Limiting Nutrient Workshop 1997

Principal investigator/Workshop organiser:

Alistar Robertson

Workshop participants:

Dominic Cheng, Richard Davis, Pat Feehan, George Ganf, David Hamilton, Barry Hart, Stuart Helliwell, Klaus Koop, Ian Lawrence, Rod Oliver, Wojciech Poplawski, Anna Porter, Alistar Robertson, Ian Smalls, Peter Thompson

MURRAY-DARLING BASIN COMMISSION

Land & Water Resources Research & Development Corporation

Published by: Land and Water Resources Research and Development Corporation
GPO Box 2182
Canberra ACT 2601
Telephone: (02) 6257 3379
Facsimile: (02) 6257 3420
Email: public@lwrrdc.gov.au
WebSite: www.lwrrdc.gov.au

Publication data: *'Limiting Nutrient Workshop 1997'*, LWRRDC Occasional Paper 7/99.

Author: Alistar Robertson
School of Science and Technology
Charles Sturt University
Wagga Wagga NSW 2678
Telephone: (02) 6933 2927
Facsimile: (02) 6933 2737
Email: arobertson@csu.edu.au

ISSN 1320-0992
ISBN 0 642 26755 3

Editing and layout: Green Words & Images, Canberra.

Printing: Panther Publishing & Printing

November 1999

Contents

Preface

Water managers throughout Australia have the task of supplying water that is safe to drink and of a quality that does not exacerbate problems of river health, such as cyanobacterial blooms. For the past two years, the National Eutrophication Management Program (NEMP), jointly managed by the Land and Water Resources Research and Development Corporation and the Murray–Darling Basin Commission, has funded research into a range of eutrophication-related issues, including the sources of nutrients that support cyanobacterial and other nuisance phytoplanktonic blooms in Australia's aquatic systems.

NEMP funded a workshop at Charles Sturt University in Wagga Wagga in November 1997 to examine recent research into the limitations to phytoplankton growth that arise from nutrient limitation. In particular, there had been increasing evidence that nitrogen could in some circumstances be as important a limiting nutrient as phosphorus. If true, this finding could have important management implications. The main aim of the meeting was to review the current state of knowledge regarding the relative importance of different nutrients and variations in the light environment in controlling phytoplankton population dynamics in freshwaters. The outcome was to be a summary of current knowledge written in plain English for managers.

Executive summary

The National Eutrophication Management Program (NEMP), funded by the Land and Water Resources Research and Development Corporation and the Murray–Darling Basin Commission, supported a workshop in Wagga Wagga in November 1997 to review the current state of knowledge regarding the relative importance of different nutrients and variations in the light environment in controlling phytoplankton population dynamics in freshwaters. The outcome was to be a report on current knowledge, written in plain English for managers. A summary is provided below. Participants provided papers on limitations to algal growth in Australian freshwater systems. These papers are available from the authors (see Appendices 1 and 2).

On their own, measurements of nutrient concentrations in water samples are of limited value in determining whether nutrients are limiting the growth and biomass yield of phytoplankton. This is because there are difficulties in measuring nutrient concentrations accurately and in determining what proportion of the measured nutrients are 'available' to support phytoplankton growth. In addition, even when 'available' nutrient concentrations are low, the resupply of nutrients from cycling within the water column, or from sediments and external sources, may be rapid. Under such circumstances, it is the rate of flux of such nutrients that controls phytoplankton biomass, not the nutrient concentrations *per se*.

The best evidence for short-term nutrient limitation of phytoplankton growth and biomass comes from a combination of algal growth bioassays and physiological measures of the response of phytoplankton cells to the addition of nutrients to water samples. Investigations in three rivers of the Murray–Darling Basin have revealed that nitrogen and phosphorus are often not limiting to phytoplankton growth. When nutrients do limit growth, nitrogen is as likely to limit phytoplankton growth as often as is phosphorus. When nitrogen is limiting, cyanobacteria may dominate the phytoplankton, as some cyanobacterial species can fix nitrogen directly from dissolved nitrogen in the water.

Over the longer term, however, in the case of nitrogen-fixing cyanobacteria, phosphorus concentrations are eventually reduced to limiting levels because all available phosphorus is incorporated into population biomass as blooms develop.

A number of factors, other than nutrient availability in the water column, influence the growth and biomass yield of phytoplankton. Australian waters can be turbid, and light availability often limits phytoplankton growth even when nutrients are present in relatively high concentrations. Conditions that decrease turbidity, such as stratification and low mixing in standing waters and low flows in running waters, increase light penetration and so potentially remove the light limitation. Even under turbid conditions, species of cyanobacteria can dominate the phytoplankton and attain bloom conditions, because they can migrate through the water column using the gas vacuoles within their cells. Sediments also play a major role in controlling the supply of nutrients in the water column. Sediments can store phosphorus which is released to the water column when bottom waters and sediments become completely anoxic.

1. Introduction

The community and waterbody managers are concerned about excessive concentrations of phytoplankton in Australian freshwaters. Phytoplankton is the collective term for all microscopic cells that are capable of photosynthesis and are suspended in the water column. Phytoplankton is used here to include algal and cyanobacterial species. Whilst phytoplankton blooms probably occurred in Australian freshwaters long before European settlement, the (assumed) increased frequency and intensity of blooms indicate that Australia's aquatic ecosystems are in poor condition. Extreme phytoplankton blooms are of concern to humans because they are unsightly, produce odours, are responsible for fish kills, and, in the case of some cyanobacterial species, because they produce toxins.

Phosphorus is one of a number of nutrients essential for phytoplankton growth. Phosphorus was chosen as the nutrient to be managed in the northern hemisphere because there was a widely accepted theory that it was the element that limited phytoplankton growth in freshwaters, whilst nitrogen was the limiting nutrient in estuarine and marine waters (Hecky and Kilham, 1988). Depressing phosphorus concentrations was also sensible from a management viewpoint because it moved ecosystems away from dominance by toxic cyanobacteria. Cyanobacteria can thrive in waters with high phosphorus and low nitrogen concentrations because some species can fix nitrogen. In addition, nitrogen was less readily controlled than phosphorus because of nitrogen's ability to change its chemical form. Consequently, the theory that phosphorus limits phytoplankton growth in freshwaters was accepted in Australia without thorough examination (Harris, 1996).

The Australian hydrological cycle is much more variable than that of any other continent (Finlayson and McMahon, 1988). Storm rainfall, floods and droughts are commonplace in Australia, and little is known about the responses of nutrient dynamics to this variability. In light of these differences and emerging Australian research that shows that turbidity and flow are important factors in algal growth (Sherman et al., 1994), there is a need to collate current understanding about the role of limiting nutrients in these systems (Harris, 1996). In particular, it is important to assess whether the phosphorus limitation in freshwaters theory holds true in Australia or not.

2. Limiting nutrients

The maximum biomass of phytoplankton that can be supported by an aquatic ecosystem is determined by nutrient supply and light availability. When a nutrient that is essential for growth is unavailable (because its store has been exhausted, or it is locked up in an unavailable form) then phytoplankton growth is limited by that nutrient.

