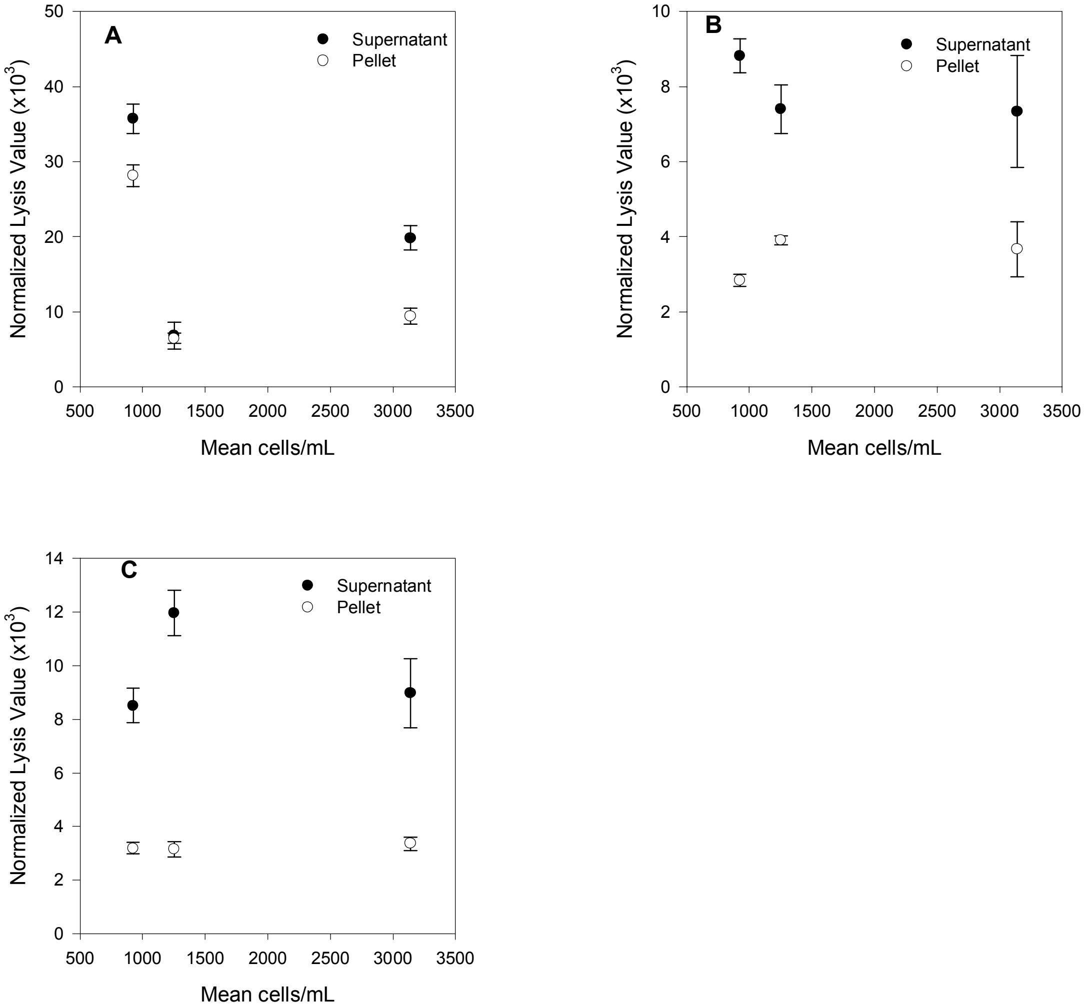
Comparison of initial cell density and hemolytic activity of Texas *P. parvum* clone under nutrient-replete conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



