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A *Synechococcus* P*glnA*::*luxAB* Fusion for Estimation of Nitrogen Bioavailability to Freshwater Cyanobacteria

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In contrast to extensive studies of phosphorus, widely considered the main nutrient limiting phytoplankton biomass in freshwater ecosystems, there have been few studies on the role of nitrogen in controlling phytoplankton populations. This situation may be due partly to the complexity in estimating its utilization and bioavailability. In an attempt to provide a novel tool for this purpose, we fused the promoter of the glutamine synthetase-encoding gene, P *glnA*, from *Synechococcus* sp. strain PCC7942 to the *luxAB* luciferase-encoding genes of the bioluminescent bacterium *Vibrio harveyi*. The resulting construct was introduced into a neutral site on the *Synechococcus* chromosome to yield the reporter strain GSL. Light emission by this strain was dependent upon ambient nitrogen concentrations. The linear response range of the emitted luminescence was 1 mM to 1 μM for the inorganic nitrogen species tested (ammonium, nitrate, and nitrite) and 10- to 50-fold lower for glutamine and urea. When water samples collected from along a depth profile in Lake Kinneret (Israel) were exposed to the reporter strain, the bioluminescence of the reporter strain mirrored the total dissolved nitrogen concentrations determined for the same samples and was shown to be a sensitive indicator of the concentration of bioavailable nitrogen.

Cyanobacteria are a dominant component of marine and freshwater phytoplankton, the productivity of which is frequently limited by nutrient availability (23, 26, 44). While it is clear that phosphorus (P) is of great importance as a limiting nutrient in freshwater ecosystems (23, 43), some studies have indicated that nitrogen (N) can act both as a primary limiting nutrient (20) and in a secondary capacity, enhancing the effect of added P (15). In all instances, however, quantification of the degree of availability of the studied nutrient, as opposed to its actual chemically determined concentration, has proved to be challenging. Several reports have presented circumstantial evidence for nutrient limitation of phytoplankton growth in a given environment: low to undetectable ambient concentrations (23), low elemental ratios in phytoplankton populations (37), enhanced uptake capacities (34, 40), or results of enrichment bioassays (15, 41). Proposed methods for a more direct assessment of N bioavailability to phytoplankton have been based on physiological parameters of the photosynthetic apparatus (25), metabolic activity (6), amino acid/protein ratios (10), chemotaxis toward nitrogenous compounds (47), enzymatic markers (8, 17, 32), or molecular and immunological probes (30, 35, 49).

Gillor et al. (18) recently proposed a different approach for estimating available nutrient concentrations: the fusion of bacterial bioluminescence (*lux*) genes to promoters of genes induced when the nutrient under study is depleted. In this manner, a cyanobacterial phosphorus-sensing strain was constructed by fusing *Vibrio harveyi* luciferase-encoding genes (*luxAB*) to the *Synechococcus* sp. strain PCC7942 alkaline phosphatase-encoding gene (*phoA*) promoter (18). Similarly, with the same cyanobacterial host, Durham et al. (14) constructed an iron limitation reporter by using the promoter of the *isiA* gene as the sensing element. In the present study, we describe the construction and application of a reporter strain for the study of N bioavailability based on the same principle.

Cyanobacteria, like most microorganisms, can use nitrate, nitrite, or ammonium and, to some extent, organic compounds as their N sources. Ammonium, either taken up from the outer medium or produced intracellularly, is incorporated into carbon skeletons mainly through the glutamine synthetase (GS)-glutamate synthase pathway (16, 24). GS (EC 6.3.1.2) catalyzes the incorporation of ammonium into glutamate to produce glutamine, which is then utilized for the synthesis of proteins and N-containing metabolites (16). In most cyanobacteria studied, the control of GS activity responds to carbon and N availability through the signal transducer P_{II} (46) and the global N regulator NtcA (24), respectively. An N deficiency results in high levels of GS activity, and when ammonium is abundant, GS activity is down-regulated (16). In the unicellular, nondiazotrophic cyanobacterium *Synechococcus* sp. strain PCC7942, the *glnA* gene encodes a typical eubacterial type I GS (11). This gene is transcribed mainly from a single NtcA-activated promoter that is severely repressed by ammonium (12, 31). A second GS isoenzyme (encoded by *glnN*) was recently identified for this strain (42). In *Synechocystis* sp. strain PCC6803, the expression of *glnN* is strongly induced by N deprivation but accounts for no more than 20% of the total GS activity measured in N-depleted cells (38).

In light of the strong dependence of cyanobacterial *glnA*

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expression on utilizable N, the activation of its promoter appears to be a good candidate as an indicator of N bioavailability. To test this idea, we fused it to bacterial luciferase-encoding genes (*V. harveyi luxAB*) and integrated the fusion into the *Synechococcus* chromosome to yield a strain that responded by dose-dependent light emission to N limitation. Following characterization of the responses of this N reporter strain to a wide range of inorganic and organic N concentrations, we attempted to use it for the estimation of the degree of N limitation in a freshwater lake.

MATERIALS AND METHODS

Constructs. To integrate a fusion of the *Synechococcus* sp. strain PCC7942 GS-encoding gene (*glnA*) promoter with *V. harveyi luxAB* into the cyanobacterial chromosome, a plasmid (pGSL) was constructed. The plasmid was based on plasmid pAM1414 (2), which contains the *luxAB* luciferase genes (*V. harveyi*) and a spectinomycin-streptomycin resistance cartridge. It also harbors the intergenic regions of a neutral site (NSI; GenBank accession number U30252) of *Synechococcus* sp. strain PCC7942 chromosomal DNA which allow for the integration of the plasmid into the chromosome by double homologous recombination (29). A 413-bp fragment that included the *glnA* promoter (GenBank accession number D26162) was cloned by PCR with a 24-base primer (5′-GATTAAGCGGCCGC TCCCGAGTG-3′) at the 5′ end of the promoter and a 25-base primer (5′-CA TTAAGGATCCAGGCCTGAGCGAC-3′) at its 3′ end. The 410-bp promoter segment was inserted into the *Not*I/*Bam*HI site of the plasmid, upstream of *luxAB*. The amplified segment included 9 bp upstream of the start codon of *glnA*, which was eliminated (T was replaced by G). The location and orientation of the fusion were confirmed by PCR and by restriction analysis with *Pvu*II.

