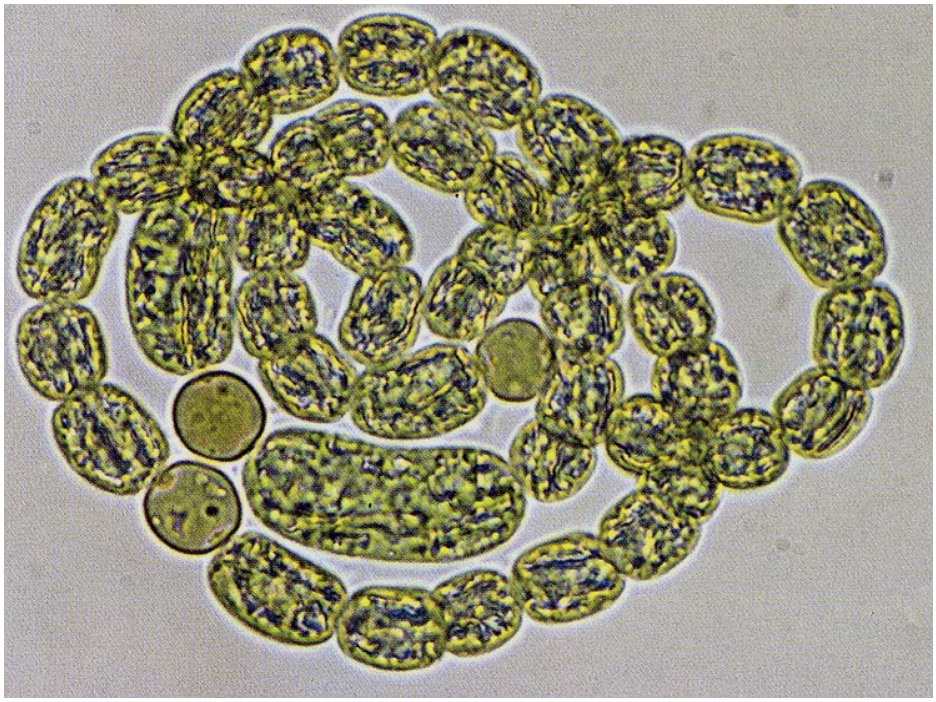
Cyanobacterial blooms in the Mildura weir pool 1996-2003

O. Scholz, N. McCasker and S. Vorwerk



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Acknowledgements

This work was supported by the Murray-Darling Basin Commission (MDBC). Thanks are extended to the Department of Land and Water Conservation (DLWC), Lower Murray Water (LMW) and Goulburn Murray Water (GMW) for their provision of cyanobacterial data and to the MDBC for supplying flow and temperature data. Nicole McCasker and Stacey Vorwerk provided much of the raw 2000-01 stratification and 2002-03 variance data through their respective summer studentships with the Lower Basin Laboratory (LBL). Thanks also to Shaun Meredith for his comments on the manuscript and to the LBL staff who assisted in the field: Ben Gawne, Bernard McCarthy, Anthony (Rex) Conallin, Clayton Sharpe, Rob Walsh, Nick Whiterod and Iain Ellis.

Cover photo: *Anabaena circinalis*- *c*ommon bloom forming cyanobacteria within the Mildura Weir pool.

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By O. Scholz, N. McCasker and S. Vorwerk

Summary

Cyanobacteria are a naturally occurring component of aquatic ecosystems that do not pose a threat to public health or stock until environmental conditions lead to the formation of blooms. The incidence of cyanobacterial blooms has been increasing this century as a likely consequence of flow regulation and land management practices. In the Mildura weir pool this is characterized by reduced flows during the warmer months.

A review of data collected between 1996-2003 from the Mildura weir pool revealed that bloom events tended to occur where periods of low flow (<2500 $ML.day^{-1}$) and high temperatures (>25 ^{o}C) coincided and persisted for several days. During such periods, flow induced water column stability facilitated the establishment of thermal and oxygen gradients in the water column. Under these conditions, the ability of cyanobacteria to function anaerobically and to regulate their buoyancy so as to exploit both surface light and benthic nutrient resources allowed them to effectively out-compete other algal taxa and develop into blooms.

Existing monitoring programs based on only a single weekly surface sample provide no measure of small-scale spatial or temporal variability, which may mask true environmental variability. This was examined during the summer of 2002-3. Spatial variability was identified as the single largest source of error. The collection of spatially replicate samples per sampling event is highly recommended.

I: Background

Cyanobacteria represent an ongoing and significant threat to water quality throughout the Murray-Darling Basin. They are a naturally occurring component of aquatic ecosystems and do not present a threat to public health or stock until environmental conditions lead to the formation of blooms. The incidence of cyanobacterial blooms appears to have been increasing this century. This is likely a reflection of the impact of river regulation, increases in catchment nutrient inputs and increased awareness and reporting of bloom events.

Typically, cyanobacterial blooms affect many of the tributaries, backwaters and slower moving reaches of the River Murray during the warmer months from November to May. Prior to 1996, significant blooms were recorded within the Mildura Weir pool in November 1991 (*Anabaena* spp.) and February-March 1993 (*Microcystis sp*.) (Baker *et al.* 1993). The occurrence of blooms at other sites throughout the Sunraysia Region (*e.g.* Hattah Lakes in January 1991, Carwap Creek d/s Nangiloc in February 1991; Baker *et al.* 1993) and a bloom in the Darling River during October-December 1991 that extended 1000km from Wilcannia to the NSW-QLD border (Bowling and Baker 1996) led to the establishment of Regional Algal Coordinating Committees in 1991 and the initiation of a coordinated monitoring program.

This review of data collected between 1996-2003 from the Mildura Weir pool examines relationships between key environmental parameters such as flow, temperature and thermal stratification that are widely acknowledged to influence the development of cyanobacterial blooms (*e.g.* Jones 1993, Webster *et al.* 2000, Mitrovic *et al.* 2003). Further, consideration is given to identifying sources of variance associated with the sampling and counting of cyanobacteria and how these might best be reduced so as to increase the resolution of routine monitoring. A clearer understanding of these relationships and issues within the Mildura Weir pool will aid the prediction of blooms and the assessment of management options such as flow manipulations or physical destratification.

II: Bloom events 1996-2003

The definition of what cyanobacterial concentrations constitute a particular threat varies depending on intended water use and species involved (refer to ANZECC 1999, Burch 1993). Until alert guidelines incorporating these issues are agreed upon, the NSW Algal Co-ordinating Committee has set indicative interim cell density ranges of 500-2000, 2000-15000 and >15000 cells.ml^{-1}, which reflect low, medium and high alert levels for management actions, respectively (refer to Jonasson 1991).

Cyanobacterial abundance data for sites within the Mildura Weir pool were provided by the agencies shown in Table 1. Modelled flow data for Mildura (MDBC Bigmod model) and Colignan water temperature data, the closest monitored upstream site to Mildura (100 km upstream), were used prior to the commencement of monitoring at Mildura by MDBC during December 2001.

