MD006025 – Synthesis of Blue Green Algae (Cyanobacteria) bloom knowledge & analysis of recent trends in the MDB

Report prepared for Murray-Darling Basin Authority

April 2023

ENQUIRIES

Name: Aleicia Holland

Title: Dr

La Trobe University

Victoria 3086

T +61 2 6024 9874

E a.holland2@latrobe.edu.au

Document history and status

VERSION	DATE ISSUED	REVIEWED BY	APPROVED BY	REVISION TYPE
Draft	06/04/2023	Aleicia Holland	Ewen Silvester	
Final	11/05/2023	Aleicia Holland	Ewen Silvester	

Distribution of copies

VERSION	QUANTITY	ISSUED TO
Final Report	1	Mike Ridley
Final communication materials	1	Mike Ridley

Report citation

Aleicia Holland, Caitlin Gionfriddo, Luke McPhan, Sam Lewis, Michael Shackleton, Ewen Silvester (2023) Synthesis of Blue Green Algae (Cyanobacteria) bloom knowledge and analysis of recent trends in the Murray Darling Basin. La Trobe Biogeochemistry and Ecotoxicology Group Publication No. 1

Traditional Owner acknowledgement

La Trobe University proudly acknowledges the Traditional Owners and Custodians of the Country. We pay our respects to the Elders past, present and emerging and respect their cultural heritage, beliefs and relationship with the land, waters and community.

Table of Contents

TABLE OF CONTENTS	3
LIST OF FIGURES	5
EXECUTIVE SUMMARY	8
GLOSSARY	10
BACKGROUND	12
LITERATURE REVIEW	13
Introduction	13
Environmental drivers of bloom formation	14
Known toxins of Cyanobacteria commonly found within the MDB	18
Emerging toxins and toxin producers	22
Environment drivers of toxin production	24
Risk of benthic cyanobacteria, not just pelagic cyanobacteria	26
Cyanobacteria and the Murray Darling Basin	28
Emerging techniques to monitor BGA blooms	29
Current management options and limitations	31
METHODS	33
Data sources	33
MDBA Provided data	33
Victoria: Goulburn-Murray Data	35
New South Wales Data	36
Queensland Data	37
Water Quality	37
Trend analysis	37
Data interpolation	39
Seasonal and/or autocorrelative de-trending	39
Data imputation	39
Generalised additive models	39
Generalised linear modelling	40
Community modeling of water quality and blue-green algae	41
RESULTS AND DISCUSSION	42
TRENDS IN CYANOBACTERIA	42
River Murray (MDBA Dataset)	42
Queensland sites	55

Victorian sites	57
New South Wales sites	60
CHANGES IN CELL COUNTS OF KEY CYANOBACTERIA OVER TIME	71
River Murray	71
Queensland sites	71
Victorian sites	72
DRIVERS OF CYANOBACTERIA IN RIVER MURRAY SITES	79
Key findings	83
Comments on sample collection, methods and identification	86
Comments on data analysis and modelling used in this study	86
Key Recommendations	87
OVERALL CONCLUSIONS	89
REFERENCES	90

List of Figures

Figure 1. Map of 14 sites included in the algal dataset provided by MDBA. Note Goolwa, Milang and Capels were removed for trend analysis due to discontinuation of monitoring. 34
Figure 2. Map of all sites included in the algal dataset for the River Murray provided by MDBA (light blue), Queensland sites provided by QLD Department Environment and Science/Sunwater (green), New South Wales sites provided by WaterNSW (yellow) and Victorian Sites provided by Goulburn Murray Water (dark blue). 36
Figure 3. Example of generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in cell counts of total toxin producing Cyanobacteria (Heywoods site). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 40
Figure 4. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in MDB River Murray Sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (1979 to 2019) and in context with the Millennium Drought (1997 to 2009). Sites are ordered from upstream to downstream on y-axis. Error bars are 1 standard deviation of the mean effect size. 43
Figure 5. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Heywoods, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 44
Figure 6. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Yarrawonga, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 45
Figure 7. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Torrumbarry, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 46
Figure 8. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Swan Hill, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 47
Figure 9. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Balranald, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 48
Figure 10. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Euston Weir, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 49

Figure 11. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Merbein, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 50
Figure 12. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Burtundy, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 51
Figure 13. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Lock 9, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 52
Figure 14. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Morgans, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 53
Figure 15. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally detrended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Tailem Bend, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data. 54
Figure 16. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Queensland sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (1979 to 2019) and in context with the Millennium Drought (1997 to 2009). Sites are ordered from upstream to downstream on y-axis. Error bars are 1 standard deviation of the mean effect size. 56
Figure 17. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Victorian sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (1979 to 2019) and in context with the Millennium Drought (1997 to 2009). Sites are ordered from upstream to downstream on y-axis. Error bars are 1 standard deviation of the mean effect size. 59
Figure 18. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Carcoar and Wyangala sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 61
Figure 19. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Macquarie River sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 62
Figure 20. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Keepit, Split Rock and Chaffey Dam sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 63
Figure 21. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Lake Inverell, Pindari and Copeton Dam sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 64
Figure 22. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Edward-Wakool sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 65

Figure 23. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Murrumbidgee sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 66
Figure 24. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Darling River sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 67
Figure 25. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in River Murray sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 68
Figure 26. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Menindee sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 69
Figure 27. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL-1) and (B) biomass (mm3 L-1) in Lachlan River sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size. 70
Figure 28. Linear trends in cell counts (cells mL-1) of the Chrysosporum and Dolichospermum. These bar graphs show the linear trends in cell counts per year for MDBA River Murray monitoring sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (1979 to 2019) and in context with the Millennium Drought (1997 to 2009). Error bars are 1 standard deviation of the mean effect size. 73
Figure 29. Linear trends in cell counts (cells mL-1) of the Raphidiopsis (Cylindrospermopsis) and Microcystis. These bar graphs show the linear trends in cell counts per year for MDBA River Murray monitoring sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (1979 to 2019) and in context with the Millennium Drought (1997 to 2009). Error bars are 1 standard deviation of the mean effect size. 74
Figure 30. Linear trends in in cell counts (cells mL-1) of Chrysosporum and Dolichospermum in Queensland Murray-Darling Basin monitoring sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (2000 to 2022) and in context with the Millennium Drought (2000 to 2009). Error bars are 1 standard deviation of the mean effect size. 75
Figure 31. Linear trends in cell counts (cells mL-1) of the Raphidiopsis (Cylindrospermopsis) and Microcystis in Queensland Murray-Darling Basin monitoring sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (2000 to 2022) and in context with the Millennium Drought (2000 to 2009). Error bars are 1 standard deviation of the mean effect size. 76
Figure 32. Linear trends in in cell counts (cells mL-1) of Chrysosporum and Dolichospermum in Victorian Murray-Darling Basin monitoring sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (2004 to 2022) and in context with the Millennium Drought (2004 to 2009). Error bars are 1 standard deviation of the mean effect size. 77
Figure 33. Linear trends in cell counts (cells mL-1) of the Raphidiopsis (Cylindrospermopsis) and Microcystis in Victorian Murray-Darling Basin monitoring sites. Colours of the bars indicate time period when linear trends were estimated for the entire period of the dataset (2004 to 2022) and in context with the Millennium Drought (2004 to 2009). Error bars are 1 standard deviation of the mean effect size. 78
Figure 34. Median taxa responses to environmental covariates. Median response value is shown as a point with 95% Highest posterior density limits. The responses coloured in red represent significantly negative responses of the associated taxa/parameter combination, while those in blue represent significantly positive responses and those in grey had HPD intervals that overlapped 0 indicating a non-significant effect of the parameter on taxa abundances. Note Raphidiopsis is reported as Cylindrospermopsis here. 81
Figure 35. Environmental Co-occurance of taxa within the community. Co-occurance represents similar responses to environmental factors between species. Significant responses that had an absolute correlation >0.7 are presented here with the darker blues or reds representing stronger positive or negative correlations respectively (shown in scale bar on right hand side of plot). 82

EXECUTIVE SUMMARY

Highlights and Recommendations

Data from five datasets: River Murray (Murray-Darling Basin Authority dataset), Queensland (Sunwater dataset), New South Wales (WaterNSW dataset) and Victoria (two datasets provided by Goulburn-Murray Water) were analysed for long-term changes in cyanobacteria.