The growth rates and the final yields of phytoplankton can be controlled by nitrogen and phosphorus. Each of these elements has the potential to limit both yields and rates of population growth. In general, growth-limiting concentrations of dissolved inorganic phosphorus and dissolved inorganic nitrogen are of the order of 10 mg m^{-3} and 100 mg m^{-3}, respectively (Sas, 1989; Reynolds, 1992).

Major research emphasis has been placed on nitrogen and phosphorus, although silicon may limit diatom growth rates and yields. In general, however, diatomaceous algae are not regarded as a nuisance group amongst the phytoplankton (except perhaps for water treatment plant operators). There is little evidence to suggest that trace elements are likely to limit phytoplankton crops in Australian freshwaters.

Three main approaches have been used to investigate nutrient limitation of phytoplankton:

- Potential nutrient limitation is estimated by using chemical analyses to compare the concentrations of nutrients in the water with the anticipated cellular requirements of the phytoplankton.
- Algal growth bioassays are used to determine experimentally the nutrient in shortest supply.
- Physiological indicators of the health of phytoplankton cells are used to identify a prevailing nutrient limitation.

These approaches are not equivalent, and each provides different information and a different perspective on nutrient conditions and nutrient limitation of phytoplankton growth. The following section summarises the evidence for nutrient limitation provided by each of these approaches.

Concentrations and elemental ratios

Nutrient availability

In theory, chemical analyses can indicate when the concentration of a nutrient is low enough to limit phytoplankton growth, but in practice these low concentrations cannot be measured reliably. Direct attempts to determine limiting concentrations of nutrients are further confounded by the difficulty of measuring, unambiguously, the forms (or species) of nutrients available to phytoplankton. The problem of nutrient speciation (which determines the different forms of element present in a sample) is more associated with the analysis of phosphorus than nitrogen, where measurements of the dissolved inorganic forms, NO_x and NH_4, seem to provide reliable estimates of the nitrogen available to support phytoplankton growth.

The difficulties with phosphorus stem largely from its reactivity with particles, which makes speciation analyses difficult, particularly in turbid waters, because of the resulting association of phosphorus with a range of particles of different composition and size and with varying degrees of reversibility (Froelich, 1988). Chemical analyses to estimate the dissolved phosphorus available to the phytoplankton are commonly carried out on samples passed through a 0.45 μm filter (filterable reactive phosphorus or FRP), but the filtrate still includes phosphorus associated with fine particles and colloids that are included in the chemical analysis but that may be unavailable to the phytoplankton (see Appendix 3 on page 16). Conversely, sample filtration removes large particles containing exchangeable quantities of phosphorus that may buffer solution concentrations (buffering refers to the maintenance of the concentration of a particular ion in solution following the principles of chemical equilibria). These large particles can eventually become available to support increases in the phytoplankton biomass as dissolved concentrations are reduced (Oliver, 1993; Oliver et al., 1993).

This buffering process is an example of a more general process of nutrient recycling and resupply that includes phosphorus from internal sources, such as

suspended particles and bottom sediments, as well as continued loading from external sources. Simple measurements of nutrient concentrations rarely reveal the effects of nutrient recycling and resupply. Consequently, low dissolved nutrient concentrations do not necessarily signify a limited supply to the phytoplankton since nutrient transformations can be made available from these internal sources on an 'as required' basis and not spend significant time in the dissolved form.

Attempts to measure the amount of phosphorus available in the water column for phytoplankton growth, including both dissolved orthophosphate and exchangeable phosphorus bound to particles, frequently have involved complex chemical extraction procedures that may not reflect availability to phytoplankton (Bostrom et al., 1988). An alternative approach uses strips of filter paper coated with iron oxyhydroxide to rapidly adsorb the dissolved phosphorus from solution, resulting in the release from particles of exchangeable phosphorus as it buffers the falling solution concentration. Adsorption of dissolved phosphorus by the iron-coated filter strips continues until all the particle-associated exchangeable phosphorus is removed into solution and adsorbed to the paper strips (Oliver, 1993; Oliver et al., 1993; Sharpley, 1993). The amount of desorbable phosphorus measured using this technique shows a one to one correspondence with the amount of phosphorus available to support phytoplankton growth estimated using quantitative growth bioassays (Oliver, 1993); see Algal growth bioassays on page 5. Thus the iron-strip measures the concentration of bioavailable phosphorus. However, McKelvie et al. (1995) and Chiswell et al. (1997) reported methodological problems with iron strips, particularly when used in marine waters and highly humic or turbid waters.

The iron-strip technique has been applied to a wide range of waters in the Murray–Darling Basin and used to demonstrate that the desorbable ('bioavailable') phosphorus can comprise between 20% and 100% of the total phosphorus concentration, with the unavailable fraction remaining associated with suspended inorganic particles. A conclusion from these measurements is that the same total phosphorus concentration can support different concentrations of phytoplankton biomass, because a variable fraction of bioavailable phosphorus will be present.

The exchangeable phosphorus can be desorbed from particles within about 24 hours if the solution concentration is held nominally at zero using the iron-coated filter paper strips. Further release of phosphorus is then very slow and insufficient to provide a significant source for continuing phytoplankton growth. Within the time scales of phytoplankton blooms (weeks), the rapidly exchangeable phosphorus can be an important source of phosphorus. The potential biomass of phytoplankton populations can be predicted if the amount of available nutrient in the system is measured prior to the growth of the cells and if the yield is known. Such predictions assume that the resupply of phosphorus to the water column from other sources (see Plants other than phytoplankton on page 8) is minimal. Attempts have been made to relate the maximum biomass of phytoplankton blooms to the preceding concentration of desorbable phosphorus in the water column, but such comparisons assume that phosphorus is limiting phytoplankton production, and this needs to be demonstrated (see Nutrient ratios below).

Nutrient ratios

One approach to predicting which nutrient will limit phytoplankton growth entails comparing the relative quantities of major nutrients with the average requirements of phytoplankton cells and then using this to identify the nutrient in shortest supply. The Redfield ratio (C:N:P of 106:15:1 by atoms) is generally used to estimate the mean elemental composition of cells growing without nutrient limitation (Hecky et al., 1993). This approach has led to the widespread use of nutrient ratios, particularly total nitrogen (TN) to total phosphorus (TP) ratios (TN:TP), to predict the nutrient that will be exhausted first, as a result of phytoplankton growth, and that will so limit biomass accumulation. Based on the Redfield ratio, nitrogen limitation is expected when the TN:TP atom ratio falls below 15, while phosphorus limitation is expected at ratios greater than 15.

In discussing any application of TN:TP ratios, the first point that needs to be made is that the ratio *per se* is meaningless as an indicator of nutrient conditions unless one of the nutrients is eventually reduced to a concentration limiting the accumulation of phytoplankton biomass. If something else is limiting phytoplankton growth (eg. light), then the TN:TP ratio is irrelevant to the phytoplankton because there will be sufficient of each nutrient available.

