# Low-Nitrate-Days (LND), a Potential Indicator of Cyanobacteria Blooms in a Eutrophic Hardwater Reservoir

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When nitrate was low in a hypereutrophic, hardwater reservoir, cyanobacteria proliferated into blooms. Based on this observation an index was developed that relates an easily measurable variable, the period of Low-Nitrate-Days (LND), to the period when nuisance cyanobacteria (blue-greens) proliferate and "bloom". The bloom indicator LND (d·yr1) was defined as the period of time during summer and early fall when nitrate concentration is below a lake-specific threshold. This concept was valuable in Fanshawe Lake, a southern Ontario reservoir of the Thames River in the Lake Erie catchment basin, where traditional bloom indicators are rare. A nitrate threshold of 1 to 2 mg·L<sup>1</sup> is supported by occasional observations of chlorophyll (Chl) concentration, blue-green biomass, visual inspection, and photographic documentation. Fanshawe Lake's water quality (phosphorus, Chl, and Secchi disk transparency) varied from summer to summer and LND ranged from 0 to 175 d·yr¹ with a long-term average of 62 d·yr¹ for 38 years. LND was positively and significantly correlated with average summer total phosphorus concentration (available for 8 years), but not Chl (n = 6) nor transparency (n = 11), possibly because of an invasion by the zebra mussel Dreissena. LND values agreed well with cyanobacteria biomass indicators predicted from other models. Significant relationships with 38 years of flows and the climatic index (winter North Atlantic Oscillation) reveal that during high-flow years estimated cyanobacteria blooms are infrequent, while during low-flow years bloom periods are extended and the water quality is poor. Investigations on other man-made lakes and river sections of the Thames River, and preliminary studies on natural lakes with differing trophic states show that LND may be a useful variable in all lakes and reservoirs where nutrient limitation switches from phosphorus to nitrogen during summer.

Key words: cyanobacteria blooms, nitrate as indicator, water quality, fast-flushed reservoir, hypertrophic, south Ontario

#### Introduction

The prevalence of cyanobacteria (also called bluegreens or, misleadingly, blue-green algae) increases with eutrophication (Cooke et al. 2005). Besides aesthetic problems from cyanobacterial blooms, toxicity from decaying cells is also worrisome (Chorus and Bartram 1999; Nürnberg et al. 2003). Unfortunately, detailed estimates of cyanobacteria abundance are rarely available for longer periods in the past since such quantification requires substantial expense, effort, and expertise. Even an estimate of general algal biomass such as the pigment chlorophyll a (Chl) is only occasionally available and prone to analytical errors and imprecision from spatial and temporal variation (Hains 1985; Gregor and Marsalek 2004). Instead, there are many chemical constituents measured in a routine monitoring program. A way that could exploit such available data for the quantification of cyanobacterial blooms would greatly increase our knowledge about these blooms spatially (in many lakes including distinct sections of large lakes and reservoirs) and temporally (for past years) and is proposed here. Once a larger knowledge base is available, hypotheses can be tested, including general relationships across lakes or specific relationships for individual lakes and reservoirs, such as nutrient, hydrological, and climatic effects on the annual variation of cyanobacterial abundance.

Much effort has been spent to determine what triggers

cyanobacteria bloom formation and many hypotheses have been put forward. While it is well accepted that cyanobacteria proliferate especially in nutrient-enriched systems and thus depend on trophic state as determined by average total phosphorus (TP) and Chl concentrations (e.g., Watson et al. 1997), the most frequent explanations are based on the concept of nutrient limitation of competing algal species.

Many cyanobacteria species can produce needed nitrogen compounds from atmospheric N<sub>2</sub> and have a competitive advantage when dissolved inorganic N (DIN) (i.e., nitrate, nitrite, and ammonium) concentration is low. In particular, all heterocystous (e.g., species of Anabaena, Aphanizomenon, and Cylindrospermopsis [Havens et al. 2003]) and most filamentous cyanobacteria are N fixers, except Oscillatoria, Lyngbia, Planktothrix, and Pseudoanabaena (Levine and Schindler 1999), and are expected to dominate in N-deficient systems (Blomqvist et al. 1994). Colonial and vacuolated nonheterocystous species like Microcystis, Oscillatoria, Lyngbia, and Planktothrix have been called low-N tolerant because of their ability to out-compete most other phytoplankton for benthic ammonium-N in nitrate depleted systems via vertical migration (Blomqvist et al. 1994). It also has been suggested that heterocystous species can migrate easily and preferably, before attempting energy-intensive N-fixing (Ferber et al. 2004). Therefore, it can be expected that blue-greens will respond positively to nitrate depletion whether heterocystous or not (as reviewed in Smith and Bennett 1999; Ferber et al. 2004).

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This hypothesis of resource limitation during periods of low nitrate (DIN-hypothesis) is supported by the argument that eukaryotes have a competitive disadvantage at low nitrate concentration, but an advantage at higher nitrate concentration because of a more efficient nitrate reductase (Ferber et al. 2004). Only when nitrate concentration decreases are the prokaryotic cyanobacteria able to reproduce faster than the eukaryotes, take advantage of other sources of N, and compete successfully for phosphorus (P).

It is important to realize that even at high total N (TN) concentration, the biologically available proportion consisting of nitrate, nitrite, and ammonia can become small over the growing season, and hence effectively limit growth of the (nonblue-green) phytoplankton. Such patterns have been observed in many shallow lakes in Europe (Weyhenmeyer et al. 2007) and the U.S. (Havens et al. 2003; Ferber et al. 2004), and in a nutrient enriched British river system (Iversen et al. 1998; Petzoldt and Uhlmann 2006). In particular, this was observed in the hypereutrophic Thames River reservoirs in southwest Ontario examined here, where nitrate and nitrite concentrations typically decline an order of magnitude throughout the summer and fall.

An older hypothesis, related to resource limitation, states that cyanobacteria proliferate at low total or inorganic N:P ratios (N:P ratio hypothesis, also reviewed in Smith and Bennett 1999; Ferber et al. 2004). N:P ratios have been used to predict cyanobacteria biomass for a long time. At a mass ratio of (total) TN:TP < 29, blue-greens proliferated but contributed less than 10% of total phytoplankton at higher ratios (Smith 1983). The exact value of this ratio was variable in different systems and could be as low as 14 (Smith and Bennett 1999). However, subsequent work determined that ratios cannot explain cyanobacteria blooms in all eutrophic lakes, that N-fixing by heterocystous cyanobacteria is far overrated, and that other characteristics including light limitation (Havens et al. 2003) and vertical migration should be taken into account (Ferber et al. 2004). Further, regression analyses determined that the separate nutrient concentrations were better predictors than their ratio (Trimbee and Prepas 1987; Downing et al. 2001). These nutrient relationships are well supported by a vast number of studies that developed significant regressions of total phytoplankton biomass (e.g., measured as growing season average Chl concentration) on both (growing season averages of) TN and TP (e.g. Watson et al. 1997; Nürnberg and Shaw 1998), and by the correlation of Chl concentration with blue-greens or its proportion of the total phytoplankton biomass (Canfield et al. 1989; Downing et al. 2001).