Trends in cyanobacteria across the Basin as a whole did not show overall increasing or decreasing trends or follow the same patterns.

Cyanobacteria abundance (cells mL^{-1}) and biomass (mm^3 L^{-1}) within the River Murray sites (MDBA dataset) were shown to be increasing over time at all sites since 1979, with greatest increases recorded since 1997, with Balranald upstream, Merbein, Burtundy, Euston Weir recording the greatest increase over the last 12 years, while Heywoods, Torrumbarry, Lock 9, Morgan, and Tailem Bend recorded highest counts during the Millennium Drought.

Victorian sites in contrast to the River Murray sites generally showed decreases in cyanobacteria cell counts (cells mL^{-1}) over the 2004-2022 period analysed, this being the full extent of this dataset. During the Millennium drought, abundance was significantly increased at most Victorian sites.

Trends in cyanobacteria within Queensland and New South Wales datasets did not show clear patterns, with trends in abundance that were site, river, or catchment specific.

Biomass generally followed a similar pattern to cell counts (abundance), however in some cases differed to trends shown for cell counts reflecting a shift in algal dominance.

Biomass in Victorian sites showed contrasting results depending on the dataset analysed and period selected. It is recommended the dataset spanning the greatest number of years be used to determine overall trends. Biomass data from 1998-2019 showed an overall increase in cyanobacterial biomass (mm^3 L^{-1}) at all but two sites (Newlyn Reservoir and Tullaroop Reservoir).

Changes in the dominant cyanobacteria genera of interest in the River Murray also occurred with *Chrysosporum* found to be increasing with highest counts recorded during 2010-2022 and *Microcystis, Dolichospermum* and *Raphidiopsis* showing site and period specific trends.

Cyanobacteria in general were reported to respond more positively to increases in total Kjeldahl Nitrogen (TKN) followed by temperature. However, responses were genera specific with some positively responding and some negatively responding to the same variable.

In regard to the four main potentially toxic cyanobacteria of interest (*Microcystis, Dolichospermum, Raphidiopsis* and *Chyrsosporum)*, all taxa varied in their responses to environmental variables. Increased abundances of *Chrysosporum* were associated with decreases in dissolved organic carbon (DOC), TKN, Soluble reactive phosphorous (SRP) and temperature. Decreases in silica (Si) and temperature were associated with higher abundances of *Microcystis*. Conversely, *Raphidiopsis* responded to increases in DOC and nitrogen oxide (NO_x) concentrations and was negatively associated with TKN and SRP. *Dolichospermum* responded to increased DOC and TKN.

The following are recommended:

- A consistent methodology for sample collection, preservation, identification, and monitoring frequency be developed for deployment across the Basin to assist in future analyses and to enhance the general utility of the datasets

- Algal monitoring be maintained at the River Murray sites and sites currently frequently monitored within the other state datasets, with the inclusion of other parameters. Specifically, we recommend that data on water quality and nutrients be collected at the same time as algal samples to improve ability to determine linkages between key parameters and increases in cyanobacteria to determine environmental drivers of blooms.

- Samples be collected to determine algal toxins, as cyanobacteria abundance and biomass is not always reflective of risk, and different toxins have different levels of risk

- The MDBA investigate new emerging techniques that will allow for monitoring of blooms in real time, and key environmental drivers such as remote sensing or deployment of analytical in-situ equipment to count algal cells, identify key taxa such as the FlowCam and measure key drivers such as nutrients

- The MDBA continue to reduce nutrient loads in the Basin and explore cost effective environmentally friendly ways to minimise bloom formation that also provide additional benefits to aquatic biota such as floating macrophyte beds or riparian planting.

- Further studies be conducted into:

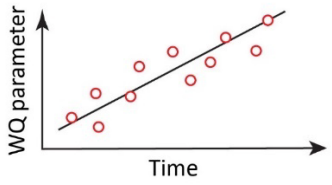
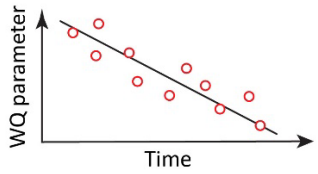
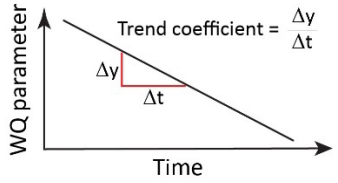
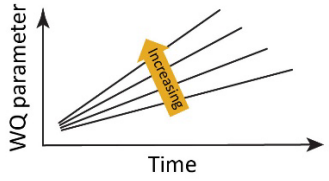
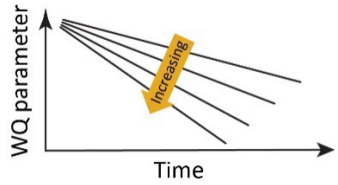
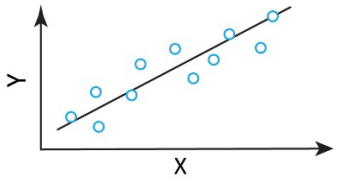
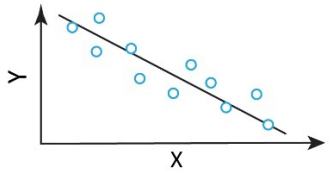
(i) the drivers of blooms in the River Murray especially regarding TKN and dissolved organic nitrogen its major constituent, temperature, and silica by undertaking fieldwork that combines both analysis of algal abundance and environmental drivers supported by appropriate laboratory experiments

(ii) succession of cyanobacteria species across the Basin

(iii) risks that dominant taxa pose to human, livestock, and aquatic organism health through measurement of algal toxins and/or use of molecular approaches to determine whether taxa contain genes for toxin production

(iv) combined with laboratory bioassays into key drivers of toxin production and toxicity.

Glossary

STATISTICAL ANALYSIS TERMINOLOGY	
Increasing trend	
Decreasing trend	
Trend coefficient / effect size (interchangeable)	
Increasing (positive) effect size	
Increasing (negative) effect size	
Positive dependence	
Negative dependence	
GLM	**Generalised Linear Model:** A linear regression which accounts for the fact that the variance of

	each measurement is dependent on its predicted value
GAM	**Generalised Additive Model:** A GLM with a smoothing function that allows for multiple changes in the direction and/or effect size of trends within that regression
Bayesian Ordination and Regression Analysis for community modelling	Analysis of multivariate abundance data, with estimation performed using Bayesian Markov chain Monte Carlo methods using the BORAL package in R. A key feature of the BORAL package is the ability to incorporate latent variables as a parsimonious method of modelling between species correlation.