If nitrogen or phosphorus does eventually limit phytoplankton growth, then the reliability of predicting the limiting nutrient based on earlier TN:TP ratios can still be compromised by other implicit assumptions. Use of the ratio as a predictive tool implies that measurements of TN and TP provide reliable estimates of the amount of each nutrient available to the phytoplankton. In clear waters with few inorganic suspended particles and insignificant concentrations of organic nitrogen or phosphorus, apart from those associated with the phytoplankton, this assumption may be acceptable. In turbid or organic waters this assumption will almost certainly be invalid (eg. see Nutrient availability on page 2). In these cases, the reliability of the ratio can sometimes be improved by replacing TN with an estimate of the dissolved inorganic nitrogen concentration (DIN) and comparing this with the TP concentration (Axler et al., 1994), or additionally replacing the TP measurements with an estimate of the concentration of phosphorus available to the phytoplankton, for example, desorbable phosphorus (DP).

Water samples from the Darling River at Bourke have been analysed for various forms of nitrogen and phosphorus and nutrient ratios have been used to predict the limiting nutrient that would develop in algal growth bioassays performed on the same samples (Hart et al., 1995); see Concentrations and elemental ratios on page 2. In five samples taken at intervals over a period of one year, the TN:TP ratio successfully predicted the limiting nutrient on only two occasions, the DIN/DP ratio predicted correctly on four occasions, and the DIN/FP ratio predicted correctly on all five occasions. In these samples the filterable phosphorus (FP) was measured on filtrates of 0.003 μm filters. Despite the success of the DIN/DP and DIN/FP ratios in predicting the limiting nutrient in bioassay cultures, their application to field populations can still be difficult because of the further implicit assumption that measured nutrient concentrations estimate the nutrient supply to the cells (see Nutrient availability on page 2). Where nutrient recycling and resupply (eg. from the sediments, see The role of sediments on page 7) occurs, this assumption may not be valid and the reliability of predictions will be compromised.

In some instances the TN:TP ratio has been used to indicate the probability of the occurrence of cyanobacterial blooms, particularly blooms of the nitrogen-fixing genera that are expected to obtain a distinct advantage over competitors when sources of combined inorganic nitrogen are limiting. Both field and culture experiments have suggested that when bloom-forming cyanobacteria, both nitrogen-fixers and non-fixers, appear then TN:TP atom ratios are less than about 60 (Tilman et al., 1986; Smith, 1983). However, there has been substantial debate about the validity of using these ratios to predict the occurrence of cyanobacteria (Pick and Lean, 1987; Harris, 1994). Indeed it has been suggested that neither laboratory nor whole-lake studies provide conclusive evidence that N:P ratios play a major role in cyanobacterial dominance (Pick and Lean, 1987). Again the problem appears to relate to whether or not nutrients actually become limiting. The N:P ratio is irrelevant to the success of nitrogen-fixing cyanobacteria unless it indicates that inorganic nitrogen levels will be reduced to low concentrations. Horne and Commins (1987) have suggested that total inorganic nitrogen concentration needs to fall below 50–100 mg m^{-3} to induce the nitrogen-fixing enzyme nitrogenase, irrespective of the N:P ratio. Perhaps this is why the most reliable demonstrations of the role of N:P ratios in leading to nitrogen-fixing cyanobacterial populations came from oligotrophic lakes where atom ratios of less than 10:1 indicated that nitrogen became limiting and resulted in the occurrence of nitrogen-fixers (Schindler, 1977; Stockner and Shortreed, 1988). As mentioned earlier, the application of these ratios will be compromised by nutrient recycling and resupply if it is not accounted for in the nutrient measurements used to calculate ratios.

Empirical chlorophyll-nutrient model

The simple model of a limiting nutrient controlling the potential phytoplankton biomass accumulation is also the basis of the well known empirical relationships relating summer chlorophyll concentrations to preceding total phosphorus concentrations (Vollenweider, 1968). In these chlorophyll-phosphorus models, the chlorophyll-*a* concentration is used as an estimate of the phytoplankton biomass, and the total phosphorus concentration as a measure of the reserves of phosphorus available to support cell growth. The broad relationship between these variables across a wide spectrum of lakes has demonstrated the key role of phosphorus in determining the maximum biomass of phytoplankton that can be supported in those waters where phosphorus becomes limiting. These relationships may not be suitable in environments where the biomass yield is limited by light, or by a nutrient other than

phosphorus, although similar models based on the appropriate limiting resource might be expected to apply in such cases. However, while nitrogen limitation is quite common in some environments, the empirical chlorophyll-phosphorus models often are still successful in predicting the phytoplankton biomass maximum in reservoirs. This is because the initial nitrogen limitation favours the growth of nitrogen-fixing species of cyanobacteria that utilise dissolved gaseous nitrogen and so continue to grow even though inorganic nitrogen in unavailable. Apparently, in many of these cases the orthophosphate concentration is eventually reduced to limiting levels by incorporation into cell biomass and the population becomes phosphorus-limited. The broad reliance of biomass yields on phosphorus concentrations even under situations of initial nitrogen limitation suggest that limitation by other nutrients and trace elements, although possible, appears to be uncommon in inland waters. This is not the case for marine and estuarine environments (eg. see Algal growth bioassays on page 5).

Although relationships between chlorophyll-*a* and phosphorus concentrations have been described for a broad range of lakes and are surprisingly congruent for a one-factor dependency, it is apparent that a single correlation does not adequately describe all situations except at the coarsest level. Specific lakes can deviate markedly from the expected relationship, resulting in disappointing responses to reductions in phosphorus in these cases. Sas (1989) found that of 18 European lakes that had undergone phosphorus reductions, seven did not show a significant decline in the phytoplankton biomass. These lakes contained excess phosphorus in solution even when the phytoplankton biomass maximum was attained, indicating that the phytoplankton biomass maximum was not dependent on the availability of phosphorus but was controlled by the availability of some other resource.

In general it was found that phosphorus limitation did not occur until the concentration of unused filterable reactive phosphorus fell below 10 mg m^{-3} for substantial periods of the growing season. In well mixed, shallow lakes the biomass declined and species shifts were observed when total phosphorus concentrations fell below about 50–100 mg m^{-3} while in deep lakes this did not occur until total phosphorus concentrations fell to 10–20 mg m^{-3}. Although these concentrations may not be generally applicable, particularly in turbid waters where the influence of suspended particles on light and phosphorus availability complicates the measurements, the simple point is that phosphorus reduction will only affect the maximum phytoplankton concentration if phosphorus supply controls the biomass yield. Consequently, before expensive nutrient reduction strategies are implemented, it is important to develop and test relationships between nutrients and phytoplankton biomass to demonstrate the reliance of phytoplankton growth on the targeted nutrient. Factors that may differ from the systems used in developing the original chlorophyll-phosphorus relationships include light availability, the resupply of nutrients from sources such as sediments (see Other factors that influence nutrient limitation on page 7), and implicit assumptions within the model, such as that total phosphorus measurements provide a reliable estimate of the store of phosphorus available for phytoplankton growth (see Nutrient availability on page 2).

The impact of internal nutrient supplies from the sediments were also demonstrated in the large dataset analysed by Sas (1989). Whereas in some lakes a reduction in the phosphorus load resulted in an immediate response in the phytoplankton biomass, the response in other lakes showed lag times of up to four years before significant reductions in biomass were achieved.