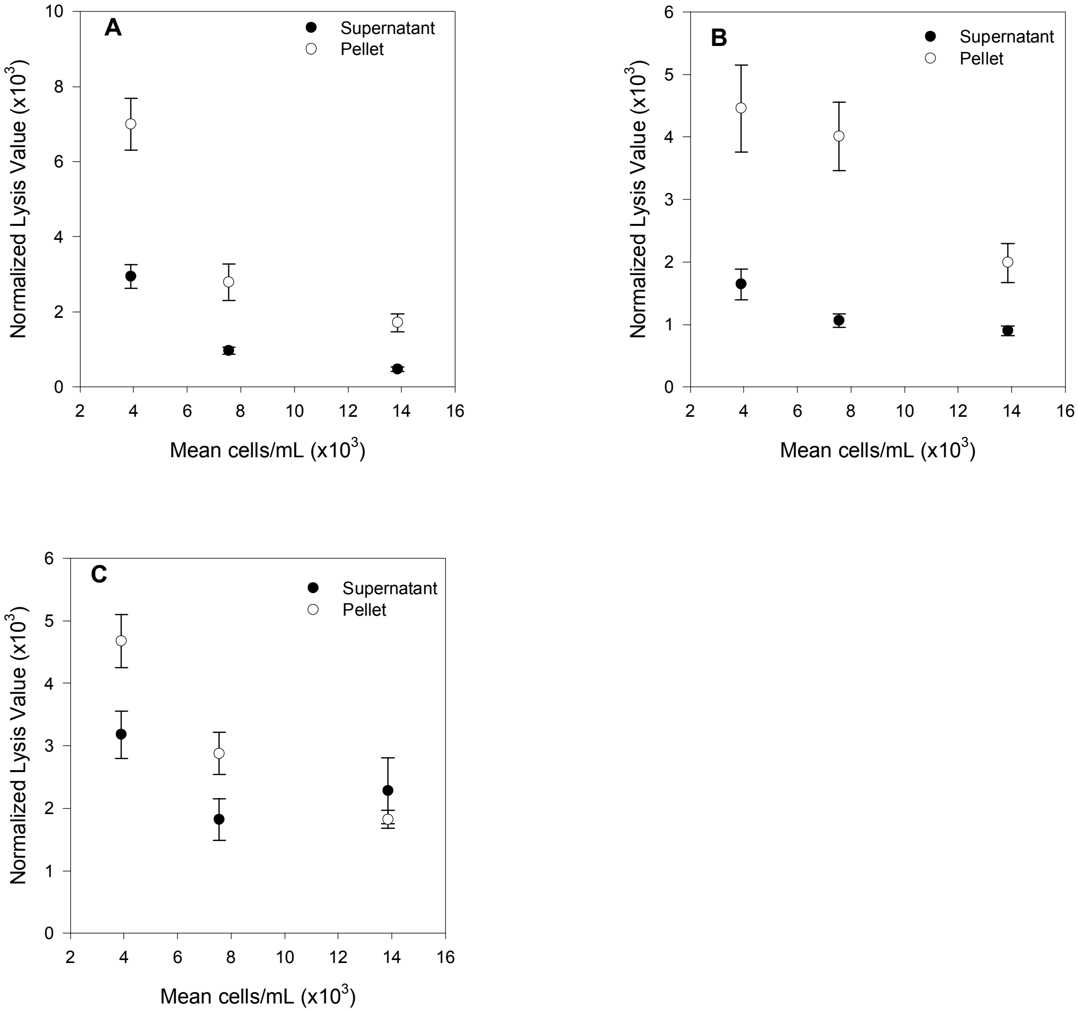
Comparison of initial cell density and hemolytic activity of *P. calathiferum* under nutrient-replete conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



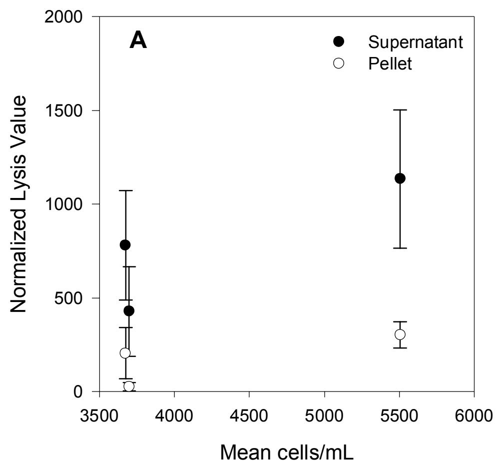
Comparison of initial cell density and hemolytic activity of North Carolina *P. parvum* clone under nitrogen-deficient conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.