Cyanobacterial sample collection and preservation procedures were generally uniform between agencies with un-concentrated surface water samples (LMW sampled at a depth of 3-4 m) being preserved with Lugol's Iodine solution. Cells were counted by DLWC, LMW and LBL using standard Sedgewick-Rafter or Lund counting protocols. LBL also counted samples provided by GMW.

Site	Gol Gol	Buronga	Midura Bridge	Mildura Water Treatment Plant (WTP)	Mildura Water Treatment Plant (WTP)	Lock 11	Mildura Weir
Agency	DLWC	DLWC	LBL	LBL	LMW	GMW-LBL	LBL
1996/7	X	X			X		
1997/8	X				X		
1998/9	X		X		X		X
1999/0	X		X		X		X
2000/1			X		X	X	X
2001/2		X	X		X	X	X
2002/3				X	X		

Table 1: Cyanobacteria sample collection sites and data sources used in this review.

1996/7

Maximum total cyanobacterial cell densities were recorded between 14th January and 3rd February 1997 at Gol Gol (9405 cells.ml^{-1}), Buronga (28555 cells.ml^{-1}) and Mildura WTP (17800 cells.ml^{-1}). Blooms during this period were dominated by *Anabaena* and *Aphanizomenon* species. Blooms coincided with River Murray flows of less than 2500 ML.day^{-1} and water temperatures above 25 ^{o}C (Figure 1).

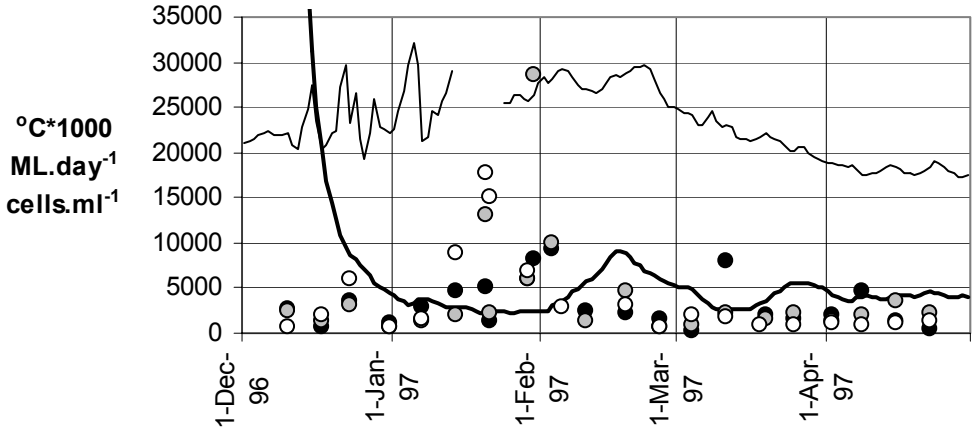

Figure 1: 1996-7 water temperatures (^{o}C*1000; thin line), River Murray flows (ML.day^{-1}; thick line) and cyanobacterial abundances (cells.ml^{-1}) recorded at Gol Gol (●), Buronga (●) and Mildura WTP (○).

1997/8

Peak cyanobacterial cell densities of 15375 cells.ml^{-1} and 15050 cells.ml^{-1} were recorded on 11th November 1997 at both Gol Gol and Mildura WTP. These blooms were preceded by several weeks (12th October to 9th November 1997) of River Murray flows below 2500 ML.day^{-1} (Figure 2). However, significant cell densities did not develop during similar periods of low flow (<3000 ML.day^{-1}) between 4th December 1997 and 17th January 1998, 3rd and 11th February 1998, and 26th February and 19th April 1998 despite temperatures exceeding 25 ^{o}C. *Anabaena* and *Aphanizomenon* species remained dominant throughout the monitored period.

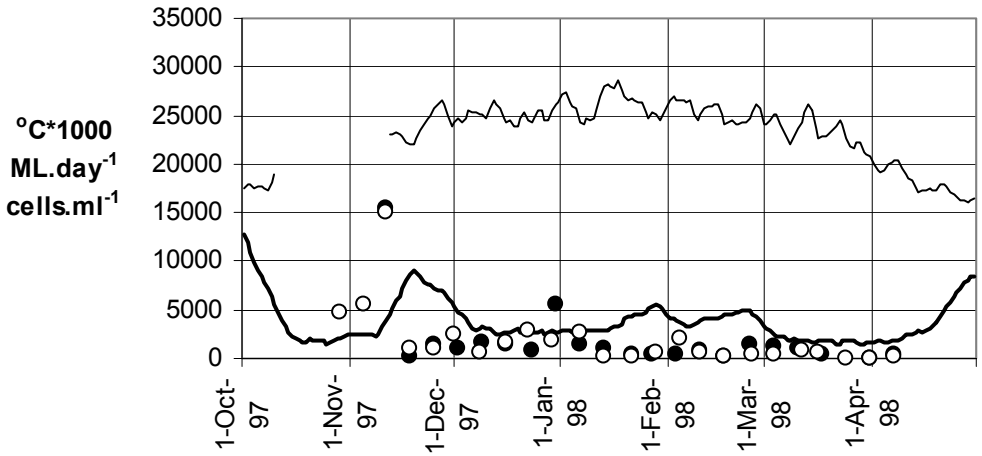

Figure 2: 1997-8 water temperatures (^{o}C*1000; thin line), River Murray flows (ML.day^{-1}; thick line) and cyanobacterial abundances (cells.ml^{-1}) recorded at Gol Gol (●) and Mildura WTP (○).

1998/9

Highest total cyanobacterial cell densities, principally *Anabaena* sp., were recorded at Gol Gol on 6th January (13430 cells.ml^{-1}) and 18th January 1999 (24260 cells.ml^{-1}) and at Mildura WTP on 1st February 1999 (10780 cells.ml^{-1}). These all occurred during periods of River Murray flows below 2,000 ML.day^{-1} and temperatures above 25 ^{o}C (Figure 3). However, cell densities remained low (<1115 cells.ml^{-1}) during a subsequent low flow (<2500 ML.day^{-1}) period between 11th and 27th March 1999 when temperatures were lower.

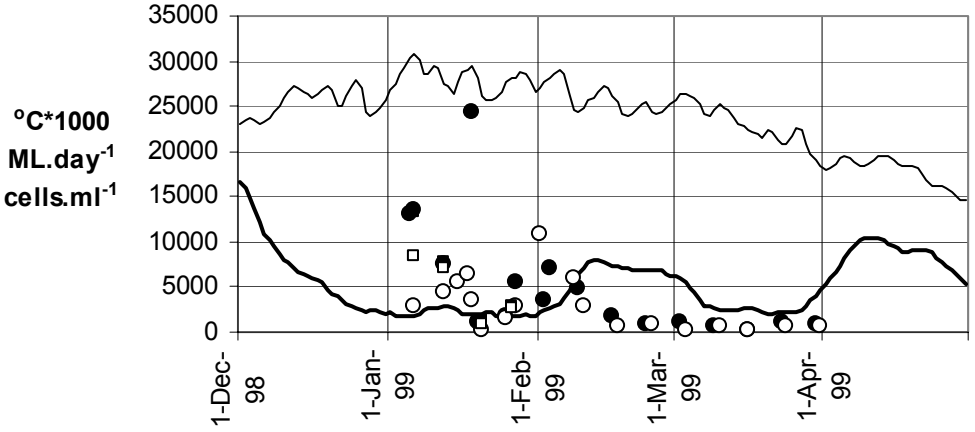

Figure 3: 1998-9 water temperatures (^{o}C*1000; thin line), River Murray flows (ML.day^{-1}; thick line) and cyanobacterial abundances (cells.ml^{-1}) recorded at Gol Gol (●), Mildura WTP (○), Mildura Bridge (■) and Mildura Weir (□).