The chlorophyll-phosphorus relationships developed for lakes will not be suitable for riverine systems, although in principle the same arguments can be applied. In a flowing river there is continual growth and loss of algae, a cycling of nutrients between organic and inorganic components and the substratum, and a constant supply of new nutrients that makes it difficult to apply the simple loading models developed for reservoirs. Although Australian rivers like the Darling may well have conditions similar to lakes when flows are low and there is significant impounding of water behind weirs, even these rivers undergo rapid changes in discharge, making it difficult to apply simple annual loading models. In riverine environments other means must be used to assess the impact of nutrient conditions on the growth of phytoplankton.

Algal growth bioassays

The limnological literature contains descriptions of a large variety of assays that have been used to assess nutrient limitation of phytoplankton growth (Hecky and Kilham 1988). The most widely used method is the 'algal growth bioassay', where the biomass yield within an isolated water sample is used to assess the potential of

the water to support phytoplankton production (see Appendix 3 on page 16). This technique may not estimate the in situ growth potential accurately because the sample is cut off from sources of nutrient resupply such as the bottom sediments. The problem is further exacerbated in bioassays that require removal of all suspended particles before the addition of a cultured alga, as suspended particles can also be a source of nutrient resupply (see Concentrations and elemental ratios on page 2).

Despite these problems, growth bioassays can provide some insight to nutrient conditions. Elser et al. (1990) reviewed a large number of datasets on North American lakes where the response of algal biomass to nutrient enrichment with nitrogen, phosphorus or nitrogen and phosphorus were compared with unenriched samples. Simultaneous nitrogen and phosphorus enrichment nearly always elicited a response while either nitrogen enrichment or phosphorus enrichment each produced positive results in about half the cases. This suggests that nitrogen was just as likely to limit phytoplankton growth as phosphorus. Similar results have been obtained for a range of rivers in the Murray–Darling Basin, indicating an important role for nitrogen limitation in these waters (Wood and Oliver, 1995; Fink and Oliver, 1998). However, the difficulty of relating the growth of isolated samples to field conditions substantially reduces the value of growth bioassays.

Physiological studies

In contrast to growth bioassays, techniques that assess nutrient limitation on the basis of either cellular composition characteristics (Healey and Hendzel, 1980) or physiological responses to environmental perturbations (Vincent, 1981; Wood and Oliver, 1995) greatly reduce the incubation time under artificial conditions, and provide a more direct measurement of the nutrient status of phytoplankton populations. Although physiological assays provide an immediate estimate of the nutrient status of cells as they exist in the environment, they are short-term measurements and require extensive repetition over time to ensure that nutrient limitation is ongoing. Physiological assay is the only method that reliably indicates immediate nutrient limitation of cell growth.

A rapid physiological assay has been devised recently to identify the occurrence of nitrogen or phosphorus limitation in phytoplankton (Wood and Oliver, 1995). The nutrient induced fluorescence transient (NIFT) assay is based on the occurrence of transient fluctuations in chlorophyll-a fluorescence caused by the addition of the limiting nutrient to a sample containing either nitrogen-limited or phosphorus-limited phytoplankton. The method has been tested in cultured phytoplankton in the laboratory and in growth bioassays and applied to field samples (Fink and Oliver, 1998). Nitrogen-limited cultures showed a perturbation in the fluorescence signal on addition of nitrate or ammonium but did not respond to phosphate. Conversely, phosphorus-limited phytoplankton showed a fluorescence perturbation in response to additions of ammonium or phosphate, but did not respond to additions of nitrate. Nutrient-replete cells did not respond to any nutrient addition. These perturbations were observed in cultures of the cyanobacteria *Microcystis aeruginosa* and *Anabaena circinalis*, the green alga *Selenastrum capricornutum*, and the diatom *Aulacoseira granulata*. Algal growth bioassays confirmed the results of NIFT assays when both were used to test natural water samples. Samples collected from three field sites in the Murray–Darling Basin showed that nitrogen and phosphorus are often not limiting to phytoplankton growth, and that nitrogen limitation occurs just as frequently as phosphorus limitation. This provides a possible explanation for the occurrence in these waters of blooms of nitrogen-fixing cyanobacteria. At a site on the Murrumbidgee River, the NIFT assay provided a direct demonstration of nitrogen limitation being associated with the development of a population of nitrogen-fixing cyanobacteria. Chemical assessment of nutrient limitation using elemental ratios (see Concentrations and elemental ratios on page 2) corresponded poorly with the results of the physiological assay, indicating that concentration data are difficult to interpret.

3. Other factors that influence nutrient limitation

Phytoplankton blooms may not occur, even under favourable nutrient conditions, when there are constraints imposed by flow, temperature, grazing by zooplankton or availability of light. Some broad generalisations are possible about how some of these factors interact in Australian aquatic habitats.

During winter in temperate Australian ecosystems, high flows, turbid water and low temperatures are more likely to be the factors that limit phytoplankton growth. Therefore the growth potential of phytoplankton may not be fully realised under these conditions.

In Australian tropical and subtropical systems dominated by summer rainfalls, high-flow events in summer can prevent the buildup of large algal biomass. However, temperature and solar radiation are often high enough during the dry season to sustain significant algal biomasses. In contrast, in temperate, lowland rivers, during periods of low flow, low euphotic to mixed depth ratios (see Light availability on page 7) and stratification enhance the abundance of phytoplankton that are capable of controlling their position in the water column (notably cyanobacteria).

In water storages, the long residence of water and the stratification of the water column enhance the potential for phytoplankton blooms. In shallow lakes and weir pools, high light availability, increases benthic nutrient regeneration (see The role of sediments on page 7) and the long residence (6 days – 30 days) of water increase the probability of conditions which favour phytoplankton blooms.

Light availability

When sediments suspended in the water settle out (eg. during periods of low mixing in standing waters, or under low flow conditions in running waters), turbidity is decreased, light penetration is increased and there is greater potential for phytoplankton growth. If sufficient nutrients are available during such conditions, algal blooms are more likely to occur.

Thermal stratification of the water column of standing waterbodies in late spring and summer restricts vertical mixing in waterbodies, which in turn exposes algal cells to higher mean light intensities. This relationship may be quantified by the use of the ratio of the euphotic depth (Z_{eu}) to the mixed depth (Z_{mix}). A rough rule of thumb often used to determine when light limitation occurs is when Z_{eu}/Z_{mix} is approximately 0.3. However, this may not always be appropriate.

Algal cells in the surface waters may grow more rapidly as the light climate improves. This can lead to low nutrient concentrations in the stratified layers. These conditions may favour the occurrence of cyanobacteria species which can migrate through the water column owing to the presence of gas vacuoles in the cyanobacterial cells, and thus access the high nutrient concentrations present in stratified bottom waters.

The role of sediments

Depending on conditions, the sediments of aquatic systems are important sources (sites of regeneration) of nutrients, as well as being temporary or near-permanent sinks (sites of loss) for nutrients for phytoplankton. Sediments can act as phosphorus sinks under aerobic conditions, but when bottom waters and the sediment surface become anoxic, sediments release phosphorus to the water column. Similar movements of nitrogen between water column and sediments occur for nitrogen, but the mechanisms are fundamentally different.