Wild-type *Synechococcus* sp. strain PCC7942 was transformed with recombinant and control plasmids pGSL and pAM1414 as previously described (19) to generate strains GSL (P*glnA*::*luxAB*) and M1414 (control), respectively. Transformed cells were selected on agar plates that contained streptomycin and spectinomycin (25 μg ml^{-1} each) in BG11 medium (39). *E. coli* DH5α, used for plasmid propagation, was grown on Luria-Bertani medium (33) supplemented with spectinomycin and/or streptomycin (50 μg ml^{-1} each) when appropriate.

Southern analysis. Southern blot hybridization, performed as described previously (18), was used to confirm the integration of pGSL into the target site (NSI) in the *Synechococcus* sp. strain PCC7942 chromosome.

Growth and experimental conditions. Wild-type *Synechococcus* sp. strain PCC7942 and its derived P*glnA*::*luxAB* engineered strain (GSL) were grown as batch cultures at 30°C in BG11 mineral medium (39) under constant fluorescent illumination (50 μmol of photons m^{-2} s^{-1}) with continuous shaking (Innova 4340; New Brunswick Scientific Co., Edison, N.J.; 125 rpm). For N deprivation experiments, the cells were pregrown to an optical density at 750 nm (OD_{750}) of approximately 0.7. Cells were then harvested by centrifugation (4,000 rpm in Eppendorf centrifuge 5810R for 10 min at room temperature), washed twice in N-free BG11 medium ($NaNO_3$ and ferric ammonium sulfate were replaced by equimolar concentrations of NaCl and ferric chloride, respectively) without antibiotics, and resuspended in the same medium to a final OD_{750} of 0.25.

For experiments with different N sources, cultures were harvested as described above and resuspended in N-free BG11 medium containing ammonium (as NH_4Cl), nitrate ($NaNO_3$), nitrite ($NaNO_2$), L-glutamine, or urea at various concentrations. All N sources used in this study were obtained from Sigma Chemical Co.

For experiments involving the readdition of N to N-deprived cells, the cells were first incubated for 10 h in N-free BG11 medium as described above and then supplemented with various ammonium concentrations.

Determination of ammonium concentrations. Ammonium concentrations were determined by using a QuikeChem 8000 flow injection analyzer (Lachat Instruments).

Measurement of bioluminescence. The bioluminescence of the cyanobacterial constructs was assayed as described previously (18). Cell aliquots were brought to a uniform cell density (OD_{750}, 0.5), and duplicate subsamples (100 μl) of the cell suspension were transferred to wells of an opaque white 96-well microtiter plate (Nunc, Roskilde, Denmark). The reaction was started by the addition of 100 μl of N-free BG11 medium containing the luciferase substrate nonyl aldehyde (0.002%) and 0.005% Igepal CA-630. Luminescence was measured with a temperature-controlled (30°C) microtiter plate luminometer (Victor^2; Wallac, Turku, Finland) every 10 s for 15 min. Light emission increased to a maximum during the first 5 min and then declined. The maximum luminescence at the peak is the value used in this communication. Light output intensity is reported either in the instrument's arbitrary relative light units (RLU) or as a percentage of the activity of the fully induced system in N-free medium. All experiments were conducted in duplicate and were repeated at least twice.

Measurement of GS activity. GS transferase activity was determined over 15 min at 37°C as previously described (48). The assay quantifies the GS-mediated transformation of glutamine into γ-glutamylhydroxamate (γ-GH), the concentration of which is determined photometrically. Culture aliquots (5 to 10 ml) were harvested by centrifugation (4,000 rpm in Eppendorf centrifuge 5810R for 10 min at room temperature) and resuspended in 0.5 mM Tris HCl (pH 7.5). Toluene was added (one-half of the cell suspension volume), and the mixture was vortexed vigorously for 60 s. Cells were then washed three times with the Tris HCl solution by centrifugation (14,000 rpm in Eppendorf centrifuge 5417C for 2 min at 4°C) and resuspended in a reaction mixture containing 135 mM imidazole HCl, 18 mM hydroxylamine HCl, 0.27 mM $MnCl_2$, 25 mM potassium arsenate, 0.36 mM sodium ADP, and 1 mg of hexadecyltrimethylammonium bromide/ml. The pH of the reaction mixture was adjusted to 7.55 with 2 M KOH, and the cell mixture was equilibrated for 15 min at 37°C. L-Glutamine (0.015 mM) was then added to the mixture, which was incubated for an additional 15 min at the same temperature. A "stop mixture" (1 ml) containing 0.2 M $FeCl_3 \cdot 6H_2O$, 0.122 M trichloroacetic acid, and 0.175 M HCl was used to terminate the enzymatic reaction, and the samples were centrifuged (4 min at 7,000 rpm in Eppendorf centrifuge 5417C) to remove the cells. The OD_{540} of the supernatant was measured, and γ-GH concentrations were calculated by using a standard curve. Protein in the cell extracts was determined by the method of Bradford (5) with bovine serum albumin as a standard. GS activity values are presented as micromoles of γ-GH produced per hour per milligram of protein or as a percentage of maximal GS activity determined with N-deprived cells at the same time point. All chemicals used in this assay were obtained from Sigma.

Field samples. Water samples were collected from Lake Kinneret, Israel, a warm monomictic freshwater lake with mean and maximum depths of 24 and 42 m, respectively. Monthly water samples were collected from November 2000 until December 2001 at the deepest point near the center of the lake (station A). The sampling depths chosen spanned both the epilimnion (1, 5, and 10 m) and the hypolimnion (30 and 37 m). To prevent biological activity that might have affected nutrient concentrations, the water samples were filter sterilized (0.22-μm-pore-size filter; Schleicher & Schuell) within 2 h of sampling and kept at 4°C until analyzed.