In conclusion, there are numerous, sometimes conflicting hypotheses about the proliferation of cyanobacteria. Simply put, it appears that on an annual basis, high average summer TP or TN concentrations likely coincide with high average cyanobacteria biomass, but on a seasonal basis, cyanobacteria tend to proliferate

during periods when available N is low, i.e., during DIN limitation.

The following study uses this observation to pragmatically determine the likely period of cyanobacterial blooms in a specific reservoir and potentially in lakes in general. While traditional concepts are not violated, this work does not intend to clarify the mechanisms of such blooms or imply any causal relationships with respect to nitrate concentration, but only describes a simple method that can possibly quantify the duration of such blooms in individual years. Hence this is a strictly empirical approach based on simultaneous observations and correlations. Although intellectually unfulfilling to some, it is the first step in the advancement of knowledge (Håkanson and Peters 1995). Such an approach seems especially appropriate with respect to N, considering that only in 2002 a basic component in the N-cycle, anammox (the formation of N, from anaerobic ammonium oxidation and nitrite) was discovered as an alternative pathway (to denitrification) for the loss of inorganic N in natural systems (Hannig et al. 2007).

In particular, this study describes the determination of a variable that seems to represent the period of cyanobacterial blooms in the hypereutrophic reservoir, Fanshawe Lake of the Upper Thames River system in southwestern Ontario, Canada. This variable, Low-Nitrate-Days (LND), is calculated as the period of days when the nitrate concentration is at or below a certain threshold, because in many lakes, nitrate concentration declines during the growing season from a spring maximum to a summer and fall minimum. Theoretically, N-limitation is certain at nitrate levels below 0.01 mg·L-1, although effective limitation may occur already at higher concentrations (Weyhenmeyer et al. 2007). In Fanshawe Lake, nitrate concentration decreased from 7 to below 1 mg·L<sup>-1</sup> from spring to fall when cyanobacteria proliferate and the lower value can serve as a threshold, as hypothesized in this study. Because this simple quantification of cyanobacteria blooms is based on longterm chemical data, information on blooms becomes available for a large number of years and can be used to identify sources of its long-term variability. In this context, water quality relationships in Fanshawe Lake, including dependencies of LND on climate, hydrology, and seasonal TP concentration were investigated.

This bloom indicator could be useful in other lakes and reservoirs as well, and therefore the general applicability of LND was investigated in other systems. In particular, it was also computed for reservoirs and sections of the Upper Thames River with varying trophic states (meso- to hypereutrophic), size, and flushing rates. Finally, the concept was tested on a shallow subtropical lake by comparing nitrate concentration with cyanobacteria biomass.

#### Methods

# Computation of Low-Nitrate-Days (LND)

Values of LND were calculated as the period of days when the nitrate concentration was below a certain threshold. This period yields a single number for each year with units of days per year and, as proposed in this paper, represents the period of cyanobacterial blooms for each year. Such periods are usually placed within the growing season, which is between the spring and late fall in temperate systems like Fanshawe Lake. In subtropical lakes, the period may extend into the winter months. The period may or may not be continuous, so that sometimes discrete periods  $(p_n)$  must be summed to yield a single value per year (Equation 1).

$$LND = p_1 + p_2 + ... + p_n$$
 (1)

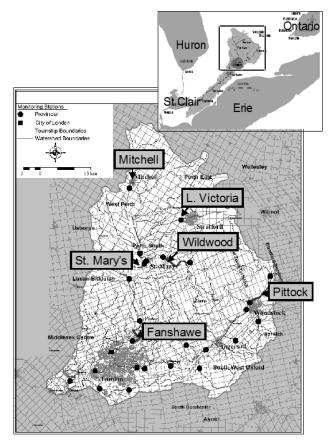
The determination of the nitrate threshold below which cyanobacteria start to proliferate is crucial and appears to differ between lakes, perhaps depending on the level of nitrate concentration in the water. The threshold was approximately 1 mg·L<sup>-1</sup> in eutrophic Fanshawe Lake which has a comparably high annual maximum nitrate concentration of 7.5 mg·L<sup>-1</sup>, while the threshold may be as small as 0.01 mg·L<sup>-1</sup> in oligotrophic lakes with low annual maximum nitrate concentrations of 1 mg·L<sup>-1</sup> (for example in a lake on the Canadian Shield, Nürnberg, unpublished studies). Theoretically, N-limitation is not expected at such relatively high nitrate levels as 1 mg·L-1, but smaller thresholds (e.g., 0.01 mg·L<sup>-1</sup>) seemed to underestimate the period of cyanobacteria bloom in Fanshawe Lake. The absolute value of the apparent threshold may be inexact and an artefact due to high analytical detection limits or divergent sampling location in space and time. Also, conditions may indeed be N-limiting even at higher N concentrations, perhaps because of the high flushing rates in the Upper Thames reservoirs and river sections, and high availability of other (potentially limiting) nutrients. In comparison, Weyhenmeyer et al. (2007) used a low threshold of 0.01 mg·L<sup>-1</sup>, below which there would be "nitrate-depleted" conditions in European lakes no matter what the P concentration, implying that the threshold could be higher under certain circumstances. The authors further speculated that these periods may coincide with "harmful algal blooms."

The potential use of LND as a measure of algal blooms can be expected to be most exact and sensitive in lakes with high nitrate concentration because, in such cases, analytical errors are small and the period of low nitrate concentration is more obvious. Therefore, Fanshawe Lake is particularly appropriate for the application of LND.

#### Fanshawe Lake

Fanshawe Lake (12.1 m maximum depth, 2.73 km<sup>2</sup>, 13.1

x 10<sup>6</sup> m<sup>3</sup>) is located in southwest Ontario, Canada, at the approximate location of 43°2' 20"N, 81°11'5"W, just north of the City of London (Fig. 1). It is a "runof-the-river" reservoir of the North Thames River, a branch of the Thames River that flows southwest into Lake St. Clair, and finally into Lake Erie via the St. Clair River. It is situated in a rural area and its watershed (1,447.4 km<sup>2</sup>) is mainly agricultural with some parks and recreational facilities immediately around the lake. The watershed to lake area ratio is large (532), as is typical for run-of-the-river reservoirs. The Upper Thames River Conservation Authority (UTRCA) constructed the dam in 1950 (filled to permanent pool height in 1952) to aid with flood control. Since then, many other purposes have been achieved, including the operation of a hydroelectric plant for 400 households, and recreation. Recreation has become an important asset as the lake was used for sculling as early as 1952, and has been the National High Performance Rowing Centre for the Canadian Women's Olympic Rowing Team since the 1980s. In addition, Fanshawe Lake's proximity to major population centres in southwest Ontario warrants its frequent use for fishing, sailing, as well as day and overnight camping in adjacent park land owned by the UTRCA.