Sediments as sources of nutrients

Under low redox conditions (–250 mV), which arise when respiration by microbial communities reduces oxygen concentrations in bottom waters during periods of temperature stratification, or when there are high organic loadings, phosphorus is released from the sediments. Until recently it was thought that this was a purely physico-chemical reaction, where phosphorus-adsorbed iron oxide was released as iron was reduced under anoxic conditions. However, it is now becoming clear that much of the release of phosphorus is mediated by sediment microbial communities, because sediments which have had their microbial communities killed release different quantities of phosphorus when compared to normal sediments (Mitchell, 1997).

Exchanges of nitrogen between sediment and water column are essentially microbial processes that are

mediated by the concentrations of oxygen at the sediment surface. Under anaerobic conditions, nitrification (the oxidation of ammonia to nitrate by certain bacteria) is stopped, resulting in an abundance of ammonia. This may in turn result in reduced rates of denitrification (the reduction of NO_x to atmospheric nitrogen by some bacteria), and hence influence the rate of loss of nitrogen to the atmosphere.

Sediments as sinks for nutrients

Permanent burial of phosphorus can occur by locking phosphorus into mineral particles through chemical reaction and subsequent physical burial of particles. In the case of nitrogen, there may be some long-term storage in the form of humic substances. In addition, while it is not a mechanism for storage of nitrogen in the sediment, denitrification (the reduction of NO_x to atmospheric nitrogen by some bacteria) that occurs under anaerobic conditions is responsible for the loss of nitrogen from aquatic systems.

Sediments and the management of phytoplankton blooms

Sediments are more important long-term sources of phosphorus than of nitrogen. Nitrogen to phosphorus ratios in sediments are highly biased towards phosphorus. If external sources of nutrients are removed, nitrogen will be depleted from sediments before phosphorus, owing to the relatively rapid rates of denitrification in sediments and lower starting concentrations of nitrogen.

Sediments can play a major role in efforts to reduce the frequency and duration of phytoplankton blooms in standing waters when managers attempt to control the supply of phosphorus. When external phosphorus inputs are removed there will be an immediate response in phytoplankton biomass unless there are significant sediment nutrient sources. In general, phytoplankton respond to phosphorus removal in four stages:

- No biomass reduction – phosphorus in excess of immediate requirements.
- Decline in available phosphorus concentration and a small reduction in phytoplankton biomass as sediment phosphorus is utilised.
- Minimal bioavailable phosphorus, phytoplankton biomass reduces approximately as described by the Vollenweider (1968) model.
- Further decline in phytoplankton biomass and change in composition of the phytoplankton.

The time sequence from stage one to stage four will vary depending upon the phosphorus buildup in the sediments and hydraulic residence times (Sas, 1989). For instance, in some lakes a reduction in the phosphorus load results in an immediate response in the phytoplankton biomass, while the response in other lakes may show lag times of up to four years before significant reductions in biomass are achieved.

Plants other than phytoplankton

Single-celled and multi-celled species of algae grow on all exposed surfaces in aquatic habitats as well as growing as phytoplankton in the water column. Such algal communities develop on woody debris, stones, large plants and on the sediment surface and are collectively known as microphytobenthos (literally, small plants on the bottom). These same surfaces also develop communities of bacteria and microbes which are not photosynthetically active. Together, the algal and other microbial communities are called biofilms.

Biofilms can play very important roles in the two-way movement of nutrients between the sediments and water column, and hence influence the nutrient loads available to phytoplankton. In oligotrophic, shallow waterbodies most of the total plant growth is by the microphytobenthos and there is little exchange of nutrients between the sediment and the water column as it is intercepted, used and recycled within the biofilm community. With increased turbidity or depth, decreasing light availability means that the biofilms are not oxygen producers, but net oxygen users, and can make bottom waters anoxic. When this happens, there can be a significant flux of phosphorus from sediments to the water column when sediments are rich in available phosphorus (see The role of sediments on page 7).

Large aquatic plants (commonly called macrophytes) also play an important role in the control of nutrient concentrations in the water column of Australian aquatic habitats. Macrophytes usually can only grow successfully in conditions where light penetrates to the sediment surface, since most macrophytes are rooted in the sediment and grow from seed or root stock.

Macrophytes can alter nutrient concentrations in water directly by moving nutrients from the sediments via their root systems. However, this is likely to be a very

minor effect, and macrophytes have greater indirect effects on nutrient concentrations in three ways. First, dense beds of macrophytes in shallow lakes and lowland rivers decrease the wind-driven mixing of sediments into the water column, thus decreasing the probability of sediment-bound phosphorus entering the water. Secondly, the abundant leaves of macrophytes provide important surfaces which support the growth of microphytobenthos. Finally, when abundant, macrophytes can be important storage sites for nutrients that would otherwise be available for phytoplankton.

4. Summary

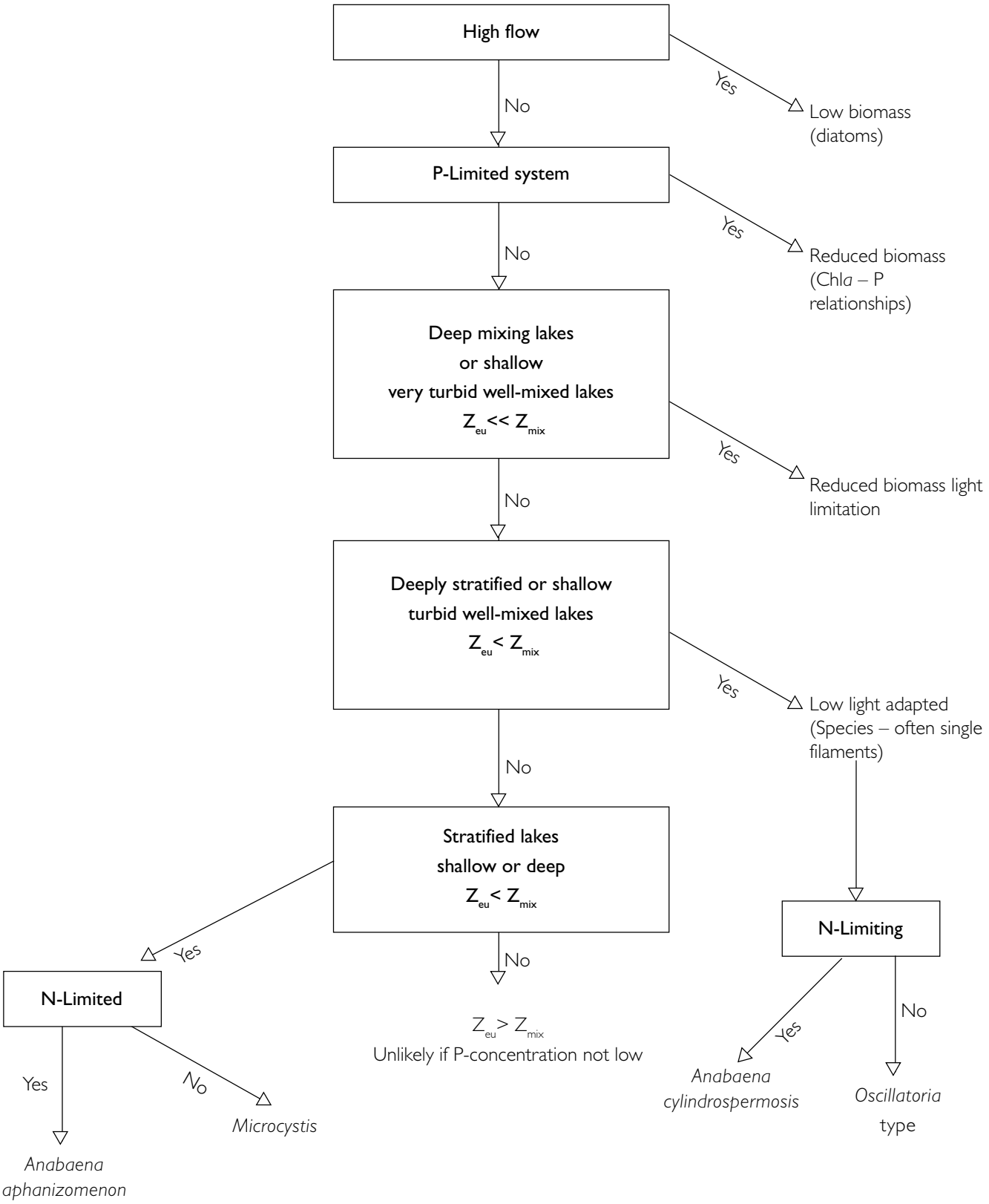
On their own, measurements of nutrient concentrations in water samples are of limited value in determining whether nutrients are limiting the growth and biomass yield of phytoplankton. This is because there are difficulties in measuring nutrient concentrations accurately and in determining what proportion of the measured nutrients are 'available' to support phytoplankton growth. In addition, even when 'available' nutrient concentrations are low, the resupply of nutrients from the cycling of nutrients within the water column, or from the sediments and external sources, may be rapid. Under such circumstances it is the rate of flux of such nutrients that controls phytoplankton biomass, not the nutrient concentrations.