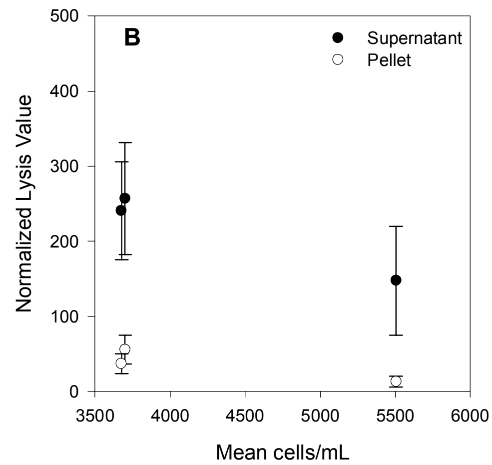


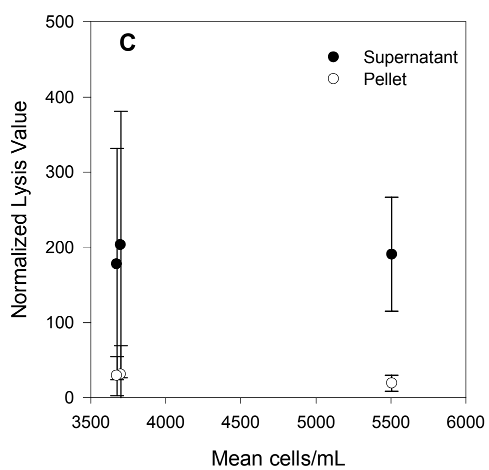
Comparison of initial cell density and hemolytic activity of South Carolina *P. parvum* clone under nitrogen-deficient conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



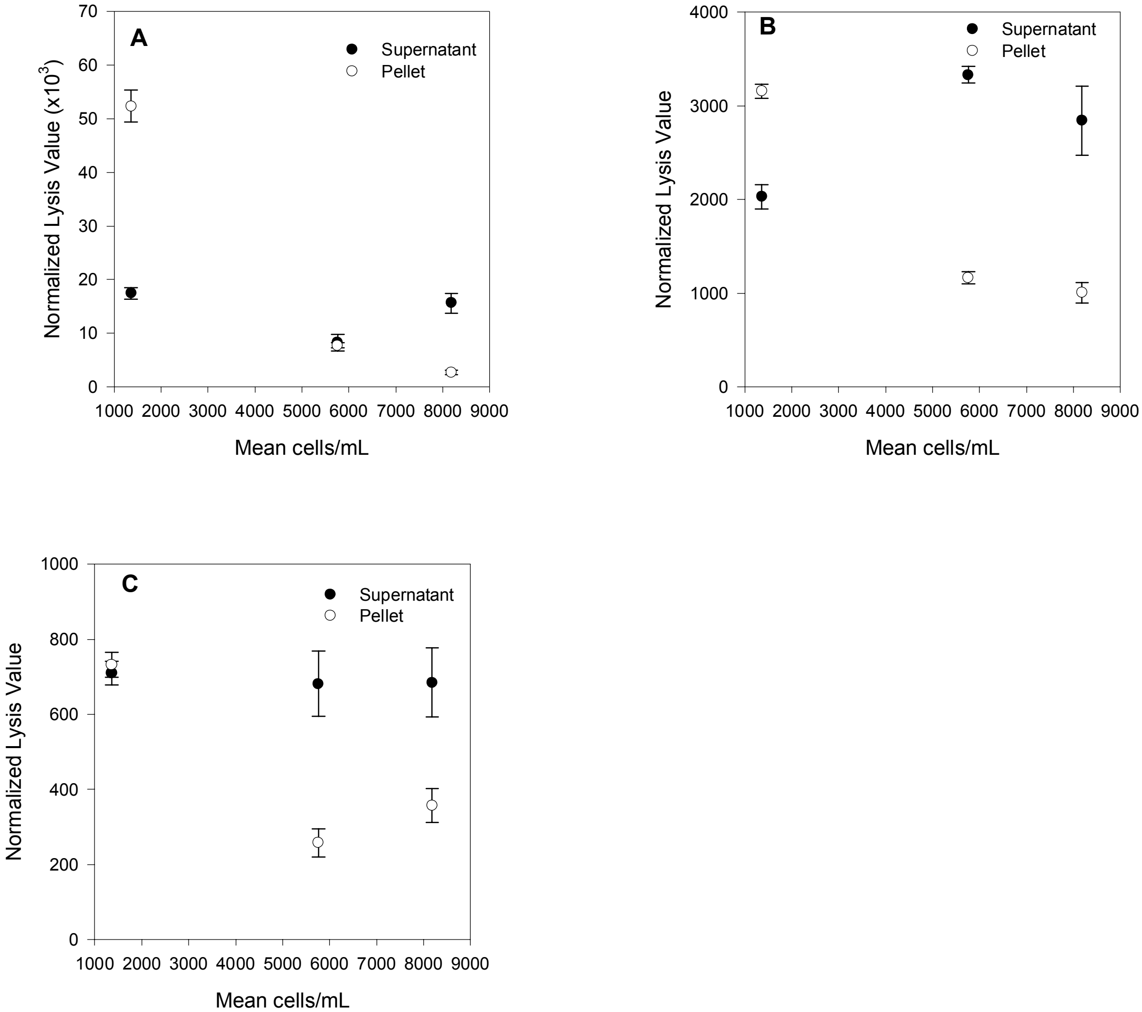
Comparison of initial cell density and hemolytic activity of Texas *P. parvum* clone under nitrogen-deficient conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