BACKGROUND

Although cyanobacteria (commonly referred to as blue-green algae (BGA)) are a natural part of most aquatic environments in the Basin, communities across the Murray-Darling Basin (MDB) are becoming increasingly concerned that BGA blooms are becoming more common, lasting longer, and becoming more severe, and communities perceive more frequent recreational use alerts and restrictions. Communities are asking what can be done to better manage BGA blooms, is the water safe to drink, is the river safe to swim in, and is the problem getting worse. Harmful algal blooms have been a regular occurrence in the River Murray in recent years with extensive blooms in 2009, 2010, 2016 and most recently in 2021. The 2016 bloom persisted from mid-Feb to early Jun. At its greatest extent in April and May, it extended from Lake Hume to Lock 8 and throughout the Edward, Wakool and Niemur River distributary system, a combined river length of ~2,360 km. Algal blooms can affect the colour and taste of water, as well as how safe it is to drink. Some BGA species produce toxins that are dangerous to humans and animals. This can potentially mean water affected by an algal bloom is: not safe for humans to drink, or for recreational activities such as swimming and boating; can poison wildlife, livestock and domestic animals, and; is difficult and expensive to treat to make it safe for drinking. This has serious consequences for Basin communities and farmers, as well as the tourism industry.

Algal blooms can also contribute to fish deaths and reduce environmental outcomes. Like plants, cyanobacteria do not photosynthesise at night. Instead, they use oxygen in a process called respiration. When large numbers of cyanobacteria respire or blooms breakdown, this removes oxygen out of the water, there may not be enough left for fish and other aquatic life to breathe. Improved understanding of the factors that promote drivers of cyanobacteria blooms and assessment of trends over time is thus needed to understand the threat cyanobacteria pose in an ever-changing world. This report therefore aims to provide a summary and analysis of BGA trends over time within the MDB by collating, analysing and synthesising data collected on algal abundance and biomass across the Basin; determining trends over time and whether BGA blooms are becoming more frequent and severe and exploring linkages with key water quality parameters. This report will also provide a literature review on environmental drivers of blooms and toxin production, emerging technologies and current management options as well as recommendations around the future monitoring of BGA and associated water quality (WQ) parameters of interest.

LITERATURE REVIEW

INTRODUCTION

Algal blooms occur when algal species grow rapidly and often results in formation of scum or discolouration of waterways. Blooms cause major problems for water quality, including oxygen depletion, turbidity and clogging of waterways, and the production of multiple toxic compounds (Steffensen 2008). Cyanobacterial blooms which are also commonly referred to as blue green algal (BGA) blooms are common in freshwaters are increasing in frequency and severity worldwide (Huisman et al. 2018). Cyanobacterial blooms in lakes and reservoirs have been promoted by the interactive effects of several anthropogenic processes, such as eutrophication of river catchments, increasing storage of fresh water in reservoir systems, and rising levels of greenhouse gases in the atmosphere. Human alteration of freshwater systems can also affect the occurrence of blooms in riverine environments, such as when cyanobacteria-rich reservoir water was discharged into slow-flowing areas of the highly regulated River Murray, south-eastern Australia, in 2009, 2010 and 2016 (Bowling et al. 2013, Crawford et al. 2017). The risk of blooms is thus particularly great in regulated river systems such as the rivers within the Murray-Darling Basin, where damming, water abstraction, and flow regulation has transformed previously variable, lotic reaches into chains of relatively stable reservoirs connected by more constant sections of lower flow. Future climate change is expected to exacerbate these conditions, resulting in longer periods of warmer temperatures and stratified water column conditions undisturbed by mixing flows. Understanding the dynamics of such blooms and how the multiple drivers of bloom formation, duration, extent, toxin production and toxicity interact under these changing climatic conditions will be key to their effective mitigation (Burford et al. 2020).

Algal blooms can be associated with one or multiple species of cyanobacteria and/or algae, with many blooms a composition of different genera, with changes in dominance of one genera over another temporally often due to abiotic factors (Zhang, Fan et al. 2021). Even blooms of the same species often include a mixture of toxic and non-toxic strains which are morphologically similar and do not differ significantly in their genotype (Kurmayer, Dittmann et al. 2002). The variation between the ratio of toxic to non-toxic strains and what strain dominates a bloom, like what species dominates a bloom, is suggested to be based on environmental conditions (Vézie, Rapala et al. 2002, Davis, Berry et al. 2009, Zhang, Fan et al. 2021). Cyanobacterial blooms within the MDB are generally caused by four main taxa *Dolichospermum circinale*, *Chrysosporum ovalisporum*, *Microcystis aeruginosa* and *Microcystis flos-aquae* all of which have the potential to produce toxins, although toxins differ between taxa. These

taxa differ in their abilities to fix nitrogen, with two of the taxa able to fix nitrogen (*D. circinale*, *C. ovalisporum*) from the atmosphere whereas the other two are not, thus different environmental drivers within the MDB may have contrasting effects on these four main taxa. To improve understanding of what factors might drive blooms within the MDB, we must first explore the effects of different environmental factors on bloom formation and toxin production.

ENVIRONMENTAL DRIVERS OF BLOOM FORMATION

In lakes and reservoirs, eutrophication of upstream rivers and groundwater with inorganic N and P has unequivocally led to an increased occurrence and extent of cyanobacterial blooms through release of nutrient limitation (Paerl and Otten 2013, Wells et al. 2015, Hamilton et al. 2016). While N and P inputs have a strong influence on bloom formation, however, different bloom-forming cyanobacterial taxa can be favoured under markedly different loads and stoichiometry (Carey et al. 2012). The most fundamental split is between taxa able to fix atmospheric N_2 (e.g., *Dolichospermum* spp.), which might predominate in systems where P is abundant relative to N (Wang et al. 2021b), and the non-N_2 fixing taxa (e.g., *Microcystis* spp.), which can proliferate when either P is limiting or when both nutrients are abundant (Gobler et al. 2016).

Adding to this complexity are interactions with other inorganic macro or micro-nutrient limitations. A key driver promoting cyanobacterial blooms over algae, which may be more effective competitors for free inorganic N and P, is either low silica (Si) loading relative to N and/or P or depletion of Si by other phytoplankton (Salmaso 2000). More specifically, diatoms generally have lower N and P requirements and higher affinities than cyanobacteria, but their growth rates are highly dependent on Si due to silicification of their cell walls (Wilhelm et al. 2006). In eutrophic systems, high loads of N and P can thus often lead to initial diatom blooms, with community composition rapidly switching through chlorophyte dominance to cyanobacteria as Si is depleted (Rocha et al. 2002). Cyanobacteria can also have higher micronutrient and trace metal requirements than algae; for example, bloom formation may often be co-limited by iron (Fe) availability (Molot et al. 2014, Facey et al. 2019). In this context, the strong dependence of cyanobacterial metabolism on Fe may have contributed to the evolution of toxin production, as certain toxins (e.g., microcystin) may play an additional biochemical role in Fe scavenging (Holland and Kinnear 2013). However, while the formation of cyanobacterial blooms is not contingent on an increase in any single inorganic nutrient, increasing nutrient loads in general are a strong predictor of bloom formation and severity (Huisman et al. 2018).

Recently the role of organic nutrients in influencing cyanobacterial blooms has been suggested (Reinl et al. 2022). Cyanobacteria are broadly described as photoautotrophs, but many taxa are clearly mixotrophic (i.e., able to use both autotrophic and heterotrophic metabolic pathways) (Matantseva and Skarlato 2013). Dissolved organic carbon (DOC) and nitrogen (DON) uptake is most well described in marine and estuarine cyanobacteria, which have been shown to directly utilise amino acids (Zubkov et al. 2003), carbohydrates (Benavides et al. 2017), and extracellular polymeric substances as a nutrient source (Stuart et al. 2016). Estuarine cyanobacteria have also been shown to use extracellular urease and alkaline phosphatase to obtain nutrients from DON and dissolved organic phosphorous (DOP), although other phytoplankton likely outcompete them for free NO_3 (Glibert et al. 2004). Freshwater cyanobacteria also directly utilise dissolved organic matter (DOM) from the environment, particularly smaller, less aromatic, more oxygenated, and nitrogen-rich molecules (Bai et al. 2017), and may be particularly good competitors for urea under favourable environmental conditions (Chaffin and Bridgeman 2014; Reinl et al. 2022). Further, cyanobacteria are able to strongly utilise dissolved organic C, N and P in wastewater (Vieira et al. 2012). Increasing DOM loads are therefore likely to increase the potential for freshwater cyanobacterial blooms to form and may form an additional nutrient source to inorganic nutrients.