The best evidence for short-term nutrient limitation of phytoplankton growth and biomass comes from a combination of algal growth bioassays and physiological measures of the response of phytoplankton cells to the addition of nutrients to water samples. Such investigations have revealed that nitrogen and phosphorus are often not limiting to phytoplankton growth, and that nitrogen is as likely to limit phytoplankton growth as is phosphorus in a range of rivers in the Murray–Darling Basin. When nitrogen is limiting, cyanobacteria may dominate the phytoplankton, as some cyanobacterial species can fix nitrogen.

However, over the longer term, phosphorus concentrations are reduced to limiting levels by incorporation into cell biomass, and populations of cyanobacteria and other phytoplankton eventually become phosphorus-limited. The broad reliance of biomass yields on phosphorus concentrations, even when nitrogen is limiting initially, suggests that limitation by other nutrients and trace elements is uncommon in Australian inland waters.

A number of factors, other than nutrient availability in the water column, influence the growth and biomass yield of phytoplankton. Australian waters can be turbid and light availability often limits phytoplankton growth when nutrients are present in relatively high concentrations. Conditions that decrease turbidity, such as stratification and low mixing in standing waters and low flows in running waters, increase light penetration, and thus modify the impact of light limitation. Even under turbid conditions, species of cyanobacteria can dominate the phytoplankton and attain bloom conditions, because they can migrate through the water column by way of the gas vacuoles within their cells. Sediments also play a major role in controlling the supply of nutrients in the water column. Sediments can store phosphorus which is released to the water column when bottom waters and sediments become completely anoxic. A simple summary of how phytoplankton biomass is influenced by nutrient limitation and other environmental factors is provided in Figure 1.

Figure 1: The influence of nutrient limitation and other environmental factors on phytoplankton biomass



References

Axler, R.P., Rose, C. and Tikkanen, C.A. 1994, 'Phytoplankton nutrient deficiency as related to atmospheric nitrogen deposition in northern Minnesota acid-sensitive lakes', *Canadian Journal of Fisheries and Aquatic Science*, 51: 1281–1296.

Bostrom, B., Persson, G. and Broberg, B. 1988, 'Bioavailability of different phosphorus forms in freshwater systems', *Hydrobiologia*, 170: 133–155.

Chiswell, R.K., Jones, G.B. and Brodie, J. 1997, 'Investigations of the speciation of phosphorus in coastal and estuarine waters of the Great Barrrier Reef, using iron strips and colorimetry', *Marine and Freshwater Research*, 48: 287–293.

Davison, W. and Zhang, H. 1994, 'In situ speciation measurements of trace components in natural waters using thin film gels', *Nature*, 367: 546–548.

Elser, J.J., Marzolf, E.R. and Goldman, C.R. 1990, 'Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments', *Canadian Journal of Fisheries and Aquatic Sciences*, 47: 1468–1477.

Finlayson, B.L. and McMahon, T.A. 1988, 'Australia vs the rest of the world: A comparative analysis of stream flow characteristics', in R.F. Warner (ed) *Fluvial Geomorphology of Australia*, Academic Press, Sydney, pp. 17–39.

Fink, M. and Oliver, R.L. 1998, 'Two techniques for assessing nutrient limitations in phytoplankton, nutrient-induced transients in chlorophyll-a fluorescence and algal growth bioassays, both indicate nitrogen limitation in waters of south-east Australia', *Journal of Phycology*, (submitted).

Froelich, P.N. 1988, 'Kinetic control of dissolved phosphate in natural rivers and estuaries: A primer on the phosphate buffer mechanism', *Limnology and Oceanograhy*, 33: 649–668.

Hamilton, D.P. and Schadlow, S.G. 1997, 'Prediction of water quality in lakes and reservoirs. Part I: Model description', *Ecological Modelling*, 96: 91–110.

Harris, G.P. 1994, Nutrient loadings and algal blooms in Australian waters – A discussion paper, LWRRDC Occasional Paper No 12/94, Canberra.

Harris, G.P. 1996, Catchments and Aquatic Ecosystems: Nutrient ratios, flow regulation and ecosystem impacts in rivers like the Hawkesbury–Nepean, CRC for Freshwater Ecology Discussion Paper, CSIRO Project Office and CRC for Freshwater Ecology.

Hart, B.T., McKelvie, I.D., Shalders, R. and Grace, M. 1995, *Measurement of bioavailable phosphorus concentrations in natural waters*, Water Studies Centre and CRC for Freshwater Ecology, Monash University, Melbourne.

Healey, F.P. and Hendzel, L.L. 1980, 'Physiological indicators of nutrient deficiency in lake phytoplankton', *Canadian Journal of Fisheries and Aquatic Science*, 37: 442–453.

Hecky, R.E. and Kilham, P. 1988, 'Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment, *Limnology and Oceanography*, 33: 796–822.

Hecky, R.E., Campbell, P. and Hendzel, L.L. 1993, 'The stoichiometry of carbon, nitrogen and phosphorus in particulate matter of lakes and oceans, *Limnology and Oceanography*, 38: 709–724.

Horne, A.J. and Commins, M.L. 1987, 'Macronutrient controls on nitrogen fixation in planktonic cyanobacterial populations', *New Zealand Journal of Marine and Freshwater Research*, 21: 413–423.