**Fig. 1.** Map indicating the location of the study area. The small map on top shows the general area with respect to the Great Lakes, the squared area is enlarged and presents the approximate locations of the reservoirs in the Upper Thames River system.

Fanshawe Lake is flushed rapidly (average water residence time, 9.5 days; annual water load, 205 m·yr¹) by the North Thames River which contributes 98% of the inflow; the entire outflow, except by evaporation, is via a dam and spillway. During the summer, most water leaves the lake at its downstream end via outlets at about 8 to 10 m depth for a hydroelectrical facility and a low flow valve. It is probably always mixed at the shallower upstream part, but occasionally stratifies in the summer at the deeper section close to the dam. On such occasions, the bottom water may become oxygen depleted.

Fanshawe Lake water is well-buffered and hard, and its quality is variable from year to year. The reservoir is usually eutrophic but occasionally hypereutrophic (based on classification by Nürnberg [1996]), with long-term median summer concentration averages of 0.063 mg·L<sup>-1</sup> TP (median of eight years, annual averages ranging from 0.036 to 0.104 mg·L<sup>-1</sup>) and of 5.5 mg L<sup>-1</sup> TN (three years) yielding a high N-P ratio (by weight) of 87, and of 16 µg·L<sup>-1</sup> Chl (six years, 7 to 73 µg·L<sup>-1</sup>), with a Secchi disk transparency of 1.2 m (11 years, 0.9 to 2.2 m) at the deep station close to the dam. At this site, Secchi disk transparency is mostly affected by algal biomass rather than nonliving seston, and the trophic state variables are smallest and most stable as compared with upper locations as is often found in run-of-the-river reservoirs.

#### **Data Sources**

Data on reservoir water chemistry (eight years of TP, three years of TN), Chl (six years), and Secchi disk transparency (11 years) were collected by staff of the Ontario Ministry of Environment (MOE) and the UTRCA and analyzed in the MOE labs according to their standard methods (MOE 1983; Janhurst 1993). In particular, nitrate was analyzed spectrometrically and recorded in weight units of the atom (e.g., mg·L¹) of nitrate-N).

Most analyses were based on depth-integrated samples of the euphotic zone that were sampled at the deep location close to the dam. Algal biomass was determined by MOE staff for 1988. Additional indications of algal blooms in summer and fall 2005 were provided by the Canadian Women's Olympic Rowing Team, who kept a lake journal with approximately weekly entries on visual water quality, and photographic evidence by the UTRCA staff. Nitrate data from the reservoir (available for 1966 to 1971 and from four short summer periods in 1988 to 1991, and 2005) were supplemented with data from the downstream station about 1,000 m below the dam (monitored at least monthly for more than 30 years). (Available nitrate data from both locations for five years were similar; in particular, the periods of nitrate concentration below 1 to 2 mg·L<sup>-1</sup> overlapped; see Table 1 for 2005.) Accordingly, the nitrate concentration in the outflow was used to determine LND for most years (between 1971 and 2005) to obtain a total of 38 years. All physical data including morphometric and hydrological characteristics and historic daily flows were provided by the UTRCA. Monthly, seasonal, and annual flow and concentration averages were computed from daily flows and interpolated concentrations. In particular, annual average flows and water loads were computed; average flows and water loads were also computed for the 12 individual months and for the four annual quarters as well as the summer period as May through September, fall as October through December, and then January through April. Data for the other Thames River reservoirs and sections were collected and analyzed in a similar fashion. More details on long-term characteristics and data sources are presented in Nürnberg and LaZerte (2005, 2006).

Climate data were used to show the application of LND in long-term studies. North Atlantic Oscillation (NAO) is an index based on normalized sea level pressure differences that applies to eastern North American and European weather patterns. Principal component based winter NAO averages of the December to March period were from Hurrel (1995).

Data for Lake Okeechobee, Fla. were collected from the U.S. Environmental Protection Agency STORET data base (U.S. EPA 2005) and amended with those provided by Therese East of the South Florida Water Management District. Only data for the northern Station 2001 were used since they were the most complete and there was no indication of light limitation due to high sediment turbidity like in other parts of the lake (Phlips et al. 1997; Havens et al. 2003).

## **Data Analysis**

All regression analyses were done on log-transformed data (to the base of 10), which improved normality in the distribution. Furthermore, LND was transformed after adding the constant "10" [then referred to as "LND(log+10)"] when necessary to prevent the log-transformation of zero values. Regressions are only reported when significant at a level of at least 95% and when alpha levels (p, and partial-p in multiple regressions) were below 0.05. The removal of significant and influential outliers is noted in the descriptive statistics of the regressions and was based on Cook's D statistic.

Using regression equations based on average summer TP concentration, cyanobacteria biomass ( $\mu g \cdot L^{-1}$ ) (Watson et al. 1997), and a blue-green index (as a surrogate of the blue-green proportion of the total algal biomass [Downing et al. 2001]) were calculated so that they could be compared with LND. In particular, the following equations were used:

Cyanobacteria biomass = 
$$-0.613 + 2.97 \log TP - 0.45 (\log TP)^2$$
 (2)

Blue-green index = 
$$-4.16 + 1.88 \log TP$$
 (3)

where the blue-green index is ln[%BG/(100 - %BG)], with %BG, cyanobacteria biomass that is expressed as a percentage of total phytoplankton biomass.