Total dissolved N (TDN) in Lake Kinneret water samples was calculated as the sum of dissolved (0.45-μm-pore-size filter) ammonium, nitrate, nitrite, and organic N; each type of N was assayed by standard methods (1), with the exception of the use of Szechrome NAS reagent (diphenylamine sulfonic acid chromogen) (45) for NO_3^- assays.

Assays of N bioavailability in lake water samples. Assaying for bioavailable N with reporter strain GSL developed in this study was performed as described previously for phosphorus bioavailability (18). Within 24 h of collection, duplicate samples (10 ml) were transferred to 50-ml sterile flasks and supplemented with N-free BG11 medium ingredients. N-replete GSL cells, pregrown and washed as described above (growth and experimental conditions), were then added to a final OD_{750} of approximately 0.25. Cells suspended in complete or N-free BG11 medium served as negative or positive controls, respectively. Samples were incubated at 30°C with continuous shaking (125 rpm) under constant fluorescent illumination (50 μmol of photons m^{-2} s^{-1}). Following 20 h of incubation, bioluminescence was assayed by using opaque white 96-well microtiter plates as described above.

RESULTS

Verification of strain GSL chromosomal integration. A P*glnA*::*luxAB* fusion was introduced into pAM1414 (2) to yield plasmid pGSL, which was then incorporated into a neutral cloning site (NSI) on the *Synechococcus* sp. strain PCC7942 chromosome (7). Homologous recombination at the target site was verified by Southern analysis with a fluorescein-labeled NSI PCR product as a probe. *Apa*I and *Mfe*I digests from the wild-type strain and strain GSL were hybridized with the probe (Fig. 1). In strain GSL, the NSI probe hybridized to a larger fragment. The difference in size (approximately 5,000 bp) corresponded to the size of the insert within the NSI in plasmid pGSL (4,894 bp), confirming that it was introduced into the

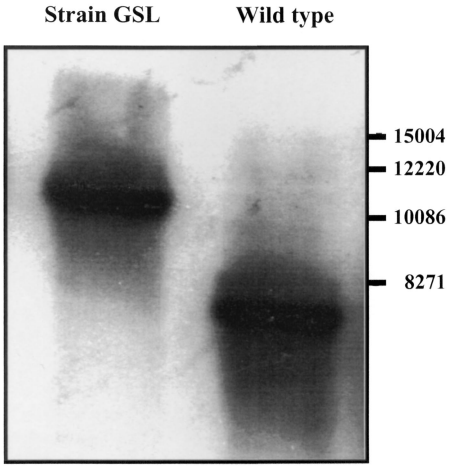

FIG. 1. Southern analysis of *Apa*I and *Mfe*I digests of genomic DNAs of wild-type *Synechococcus* sp. strain PCC7942 and strain GSL. The blot was hybridized with a fluorescein-labeled probe based on a 382-bp NSI PCR product. Numbers on the right indicate molecular weights.

genome at the NSI by a double-recombination event. The location and orientation of the insert were further confirmed by PCR (data not shown).

Response of *Synechococcus* sp. strain GSL to N availability. Upon transfer to an N-free medium, both wild-type and GSL (harboring the P*glnA*::*luxAB* fusion) *Synechococcus* cultures underwent one slow cell division within 8 to 12 h and then ceased to grow. The N-deprived cultures turned from green to yellowish green approximately 12 h after N depletion as previously described (11, 13). In spite of the obvious stress exhibited under these conditions, the cells responded to N deprivation by luciferase synthesis, as detected from the emission of luminescence within 2 to 5 h after transfer. Luminescence continued for at least 20 h (Fig. 2A). When the cells were transferred to an N-replete medium (1 mM ammonium), no luminescence above the background level (approximately 10% the maximum luminescence measured) was observed. Lower ammonium concentrations led to a progressively higher degree of bioluminescence (Fig. 2A). The N dose dependence is shown in Fig. 2B, in which luminescence at 20 h is plotted as a function of ammonium concentration. Strain GSL appeared to be responsive to ammonium concentrations of between 1 mM and 1 μM. Figure 2B also shows the GS activities of the same cultures at the same time point, indicating that the activity of this enzyme was a far less sensitive indicator of N availability: activities were similar at all tested concentrations below 1 mM. No luminescence was observed for *Synechococcus* sp. strain M1414 control cells, which lacked the *glnA* promoter upstream of the *luxAB* genes. Neither did phosphorus limitation induce luminescence in strain GSL (data not shown). In all cases, the inorganic carbon (240 μM) present in BG11 mineral medium ensured that GS induction and activity were not affected by carbon limitation.

To better characterize the response of strain GSL to ammonium, the cells were exposed to a 100 μM concentration of this ion; luminescence, GS activities, and ammonium concentrations were monitored at short time intervals (Fig. 3). Within

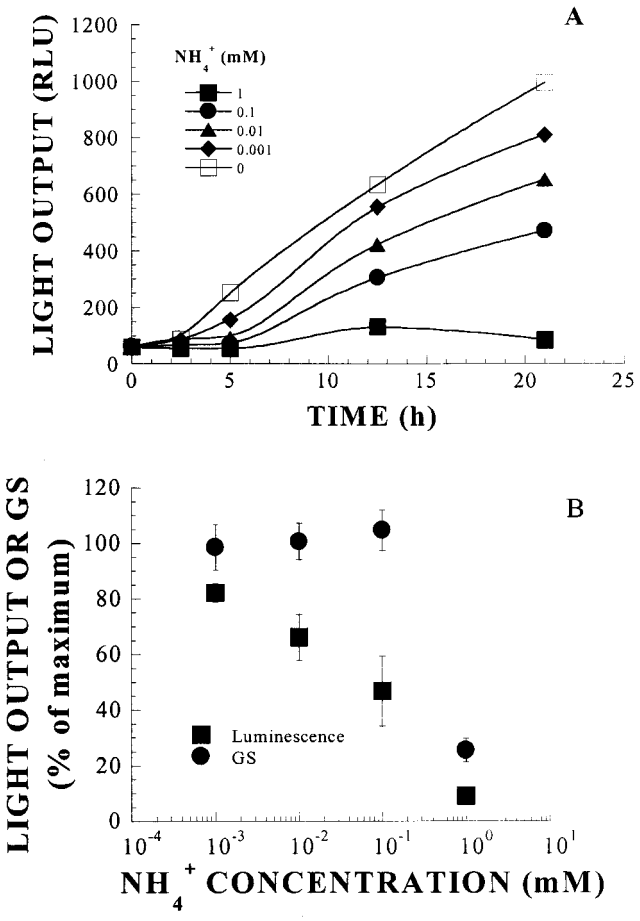

FIG. 2. Bioluminescence in *Synechococcus* sp. strain GSL. (A) Luminescence development in the presence of different NH_4^+ concentrations. (B) Light emission and GS activity as a function of NH_4^+ concentration following 20 h of exposure. Data in panel B (averages and standard deviations) represent the percent maximal luminescence or GS activity obtained at that time point in N-free medium in three replicate experiments (917.9 ± 120.4 RLU or 443.4 ± 34.0 μmol of γ-GH h^{-1} mg of $protein^{-1}$, respectively).