As photoautotrophs, cyanobacteria are also constrained by the availability of inorganic carbon (carbon dioxide, $CO_{2;}$ and bicarbonate, HCO_3^-) (Shapiro 1997). Cyanobacterial productivity increases with concentrations of inorganic carbon, whether atmospheric or dissolved (Ibelings and Maberly 1998), and may thus be an important limiting nutrient in bloom development (Verspagen et al. 2014). Cyanobacterial blooms can rapidly deplete dissolved CO_2 concentrations, which also shifts the pH balance of the water column towards alkalinity and a predominance of HCO_3^- in the pH-driven dissolved inorganic carbon equilibrium, as well as an overall reduction in the availability of inorganic carbon (Wilhelm et al. 2020). Yet cyanobacteria have a range of HCO_3^- and CO_2-concentrating biochemical mechanisms that enable them to function under low inorganic carbon availability (Huisman et al. 2018). Different cyanobacterial strains also have a wide range of affinities for CO_2, as well as HCO_3^-; cyanobacterial community composition within blooms may thus shift rapidly in response to changing inorganic carbon availability and composition (Sandrini et al. 2016). Increases in water column pH may also increase atmospheric invasion of CO_2, particularly as atmospheric CO_2 concentrations continue to rise, leading to overall low concentrations but a high flux of dissolved CO_2 to cyanobacterial blooms (Kragh and Sand-Jensen 2018). Inorganic carbon availability is therefore unlikely to act as a threshold to cyanobacterial bloom formation. Instead, indirect effects of increasing

CO_2 concentrations, such as its role as a greenhouse gas (and thus driver of ambient temperature), may have larger effects on bloom occurrence and severity.

Increasing temperatures are likely a key factor in the occurrence of cyanobacterial blooms, but there are a number of indirect and direct effects of temperature that can act idiosyncratically on different bloom-forming taxa. Many bloom-forming cyanobacteria have higher temperature optima for growth than algae (Paerl et al. 2016), although this trend is dependent on the taxa being examined. For example, *Planktothrix* exhibit maximum reproductive rates at approx. 20 °C, similar to the general temperature optima for diatoms (Jöhnk et al. 2008), while *Microcystis* has a substantially higher maxima at approx. 28 °C (Reynolds 2006). However, many algal taxa might also increase their growth rates at higher temperatures (Lürling et al. 2013). Temperature might also be a strong driver in the rates of biochemical processes which might favour cyanobacterial blooms (e.g., toxin or enzyme production), particularly N_2-fixation (Carey et al. 2012). Yet these responses are, again, taxon-specific. For example, increasing temperatures can decrease toxin production by *Microcystis* (Peng et al. 2018). In addition, increasing temperatures affect several other key processes such as oxygen flux and respiration which can limit cyanobacterial growth and biochemistry (Staal et al. 2003). Temperature, in isolation, is thus often not directly associated with the frequency or severity of cyanobacterial blooms. Instead, the primary effects of increasing temperature might be increased thermal stability of stratified water column layers, earlier onset of stratification and lower water viscosity. Cyanobacteria often have high buoyancy due to gas vesicle production within their cells (Huisman et al. 2018), and stable stratification thus generates ideal conditions for cyanobacteria to float into the upper water layers where they can obtain greater access to light and nutrients (e.g. Ibelings and Maberly 1998). Buoyancy also aids cyanobacteria in competition for CO_2, as it allows them greater access to atmospheric rather than dissolved forms (Ibelings and Maberly 1998). The effect of increasing temperatures on bloom severity is often dependent on both the dominant bloom-forming taxa and the trophic state of the environment; for example, warming likely only increases the volume of blooms when inorganic nutrients are not limiting (Kosten et al. 2012, Rigosi et al. 2014). Regardless, warmer temperatures are, in general, associated with a higher proportion of cyanobacteria than algae in freshwater phytoplankton assemblages (Kosten et al. 2012).

Increasing temperatures are unable to result in thermal stratification in isolation, however. Promotion of cyanobacterial blooms through stratification is also dependent on low water column mixing rates, which are strongly driven by wind-driven turbulence of lakes and reservoirs (e.g., Bocaniov et al. 2014) and flows from – and within – river networks (e.g., Niemeyer et al. 2018). Mixing rates also usually

correlate with shorter water residence times in lakes and reservoirs, where greater riverine inflows are strongly associated with lower nutrient concentrations through regular flushing and reduced sedimentation rates, as well as less rapid depletion of key micronutrients such as Si (Brooks et al. 2014, Maavara et al. 2020). Rates of mixing within stratified layers can also constrain cyanobacterial blooms, as highly buoyant taxa may be unable to flocculate and cause light limitation under turbulent conditions (Huisman et al. 2004). Yet mixing is also not a universal constraint on cyanobacterial bloom formation. Short-term mixing after a period of stratification can draw anoxic, relatively nutrient-rich hypolimnetic water into shallower, nutrient-depleted upper layers (e.g., MacIntyre and Jellison 2001), thus releasing nutrient limitations on phytoplankton. For example, periodic wind-driven mixing during drawdown periods in the Lake Hume reservoir, south-eastern Australia, likely drives the expansion of otherwise nutrient-limited cyanobacterial blooms (Baldwin et al. 2008, Bowling et al. 2018). Even extended periods of mixing do not necessarily deplete cyanobacterial populations. Many taxa can persist as dormant resting cells (akinetes), or reduce their metabolic activity to remain as vegetative colonies, remaining on the benthos until environmental conditions favour returning to the water column (Cottingham et al. 2020). Benthic cyanobacterial colonies are also common as biofilms in shallow, photic zones, including the littoral areas of lakes and reservoirs (Quiblier et al. 2013). Increased mixing to control pelagic blooms may therefore not be a universal control to cyanobacterial blooms in regulated river networks, as increasing temperatures and frequency of low flows are also driving proliferation of these potentially toxic cyanobacterial mats in wetlands and flowing areas (Burford et al. 2020).

The ability of cyanobacteria to photosynthesise, and their adaptations towards increased buoyancy, suggests light availability as a strong co-limiting factor on bloom formation. Some cyanobacteria (e.g., *Microcystis*) produce extracellular metabolites which protect against UV radiation (Xu and Jiang 2013), and may therefore have a competitive advantage over algae under high light conditions (Stockenreiter et al. 2021). As with inorganic carbon availability, however, there exists marked variation among different cyanobacterial species in their affinity for light (Carey et al. 2012). In addition, many taxa are unable to acclimate quickly to high light intensities, and may become photo-inhibited at high light levels due to the necessity of protecting their cell mechanisms from damage by excess irradiance (Carey et al. 2012). Rather than being more efficient competitors for light *per se*, cyanobacteria may largely outcompete other phytoplankton for light due to their high buoyancy, high UV tolerance, and the dense biomass of cyanobacterial mats, which can result in substantial light limitation for photoautotrophs below the bloom (Kosten et al. 2012).