TABLE 1. Comparison of nitrate concentrations in the outflow and within the lake with observations noted in the "Rower's Journal," and with occasional surface Chl concentrations

	Nitrate						
Date	(mg·L	1) a	Chl ( $\mu g{\cdot}L^{ ext{-}1}$ ) $^b$	Journal entry <sup>c</sup>			
Weekly to me	onthly nitra	te data	a average 6.2 mg·I	L <sup>-1</sup> (3 - 9 mg L <sup>-1</sup> ) from 10-Jan to 13-Jun			
20-Jun	8.36			Start of Journal: no algae			
23-Jun				Cryptomonas sp. bloom, a flagellate			
27-Jun	4.84			no algae			
11-Jul	2.11			some, green-brown scum			
13-Jul	3.2	*	4.5; 3.9	no algae			
18-Jul	3.4			no algae			
29-Jul	2.9	*	4.7; 6.7	no algae			
02-Aug				no algae			
03-Aug				Blue-green coloured clumps throughout the lake			
05-Aug				Blue-green coloured clumps throughout the lake			
08-Aug	1.76						
10-Aug	2.2	*	3.2; 12.5				
15-Aug	1.15						
22-Aug	1.39						
25-Aug	1.4	*	16.2; 26.8	Photographic evidence of lots of blue-greens			
30-Aug	1.64			Lot of algae			
08-Sep	1.1	*	9.7; –	Some algae			
12-Sep	1.25			Some algae clumps, smelly			
19-Sep	3.42			Some algae clumps, froth			
22-Sep	0.6	*	2.3; 19.1	Some algae, poor clarity			
26-Sep				No algae			
15-Oct				Some algae			
17-Oct	2.96						
24-Oct	3.12						
27-Oct				No algae, poor clarity			
31-Oct	1.72						
07-Nov	2.11			No algae, good clarity, end of Journal			
14-Nov	1.86						
21-Nov	11.8						
28-Nov	11.2						

<sup>&</sup>lt;sup>a</sup> Asterisk (\*) indicates the within lake observations; the remaining are from the outflow

# **Results and Discussion**

### LND in Fanshawe Lake

Nitrate is usually present in large quantities in Fanshawe Lake, but often decreases in the late fall and sometimes even in the summer to below detection limits. There are a few years when both nitrate and Chl were simultaneously determined in the reservoir, one year (2005) when bloom conditions were determined by visual inspection, and one year (1988) when complete algal biomass was measured. In a study of 1988 (Vandermeulen and Gemza 1991), simultaneous measurements of phytoplankton and cyanobacteria biomass, Chl concentration,

Secchi transparency, and various chemicals, including nitrate, indicate that the proportion of algae that are cyanobacteria (blue-green, %) increased at the same time that nitrate concentration was low (< 1 mg·L<sup>-1</sup>) in late summer and fall (Fig. 2). Conversely, in the spring and beginning of the summer, high Chl concentration, total algal biovolume, and low Secchi transparency were also observed at higher nitrate concentration, but then blue-green biomass was low. The two subsequent years (1989 and 1990) had extreme water quality conditions with respect to Chl concentrations: in the hypereutrophic year 1989, Chl was exceptionally high (indicating bloom conditions) as nitrate levels dropped from several milligrams per litre to detection limits in the summer

<sup>&</sup>lt;sup>b</sup> Surface (0–1 m) Chl samples are for the station close to the dam (first value) and a more eutrophic mid reservoir station (second value). "–": not available.

<sup>&</sup>lt;sup>c</sup> Observations as noted in the "Rower's Journal," Fanshawe Lake 2005.

and fall. In the succeeding year of 1990, Chl was low so that there were no blooms, while nitrate remained high (Fig. 3). In 1991, Chl concentration increased in the late summer, indicating blooms, and nitrate concentration decreased.

More detailed observations were made in 2005 when members of the Canadian Women's Olympic Rowing Team recorded almost weekly visual aspects of water quality in the summer and fall. The observations of poor transparency and fluorescent green "scum" coincided with periods of nitrate concentrations below 1.5 to 2.0 mg·L<sup>-1</sup> (measured in the surficial lake water and outflow), while the occurrence of noncyanobacterial blooms of flagellates occurred earlier in the summer at nitrate concentration above 5 mg·L<sup>-1</sup> (Table 1).

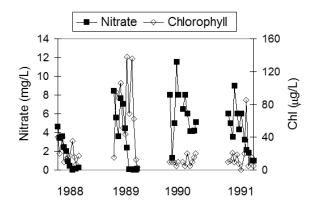
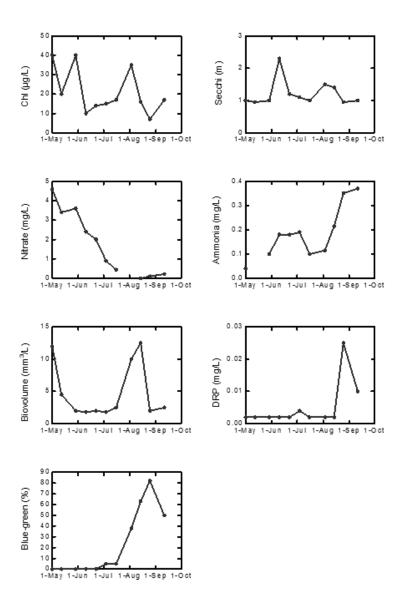


Fig. 3. Nitrate and Chl in Fanshawe Lake for selected years.



**Fig. 2.** Cyanobacteria (% of total algal biovolume) compared to chlorophyll, Secchi transparency, nitrate, ammonia, and dissolved reactive P (DRP) in Fanshawe Lake 1988.

Chl measurements of the midstation supported the journal entries, while the concentration at the dam was often low, underestimating algae proliferation; such patchiness is found frequently in run-of-the-river reservoirs. Photographs taken throughout the reservoir on August 26, 2005 showed the distinct blue-green colour on "surface scum" typical of cyanobacterial blooms.

In summary, although transparency was generally low and Chl high when nitrate concentration was low, the reverse was not true; Chl was also high during blooms of other algae, such as diatoms in the spring (probably in 1988, Fig. 2) and flagellates or green algae in the summer (Table 1). Therefore, low nitrate appears to specifically indicate the occurrence of bloom-forming blue-greens in Fanshawe Lake. Because there is no increased ammonia at the time of decreased nitrate until hypolimnetic entrainment in the fall (e.g., Fig. 2), and often total N is decreased as well, it is not plausible to assume that low nitrate is simply a consequence of chemical reduction under hypoxic conditions. Furthermore, there is no inverse relationship between ammonia and nitrate concentration throughout the study years (data not shown). But it is uncertain whether ammonia has any influence on bloom proliferation in Fanshawe Lake. Ammonia average summer concentration was an order of magnitude smaller than average nitrate, making it unlikely to be of major importance. On the other hand, cyanobacteria increased in parallel with low concentrations of ammonia in the fall of 1988 (Fig. 2). Fall increases of ammonia can be expected in many years since P concentration in the hypolimnion and the deep outflow typically increases in the fall, indicating anoxic conditions. A fertilizing role of ammonia has been described in a hypereutrophic Vermont lake where up to 98% of N was acquired as ammonium, according to <sup>15</sup>N-tracer estimates, and less than 5% was taken up as nitrate in the summer by the phytoplankton that consisted mainly of cyanobacteria (Ferber et al. 2004).

Bloom proliferation during nitrate depletion may not indicate any causal relationship. Above all, it is not suggested that an artificial increase in nitrate would combat algal blooms as has been found in the unsuccessful N-fertilization of a Wisconsin hypereutrophic lake (Lathrop 1988); instead, the nitrate decrease is simply a correlation possibly due to the high uptake of nutrients by the large number of cells.