approximately 5 h, the ammonium concentration decreased to ca. 10 μM, at which time both luminescence and GS activity started to increase. These results indicate that during the increasingly longer lag periods observed earlier (Fig. 2A) in the presence of higher ammonium concentrations, free ammonium was first consumed and only then was P*glnA*-driven bioluminescence induced. The ammonium concentration below which induction occurred under these conditions was about 10 μM.

Synechococcus sp. strain PCC7942, like other cyanobacteria, can utilize diverse N sources in addition to ammonium. These include nitrate, nitrite, and various organic N compounds (39). Figure 4 summarizes the bioluminescence and GS activity of strain GSL cells 20 h after exposure to four different N sources at various concentrations: nitrate (Fig. 4A), nitrite (Fig. 4B), glutamine (Fig. 4C), and urea (Fig. 4D). In all cases, a clear dependence of bioluminescence intensity on the initial N concentration was observed; the detection range appeared to be broader for the inorganic N sources, extending from 1 mM to

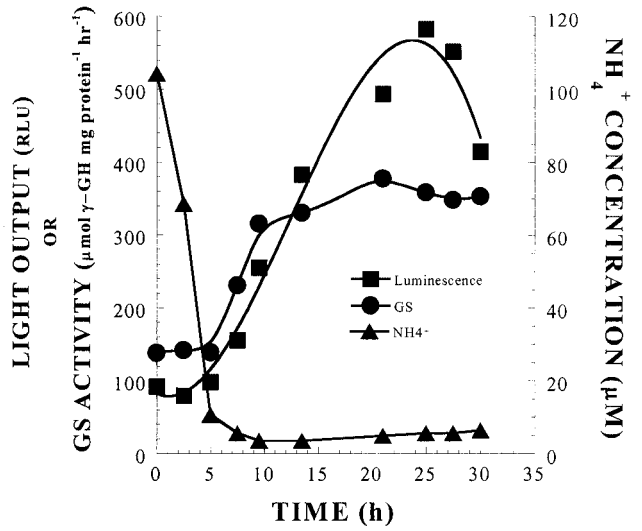

FIG. 3. NH_4^+ uptake, bioluminescence, and GS activity induction in *Synechococcus* sp. strain GSL. Mid-log-phase cells grown in complete BG11 medium were transferred at time zero to N-free medium containing 100 μM NH_4^+. The remaining NH_4^+, bioluminescence, and GS activity were determined at intervals.

1 μM. For glutamine and urea, the lowest concentrations detected in this manner were about 10 and 50 μM, respectively. GS activity, as in the case of ammonium, was a far less sensitive indicator of N source concentration; in most cases, with the possible exception of urea, below a certain high concentration GS activities were uniformly high and thus could not serve as an indicator of N levels.

The experiment shown in Fig. 5 was designed to characterize the response reciprocal to that shown in Fig. 2: N-depleted (and thus preinduced) cultures were confronted with a renewed NH_4^+ supply. The cells were prestarved for N for 10 h and then transferred to media with different NH_4^+ concentrations (Fig. 5A). In an N-free medium, luminescence continued to increase for approximately an additional 10 h, whereas at an NH_4^+ concentration of 1 mM, light emission was completely inhibited within the same time period. For intermediate concentrations, a temporary decrease in luminescence was followed by a renewed increase after more than 5 h (0.1 and 0.01 mM) or 15 h (0.5 mM). The decline, representing a temporary cessation of *glnA* promoter activation and a lack of luciferase protein synthesis, probably lasted for as long as NH_4^+ was available. The observed luminescence at each time point reflected the balance between the deterioration of "old" luciferase activity from the preinduction period and the synthesis of "new" enzyme in response to renewed N depletion. GS activity, monitored in parallel in the same samples, also responded to the renewed presence of ammonium, but in a significantly less sensitive manner. This difference between the two activities is demonstrated in Fig. 5B, which shows luminescence and GS activity 10 h after ammonium readdition. From the kinetic data in Fig. 5A, luciferase and GS half-lives of approximately 3 to 4 h and 10 h, respectively, were calculated.

N bioavailability in Lake Kinneret (Sea of Galilee). To test the applicability of the developed method, water samples were collected monthly from Lake Kinneret, Israel's largest lake and main water reservoir. The lake, 42 m at its deepest, is isothermal during the winter and stratified in spring, summer, and fall, with a thermocline at a depth of 18 to 25 m (22). Over a 12-month period, a profile was sampled monthly along the upper (1, 5, and 10 m) and lower (30 and 37 m) layers of the lake.

N-replete GSL cells were exposed to these samples, and luminescence was recorded 20 h later. Representative examples of two such profiles are shown in Fig. 6A and C, along with the total dissolved N (TDN) measured chemically in the same water samples. Also shown are the temperature profiles (Fig. 6B and D) in the lake at the time of sampling. N bioavailability, as indicated by the luminescence pattern in both sampling periods, clearly mirrored both the stratification (as evidenced by the temperature profile) and the TDN concentrations. The latter parameter encompasses ammonium, nitrate, nitrite, and organic N sources; none of these individual N compounds displayed the same degree of correlation to the bioluminescence profiles as TDN (data not shown). This result is understandable in view of the fact that strain GSL responds to a different degree to each of the potential N sources; since all of them were represented to some extent in the water samples, only a global parameter may reflect the total potentially available N.