Ultimately, the wide variation in environmental preferences and biochemistry among different cyanobacterial taxa precludes the identification of any single factor as a universal control on bloom formation. For example, despite the driving role of increasing nutrient loads in more frequent cyanobacterial blooms worldwide (Paerl and Otten 2013), cyanobacterial blooms do still occur in low-nutrient systems (Reinl et al. 2021), such as the generally N and P co-limited freshwater environments of south-eastern Australia (Müller and Mitrovic 2015). In this context, a further factor in bloom dynamics are internal feedback mechanisms which can extend bloom durations and volume, often through rapid and widespread transitions between the dominant bloom-forming taxa. For example, N_2-fixing cyanobacteria are often replaced by non-diazotrophic taxa as N limitation eases (Facey et al. 2022). N_2-fixing taxa also increase internal nutrient loading over time through sedimentation, increasing the potential for future blooms through higher trophic states and the potential for respiration-driven, anoxic release of nutrients from the benthos (Cottingham et al. 2015). In particular, the ability of cyanobacteria to produce extracellular toxins creates a complex interplay between inhibition of other taxa and the role of some potential toxins as secondary metabolites (Holland and Kinnear 2013). Yet the environmental conditions generated by blooms (light attenuation, higher pH, lower dissolved O_2) can also act as limiting factors. For example, depletion of CO_2 by cyanobacterial blooms decreases the bioavailability of inorganic nutrients, such as P and trace metals, as they precipitate under higher pH. Consequently, while controlling for factors such as nutrient loads and stratification can aid in restricting cyanobacterial bloom formation, understanding the complexity of bloom community composition and its variable interactions with the physical and chemical environment is increasingly a priority for management (Mantzouki et al. 2016).

In conclusion not one environmental parameter has been shown to be the major driver of cyanobacterial blooms, with the majority of blooms caused by a combination of factors. The main parameters associated with cyanobacteria are inorganic and organic nutrients, temperature, stratification, micronutrients such as Si and Fe, inorganic carbon and light availability.

KNOWN TOXINS OF CYANOBACTERIA COMMONLY FOUND WITHIN THE MDB

As mentioned above cyanobacterial blooms within the MDB are generally caused by four main toxin producing taxa *Dolichospermum circinale*, *Chrysosporum ovalisporum*, *Microcystis aeruginosa* and *Microcystis flos-aquae*. These taxa can produce a suite of toxins including microcystins (MC), cylindrospermopsin (CYN), saxitoxins (STX) and anatoxins (ATX). Another species that has been reported for the MDB is *Nodularia spumigena* which also has the potential to produce the toxin Nodularin.

Microcystins (MC)

One of the most widespread cyanotoxins, microcystin (MC) is the most commonly identified toxin in Australia, produced by species like *Microcystis, Anabaena, Anabaenopsis, Aphanizomenon, Dolichospermum, Plantothrix, Pseudoanabaena* and *Nostoc.* MC comprise of more than 240 known variants each with a slightly different chemical structure, with more analogues still being discovered (Spoof and Catherine 2017). MC is a hepatotoxin and as such, the main site effected by MC is the liver (Campos and Vasconcelos 2010). As MC exists as cyclic heptapeptides they commonly target the inhibition of serine and threonine protein phosphatases disrupting a cell's homeostasis (Campos and Vasconcelos 2010). One of the main mechanisms of toxicity by MC is this inhibition of protein phosphatases causing cytotoxic and genotoxic effects along with the induction of oxidative stress (Campos and Vasconcelos 2010). The provisional guideline values (GV) for MC provided by the world health authority are 1 μg/L for chronic (lifetime) drinking water exposure, 12 μg/L for short-term drinking water exposure and 12 μg/L for recreational water exposure (World Health Organization 2020). The drinking water exposures were calculated using the average weight of 60 kg body weight of adults and the recreational water exposure was calculated using a 15 kg body weight average of a child (World Health Organization 2020). MC has shown to adversely impact human health, with the majority of reported incidents occurring in Europe, however, known cases have been reported in Australia. In 1981 a bloom of *M. aeruginosa* recorded in the Malpas Dam, Armidale was associated with increased liver damage.

MC can also have adverse effects on aquatic organisms with decreases in survival of fish reported with earlier developmental stages more affected (Palikova, Navratil et al. 2003). Eggs of the common carp (*Cyprinus carpio*), exposed to the crude extract of MC at 130 μg/L showed acute toxicity observed through embryo mortality, prolonged hatching, malformed and dead larvae (Palikova, Navratil et al. 2003). Lower concentrations of 13 μg/L caused toxicity after 8 days of long-term exposure and 1.3 μg/L caused toxicity after 30 days with increases in malformed and dead larvae reported (Palikova, Navratil et al. 2003). A New Zealand study by Clearwater, Wood et al. (2014) observed decreased survival of juvenile freshwater mussels (*Echyridella menziesii*) and crayfish (*Paranephrops planifrons*) after 4 days of exposure to environmentally realistic concentrations of MC extracts. The crayfish exhibited behavioural changes at >2,426 μg/L and the lethal concentration to cause 50% decrease in survival (LC50) was 11,146 μg/L suggesting MC to be a stressor in only severe MC blooms (Clearwater, Wood et al. 2014).

Cylindrospermopsin (CYN)

Cylindrospermopsin (CYN) was first identified in 1992 (Ohtani, Moore et al. 1992) and with 4 decades of research is the second most studied cyanotoxin (behind MC), in part due to its global distribution and bioaccumulation (Scarlett, Kim et al. 2020, Yang, Yu et al. 2021). CYN was first identified in a species of *Cylindrospermopsis*, which after the unification of the *Raphidiopsis* genera, was renamed to *R. raciborskii* (Aguilera, Gómez et al. 2018). Both toxic and non-toxic strains of this species exist and coexist together. Other genera that produce CYN include; *Anabaena* (Spoof, Berg et al. 2006), *Aphanizomenon* (Preußel, Stüken et al. 2006), *Dolichospermum* (Akçaalan, Köker et al. 2014), *Chyrosporum and Lyngba (Seifert, McGregor et al. 2007).* Unlike other cyanotoxins, CYN is classed as a cytotoxin as it elicits hepatoxic and nephrotoxic effects (Funari and Testai 2008). Additionally, CYN attacks other organs (Terao, Ohmori et al. 1994) and genotoxicity has also been reported (Humpage, Fontaine et al. 2005, Funari and Testai 2008). Primarily, CYN causes toxicity by inhibiting protein synthesis and inducing oxidative stress through interactions with cytochrome P450 (Humpage, Fontaine et al. 2005, Yang, Yu et al. 2021). The World Health Organization (2020) states the provisional guideline values (GV) for cyanotoxins in drinking water are 0.7 μg/L for chronic (lifetime) exposure, 3 μg/L for short-term exposure and 6 μg/L for recreational water exposure. This was calculated using the average weight of 60 kg body weight of adults and is based on toxicological data for CYN exposure that effects the kidneys (World Health Organization 2020). In 1979 a bloom of *R. raciborskii* cause 148 people on Palm Island to become ill with hepatoenteritis-like symptoms after drinking water containing CYN.

Exposure to aquatic organisms varies greatly. In the aquatic snail *Melanoides tuberculate*, CYN at >200 μg/L were toxic in hatchlings, but not adults (Kinnear, Duivenvoorden et al. 2007). Moreover, exposure to both intracellular and extracellular forms posed a greater risk than extracellular toxins alone (Kinnear, Duivenvoorden et al. 2007). As CYN acts as a protein synthesis inhibitor this suggests why in many species, developing organsims were susceptible to the toxin while the adults could remain unharmed (Kinnear, Duivenvoorden et al. 2007). In *Danio rerio* embryos, direct injection of CYN significantly caused mortality at an LC50 of 8.77 μg/L, but the water-soluble form of the toxin did not elicit the same effects (Berry, Gibbs et al. 2009, Scarlett, Kim et al. 2020). No consistent pattern in the developmental pathways were identified (Berry, Gibbs et al. 2009). Scarlett, Kim et al. (2020) summarizes the literature of the observed acute and chronic effects by CYN to aquatic organisms. However, a very limited number of studies analytically verified treatment levels or determine the CYN purity and suggest future work is imperative to determine toxicity limits of CYN to aquatic organisms and the mechanistic action (Scarlett, Kim et al. 2020).