It has long been suggested that N:P ratios control cyanobacteria growth, such that low ratios (below approximately 15, TN:TP by mass) facilitate bloom conditions (Smith and Bennett 1999). In Fanshawe Lake, the summer long-term average (total) N:P ratio is quite large at 87 (see above). For example, it decreased from 177 in the spring (April 14) to 15 at the height of the bloom (August 16) in 1989 when LND was high at 103 d·yr¹. During the same period, ratios of mineral nutrients (nitrate-nitrite plus ammonia versus dissolved reactive P) declined two orders of magnitude from 1,692 to 17. At the same time, surface-layer dissolved reactive P was

below the detection limit throughout the summer until it increased in late fall to measurable concentrations, when hypolimnetic entrainment occurred (e.g., in 1988, Fig. 2). The decline in N:P ratios throughout the summer is indicative of blue-green abundance in Fanshawe Lake, and is therefore consistent with observations of many lakes world wide, as discussed in the Introduction.

The observations presented in Fig. 2, Fig. 3, and Table 1 indicate that there is a threshold with respect to nitrate below which cyanobacteria proliferate, and that this threshold is between 1 and 2 mg·L<sup>-1</sup> in Fanshawe Lake. Accordingly, nitrate data were used to estimate cyanobacteria bloom occurrences by computing 38 annual periods (between 1966 and 2005) of LND, when nitrate was at or below a threshold of 1 mg·L<sup>-1</sup> (Fig. 4). This choice is further supported by occasional observations of lake users who noted extreme algal blooms and low transparency in years of high LND (Nürnberg and LaZerte 2006).

Although usually the period of low nitrate starts in late summer and reaches far into fall, it is highly variable between years. Accordingly, LND covers a wide range, from no days to the entire summer and early fall (0 to 175 d·yr<sup>1</sup>, Fig. 4). Values of LND were high in the past at about 125 d·yr<sup>1</sup>, but decreased to about 30 d·yr<sup>1</sup>, with occasional high-bloom years. The regression of logtransformed LND on year is negative and significant and explains 21% of the variance (n = 38,  $R^2 = 0.21$ , p < 0.210.005), indicating reduced bloom periods and, therefore, improved water quality with time. Improved conditions are probably due to enhanced wastewater treatment and the P ban in detergents in the seventies, which helped decrease trophic state in general, including the frequency of algal blooms. However, the largest part of the variance in LND is due to river and reservoir characteristics and is discussed next.

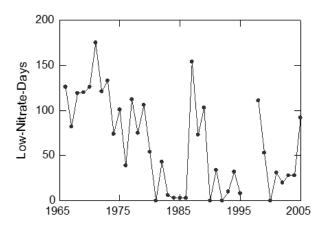


Fig. 4. Algal bloom indicator LND (days) in Fanshawe Lake outflow.

# Relationships of LND with Water Quality Indicators

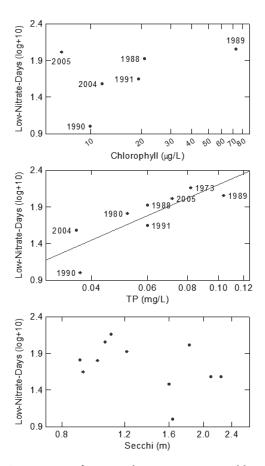
The long-term knowledge about algal bloom periods in Fanshawe Lake, expressed as LND, renders a more detailed study of relationships with other lake characteristics possible. In particular, indicators of water quality and trophic state should be correlated to such blooms, and accordingly, relationships with algal biomass indicators and nutrients will be examined first. Furthermore, water quality in reservoirs and lakes has been found to depend on hydrological and climatic conditions (Nürnberg 2002; Thierfelder 1999), and therefore such variables will be tested next.

Average summer Chl concentration is often used as an indicator of algal biomass, and if its value is above a certain threshold, it has been used to mark algal blooms (e.g., 10 µg·L-1 in South African reservoirs [Walmsley 1984]). However, as explained before, Chl is not specific to cyanobacteria but also indicates less harmful algae, so that cyanobacteria abundance can be low and nitrate concentration high on specific dates, even when Chl concentration is high. Furthermore, it is difficult to achieve representative summer average Chl estimates because of analytical complications (especially the applicability of a pheophyton correction) and the large spatial variation of the pigment in the water (Table 1); also, there are only six years of data available for Fanshawe Lake. In addition, there was a recent invasion of the zebra mussel (Dreissena polymorpha) in the winter of 2003/2004 that may have been responsible for an increase in cyanobacteria biomass as observed in several Michigan Lakes (Raikow et al. 2004) and Lake Erie (Conroy et al. 2005). Thus, it is not surprising that there is no significant relationship of LND with average summer Chl concentration in Fanshawe Lake (n = 6, Table 2, Fig. 5).

Another potential indicator of algal biomass is Secchi transparency, but in Fanshawe Lake, summer average transparency is not significantly correlated with LND either (n = 11, Table 2, Fig 5). Again, it can be argued that low transparency is not specific to cyanobacteria and that the recent invasion of the zebra mussel in the winter of

2003/2004 caused a dramatic increase in transparency and is responsible for the lack of a correlation.

Cyanobacteria are more abundant in eutrophic waters, and a positive relationship with total nutrient concentration of TP and TN can be expected as discussed in the Introduction (Downing et al. 2001). Indeed, the log-log regression of LND on summer average TP is



**Fig. 5.** Comparison of LND with summer average Chl (top), TP (centre), and Secchi disk transparency (bottom). Line represents regression line and numbers represent years.

TABLE 2. Regression results for relationships between LND and water quality or flow in Fanshawe Lake  $^a$ 

Dependent	Independent	n	$R^2$	Sign <sup>b</sup>	Significance <sup>c</sup>
LND	Chlorophyll	6	0.18		n.s.
LND	Transparency	11	0.17		n.s.
LND	TP	8	0.68	+	p < 0.02
LND	Year	38	0.21	-	p < 0.005
LND	Summer inflow	38	0.43	-	p < 0.0001
LND	Annual inflow	38	0.22	-	p < 0.005
LND	Summer inflow, Year	38	0.61	-, -	p < 0.0001
LND	NAO	38	0.22	-	p < 0.005
LND	Summer inflow, NAO	38	0.51	-, -	p < 0.001
Summer inflow	Year	38	0.004	·	n.s.
Summer inflow	NAO	52	0.09	+	p < 0.05

<sup>&</sup>lt;sup>a</sup> All values, except for NAO, were log-transformed before analysis, as explained in Methods.