To present a more comprehensive profile of N availability in Lake Kinneret throughout the year, bioluminescence and TDN results for all upper-layer (1, 5, and 10 m) and lower-layer (30 and 37 m) samples were pooled, averaged, and plotted as a function of the sampling month (Fig. 7). Also depicted in Fig. 7 are the average chlorophyll *a* concentrations for the same water samples, representing the density of the phytoplankton populations. In the upper layer (Fig. 7A), TDN concentrations were comparatively low (0.36 to 0.73 mg $liter^{-1}$) throughout the year, yielding correspondingly high bioluminescence. In the lower layer (Fig. 7B), N concentrations were high during stratification (March to December) and low during the mixed period (January and February), changes that were consistently reflected by the bioluminescence of strain GSL in the same samples. The seasonal chlorophyll development in the lake followed a clear monomictic pattern with a single annual peak: concentrations were low for most of the year, with a single significant increase during the mixed period in January and February. The mixing effect led to a substantial decrease in TDN concentrations in the hypolimnion but only to a moderate increase in the epilimnion. Both of these effects were clearly mirrored by strain GSL bioluminescence.

While the correlation between TDN and bioluminescence was obvious, it was not sufficient for assessing the degree of availability of the chemically determined nutrient. For this purpose, we used the calibration curve plotted in Fig. 2B to calculate the concentration of "available N" indicated by the measured bioluminescence of strain GSL. We carried out this calibration by using the most available N species, ammonium. The equation used was based upon the data in Fig. 2B: $y = -10.89\ln(x) + 46.155$ ($R^2 = 0.96$); in this equation, y corresponds to light output (as a percentage of the maximum luminescence obtained in N-free medium) and x corresponds to initial NH_4^+ concentration (in milligrams $liter^{-1}$). Because ammonium was used as the reference, our availability results may be considered in terms of "ammonium equivalents." The

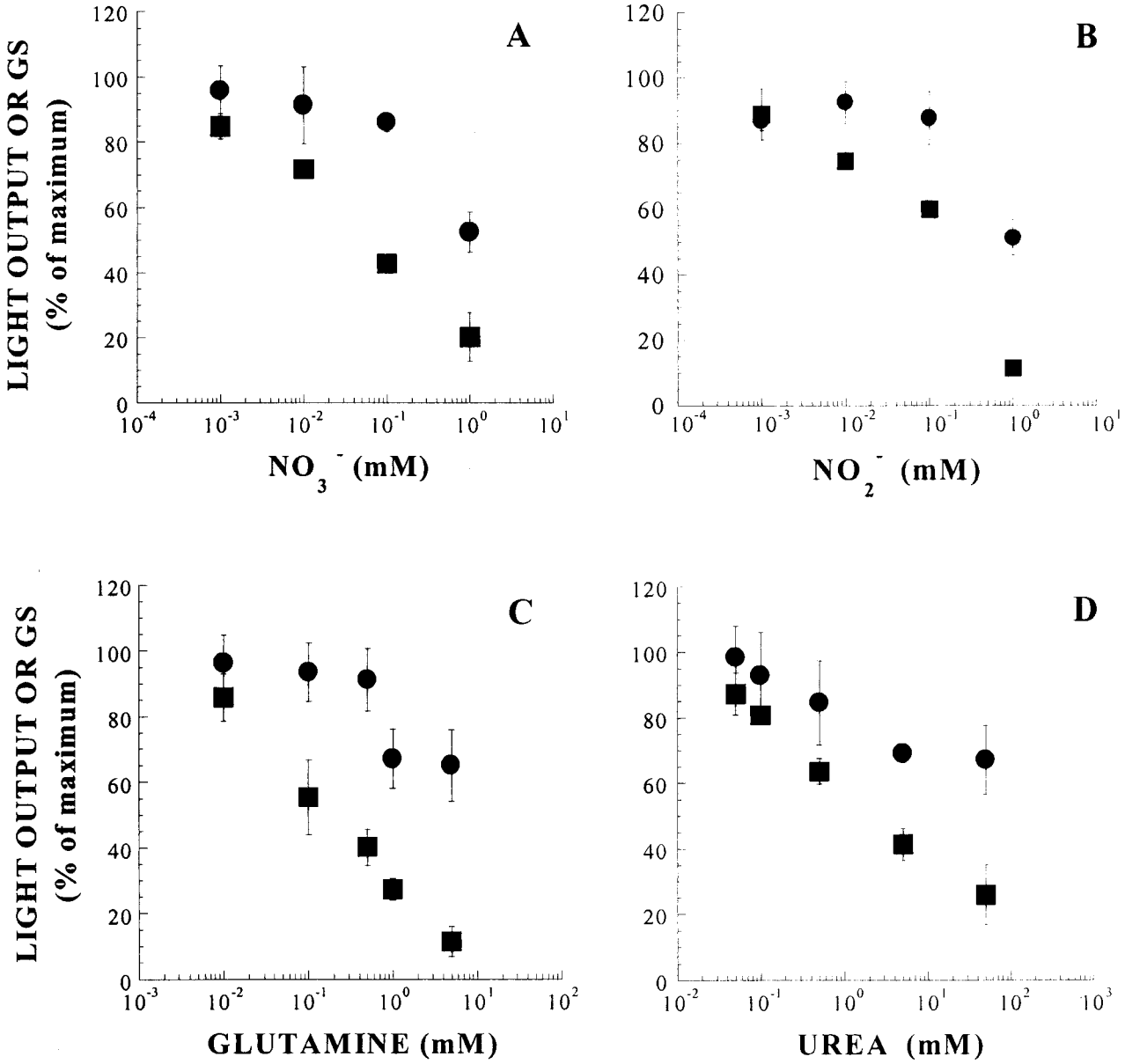

FIG. 4. Bioluminescence and GS activity in GSL cells 20 h after transfer to N-free BG11 medium supplemented with nitrate (A), nitrite (B), glutamine (C), or urea (D). Data (averages and standard deviations; three replicate experiments) represent the percent light output (■) or GS activity (●) obtained at that time point in the maximally induced (N-free) control. Average bioluminescence and GS control (100%) values were 576.8 ± 60.0, 782.8 ± 166.6, 685.8 ± 235.7, and 1,056.8 ± 98.6 RLU and 325.4 ± 12.1, 403.2 ± 62.8, 330.3 ± 16.1, and 368.3 ± 32.4 μmol of γ-GH h^{-1} mg of protein^{-1} for nitrate, nitrite, urea, and glutamine, respectively.