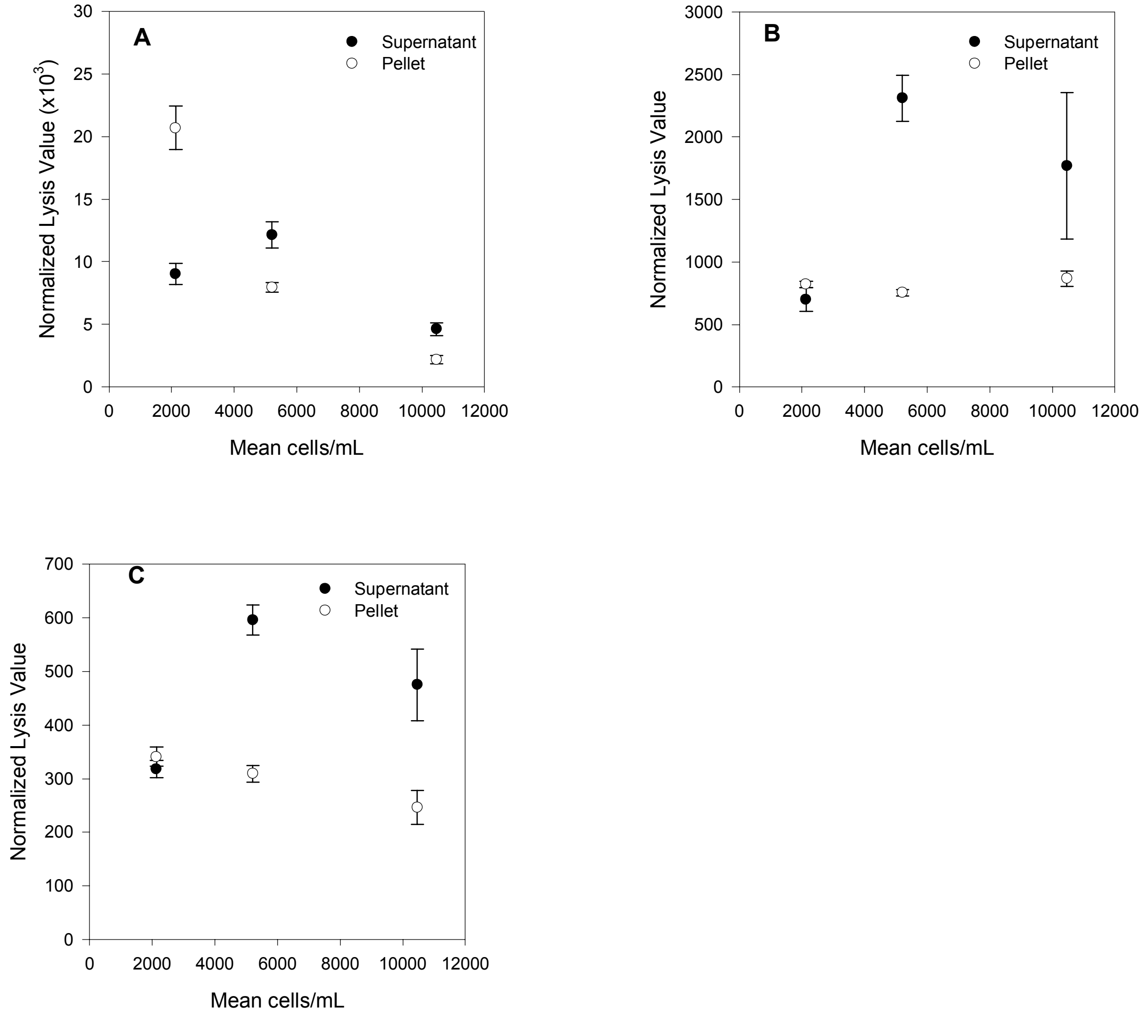




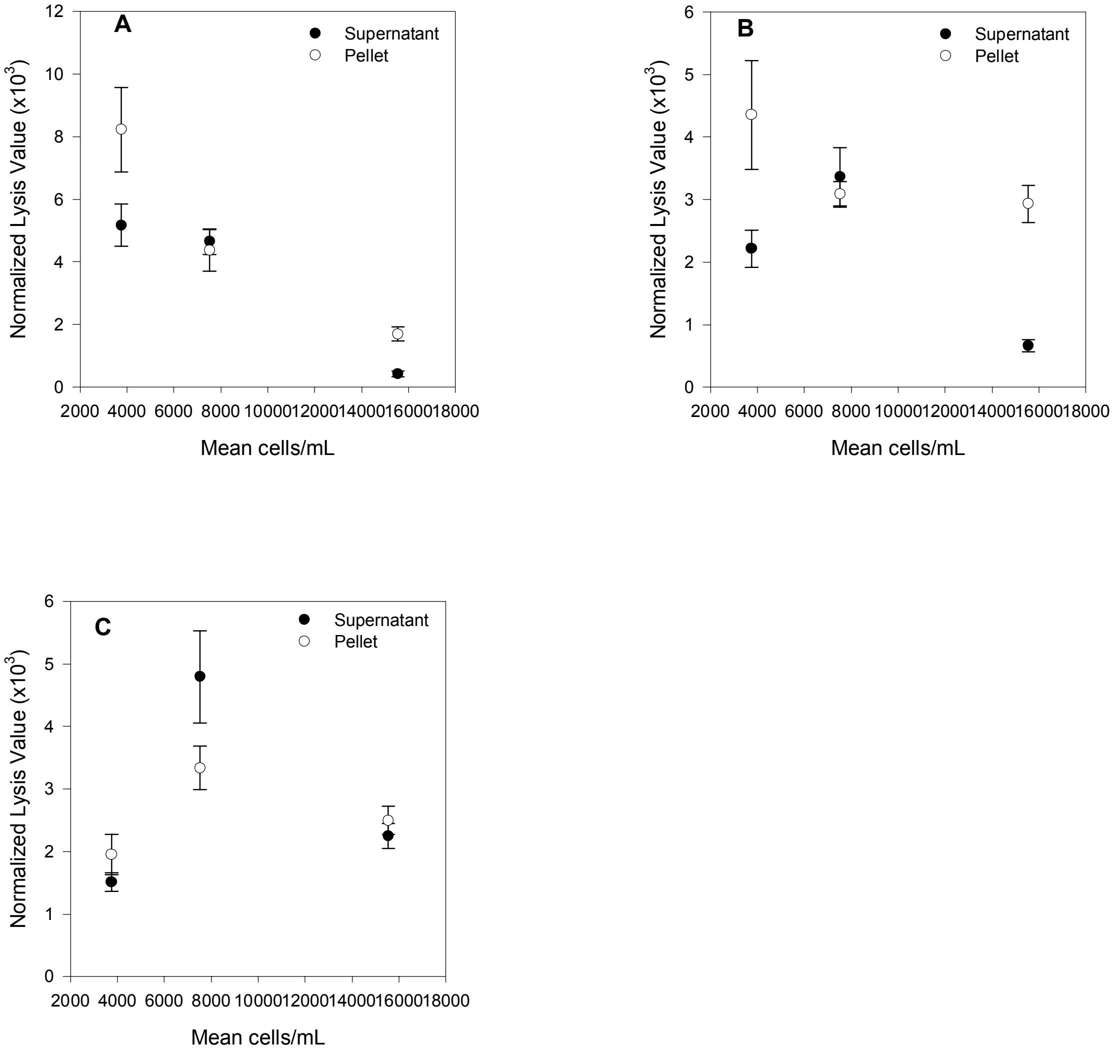
Comparison of initial cell density and hemolytic activity of *P. calathiferum* under nitrogen-deficient conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