Saxitoxins (STX) and Anatoxins (ATX)

Saxitoxins and Anatoxins are a group of neurotoxins and whilst studied heavily in marine environments, STX and ATX produced in freshwater has been connected to animal poisonings (Rutkowska, Płotka-Wasylka et al. 2019, Christensen and Khan 2020). STX and its derivatives produce neurotoxic alkaloids that cause paralytic shellfish poisoning more commonly associated with being produced by marine dinoflagellates. Cyanobacteria such as from the genera *Dolichospermum, Aphanizomenon, Chyrosporum and Lyngba* also produce the paralytic shellfish toxins in freshwater blooms (Wiese, D'Agostino et al. 2010). In Australian wastewater treatment plants, *Dolichospermum circinale* (previously *Anabaena circinalis)* was found to produce significant concentrations of paralytic shellfish poisons up to 17 μg/L (Hoeger, Shaw et al. 2004). However, after treatment, < 1 μg/L of the poison was detected in tap water (Hoeger, Shaw et al. 2004). As STX is very potent, no human cases of chronic exposure have been recorded (World Health Organization 2020). The acute toxicity GV are 3 μg/L based on a 5kg infant and the recreational limit GV is 30 μg/L based on a 15kg child (World Health Organization 2020). STXs disrupts the action potential of neurons by reversibly binding to the voltage-gated sodium channels (Aráoz, Molgó et al. 2010, Rutkowska, Płotka-Wasylka et al. 2019). By blocking the axonal conduction, it impacts the neural pathways required for muscular and neural function, ultimately leading to paralysis or death by suffocation (Aráoz, Molgó et al. 2010).

Anatoxins are another group of understudied neurotoxins in freshwater environments, made up of; anatoxin-a, homoanatoxin-a and anatoxin-a(s), however there has been a recent proposal to reclassify anatotoxin-a(s) to guanitoxin as it is structurally unrelated to the others (Rutkowska, Płotka-Wasylka et al. 2019, Fiore, de Lima et al. 2020). Like STX, anatoxin-a and homoanatoxin-a are neurotoxic alkaloids which target nicotine acetylcholine receptors (Rutkowska, Płotka-Wasylka et al. 2019). They are agonists but homoanatoxin-a is even more potent as it releases more acetylcholine into neuromuscular synapses with overstimulation leading to neuromuscular blockade (Aráoz, Molgó et al. 2010, Rutkowska, Płotka-Wasylka et al. 2019). Some genera that produce anatoxin-a include *Dolichospermum, Aphanizomenon, Chyrosporum, Cylindrospermum, Microcystis* and *Planktothrix* (Aráoz, Molgó et al. 2010). Synthesis of both anatoxin-a and homoanatoxin-a occurs in species of *Raphidiopsis* and *Oscillatoria* (Aráoz, Molgó et al. 2010). Homoanatoxin-a is produced by some species of *Dolichospermim* and *Phormidium* (Aráoz, Molgó et al. 2010). The World Health Organization (2020) states the short-term drinking water GV of ATX is 30 μg/L (based on 60kg adult) and 60 μg/L in recreational water for a 15kg child. There is no GV on long term effects of ATX due to insufficient information (World Health Organization 2020). However, as the knowledge of neurotoxicity from ATX

is still limited, it has been suggested that links between ATX and neurodegenerative diseases like Alzheimer's Disease need to be explored (Mello, Braidy et al. 2018, Rutkowska, Płotka-Wasylka et al. 2019). No human fatalities or illness in Australia have been linked to freshwater production of STX or ATX, however, there has been numerous reports of livestock deaths especially within the MDB (McBarron et al. 1975, Negri et al. 1995)

ß-N-methylamino-l-alanine (BMAA)

BMAA is a non-protein amino acid. BMAA can substitute itself with the amino acid L-serine during protein formation creating faulty proteins, which change shape and no longer perform their normal function, leading to cell death (Dunlop et. al 2013). BMAA has been reported to be produced by *Raphidiopsis, Anabaena, Microcystis, Lyngbya,* and *Oscillatoria* (Violi et al. 2019). It has been suggested that it may play a role in causing neurodegenerative diseases such as motor neuron disease (MND) (Dunlop et. al 2013). BMAA has been found in 70% of samples collected from NSW waterways including the Darling and Murrumbidgee rivers and has been suggested to be associated with hotspots of MND (Main et al. 2018).

EMERGING TOXINS AND TOXIN PRODUCERS

It has only been a little over a decade ago that *Limnothrix* was revealed to be toxic (Bernard, Froscio et al. 2011). Isolated from Central Queensland, the strain closely aligned to *Limnothrix redekei* but the toxin that it produces did not behave like that of previously described cyanotoxins (Bernard, Froscio et al. 2011). Further investigation revealed the toxin to inhibit protein synthesis, but is unlike other toxins, suggesting a novel toxin (Bernard, Froscio et al. 2011). Humpage, Falconer et al. (2012) exposed mice to the cultured strain which caused acute neuropathy and gastrointestinal damage and described this new toxin as "Limnothrixin". Limnothrix has also been previously reported in the MDB.

Formally known as anatoxin-a(S), Guanitoxin is one of the most harmful cyanotoxins, yet due to its instability and incompatibility with current analytical methods (Fiore, de Lima et al. 2020), it is not monitored in the environment. Commonly, biosynthetic gene clusters (BGC) for other cyanotoxins like anatoxin (Méjean, Mann et al. 2009) are used for detection, but the BGC of Guanitoxin were previously unknown. Understanding the genetic basis of this neurotoxin is imperative to not exclude it from environmental monitoring. Lima, Fallon et al. (2022) deciphered the biosynthesis and nine-step metabolic pathway of Guanitoxin in *Sphaerospermopsis torques-reginae* from its starting molecule of L-arginine. Being an organophosphate cyanotoxin (the only known one), it shares the mechanism of action seen in other organophosphates like a chemical warfare agent and a banned pesticide (Lima,

Fallon et al. 2022). It causes lethal neurological toxicity and has been identified in blooms responsible for the deaths of animals (Henriksen, Carmichael et al. 1997). Although the chemical structure of guanitoxin has already been known for decades (Matsunaga, Moore et al. 1989), this study elucidated the BGC and thus, environmental gene monitoring can identify its presence and combat the problem of being under-represented in monitoring. Lima, Fallon et al. (2022) accessed raw environmental sequencing data to map its distribution to gain some insight into the environments that cyanobacteria are producing guanitoxin in. Using metatranscriptomics, several freshwater sources in the USA returned positive hits of the transcripts unique to guanitoxin, including longer-term data sources of publicly accessible freshwater water, where a particular source had guanitoxin detected over many years (Lima, Fallon et al. 2022). This study analysed the guanitoxin from a *Sphaerospermopsis* species but the extent of the genera capable of producing the neurotoxin is limited (Lima, Fallon et al. 2022). The research also explored the capability of other genera to produce guanitoxin and identify it in the environment and data sets of *Cuspidothrix* and *Aphanizomenon,* species known to produce other cyanotoxins (Lima, Fallon et al. 2022). The BGC of guanitoxin matched up to these genera which warrant future environmental surveying for guanitoxin, regardless that they have not yet been reported to produce gunaitoxin (Lima, Fallon et al. 2022).