luminescence data in Fig. 7 were transformed in this manner and are plotted in Fig. 8 against chemically determined TDN concentrations in the same samples. The emerging general pattern is clear: the lower the overall N concentration, the lower the available fraction out of this total. Thus, in the lower layer, characterized by high N concentrations, calculated availability values were similar to or even higher than measured ones, indicating that under these conditions, practically all of the nutrient was in a form available to the reporter strain. TDN concentrations were up to 10-fold lower in the epilimnion than in the hypolimnion. This depletion, probably caused by uptake of the more available N species, led to an increase in the fraction of less available N. Even under these conditions, however, N availability was rarely estimated to be lower than 5% of the TDN, or 0.04 mg liter^{-1}.

DISCUSSION

In contrast to extensive studies of phosphorus, widely considered to be the main limiting nutrient for algal growth in freshwater ecosystems, fewer studies have addressed the role of N in controlling phytoplankton biomass (32). A chemical determination, as precise as it may be, presents only a short-term profile of ambient N concentrations, without providing

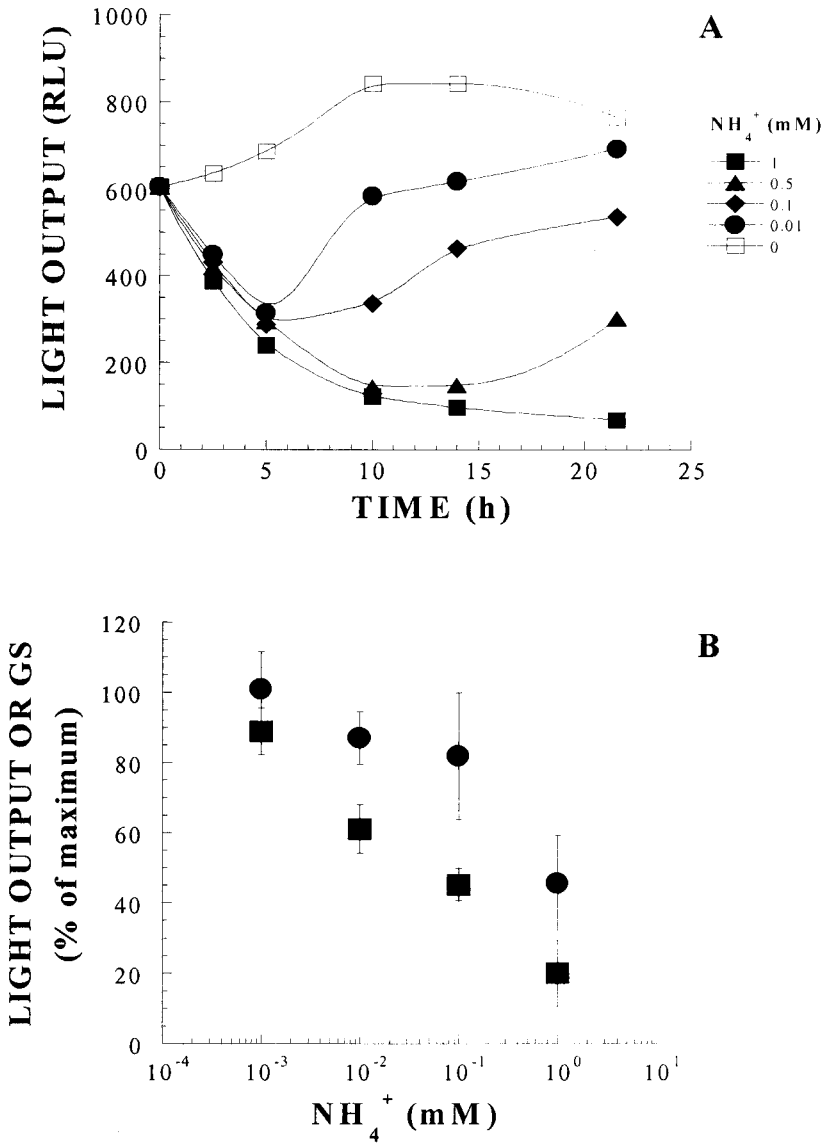

FIG. 5. Effects of the addition of NH_4^+ to preinduced (10 h in N-free medium) GSL cells. (A) Luminescence development in the presence of different NH_4^+ concentrations. (B) Light emission (■) and GS activity (●) as a function of NH_4^+ concentration following 10 h of exposure. Data in panel B (averages and standard deviations) represent the percent maximal luminescence or GS activity obtained at that time point in N-free medium in three replicate experiments (661.7 ± 117.4 RLU and 360.7 ± 77.3 μmol of γ-GH h^{-1} mg of $protein^{-1}$, respectively).

any information on flux or availability. The fact that organic and inorganic forms of N both can be taken up by phytoplankton (36) further complicates traditional approaches for determining N limitation (3).

It is not surprising, therefore, that there is a constant search for alternative methods to assay N bioavailability as sensed by cells (28). One such approach is the use of enzymes associated with N assimilation, such as nitrate reductase, GS, and glutamate dehydrogenase, as possible indicators of N bioavailability (3). However, nitrate reductase activity was reported to be independent of inorganic N substrate concentrations (32, 50), while N assimilation rates estimated by GS and glutamate dehydrogenase activities were lower than those obtained through isotopic experiments (8, 9). The applicability of N assimilation enzyme levels as indicators of N limitation thus appears to be limited.

Here we report a different approach to N bioavailability assessment: fusion of the promoter of an N assimilation gene (*glnA*) to reporter genes and monitoring of the induction of this construct in real time. In recent years, there have been several reports on the use of *luxAB* reporter genes in cyanobacterial constructs for monitoring phosphate (18) or iron (14, 27) bioavailability. To our knowledge, this approach has not been implemented for assaying the N status of photosynthetic organisms.