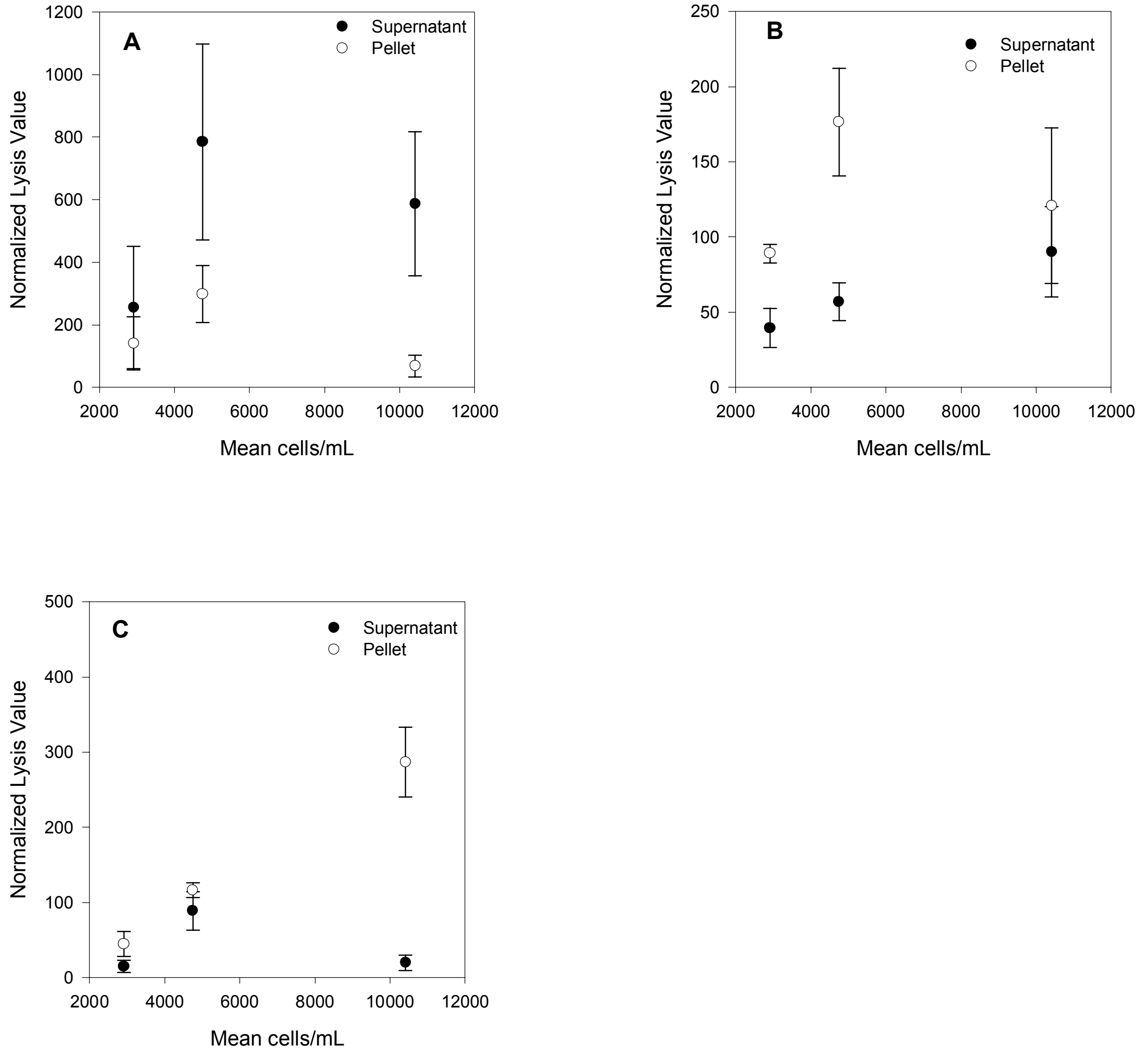
Comparison of initial cell density and hemolytic activity of North Carolina *P. parvum* clone under phosphorus-deficient conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



Comparison of initial cell density and hemolytic activity of South Carolina *P. parvum* clone under phosphorus-deficient conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



Comparison of initial cell density and hemolytic activity of Texas *P. parvum* clone under phosphorus-deficient conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.



Comparison of initial cell density and hemolytic activity of *P. calathiferum* under phosphorus-deficient conditions for three trials. (A) Lag phase. (B) Log phase. (C) Stationary phase.