1999/0

No significant cyanobacterial blooms were recorded at either Gol Gol (maximum 5205 cells.ml^{-1} 7th December 1999), Mildura Bridge (maximum 7220 cells.ml^{-1} 13th December 1999) or Mildura WTP (maximum 2970 cells.ml^{-1} 19th January 2000) during the monitored period. Cell densities remained low despite periods of flows below 2500 ML.day^{-1} (4th to 29th December 1999, 30th January to 10th February 2000, and 23rd to 28th March 2000). Maximum temperatures recorded during these low flow periods were 26.1 ^{o}C, 28.8 ^{o}C and 22.7 ^{o}C, respectively (Figure 4).

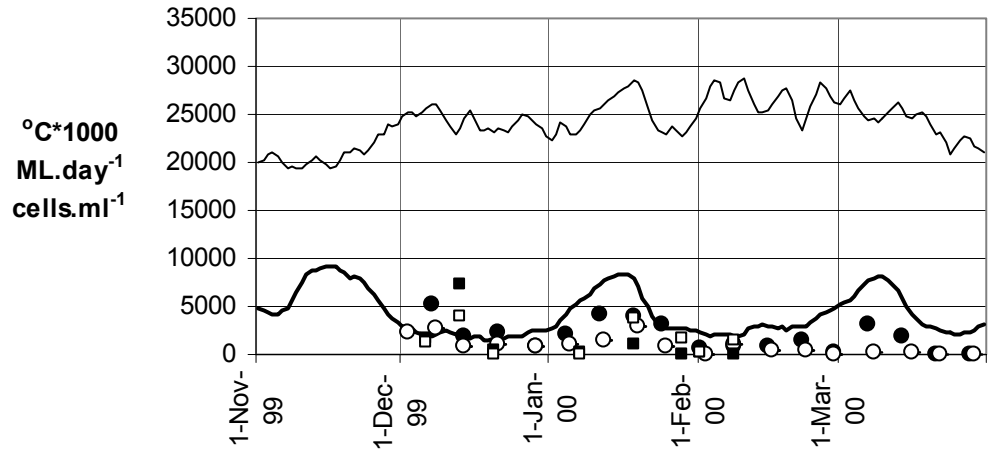

Figure 4: 1999-0 water temperatures (^{o}C*1000; thin line), River Murray flows (ML.day^{-1}; thick line) and cyanobacterial abundances (cells.ml^{-1}) recorded at Gol Gol (●), Mildura WTP (○), Mildura Bridge (■) and Mildura Weir (□).

2000/1

Cyanobacteria were monitored at four locations within the Mildura Weir pool. Peak *Anabaena* spp. cell densities of 18248 cells.ml^{-1} (29th January 2001) were recorded at Mildura Bridge, 25950 cells.ml^{-1} (8th February 2001) at Mildura WTP, 11100 cells.ml^{-1} (6th February 2001) at Lock 11, and 8841 cells.ml^{-1} (22nd January 2001) at Mildura Weir. These cell densities coincided with flows below 2500 ML.day^{-1} and temperatures between 25.9-30.0 ^{o}C (Figure 5). As in previous years, no cyanobacterial blooms developed during subsequent low flow (<2500 ML.day^{-1}) periods (10th to 25th March 2001 and 21st to 28th April 2001) when water temperatures had declined.

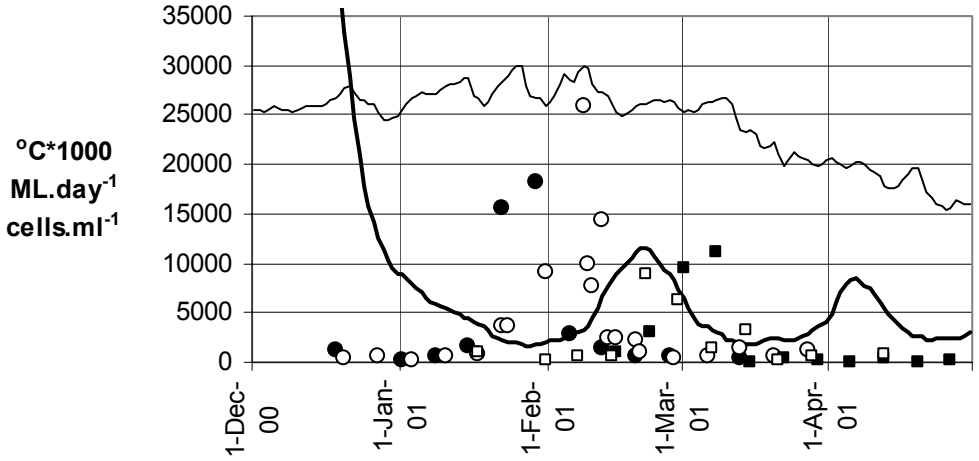

Figure 5: 2000-1 water temperatures (^{o}C*1000; thin line), River Murray flows (ML.day^{-1}; thick line) and cyanobacterial abundances (cells.ml^{-1}) recorded at Mildura Bridge (●), Mildura WTP (○), Lock 11 (■) and Mildura Weir (□).

2001/2

Cyanobacterial cell densities did not exceed 1200 cells.ml^{-1} at the Lock 11 site. Abundances were generally greater at Buronga, with a maximum of 10760 cells.ml^{-1} (excluding *Aphanocapsa* sp.) recorded on 29th January 2002 after five days of warm water temperatures (25.9-27.2 ^{o}C) and flows ranging from 3450-3800 ML.day^{-1}. No cyanbobacterial response to a subsequent period of flows below 2500 ML.day^{-1} (19th to 25th March 2002) was observed, presumably due to somewhat cooler water temperatures (22.7-23.8 ^{o}C) (Figure 6).