<sup>&</sup>lt;sup>b</sup> Sign of regression: +, positive; -, negative.

<sup>&</sup>lt;sup>c</sup> n.s., not significant.

significant and explains more than half of the variance in LND, despite its small sample size of eight years (p < 0.02, Table 2, Fig. 5). There are only three years of summer averages of TN available and its influence on LND cannot be tested.

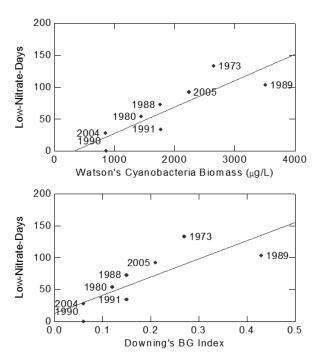
Models that were developed in other studies to predict cyanobacteria from lake characteristics can serve to test the concept of LND in Fanshawe Lake. Using regression equations (Equations 2 and 3) based on average TP concentration, cyanobacteria biomass (Watson et al. 1997), and a blue-green index (as a surrogate of the blue-green proportion of the total algal biomass [Downing et al. 2001]) were calculated and compared with LND. Regressions of LND on both variables were highly significant for the eight years for which they could be determined (Fig. 6, n = 8, p < 0.01,  $R^2 = 0.72$ ; p < 0.010.02,  $R^2 = 0.63$ ), and significance is improved when the significant and influential outlier of 1989 is removed (n = $7, p < 0.003, R^2 = 0.85; p < 0.002, R^2 = 0.88$ ). Using these models, the predicted summer average of cyanobacteria biomass fluctuates between approximately 850 µg·L<sup>-1</sup> when LND was minimal (0 and 20 days), to 3,500 µg·L<sup>-1</sup> in 1989 when LND was more than 100 d·yr-1. Predicted blue-green indices ranged from 0.06 to 0.43.

# Relationships of LND with Climate-Related Variables

Climatic conditions were highly variable; for example, average summer flows fluctuated by 20-fold in the years of reservoir operation. Similarly, the winter NAO fluctuated between -2.6 and +2.6 (Fig. 7). Climatic influences on water quality and specifically algal blooms could be large, and LND provides a long-term quantification of algal blooms and water quality in Fanshawe Lake. Therefore, LND values were compared with various flows, summer temperature, and the winter NAO as an example of a climate index.

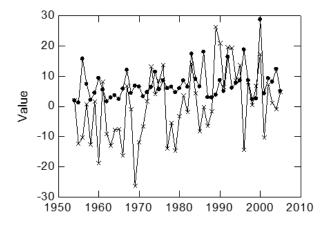
LND was significantly negatively correlated to inand outflows for most of the time periods of the summer, including individual summer months. The highest regression coefficient was found for the average summer flows, both for the inflow (p < 0.0001, Fig. 8, Table 2) and the outflow (not shown). In other words, the higher the summer flushing rate, the better is the water quality. This result is supported by similar relationships with the trophic state variables summer average TP (n = 7, p < 10.01,  $R^2 = 0.75$ , without the significant outlier of 1988), and Secchi disk transparency ( $n = 11, p < 0.05, R^2 = 0.43$ ), but not Chl (n = 6,  $R^2 = 0.26$ ). These relationships also indicate that runoff and fertilizer associated with high flows may be of less importance, because otherwise such high flows should be positively correlated with reservoir TP and decrease its water quality.

Summer surface water temperatures between 1988 and 2005 (available for eight years) were not significantly correlated with LND, although the lowest recorded temperature average (20°C in 1990) coincided

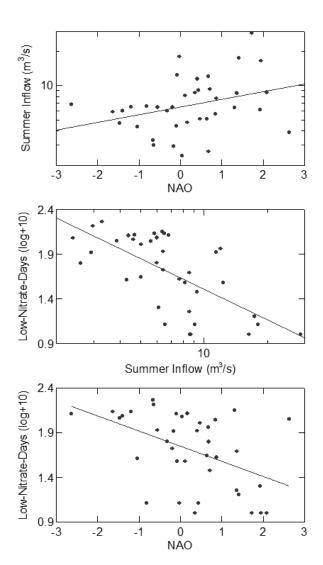


**Fig. 6.** Comparison of LND with predicted cyanobacteria biomass (Watson et al. 1997) and blue-green index (Downing et al. 2001). Regression lines and years are indicated.

with a LND of zero, and the highest temperature (24.3°C in 2005) coincided with the second highest LND, 92 d·yr¹, for the years with available temperature data. In comparison, the number of occasions with nitrate depletion in two eutrophic and 10 nutrient-poor European lakes was positively correlated with both N from atmospheric deposition and surface water temperatures (Weyhenmeyer et al. 2007). The authors speculated that such increased nitrate-depleted periods could "favour the occurrence of potentially toxic N-fixing cyanobacteria." An effect from changes in N deposition is not expected



**Fig. 7.** Summer inflow into Fanshawe Lake (May to September average in m³•s⁻¹, filled circles) and the winter Northern Atlantic Oscillation (NAO, multiplied by 10) with time.



**Fig. 8.** Relationships between the North Atlantic Oscillation Index (NAO), log-transformed LND, and main summer inflow (see statistics in Table 2).

in the Upper Thames watershed because of the overwhelming influence of N from agricultural runoff, and was not tested.

LND was also significantly negatively correlated with winter NAO, which signifies wetter and warmer conditions at higher values, basically reflecting the flow relationships with LND (Table 2, summer inflow and NAO are marginally correlated as well). The winter NAO index influences winter stagnation periods and iceout times and has been found to impact lake limnology (Straile et al. 2003). Other climatic indexes may yield relationships of higher significance in Fanshawe Lake; this example should just serve as a possible application of LND. Often flows or other specific long-term climate data are not available for a water body; a regional climate index could be used in these instances to at least partially explain variations in cyanobacteria blooms.

As described above, there is a significant decrease of LND with time. Therefore the influence of both variables,

flows and years, was tested. In fact, the variable "year" improves the flow relationships significantly, and summer inflow effect on LND is improved by 14% (from 43 to 57%, Table 2; there is no evidence of a spurious correlation because inflow is not significantly correlated with "year"). The influence of "year" could be due to decreasing TP concentration in the inflow that is marginally significant with "year" as well (n = 23, p < 0.05,  $R^2 = 0.19$ ). Such a decrease is possibly the result of improved agricultural management practices and of decreased P concentrations in wastewater effluents because of a P ban in detergents in the last twenty years.