McKelvie, I.D., Peat, D.M.W. and Worsfold, P.J. 1995, 'Techniques for the quantification and speciation of phosphorus in natural waters', *Analytical Procedure*, 32: 437–445.

Miller, W.E., Greene, J.C. and Shiroyama, T. 1978, The Selenastrum Capricornutum Printz Algal Assay Bottle Test: Experimental design, application and data interpretation protocol, No. EPA-600/9-78-018. USEPA, Corvalis, Oregon.

Mitchell, A. 1997, The effects of hydrological regime on the potential for bacterially-mediated phosphorus release from freshwater sediments, Bachelor of Applied Science (Honours) Thesis, Charles Sturt University.

Oliver, R.L. 1993, Phosphorus Dynamics and Bioavailability in Turbid Waters, LWRRDC Occasional Paper No. 03/93, Canberra.

Oliver, R.L., Hart, B.T., Douglas, G.B. and Beckett, R. 1993, 'Phosphorus speciation in the Murray and Darling Rivers', *Chemistry Australia*, 60(8): 392–397.

Pick, F.R. and Lean, D.R.S. 1987, 'The role of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes', *New Zealand Journal of Marine and Freshwater Research*, 21: 425–434.

Reynolds, C.S. 1992, 'Eutrophication and the management of planktonic algae: What Vollenweider couldn't tell us', In D.W. Sutcliffe and J.G. Jones (eds) *Eutrophication: Research and application to water supply*, Freshwater Biological Association, Amblefied, United Kingdom, pp. 4–29.

Sas, H. 1989, 'Lake restoration by reduction of nutrient loading: Expectations, experiences, extrapolations', Academia Verlag Richarz, St Augustin, p. 497.

Schindler, D.W. 1977, 'Evolution of phosphorus limitation in lakes', *Science*, 195: 260–262.

Sharpley, A.N. 1993, 'An innovative approach to estimating bioavailable phosphorus in agricultural run-off using iron-oxide impregnated paper', *Journal of Environmental Quality*, 22: 597–601.

Sherman, B.S., Jones, G.J. and Webster, I. 1994, Flow, Stratification and the Growth of Algae in the Maude Weir Pool on the Murrumbidgee River. Proceedings of the AWWA Conference on Environmental Flows, Canberra, August 1994.

Smith, V.H. 1983, 'Low nitrogen to phosphorus ratios favour dominance by blue-green algae in lake phytoplankton', *Science*, 221: 669–671.

Stockner, J.G. and Shortreed, K.S. 1988, 'Response of Anabaena and Synechococcus to manipulation of nitrogen: Phosphorus ratios in a lake fertilization experiment', *Limnology and Oceanography*, 33: 1348–1361.

Tilman, D., Kiesling, R., Sterner, R., Kilham, S.S. and Johnson, F.A. 1986, 'Green, bluegreen and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen', *Archiv Fuer Hydrobiologie*, 106: 473–485.

Vincent, W.F. 1981, 'Rapid physiological assays for nutrient demand by the plankton: II. Phosphorus', *Journal of Plankton Research*, 3: 699–710.

Vollenweider, R.A. 1968, Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication, Organization for Economic Cooperation and Development, Paris, Technical Report DAS/SCI/68.27: 182P.

Wood, M.D. and Oliver, R.L. 1995, 'Fluorescence transients in response to nutrient enrichment of nitrogen and phosphorus limited *Microcystis aeruginosa* cultures and natural phytoplankton populations: A measure of nutrient limitation', *Australian Journal of Plant Physiology*, 22: 331–340.

Zhang, H. and Davison, W. 1995, 'Performance characteristics of DGT (diffusion gradients in thin films) for the in situ measurement of trace metals in aqueous solutions', *Analytical Chemistry*, 67: 3391–3400.

Appendices

Appendix 1: Addresses of participants

Professor Dominic Cheng
Department of Environmental Biology and Horticulture
University of Technology
PO Box 123
SYDNEY NSW 2007

Dr Richard Davis
CSIRO Land and Water
PO Box 1666
CANBERRA CITY ACT 2601

Mr Pat Feehan
Goulburn–Broken Water Quality Coordinator
Goulburn Murray Water
PO Box 165
TATURA VIC 3616

Dr George Ganf
Department of Botany
University of Adelaide
ADELAIDE SA 5000

Dr David Hamilton
Centre for Water Research
University of Western Australia
NEDLANDS WA 6907

Professor Barry Hart
Water Studies Centre and CRC Freshwater Ecology
Monash University
PO Box 197
CAULFIELD EAST VIC 3145

Dr Stuart Helliwell School of Science and Technology
Charles Sturt University
PO Box 588
WAGGA WAGGA NSW 2678

Dr Klaus Koop
Environment Protection Authority
Locked Bag 1502
BANKSTOWN NSW 2200

Mr Ian Lawrence
CRC Freshwater Ecology
University of Canberra
PO Box 1
BELCONNEN ACT 2616

Dr Rod Oliver
Murray–Darling Freshwater Research Centre
and CRC Freshwater Ecology
PO Box 921
ALBURY NSW 2640

Dr Wojciech Poplawski
Principal Engineer
Resource Sciences Centre
Department of Natural Resources
80 Meiers Road
INDOOROOPILLY QLD 4068

Ms Anna Porter
Department of Land and Water Conservation
PO Box 550
TAMWORTH NSW 2340

Professor Alistar Robertson
School of Science and Technology
Charles Sturt University
PO Box 588
WAGGA WAGGA NSW 2678

Dr Ian Smalls
Principal Scientist
Department of Land and Water Conservation
PO Box 3720
PARRAMATTA NSW 2124

Dr Peter Thompson
Department of Aquaculture
University of Tasmania
LAUNCESTON TAS 7250

Appendix 2: List of papers presented at the Limiting Nutrient Workshop – held at Charles Sturt University on 27 and 28 November 1997

Professor Dominic Cheng – Solving the Blue-Green Algae Problem Through Reduction of Internal and External Nutrient Loading: A success story.

Mr Pat Feehan – Factors Influencing Algal Growth: A management perspective.

Dr George Ganf – Conditions which Limit Algal and Cyanobacterial Growth in Freshwater.

Dr David Hamilton – Chemical Factors Affecting Phytoplankton Growth.

Professor Barry Hart – Measurement of Bioavailable Phosphorus Concentrations in Natural Water.

Dr Klaus Koop – Nitrogen and Phosphorus Limitation of Phytoplankton and the Role of Light on Production.

Mr Ian Lawrence – P Limitation of Sediment Redox Processes.

Dr Rod Oliver – Identifying Light and Nutrient Limitation of Phytoplankton Growth.

Dr Wojciech Poplawski – Nutrient Monitoring Requirements.

Ms Anna Porter – Nutrient Management in the Namoi Catchment.

Dr Peter Thompson – Nutrient Limitation of Phytoplankton: The case of the Swan River, Western Australia. Nutrient Limitation in Periphyton: The case of the Thompson River, Western Canada.

For further information regarding any of the above papers, please contact the author directly at the addresses in Appendix 1 on page 14.