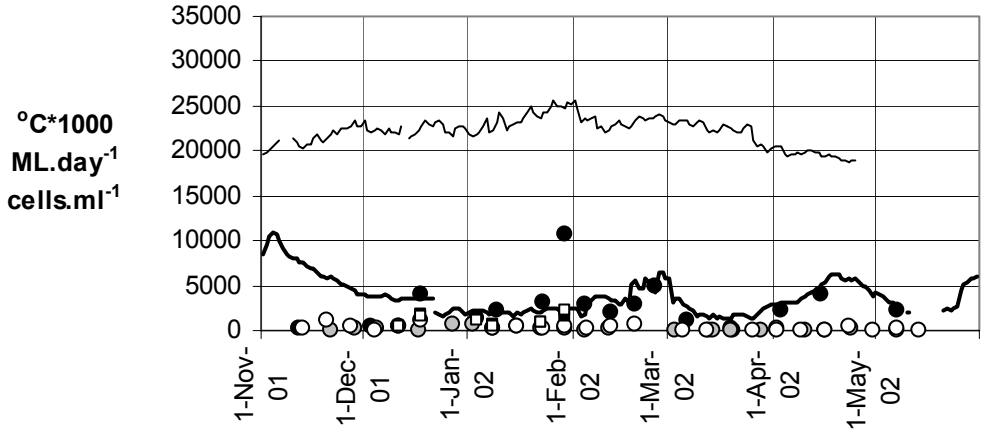

Figure 6: 2001-2 water temperatures (^{o}C*1000; thin line), River Murray flows (ML.day^{-1}; thick line) and cyanobacterial abundances (cells.ml^{-1}) recorded at Buronga (●), Mildura WTP (●), Lock 11 (○), Mildura Bridge (■) and Mildura Weir (□).

2002/3

A single *Anabaena* sp. dominated bloom was observed at the Water Treatment Plant during late December 2002. Maximum cell densities of 9575 (23rd December) and 15565 cells.ml^{-1} (30th December) were preceded by at least 5 days of surface water temperatures above 25 ^{o}C. Flows during this period, which ranged between 8000-10000 ML.day^{-1}, were much higher than flows recorded during bloom events in previous years.

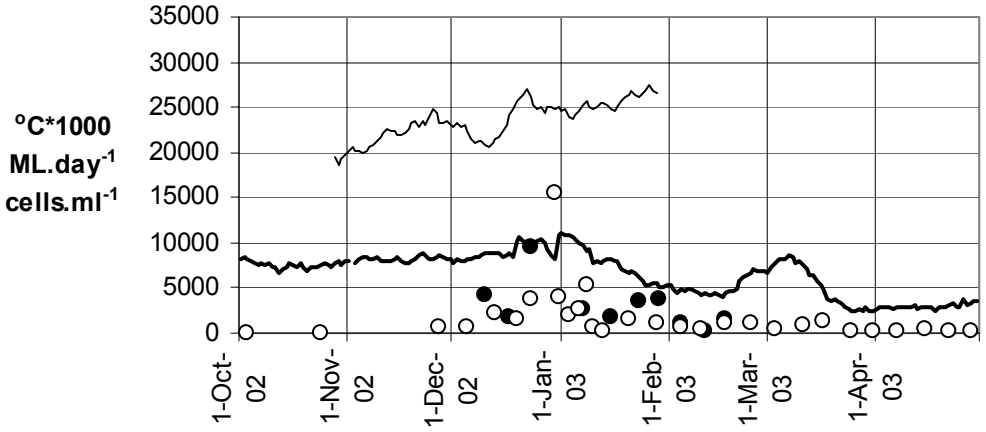

Figure 7: 2002-3 water temperatures (^{o}C*1000; thin line), River Murray flows (ML.day^{-1}; thick line) and cyanobacterial abundances (cells.ml^{-1}) recorded at Mildura WTP (LBL) (●) and Mildura WTP (LMW) (○).

III: Bloom development

Introduction

Studies that have successfully modelled the development of cyanobacterial blooms in regulated systems have identified flow induced water-column stability as a key determinant (*e.g.* Jones 1993, Bormans *et al.* 1997, Bormans and Condie 1998, Webster *et al.* 2000, Mitrovic *et al.* 2003), although other contributory factors, such as wind, turbidity and nutrient availability have also been implicated (Herath 1997, Grace *et al.* 1997, Havens *et al*. 1998, Maier *et al*. 2001). This section examines the links between the physico-chemical environment and cyanobacterial abundance using data collected between 1996-2003 from the Mildura Weir pool, and data collected during a more detailed investigation of a bloom event that occurred during the summer of 2000-1.

Methods

Water-quality and cyanobacterial abundances were monitored weekly from 18th December 2000 to 26th March 2001 at two sites within the Mildura Weir pool: close to the Mildura Bridge and close to the weir structure. Modelled flow data for Mildura was sourced from the MDBC (Bigmod model). Water temperature, turbidity (NTU), dissolved oxygen (mgO.L^{-1}) and pH were measured *in situ* using a U-10 multi-probe (Horiba Ltd., Aust.). 0.45µm filtered reactive phosphorus (FRP) samples were stored frozen until analysis using ascorbic acid (APHA 1995). Field data were collected at midday from the mid-stream both just below the water's surface (approximately 0.25 m depth) and close to the bottom sediments. Cyanobacterial samples were preserved in the field with Lugol's Iodine solution (1% v/v). 1 ml sub-samples were transferred to a Sedgewick-Rafter (S-R) cell for counting. Pearson correlation r values and Bonferroni-adjusted probabilities were determined using SYSTAT™ V10.2 (SYSTAT Software Inc., USA).

Results

The relationships between cyanobacterial abundance and both flow and surface water temperature for data collected between 1996-2003 by various agencies from the Mildura Weir pool are shown in Figure 8. These clearly demonstrate the increased probabilities of cell densities exceeding the management high alert threshold (>15000 cells.ml^{-1}) at flows below 2500 ML.day^{-1} and temperatures above 25 °C.

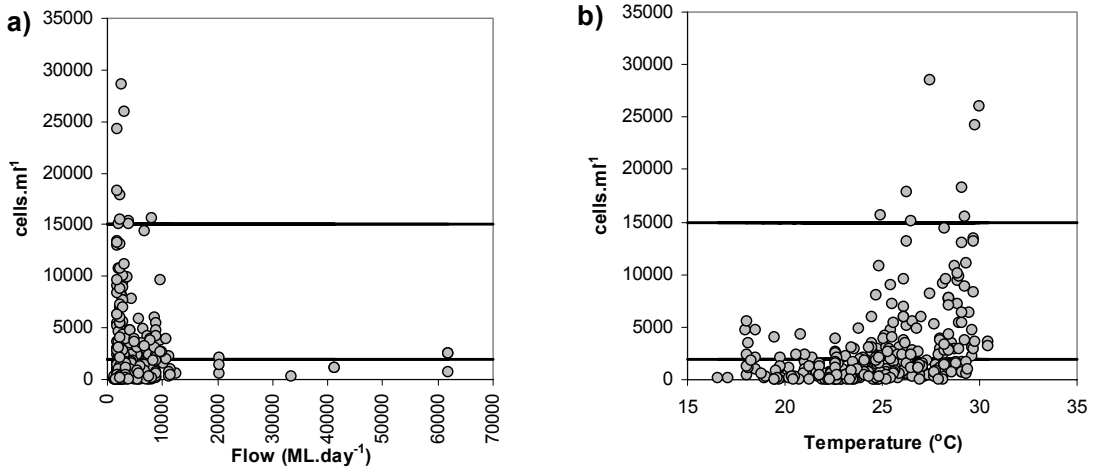

Figure 8: a) Cyanobacterial abundance (cells.ml^{-1}) vs flow (ML.day^{-1}), and b) cyano-bacterial abundance (cells.ml^{-1}) vs surface water temperature (°C) relationships for data collected between 1996-2003. Horizontal lines indicate medium and high management alert thresholds of 2000 cells.ml^{-1} and 15000 cells.ml^{-1}, respectively.