The P*glnA*::*luxAB* fusion was successfully integrated into the chromosome of *Synechococcus* sp. strain PCC7942 (Fig. 1), and light was emitted by the engineered strain under N-limit-

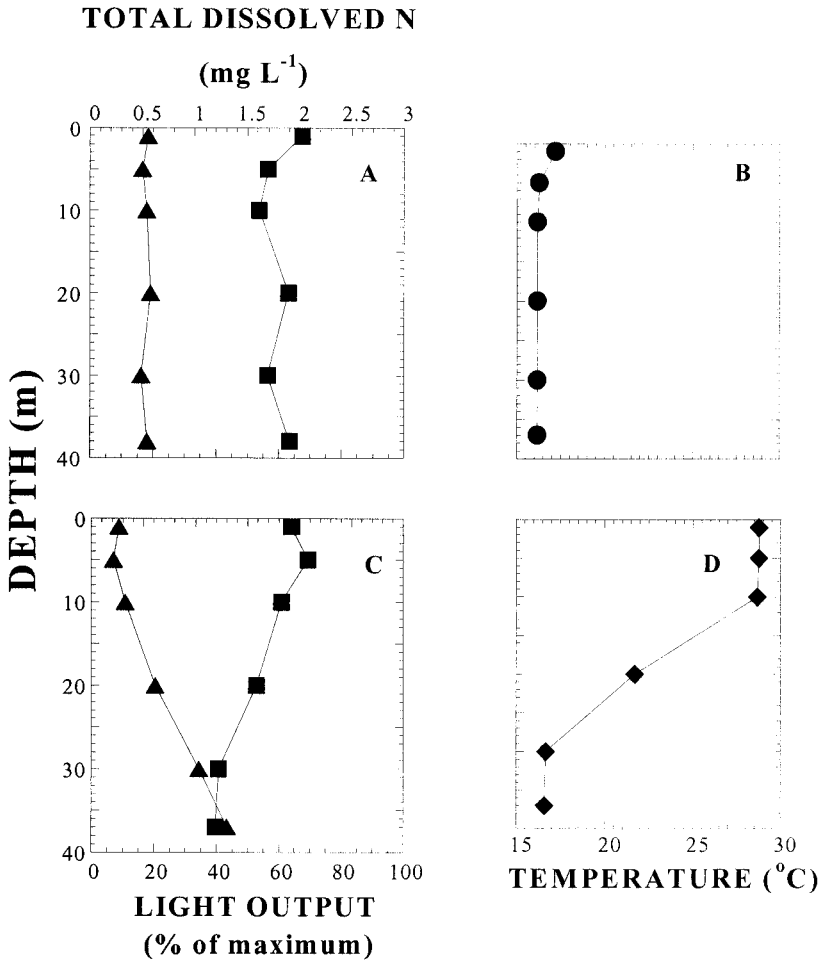

FIG. 6. Bioluminescence in *Synechococcus* sp. strain GSL in water samples collected from Lake Kinneret in February 2001(A and B) and September 2001 (C and D). Bioluminescence (■) measured 20 h after inoculation is plotted along with TDN (▲) and temperature (●, ◆). The temperature data (B and D) demonstrate the stratification (September) and mixing (February) of the lake at the time of sampling.

ing conditions. As expected, the magnitude of the luminescence was inversely dependent upon the N concentration, with detection thresholds as low as 1 μM for ammonium, nitrate, and nitrite (Fig. 2B, 4A, and 4B, respectively) and 10- to 50-fold higher for organic N compounds (Fig. 4C and D). These differences are consistent with the understanding that inorganic N sources are preferred by photosynthetic microorganisms, although many of them have the ability to use a variety of organic N sources for growth (4, 24, 28).

In contrast to the gradual N-dependent increase in bioluminescence, GS activities were mostly independent of inorganic N concentrations below 0.1 mM (Fig. 2B, 4A, and 4B) and organic N concentrations below 0.5 mM (Fig. 4C and D). This difference in dose-response pattern demonstrates the advantage of monitoring *glnA* promoter activation rather than the activity of the enzyme encoded by the same gene. Part of the reason may be the much higher stability of GS than of bacterial luciferase, which may render it less sensitive. Another explanation may stem from the fact that GS activity is the outcome of the transcription of two genes, *glnA* and *glnN* (11, 42); thus, the kinetics of overall GS activity differ from those of the bioluminescence reaction, the induction of which is controlled only by the *glnA* promoter.

In an attempt to estimate N bioavailability to our reporter strain in a natural environment, samples obtained monthly from the upper and lower layers of Lake Kinneret were monitored in 2000 and 2001. In both layers of the lake, light output emitted by the cyanobacterial sensor strain correlated well with TDN. In the hypolimnion, bioluminescence represented a clear mirror image of TDN concentrations (Fig. 7B). Following the winter mixing event, N concentrations in that layer decreased to a minimum, and GSL luminescence was enhanced correspondingly. Starting in April, stratification was reestablished, and hypolimnion TDN concentrations started to build up again. Since all of the "new" N appeared to be readily available, this activity was accompanied by a corresponding inhibition of luminescence.