But more summer flushing also means elevated nutrient loads, which were negatively correlated with LND (e.g., n = 23,  $R^2 = 0.19$ , R = -0.44, p < 0.05, for summer TP loads of the main inflow). Therefore, low water quality in Fanshawe Lake is correlated with low loads and high TP concentration (see previous section). In natural lakes, high TP loads are usually associated with high lake-TP concentration and low water quality. But in run-of-the-river reservoirs like Fanshawe Lake, water quality increases with flushing; this has also been observed in the large Snake River reservoir, where hypoxia decreased with increased flow (Nürnberg 2002). Such phenomenon can be explained by increased flushing of nutrients, organic substances, and algae out of the reservoir (Soballe and Kimmel 1987).

#### LND in Other Sections of the Thames River

This bloom indicator could be useful in other lakes and reservoirs as well, and therefore the general applicability of LND was investigated in other systems. In particular, it was computed for smaller reservoirs and sections of the Upper Thames River with varying TP concentration and flushing (Fig. 1, Table 3).

In the Thames River impoundments, nitrate concentration fluctuated similarly to Fanshawe Lake, typically between 7 mg·L<sup>-1</sup> and the detection limit each year, as shown for Wildwood Reservoir outflow in Fig. 9. Sections were mesotrophic to hypereutrophic, with average TP concentration ranging between 0.026 to 0.208 mg·L<sup>-1</sup>, and reservoir sizes ranged from 2 to 200 ha (Table 3). Visual inspection in the summer of 2005 revealed blue-green blooms in many impoundments and river sections. Most stations were in the North Thames River watershed and are thus related, although several were located on tributaries (Avon River and Trout Creek), except for Pittock Reservoir which was independently located on the south branch of the river. Therefore, comparison of Thames River sections should make it possible to evaluate whether size, flow rate, and trophic state impacts the usefulness and applicability of LND.

Comparison of nitrate concentrations with visual observations of blue-green algal blooms in the summer of 2005 supported a threshold of 1 mg·L<sup>-1</sup> for the determination of LNDs throughout this watershed.

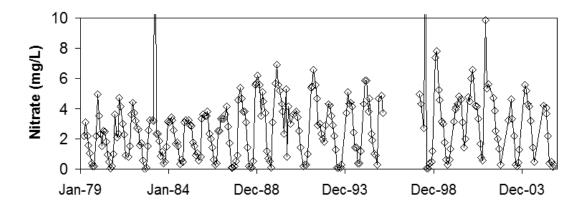


Fig. 9. Nitrate concentration in the outflow of Wildwood reservoir. Nitrate values of 26 and 15 mg·L<sup>-1</sup> nitrate are off-scale.

Furthermore, in most reservoirs, the outflow nitrate concentration resembled the reservoir concentration and could be used instead. In one reservoir, Lake Victoria at the City of Stratford, outflow nitrate concentration was usually higher than in-lake concentration, likely because the effluent of a wastewater treatment plant is discharged just above the downstream monitoring site. Therefore, the calculated LNDs in Lake Victoria are minimum estimates (Table 3).

Accordingly, LND values could be determined for more than 20 years for many stations throughout the Thames River watershed (Table 3, Fig. 10), and were compared with each other and other characteristics. Such a wealth of information on estimated bloom variability has not been available for this many sections

and reservoirs before. In fact, only the 2005 monitoring program included Chl measurements. Consequently, effects of flow alterations could be examined. For example, the dam of Wildwood Reservoir (here, annual average water residence time is the highest of the system at 47 days, and residence time is even longer in the summer) creates favourable conditions for algal growth so that LND increases on average from 9 to 76 d·yr¹ between its up- and downstream stations. In Fanshawe Lake, such a pattern has not been observed, probably because its residence time of about 10 d·yr¹ is not long enough for these conditions to occur.

In addition, hydrological dependencies were tested and found to be similar to that for Fanshawe Lake. In particular, LNDs were negatively correlated with

TABLE 3. Characteristics of reservoirs and river sections of the Thames River watershed (Fig. 1)

Rkm <sup>a</sup>	River <sup>b</sup>	Location/ Reservoir <sup>c</sup>	Flow (10 <sup>6</sup> m³ ) Annual	Size <sup>d</sup> (ha)	TP (mg·L <sup>-1</sup> ) May-Sep	$LND (d \cdot yr^{-1})$	WQ <sup>e</sup> Year
					, , , ,		
81.8	NTR	d/s Mitchell	146.7	15	0.108	48	1986–05
73.5	Neil Drain	Fullarton	1.4	1.8	0.208	121	2005
73.0	Black Creek		57.7		0.044	81	2003-05
56.0	Avon River	d/s Victoria	63.6	16	0.120	17	1986–05
48.9	Trout Creek	u/s Wildwood	18.1		0.082	9	1986-05
48.9	Trout Creek	d/s Wildwood	64.3	200	0.077	76	1986-05
48.3	NTR	d/s St. Mary's	460.1	14	0.077	61	1986–05
60.5	Otter Creek		22.1		0.026	39	2003-05
61.0	Flat Creek		34.1		0.051	27	2003-05
39.0	Fish Creek		59.9		0.056	10	2003-05
40.0	Gregory Creek		22.1		0.085	81	2003-05
37.5	NTR	u/s Fanshawe	577.4		0.057	70	1986–05
21.6	NTR	d/s Fanshawe	569.0	273	0.093	45	1986–05
	STR	d/s Pittock	98.1	148	0.094	77	1986–05

<sup>&</sup>lt;sup>a</sup> Rkm, River kilometer upstream of the Byron Gage, below the confluent with the main stem. In the case of the tributaries, Rkm indicates the location of confluence with the NTR.

<sup>&</sup>lt;sup>b</sup> NTR, North Thames River; STR, South Thames River.

<sup>&</sup>lt;sup>c</sup> d/s, downstream; u/s, upstream.

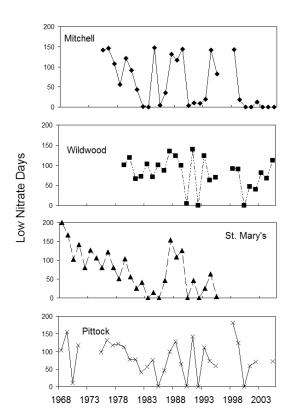
<sup>&</sup>lt;sup>d</sup> Average surface area of the (upstream) reservoir.

<sup>&</sup>lt;sup>e</sup>There are no water quality (WQ) data for 1996 and 1997.

summer flow in the downstream stations of the reservoirs Wildwood (n = 24,  $R^2 = 0.34$ , p < 0.01), Pittock (n = 22,  $R^2 = 0.49$ , p < 0.001), Lake Mitchell (n = 29,  $R^2 = 0.17$ , p < 0.03), and Lake Victoria (n = 36,  $R^2 = 0.18$ , p < 0.01) even though LND is underestimated in Lake Victoria because of wastewater treatment effluents. At the St. Mary's downstream station, the relationship is more simple, so that at summer flows of above about  $80 \times 10^6 \, \text{m}^3$  (or  $6 \, \text{m}^3 \cdot \text{sec}^{-1}$ ), LND is low; at low flows of below  $70 \times 10^6 \, \text{m}^3$  (or  $5 \, \text{m}^3 \cdot \text{sec}^{-1}$ ), LND is high at around  $100 \, \text{d·yr}^1$ ; and at intermediate flows, LND is variable (Fig. 11).