The *Anabaena* sp. dominated bloom observed at both sites within the Mildura Weir pool during the summer of 2000-1 coincided with a period of low flows (<5000 ML.day^{-1}) (Figure 9) and warm temperatures (>28 °C) (Figure 10). Low flows facilitated the settling of suspended particulate matter, reducing water column turbidity, increasing light penetration and the potential for photosynthetic

growth (Figure 11). Warmer temperatures would have further stimulated metabolic rates and cell growth.

Incomplete mixing of the water column under low flow and warm conditions led to the development and persistence of thermal stratification (cooler bottom than surface waters, expressed as delta temperature) (Figure 12). The onset of thermal stratification was closely associated with oxygen stratification, with greatly reduced dissolved oxygen concentrations in the deeper waters (Figure 13) (Pearson correlations; Mildura Bridge r=0.840, P=0.001, n=12 and Mildura Weir r=0.741, P=0.006, n=12). Increases in the sedimentation rate of suspended particulate matter during periods of low flow results in the benthic accumulation of organic matter (*cf.* Bormans and Condie 1998, B. McCarthy unpublished data). Subsequent aerobic decomposition of this material near the sediments would have contributed further to hypolimnetic oxygen depletion and accounted for the concomitant hypolimnetic decreases in pH (Figure 14) (Pearson correlations; Mildura Bridge r=0.848, P<0.001, n=12 and Mildura Weir r=0.796, P=0.002, n=12). Anoxia of the hypoliomnion often stimulates the release of significant amounts of inorganic phosphorus (*e.g.* Driscoll *et al.* 1993, Webster *et al.* 2001). Measured as filterable reactive phosphorus (FRP), such releases following oxycline establishment were apparent at both sites (Figure 15) (Pearson correlations; Mildura Bridge r=0.914, P=0.001, n=9 and Mildura Weir r=0.687, P=0.028, n=10).

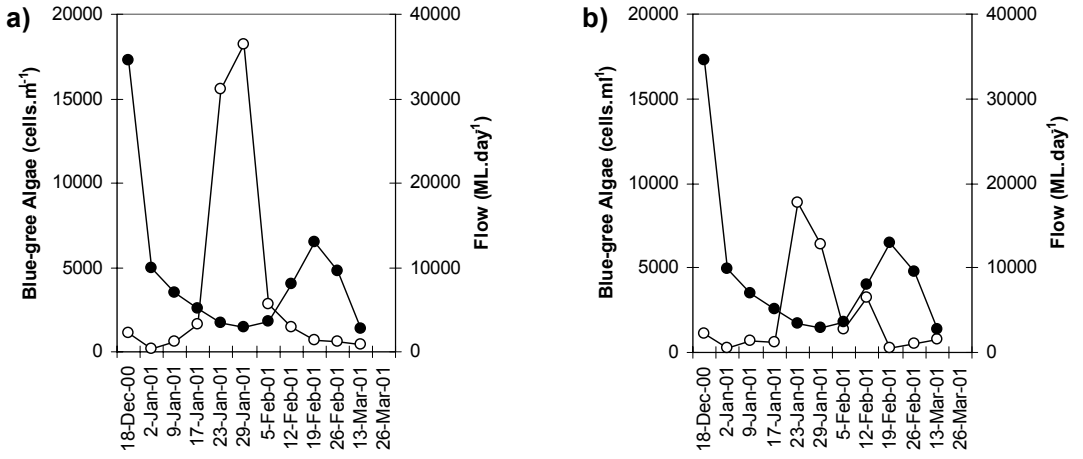

Figure 9: Flows (ML.day^{-1}) (●) and cyanobacterial densities (cells.ml^{-1}) (○) for a) Mildura Bridge and b) Mildura Weir sites during the summer of 2000-1.

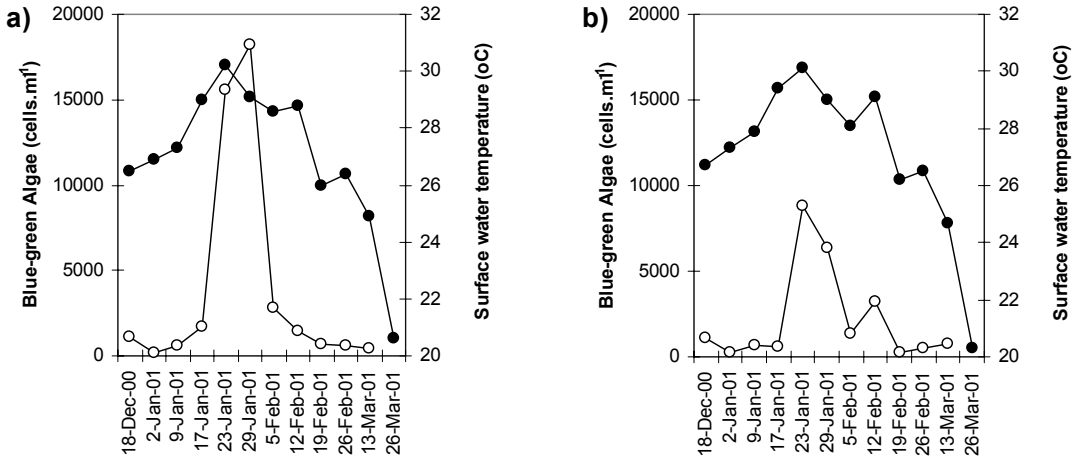

Figure 10: Surface water temperatures (^{o}C) (●) and cyanobacterial densities (cells.ml^{-1}) (○) for a) Mildura Bridge and b) Mildura Weir sites during the summer of 2000-1.

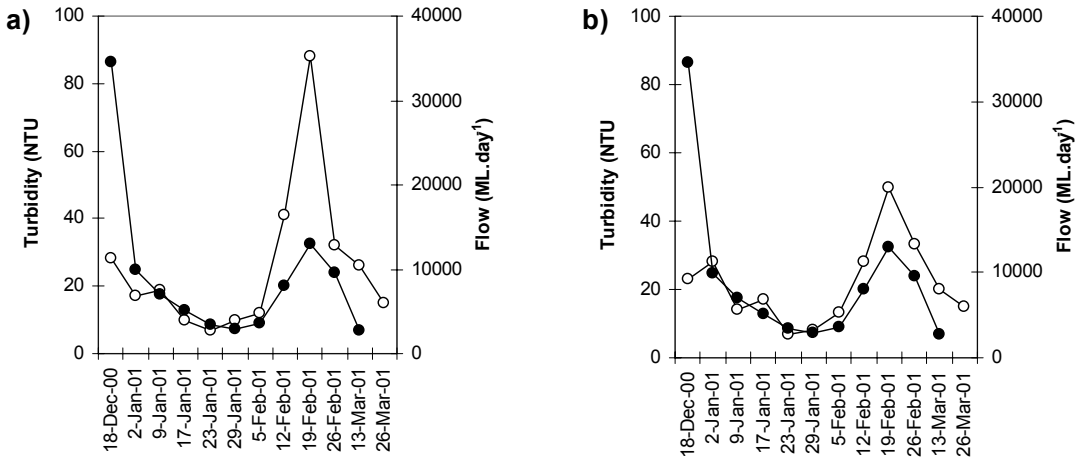

Figure 11: Flows (ML.day^{-1}) (●) and turbidities (NTU) (○) for a) Mildura Bridge and b) Mildura Weir sites during the summer of 2000-1.