In the epilimnion, TDN concentrations were lower throughout the year, and the bioluminescence emitted by the reporter strain was correspondingly high (Fig. 7A). These low concentrations, however, in the range of 0.3 to 0.8 mg liter^{-1}, are relatively high compared to those in oligotrophic aquatic hab-

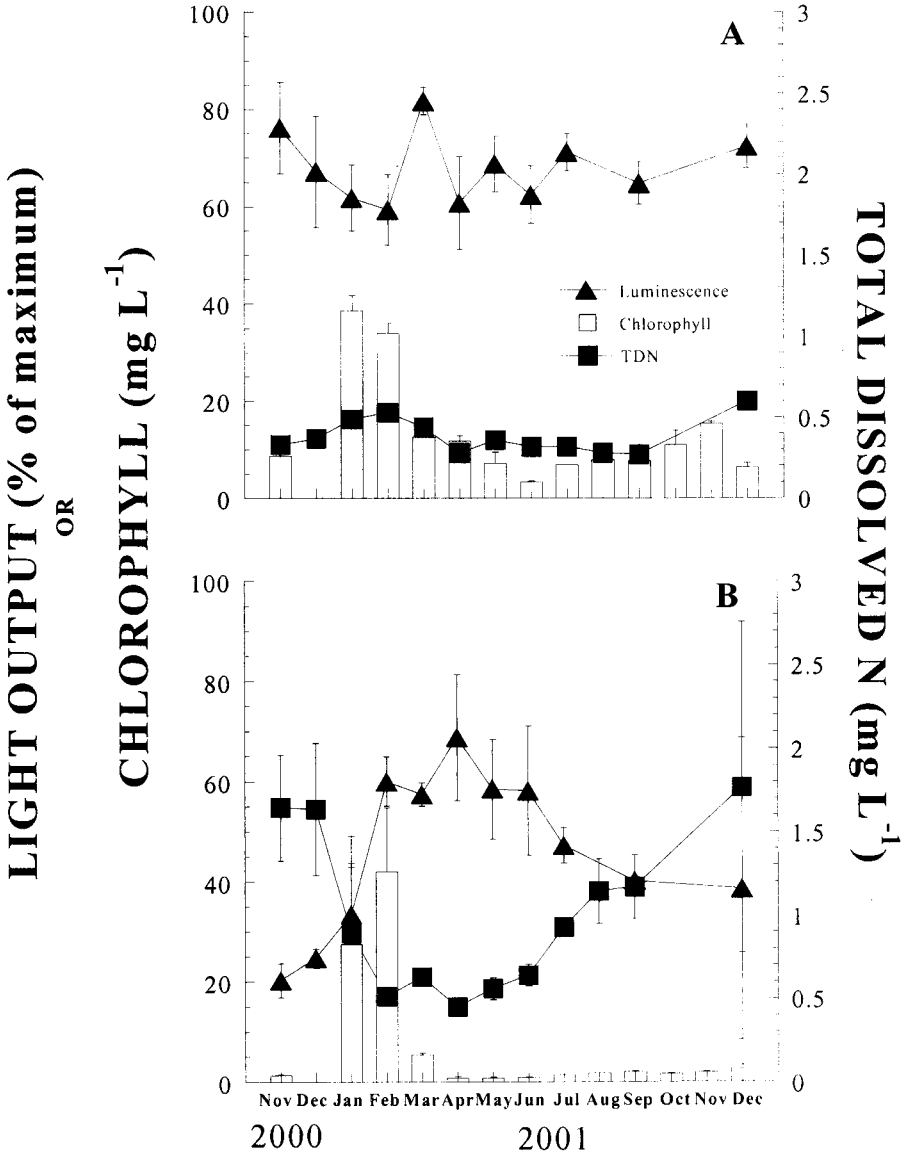

FIG. 7. Light output of GSL cells measured 20 h after inoculation and chlorophyll and TDN concentrations in monthly epilimnion (A) (average of 1-, 5-, and 10-m samples) and hypolimnion (B) (average of 30- and 37-m samples) Lake Kinneret samples. Data are averages and standard deviations.

itats (21); it is thus not surprising that luminescence, although clearly apparent, was only 60 to 70% the maximum for most of the year. A temporary increase in March, indicating a possible decrease in N bioavailability in that sample, might have been the result of the considerable increase in chlorophyll concentrations detected during the mixed period (January and February), which may have led to a depletion of bioavailable nutrients from the water column. Except for that particular sample, at least as judged from the *Synechococcus* reporter studies, there appeared to be no severe N limitation in the lake. These results are in contrast to those for phosphorus, the availability of which in the photic zone appears to control primary productivity in the same time period (18; O. Gillor et al., submitted for publication). A further indication of the general availability of N in the lake is provided by the calculation shown in Fig. 8. For most of the samples, the magnitude of calculated available N concentrations is close to that of the chemically determined TDN concentrations. Even for the epilimnion samples exhibiting high luminescence and low availability, available N was generally not lower than 10% of the total. Since organic N constitutes a large fraction of the TDN measured in the lake, our results suggest that this fraction may serve as a significant nutritional source; indeed, the ability of phytoplankton species in the lake to utilize organic N sources has been demonstrated (4).

This communication constitutes the first report in which promoter-fused reporter activity was used to evaluate changes in N availability in a freshwater lake. We have demonstrated the applicability of the approach and have used it to further our understanding of the N regimens of Lake Kinneret. In

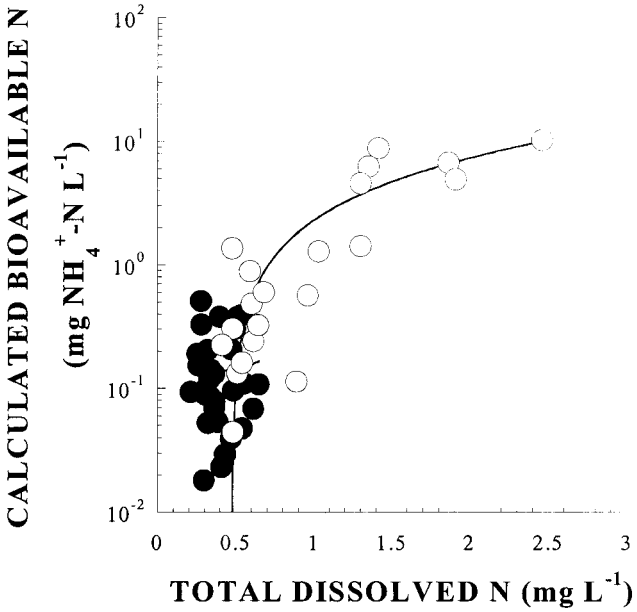

FIG. 8. Available N concentrations, calculated as ammonium equivalents and plotted as a function of TDN in the same samples. Symbols: ●, epilimnion samples; ○, hypolimnion samples.

combination with additional enzymatic and molecular assays, strain GSL and similarly constructed reporter strains may serve as important tools for assessing the N status of phytoplankton in aquatic environments.

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