A study exploring a known, generally non-toxic cyanobacteria *Synechococcus* identified it to produce gene homologues of toxins, and therefore, identify it as a potential toxin producer that needs to be monitored (Gin, Sim et al. 2021). The picocyanobacterial species *Synechococcus* have been known to produce MC (Furtado, Calijuri et al. 2009) and BMAA (Cox, Banack et al. 2005), but was not known to contribute to harmful algal blooms (Fukushima, Tomioka et al. 2017). Upon further investigation of *Synechococcus,* Gin, Sim et al. (2021) noticed the production of CYN and anatoxin-A by *Synechococcus* species. However, the total concentrations of toxins detected from *Synechococcus* were relatively low compared to other toxic species isolated from the tropical lake, like *Raphidiopsis* sp (Gin, Sim et al. 2021). Gin, Sim et al. (2021) suggests that the CYN produced by *Synechococcus* utilises a novel synthesis pathway as strains that did not have all of the gene cluster for CYN as in other cyanobacteria such as *Anabaena* sp. and *Raphidiopsis* sp., but still ultimately produced CYN. Additionally, the emerging threat of climate change benefits species like *Synechococcus* for reasons such as having a wide thermal tolerance, tolerance for varying nutrient availability, outcompeting other species of cyanobacteria and being able to thrive in both eutrophic and oligotrophic blooms (Xia, Guo et al. 2017). Despite being a small toxin producer, it has the potential to dominate freshwater environments and multiply rapidly.

ENVIRONMENT DRIVERS OF TOXIN PRODUCTION

Like bloom formation toxin production has been linked with both abiotic and biotic environmental drivers. However what triggers toxin production and their ecological role is still unknown. Holland and Kinnear (2013) explored how environmental conditions, biotic and abiotic, support the production of toxins and how understanding the ecological role of these metabolites might help elucidate key drivers. It is thought that toxins evolved to provide cyanobacteria either with a competitive advantage or to act as a physiological aid or both. Even known toxin producers do not produce toxins at all times, with the same species containing strains that produce toxins and strains that do not. Even within a strain that has the capacity to produce toxins sometimes toxins are not produced whereas at other times toxin production is greatly enhanced. Cyanobacteria species can also produce a variety of different toxins, making it difficult to determine why they do this and what drives toxin production.

Different environmental and nutrient conditions, particularly the concentrations of phosphorus and nitrogen, promote the dominance of specific taxa, and as cyanotoxins are species-specific, toxins vary with succession (Paerl and Otten 2016, Wang, Akbar et al. 2021). For example, higher concentrations of nitrogen will shift the community composition to non-nitrogen fixing cyanobacteria species like *Microcystis* and *Planktothrix* (Kaebernick and Neilan 2001), and towards the production of microcystin (Beversdorf, Miller et al. 2015). The dominance of *Microcystis* is also seen in low phosphorus environments (Gobler, Burkholder et al. 2016, Wan, Chen et al. 2019), whereas *Planktothrix* requires higher phosphorus conditions (Davis, Bullerjahn et al. 2015, Harke, Davis et al. 2016). Conversely, lower concentrations of nitrogen support diazotrophic harmful cyanobacteria like *Nostoc* and *Dolichospermum,* which generally favour low-phosphorus conditions as well (Wan, Chen et al. 2019). Different taxa are more sensitive to variations in different environmental properties, with *Dolichospermum* being more sensitive to changes in nutrients, whereas *Microcystis* has been reported to respond greater to changes in temperature (Rigosi, Carey et al. 2014, Deutsch, Alameddine et al. 2020). This leads to changes in toxin profiles with different nutrient dynamics.

The contribution of nitrogen-fixing and phosphorus scavenging from the cyanobacterial bloom species can also drive the shift in nutrient composition from low to high, and along with seasonal changes means that changes in the dominant species in one body of water are frequent (Beversdorf, Miller et al. 2013, Cottingham, Ewing et al. 2015, Harke, Davis et al. 2016). The understanding of the response of cyanotoxin production to the nitrogen production from nitrogen-fixing cyanobacteria is limited. Moreover, at any one-time, different dominant species can occupy different niches in the same body of water (Shan, Song et al. 2019).

Being able to predict a species' succession is imperative to devise management plans amid climate change, especially as eutrophication in response to climate change will promote toxin production (O'Neil, Davis et al. 2012). Eutrophic freshwaters with different ratios of nitrogen and phosphorus affect the biomass of the bloom and therefore, management solutions focusing on the reduction of a singular nutrient may not reduce the cyanobacterial blooms (Gobler, Burkholder et al. 2016). Other research disagrees and states that bloom biomass can be reduced if either N or P can be reduced significantly (Chorus, Fastner et al. 2021). A study by Li, Hansson et al. (2018) says that it is the dissolved inorganic nitrogen and total phosphorus (DIN:TP) ratio that is the key to reducing bloom biomass. Regardless, a 2022 study by Hellweger, Martin et al. (2022) states that a planned reduction of phosphorus will reduce the overall biomass of *Microcysits*, however will make the lake even more toxic due to an increase in the availability of nitrogen causing a greater production of microcystin.

There is a plethora of literature outlining the effect of temperature on taxa dominance and toxin production (Wu, Li et al. 2016, Shan, Song et al. 2019, Zhang, Fan et al. 2021). However, nutrient changes are generally a better use for prediction of dominance than temperature (Rigosi, Carey et al. 2014). Regardless, the combined effect of the synergistic properties on dominance and toxin production must not be negated and understanding the collaboration between properties is ultimately the best predictor of what drives toxin production (dos Santos Silva, Chia et al. 2022). For example, dos Santos Silva, Chia et al. (2022) found that the biomass of *R. raciborskii* increased with higher nutrient levels (N and P) and temperature (24°C v 30°C), however the synthesis of SXT was elevated in the lower temperature group of 24°C. This result supports some previous studies (Mesquita, Lürling et al. 2019) and opposes others (Vico, Bonilla et al. 2020). Not only the synergy between the abiotic factors but also between biotic and abiotic factors needs to be considered.

Independently, biotic factors influence toxin production, for example, in the presence of a green alga *Chlamydomonas reinhardtii, Anabaena flos-aquae* produced anatoxin-*a* and ceased the production of microcystin (Kearns and Hunter 2000). Likewise, Cladocera and Rotifer presence stimulates MC production by *microcystis sp.* (Pérez-Morales, Sarma et al. 2015). Competition within taxa via allelopathy will also influence the species succession and toxin production (Chia, Jankowiak et al. 2018). For example, a study by Ma, Wu et al. (2015) observed that *Aphanizomenon flos-aquae* growth was inhibited by some strains of *Microcystis.* A high amount of MC production was then produced but further analyses on the toxin revealed it is not an allelochemical, and it was the species succession not the toxin production that inhibited *A. flos-aquae* growth (Ma, Wu et al. 2015). Despite an increase in

studies focusing on what drives toxin production, it is still unclear on the combined effects and the synergistic effects of abiotic and biotic factors on toxin production.

It is clear that the combination of abiotic and biotic environmental drivers on toxin production means that management interventions focusing on one driver may not necessarily decrease the production of toxins. As the limiting factor can be site and species specific, a standardised approach to manage biomass and toxins is impractical due to the variability that exists between water sources, species, variants within species and toxins (Erratt, Creed et al. 2022). Furthermore, as there is not a positive correlation between bloom biomass and toxin production, measuring algal biomass and reducing the biomass of cyanobacteria is not an effective strategy at determining and mitigating risk from toxins, and other variables need to be considered (Hellweger, Martin et al. 2022).