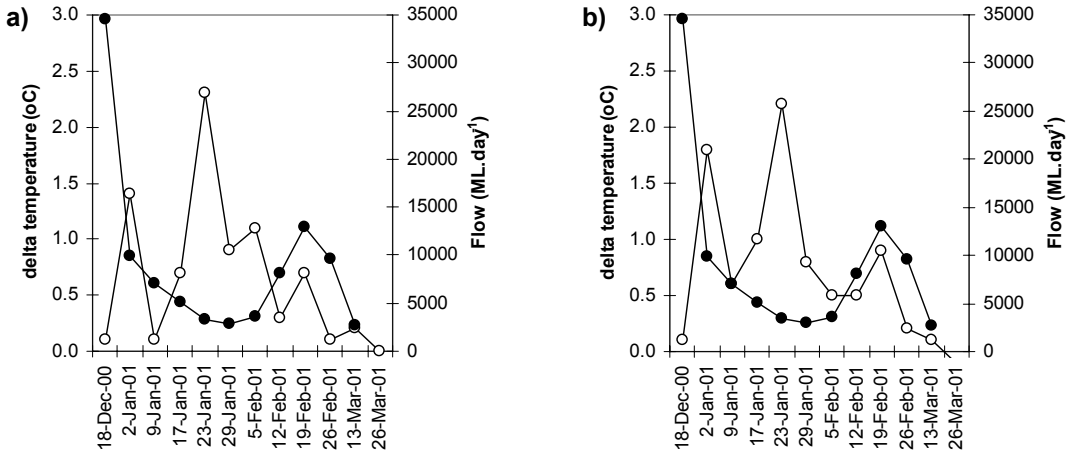

Figure 12: Flows (ML.day^{-1}) (●) and surface-bottom temperature differences (^{o}C) (○) for a) Mildura Bridge and b) Mildura Weir sites during the summer of 2000-1.

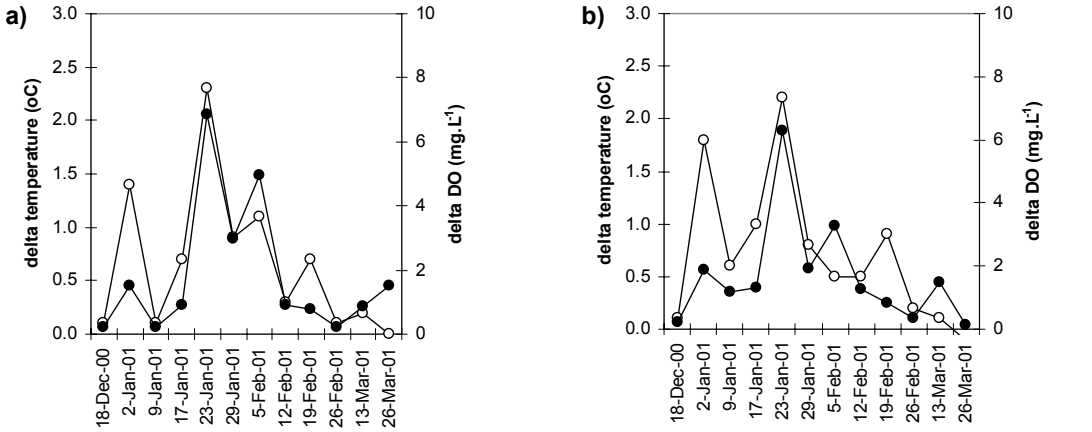

Figure 13: Thermal stratification (delta temperature) (○) and oxygen stratification (delta mgDO.L^{-1}) (●) for a) Mildura Bridge and b) Mildura Weir sites during the summer of 2000-1.

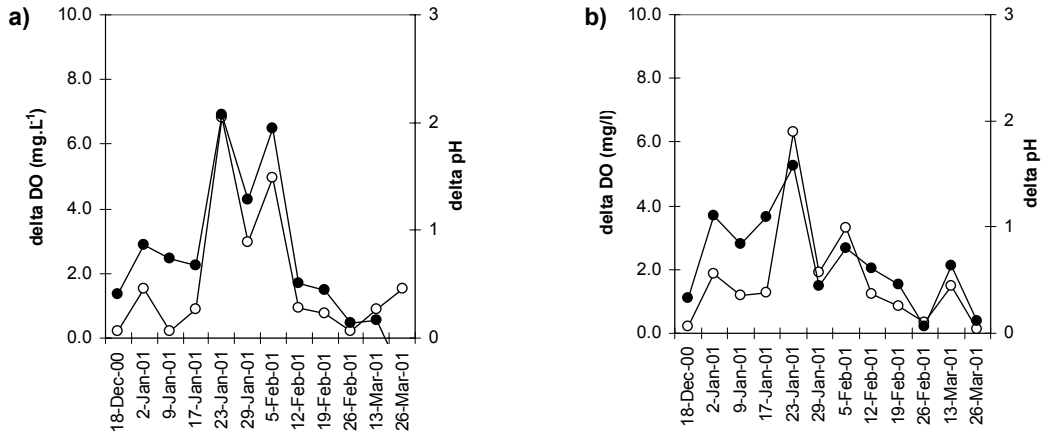

Figure 14: Oxygen stratification (delta mgDO.L^{-1}) (○) and delta pH (●) for a) Mildura Bridge and b) Mildura Weir sites during the summer of 2000-1.

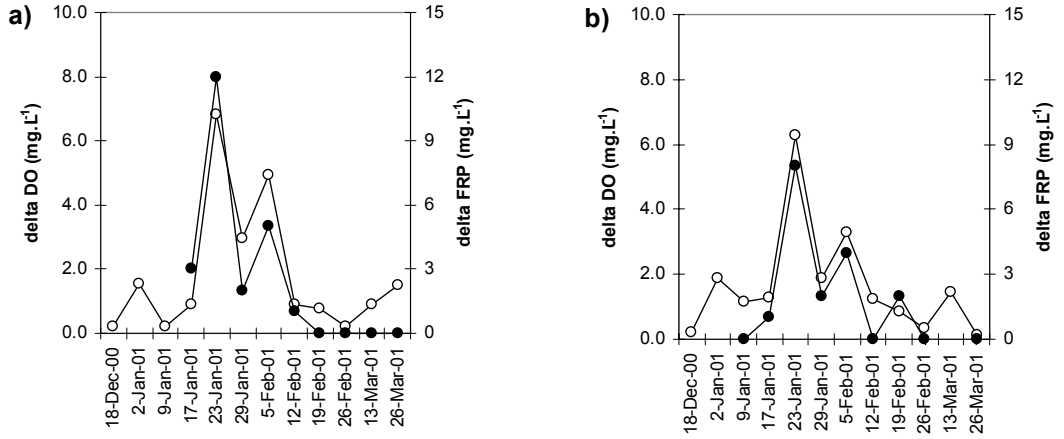

Figure 15: Oxygen stratification (delta mgDO.L^{-1}) (○) and filterable reactive phosphorus (delta mgP.L^{-1}) (●) for a) Mildura Bridge and b) Mildura Weir sites during the summer of 2000-1.