In river sections that are not impounded, LND values may be low despite cyanobacteria blooms, especially where point sources provide nitrate, and blooms may occur even if the nitrate concentration is higher than 1 mg·L<sup>-1</sup>. Therefore, the LND of creeks and river sections may underestimate bloom periods. Nonetheless, they probably indicate a minimum of blue-green bloom periods and were computed for all sites with available nitrate data.

The reservoirs in the agricultural Thames River watershed have high maximum nitrate concentrations with extreme fluctuations, facilitating the computation of LND. Size, flushing rate, and trophic state, as long as it is in the mesotrophic and higher range, did not compromise the applicability of the LND concept. Therefore LND is

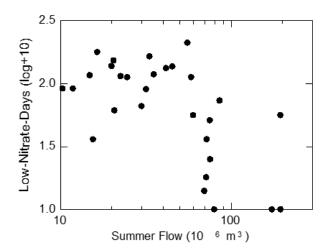


**Fig. 10.** LND in outflows of four reservoirs in the Thames River watershed (Table 3).

probably also applicable in the important downstream waters like Lake St. Clair, the St. Clair River, and finally Lake Erie. These systems are known to be highly eutrophic and exhibit cyanobacteria blooms; toxicity above the provisional guideline concentration of 1.0 µg of microcystin per litre, set by the World Health Organization (1998) from high biovolumes of *Microcystis spp.*, has been detected recently in the south-western part of Lake Erie (Rinta-Kanto et al. 2005). An estimation of past blooms by the algal bloom indicator LND, as presented here, would help determine trends with time, relationships with driving factors including hydrology and climate, and potential causes.

#### Potential Application of LND to Natural Lakes

As total N concentrations in natural lakes are often much lower than found in the Thames River, the nitrate threshold below which blooms occur is also lower and approaches "reasonable" N-limitation concentrations. However, because of different lake characteristics (including hydrology and other potentially limiting conditions) as well as analytical and sampling methods, it seems that thresholds may differ between lakes. That means that, as observed in the Upper Thames, cyanobacteria may proliferate also above 0.01 mg·L<sup>-1</sup> of nitrate, a value below which N-depletion is expected for certain (Weyhenmeyer et al. 2007). Consequently, thresholds need to be evaluated for each lake individually to avoid underestimating cyanobacterial abundance. Thresholds can best be determined when multiple years of coinciding nitrate and cyanobacteria biomass data are available as in the shallow, sub-tropical Lake Okeechobee, Fla. (Fig. 12). In the northern open water region of this lake, nitrate concentration below a threshold of 0.1 mg·L<sup>-1</sup> appear to best coincide with cyanobacterial blooms, and the lake-specific LND and annual blue-green biomass were significantly correlated (Fig. 13).



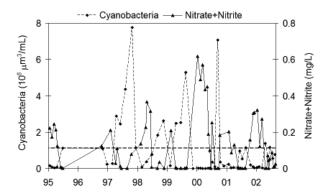
**Fig. 11.** Log-transformed LND versus May to September flows at the station downstream of St. Mary's.

Further preliminary results indicate that LND is significantly positively correlated to cyanobacteria abundance in several other natural lakes with varying trophic state, N:P ratio, and temperature, supporting the applicability and usefulness of LND as an algal bloom indicator in many lakes and reservoirs. (Nürnberg unpublished studies, 2006). Nitrate concentration and therefore the threshold in these lakes was an order of magnitude lower than in the Upper Thames River system, but nonetheless fluctuated similarly between a high and a low value throughout the year.

The high N concentration in the Thames River and its reservoirs is more similar to those in European rivers and lakes where it is frequently above 10 mg·L<sup>-1</sup> and seasonal nitrate concentration fluctuates as much as in the Thames (Iversen et al. 1998; Petzoldt and Uhlmann 2006). Such systems are quite infested with cyanobacteria, and the application of LND would increase quantification, knowledge, and understanding of blooms in these highly eutrophic systems as well.

It would be interesting to determine whether only certain cyanobacteria species proliferate at low nitrate concentration and can be predicted by LNDs. For example, when and where the open water of Lake Okeechobee was not turbid and not light limited, blooms consisted mainly of the heterocystous Anabaena circinalis (Phlips et al. 1997). Response to nitrate depletion may depend on whether or not blue-greens are N-fixers, as discussed in the Introduction. In this context, the role of ammonia is uncertain because the widespread occurrence of mixed blooms of heterocystous and nonheterocystous blue-greens has been explained by vertical migration to benthic ammonia (Blomqvist et al. 1994; Ferber et al. 2004). Ammonia in Fanshawe Lake increased slightly in the fall (e.g., in 1988, Fig. 2), and occasional oxygen depletion in the bottom water indicates that it may also have increased in some of the other study reservoirs.

In summary, it can be expected that blue-greens respond positively to nitrate depletion whether

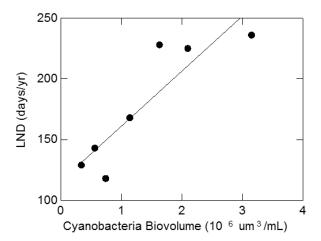


**Fig. 12.** Lake Okeechobee (Stn L001), Florida, variability of cyanobacteria biovolume and combined nitrate and nitrite concentration with time. A threshold of 0.1 mg•L¹ nitratenitrite is indicated as broken horizontal line.

heterocystous or not and whether ammonia is involved or not. Despite open questions with respect to mechanistic relationships, the study shows that LND is a useful quantifier of past and present cyanobacteria booms in the Upper Thames Reservoirs. This conclusion is supported not only by limited observations in Fanshawe Lake, but also by the highly significant correlation of LND with blue-green biomass indicators predicted from models developed with numerous temperate lakes (Watson et al. 1997; Downing et al. 2001). Therefore, the concept of LND may apply to many systems with cyanobacteria blooms, where nitrate concentrations decrease when nutrient limitation switches from P to N during summer.

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**Fig. 13.** Lake Okeechobee (Stn. L001), Florida, LND (threshold of 0.1 mg•L<sup>-1</sup> nitrate–nitrite) versus cyanobacteria biovolume (Regression line is shown for  $R^2 = 0.79$ , p < 0.01, n = 7).

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