Appendix 3: Techniques for analysis of nutrient limitation

Chemical: Dissolved

A large number of chemical methods exist for estimating the concentration of various forms of phosphorus in natural waters (McKelvie et al., 1995). These can be broadly classified on the basis of size separation (filtered or unfiltered) and whether any chemical treatment is involved. The two common methods are total phosphorus (TP – unfiltered sample, chemical digestion, analysis of liberated orthophosphate) and filterable reactive phosphorus (FRP – filtered sample, no digestion, analysis using acidic molybdate). If the filtered solution is also digested and then analysed to give a measure of the filterable total phosphorus (FTP), an estimate is provided of the phosphorus associated with colloidal matter or in filterable forms not reactive to the acidic molybdate solution used to detect orthophosphate.

There has been much debate over the most appropriate filter size to use in assessing the 'dissolved' or readily bioavailable phosphorus concentration. The usual reason for filtering a water sample is to provide an estimate of the dissolved nutrient that is immediately available to the phytoplankton, and in particular to estimate the concentration of orthophosphate. The value of this measurement is that both the phosphorus uptake rate and the phosphorus regulated growth rate of phytoplankton cells are a function of the orthophosphate concentration.

Conventionally, 'dissolved' phosphorus has been determined using 0.45 µm filters. However, data collected from the Darling River at Bourke showed that the FRP concentration passing through a 0.45 µm filter was always greater than that passing through a 0.003 µm filter (closer to the 'true' dissolved fraction), by as much as 40 µg per litre (or 60–70%). In the Ovens, Murray and Goulburn Rivers, estimates of the orthophosphate concentration obtained using 0.003 µm filters have often been significantly reduced below estimates made using 0.45 µm filters, sometimes by as much as 95%, with the improved filterable reactive phosphate measurements giving concentrations less than 5 µg P/L when the standard technique indicated concentrations in excess of 10 µg P/L.

The lower concentrations suggest that resource competition could be a reality in these waters and that the success of particular phytoplankton species may rely on their ability to capture and utilise phosphorus, a possibility not apparent from the standard 0.45 mm measurements.

Ideally, the 0.003 µm filtered solution should be used to estimate dissolved nutrient concentrations. At present there are difficulties in application, but improvements in technology should overcome these.

A practical compromise in some situations is to use 0.2 µm filters. McKelvie et al. (1997) found almost no difference between the 0.2 µm and 0.003 µm FRP concentrations in 14 different natural water samples, suggesting that most of the colloidal matter in these samples was in the 0.2–0.45 µm fraction.

Chemical: Particle bound

Considerable amounts of phosphorus can be associated with particulate and colloidal matter present in most natural water samples. Some of this is easily exchanged or desorbed, and failure to measure this easily exchangeable particle-bound fraction may lead to a serious underestimate of the actual 'readily bioavailable' phosphorus pool, especially in water columns with high suspended particulate matter concentrations.

Attempts to measure the entire amount of phosphorus in the water column that is available for phytoplankton growth, including both dissolved orthophosphate and the exchangeable phosphorus bound to particles, often involve complex chemical extraction procedures that are poorly linked to phytoplankton availability (Bostrom et al., 1988).

Iron strip

A better approach is the iron-strip desorption method. This uses strips of filter paper coated with iron oxyhydroxide to rapidly adsorb the dissolved phosphorus from solution, resulting in the release of exchangeable phosphorus from particles. This phosphorus buffers the falling solution concentration. Adsorption of dissolved phosphorus by the iron-coated filter strips continues until all the particle-associated exchangeable phosphorus is removed into solution and adsorbed to the iron filter-paper strips (Oliver, 1993; Oliver et al., 1993; Sharpley, 1993). The standard incubation lasts 24 hours.

The desorbable phosphorus (DP) measured using the iron-strip technique shows a one to one

correspondence with the phosphorus available to support phytoplankton growth estimated using quantitative growth bioassays (Oliver, 1993), that is, bioavailable phosphorus.

A variable proportion of the particle-associated phosphorus is desorbed by the iron strips. In the Murray–Darling Basin, the desorbable ('available') phosphorus can comprise between 0% and 80% of the total phosphorus concentration, with the unavailable fraction remaining associated with suspended inorganic particles. A conclusion from these measurements is that the same TP concentration can support different phytoplankton biomass concentrations.

Isotope exchange

Isotope exchange using radioactive phosphorus (eg. ^{33}P) provides information on the readily exchangeable particle-associated phosphorus concentration. However, this technique is unlikely to become generally available.

Gel probes

Gel probes are a promising new technique that should provide similar information to the iron-strip technique for phosphorus, but can also be extended to other nutrients. The technique is based on a simple device that accumulates ions on a binding agent (ion exchange resin or iron oxyhydroxide) after passage through a hydrogel which acts as a well defined diffusion layer (Davison and Zhang, 1994; Zhang and Davison, 1995). The method relies on the establishment of a steady state concentration from the solution to the binding agent. The solution concentration is then calculated using the mass of solute accumulated in the binding agent after a known deployment time, and the diffusion characteristics of the gel.

The gel probes should be less vulnerable to humic substances and particulate matter than iron strips, but this needs to be proven.

Biological

Algal growth bioassays

The most widely used method is the algal growth bioassay, where the response of algal biomass to nutrient enrichment with nitrogen, phosphorus or nitrogen and phosphorus are compared with unenriched samples. The growth of cultures is monitored over 7 to 14 days by measurement of parameters such as cell numbers, biomass or chlorophyll-a concentration (Miller et al., 1978; Elser et al., 1990). The results enable identification of the nutrient (nitrogen or phosphorus) in shortest supply in the isolated sample.

In some cases, the sample is filtered and re-inoculated with natural or test algae. This removes particles that may supply nutrients and is not recommended for estimating total bioavailable nutrients.

Algal bioassays are time-consuming, labour-intensive and subject to considerable variability. Additionally, they may not accurately estimate the in situ growth potential, because the sample is cut off from sources of nutrient resupply, such as sediments and particulate matter.

Physiological assays

NIFT

A new technique that can provide information on nitrogen and phosphorus limitation of phytoplankton is the nutrient induced fluorescence transient (NIFT) assay.

The NIFT assay is based on the occurrence of transient fluctuations in chlorophyll-a fluorescence caused by the addition of the limiting nutrient to a sample containing either nitrogen-limited or phosphorus-limited phytoplankton. The method has been tested in culture and in growth bioassays and applied to field samples (Fink and Oliver, submitted). Algal growth bioassays confirmed the results of NIFT assays when both were used to test natural water samples. It is likely that within 12 months this technique will have been introduced to Australian water agencies as a monitoring tool.

Flow-cytometry/fluorogenic probes

Flow cytometry provides the ability to analyse individual algal cells. When used with fluorogenic probes, the nutrient status of cells can be assessed.

Potentially bioavailable nutrients in sediments

There are a number of methods available for assessing the potentially available phosphorus concentration in sediments, for example, nutrient uptake-release experiments using sediment slurries and sediment cores (Mitchell, 1997), measuring nutrient fluxes in benthic chambers, and using process-based computer models (Hamilton and Schadlow, 1997).

These are active areas of research by several organisations and techniques are still being developed.