Discussion

Under certain environmental conditions, such as those described above, the ability of cyanobacteria to function anaerobically to regulate their buoyancy so as to exploit both surface light and benthic nutrient resources, and their capacity to fix atmospheric nitrogen provides a distinct competitive advantage over other algal taxa, and allows them to develop into blooms (Bormans *et al.* 1999).

Our data illustrate that low flows facilitate the initiation of thermal and oxygen stratification and the release of nutrients from the sediments. These , in turn, stimulate cyanobacterial growth. As indicated above, the establishment of cyanobacterial blooms was closely associated with water column stability, which is largely influenced by flow-rate, depth of turbulent mixing due to wind and solar radiation. As these variables are site-specific, identified threshold values for flow will not be transferable to other water bodies.

Over the period 1996-2003, observations of cyanobacterial cell densities greater than 15000 cells.ml^{-1} most commonly occurred during January and early February when water temperatures exceeded 25 ^{o}C. Surface water temperature profiles vary between years and thus will the risk of bloom development. Although growth rates of common bloom-forming cyanobacteria, such as *Anabaena circinalis*, have been shown to increase above 20 ^{o}C and to peak around 35 ^{o}C (Winder and Cheng 1995), *Anabaena* blooms have been reported during much colder periods, for example in the Darling River during July-September 1992 when temperatures were only 9-11 ^{o}C (Harriss and Reid 1992). As a guide, the NSW

Blue-Green Algal Task Force (1992) has indicated that periods during which water temperatures exceed 20 ^{o}C elevate the risk of bloom establishment. However, as blooms are not restricted to warmer periods, temperature alone provides little predictive insight.

Given the suggested causative influence of flow on bloom formation, the ability to predict periods of low flow provides further opportunity for bloom prediction and mitigation. The efficacy of flow-oriented mitigation efforts revolves around the identification of a threshold flow below which the risk of bloom development is substantially increased. Such a threshold used in conjunction with the predictive modeling of River Murray flows available through the MDBC can be a useful management tool. Throughout this examination of Mildura Weir data, a 'working' threshold flow of 2500 ML.day^{-1} was identified, although elevated cyanobacterial densities were also noted during flows of 3450-3800 ML.day^{-1} in 2001-02 and 8000-10000 ML.day^{-1} in 2002-3. A more accurate determination of this threshold will require more extensive modeling of flow-stratification characteristics within the Mildura Weir than is currently available.

Cyanobacterial blooms are not directly caused by any single environmental variable, but rather by the simultaneous occurrence of co-determinants. Mitrovic *et al.* (2003), in their work on the Darling River, indicate that the growth of cyanobacteria to nuisance concentrations involves a time lag of at least five days once persistent stratification has become established. A similar time lag for bloom establishment in the Mildura Weir pool is supported by the historical data reported above. Thus, whilst temperature and flow data alone may provide and indication of periods of elevated risk, it is periods during which both high temperatures and low flows coincide to facilitate the formation of persistent thermal stratification and reductions in turbidity that represent periods of greatest risk.

Whilst temperature itself is not a manageable variable, the disruption of thermal stratification through flow management is. Webster *et al.* (2000), in their evaluation of flow management options for the Maude Weir pool, suggest that whilst maintaining base flows above a critical level may be the most effective means of preventing bloom formation, it has a higher water demand than other options, such as pulsed flows. Though weekly pulsing was trialed during 1999-0 (Reid *et al*. 2000), the efficacy of flow management options in the Mildura Weir pool has not as yet been adequately examined.

IV: Bloom monitoring

Introduction

DLWC, LMW and GMW monitor cynobacterial abundances within the Mildura Weir pool (and elsewhere) by collecting a single surface sample (LMW sample at a depth of 3-4 m) per week. No information is currently available regarding the ability of these programs to accurately reflect the spatial and temporal distribution of cyanobacteria. This study was undertaken to assess the magnitude of spatial, temporal and analytical sources of variance associated with the estimation of cyanobacterial abundances, and to examine the influence of sampling effort on detection limits. This information is essential to ensure that sample collection and counting errors do not swamp environmental variability, and to identify the most efficient allocation of resources.

Methods

Three unconcentrated 200 ml surface water samples were collected every 7 days (10th December 2002 to 17th February 2003) from 1 site adjacent to the Mildura water treatment plant. Distance between samples was approximately 50 m. On one occasion (4th February 2003) cyanobacteria were sampled every 6 hrs (6 am, midday, 6 pm, midnight) at 1 m depth intervals. Surface samples collected at these times were used to assess small-scale (<24 hr) temporal variation and the influence of sample numbers and cell densities (cyanobacterial and total algal abundances) on detection limits. Analytical variance at the sub-sampling level was examined by repeatedly sub-sampling a surface sample collected on 10th December 2002. Samples were preserved in the field with Lugol's Iodine solution (1% v/v). 1 ml sub-samples were transferred to a Sedgewick-Rafter (S-R) cell for counting. Cyanobacteria were counted from 10 transects of the counting cell (*i.e.* 20 % of the sub-sample). This was kept constant throughout the study.

Magnitudes of sources of variation were assessed as co-efficients of variation (CV=SD/mean x 100). Normalising the variance ($SD^2=s^2$) to the mean allows standardized comparison of the variance associated with means of different magnitude. Analyses of variation using ANOVA were precluded by the presence of empty data cells at some levels of sampling. The resources available to this study necessitated this compromise. Minimum detectable differences δ (the minimum difference between observed and real cell abundance that is detectable 75% of the time) were calculated as:

$$\delta = \sqrt{(s^2/n)} \times (t_{0.05(2),n-1} + t_{0.1(1),n-1}) \text{ (Zar 1984).}$$

Results and Discussion

Over the 10-week study period weekly mean cyanobacterial abundances of three surface samples ranged from 167–9575 cells.ml^{-1} (Figure 7 in Section II). Highest cell densities were recorded towards the end of December 2002.

The magnitude of spatial, temporal and analytical variation (CV) determined during this study is summarized in Figure 16. Horizontal spatial variability (surface cell densities measured at 3 sites on 5 occasions) produced the greatest error CV range 9.3-86.6 %. Vertical spatial variability (cell densities measured at 7 depths on 4 occasions over 24 hours at 1 site) ranged from 37.3-66.0 % (CV) (Figure 17). Changes in the vertical distribution of cyanobacteria observed during this period were consistent with established models of cyanobacterial vertical migration and bloom development (Figure 18). These suggest that during periods of stratification cyanobacteria regulate their buoyancy so as to exploit light near the surface during the day and higher nutrient concentrations near the sediments during the night (*e.g.* Bormans *et al.* 1999). Accordingly, both vertical spatial and temporal (<24 hr) variation are likely to increase as thermal stratification develops and persists. Temporal (<24 hr) variation of surface samples had a CV of 23.6% (n=4).

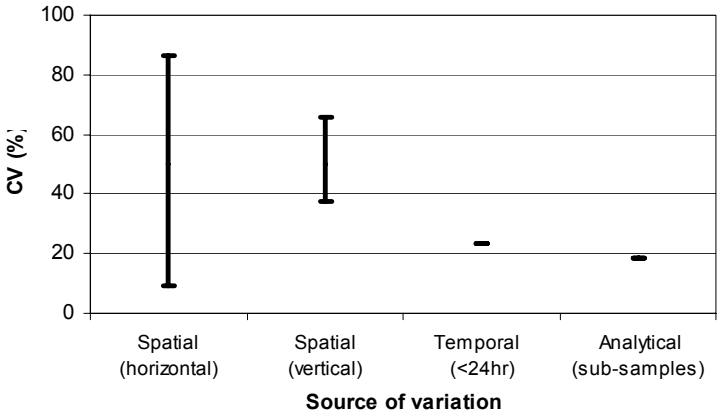

Figure 16: Sources and range of variation (CV) encountered during the study. Note that temporal and analytic CVs are based on only a single value.

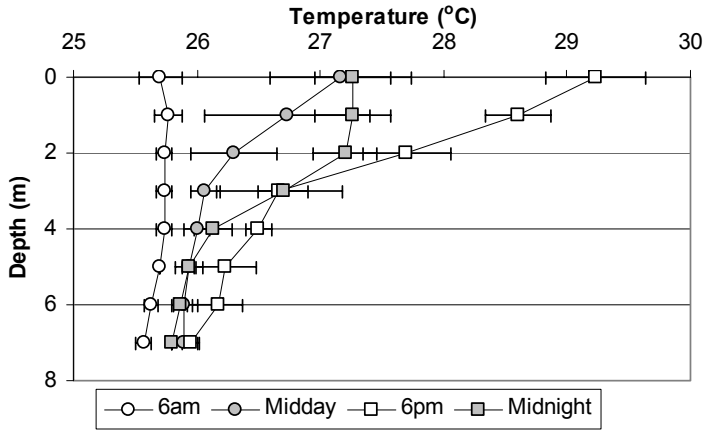

Figure 17: Water temperature depth profiles measured within the Mildura Weir on 4th February 2003 at 6 hour intervals (mean±SD).

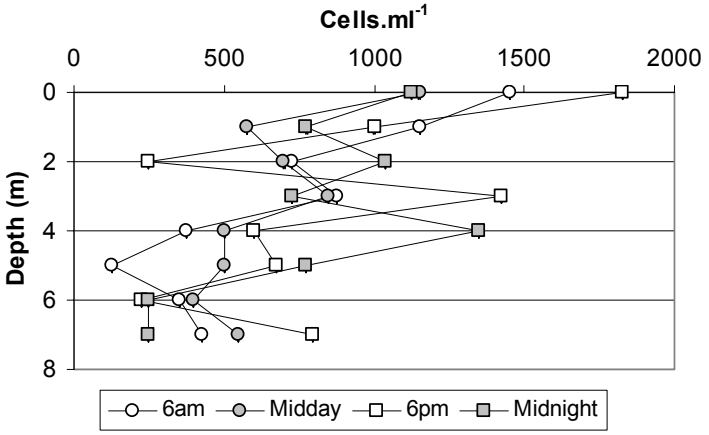

Figure 18: Blue-green algal cell density depth profiles measured on 4th February 2003 at 6-hour intervals.

Existing monitoring programs base estimates of cyanobacterial abundances on a single weekly sample per site, from which a minimum of 200 cells or 10% of the sample are counted, whichever is greater. Analytical CV (18.3%, 7 counts from 1 sample) was generally lower than was that between spatially (horizontally) replicated samples (9.3-99.8%), suggesting that increasing the number of samples collected at a site will reduce overall sampling variability more than would the counting of more sub-samples per replicate. Woelkerling *et al.* (1976) report similar findings.

The influence of sample number on precision was examined using 4 surface samples collected over 24 hours on 4th February (Table 2). Whilst this provides an indication of the sampling effort needed to achieve a desired precision over temporal (<24hr) scales, less precision for the same effort is likely over spatial (horizontal) scales due to greater variability at this level (Figure 15).

At low cell densities counting 3 samples produced a mean that did not differ from the true mean by more than about 1000 cells.ml^{-1} (*i.e.* δ) 90% of the time at the 5% significance level (Table 2). That is, for a true cell density of 1388 cells.ml^{-1} counting 3 samples is likely to provide a mean ranging from 422 to 2354 cells.ml^{-1}. As detection limits (δ cells.ml^{-1}) increase with sample cell density, it is more useful to express precision as δ % of mean (*i.e*. CV) which varies less with cell density. Extrapolation of these detection limits to a true density of 20000 cells.ml^{-1} indicates that whilst counting 3 samples will return a range of 5000-35000 cells.ml^{-1} (δ % = 75 %), counting 10 samples will return a range of 15000-25000 cells.ml^{-1} (δ % = 25 %). Admittedly, these detection limits are based on data obtained from only 4 samples, however, they do illustrate the effect of sample number on precision and that the collection and counting of only a single sample per sampling event may be inadequate where clear management thresholds exist.

	Low cell densities		High cell densities	
Mean cell density (cells.ml^{-1})	1388		8549	
SD (cells.ml^{-1})	327		2190	
No. of samples counted	δ cells.ml^{-1}	δ % of mean	δ cells.ml^{-1}	δ % of mean
1	--	--	--	--
2	3168	228.3	21229	248.3
3	966	69.6	6474	75.7
4	645	46.5	4323	50.6
5	514	37.1	3445	40.3
6	440	31.7	2949	34.5
7	391	28.2	2620	30.6
8	356	25.6	2382	27.9
9	328	23.7	2199	25.7
10	307	22.1	2054	24.0

Table 2: The influence of sampling effort and sample cell densities on minimum detection limits (δ cells.ml^{-1}) and the percentage δ represents of the mean.

Conclusion

This study suggests that the current monitoring programs based on only a single weekly surface sample are subject to significant levels of sampling variation that may mask true environmental variability. Lack of horizontal spatial replication was identified as the single largest source of error. Vertical spatial variability is likely to be greatest during bloom periods as a consequence of vertical migration by cyanobacterial populations. The collection of replicate samples has been recommended previously (Mitrovic *et al.* 1997) and is again recommended here.

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