RISK OF BENTHIC CYANOBACTERIA, NOT JUST PELAGIC CYANOBACTERIA

When we think of algal blooms we tend to think of pelagic blooms that float on the water surface or within the water column that are generally associated with taxa such as *Dolichospermum* and other key species, however, blooms of freshwater benthic cyanobacteria have been reported in many countries, and most often contain toxins such as AXT or MC (Wood, Kelly et al. 2020). However, there is still limited knowledge on the risk of toxic benthic cyanobacteria, and the existing literature largely focuses on select species and habitats (Wood, Kelly et al. 2020). A review of the risk of benthic cyanobacteria by Wood, Kelly et al. (2020) highlights the large knowledge gaps that has rendered a lack of a standardised approach to monitor and manage the benthic cyanobacteria proliferations. Wood, Kelly et al. (2020) shows how toxic benthic cyanobacteria have been found in a variety of freshwater sources in numerous countries i.e., lakes, rivers, geothermal pools and grow on all sorts of substrate such as sand, wood, aquatic plants. Many species have been reported to produce toxins and even though non-toxic taxa are also contained in benthic proliferations, these toxic species tend to dominate the mats (Wood, Kelly et al. 2020). The effect of environmental factors on toxin production from benthic cyanobacteria has not been studied, however, culture studies have revealed that the production of toxin varies with the growth stage of the cyanobacteria (Harland, Wood et al. 2015) and availability of nutrients (Heath, Wood et al. 2014). As the benthic cyanobacterial mats contain both toxic and non-toxic taxa the concentration of toxins produced may depend on the ratio of abundance of each (Heath, Wood et al. 2010). What causes a genotype to be dominant in the mat is unknown.

Cyanotoxins released by benthic cyanobacteria pose a risk of moving up trophic levels, for example, mayfly larvae exposed to high concentrations of ATX in a laboratory setting, accumulated high

amounts of the toxin, and being prey to a number of fish species, the possibility of trophic transfer is high (Kelly, Puddick et al. 2020). Given biofilms containing benthic cyanobacteria are food for a number of scraper organisms including mayflys, snails etc. the risk these benthic taxa pose to aquatic organisms cannot be ruled out. Moreover, nodularin produced by mats of benthic cyanobacteria were found in freshwater crayfish, a cultural source of food in New Zealand (Wood, Phillips et al. 2012). Additionally, a species of *Chironomidae* larvae accumulated MC and AXT and comparisons between crude extract and the purified toxin showed the crude extract to be more toxic, suggesting compounds other than the known cyanotoxin itself have a negative effect on aquatic biota (Toporowska, Pawlik-Skowronska et al. 2014). Likewise, the mortality of three macroinvertebrate species exposed to crude extracts of AXT (Anderson, Voorhees et al. 2018) support the evidence of other compounds produced by the cyanotoxin also to be toxic. Benthic cyanobacteria have also been responsible for the deaths of animals; dogs being the most frequently reported (Wood, Selwood et al. 2007, Puschner, Hoff et al. 2008, Faassen, Harkema et al. 2012). In most of the animal-related deaths caused by exposure to benthic cyanobacteria toxins it is AXT that is the culprit, however MC has also been responsible (Wood, Heath et al. 2010). These deaths have all assumingly been due to direct ingestion of the mats as a New Zealand study investigated the toxins released into surrounding water and concluded these concentrations were not significant enough to cause death alone (Wood, Biessy et al. 2018).

The risks of benthic proliferations are amplified by the underreporting of blooms as it is commonly taught to the public to identify blooms of cyanobacteria through the presence of discoloured water (Wood, Kelly et al. 2020). As the benthic blooms are not in the water column, therefore not visible, identification becomes difficult and monitoring of growth typically requires divers (Wood, Kelly et al. 2020). Also, common methods for determination of algal abundance utilise the collection of water samples and not benthic grabs, so the estimation of algal abundance might not be representative of true algal abundance especially when benthic blooms are present. Moreover, the recreational use of rivers concurs with the highest levels of benthic blooms as growth occurs when the water is more stable, in summer (Ibelings et al., 2014; (Wood, Kelly et al. 2020). The education surrounding the risks of benthic blooms to both the public and water managers is seriously lacking and the recreational guidelines are limited with thresholds almost being non-existent; only existing in Cuba and New Zealand (Ibelings, Backer et al. 2014). Although these thresholds need to be refined with more research required to improve the monitoring techniques (Wood, Hamilton et al. 2009), greater education of benthic blooms of toxic cyanobacteria and recreational guidelines with thresholds also should be implemented globally (Wood, Kelly et al. 2020).

The concerning risk of cyanobacterial blooms, not only pelagic, or benthic, is exacerbated by the risk of climate change (Paerl and Huisman 2009). Anthropogenic-induced eutrophication and increasing temperatures create environments that allow cyanobacteria, above other phytoplankton, to thrive (Paerl and Huisman 2009, Lürling, Mello et al. 2018). Potential positive feedback between the cyanobacterial blooms further increasing the water temperatures through light absorption also cause blooms to get worse over time, an added risk to the already existing threat of climate change (Kahru, Leppanen et al. 1993, Kahru, Savchuk et al. 2007).

CYANOBACTERIA AND THE MURRAY-DARLING BASIN

Blue-green algae (or Cyanobacteria) blooms represent a significant threat to water quality in the Murray-Darling Basin (MDB). The MDB covers 1,059,000 square kilometres of south-east Australia and drains one-seventh of Australia's land area. The Basin incorporates the Australian Capital Territory, and parts of Queensland, New South Wales, Victoria, and South Australia. The MDB contains Australia's three longest rivers, the Darling (2,740 km), Murray (2,530 km) and Murrumbidgee (1,690 km) and incorporates a vast network of tributaries, creeks, and watercourses. Freshwater flows through the Basin support a wide range of estuarine, floodplain, and wetland ecosystems, including 16 internationally significant wetlands that contain endangered species. The MDB is of significant environmental, cultural, and economic value to Australia and supports drinking water, agriculture, tourism, and other primary industries for over 3 million people. Around 40% of Australia's agricultural production comes from the Basin, with water used for livestock, piggeries, wineries, dairies, and irrigation of crops (e.g., rice, grapes, almonds, stone fruit) (https://www.mdba.gov.au/importance-murray-darling-basin). Furthermore, freshwater flows down the Murray-Darling system are central to the cultural, social, and spiritual identity of Australia's First Nations people and seen as the lifeblood of their Country (Jackson et al. 2021). Numerous cyanobacteria blooms have been recorded over the last 20 years and have extended across large areas of the Basin (Bowling et al. 2013; 2018). The key toxic species of interest in Australian freshwaters and those relevant to the MDB are *Microcystis aeruginosa*, *Dolichospermum circinale*, *Cylindrospermopsis raciborskii* and *Chrysosporum ovalisporum*. National Health and Medical Research Council (NHMRC) Guidelines for Managing Risks in Recreational Waters have been developed regarding alerts associated with cyanobacteria and are provide in Table 1.

Table 1. Algal Alert levels

Alert	*Microcystis aeruginosa* (cells mL-1)	Biovolume (mm3 L-1) where toxin producing cyanobacteria are dominant (>75%)	Biovolume (mm3 L-1) where toxin producing cyanobacteria are not dominant (>75%)	Definition
Red	>50,000	>4	>10	Bloom conditions; water may appear green, contain visible scums or have a strong odour; water should not be used for drinking, stock watering or for recreation; people should not eat mussels or crayfish; recommended not to eat fish
Amber	5,000-50,000	0.4-4	0.4-10	BGA are multiplying; water may have a green tinge or odour; should be considered unsuitable for potable use; may be unsuitable for stock; water remains suitable for recreation however users should use caution and avoid areas where obvious signs of BGA are visible
Green	<5,000	>4 of total cyanobacteria biovolume but <amber level	>4 of total cyanobacteria biovolume but <amber level	BGA present in low amounts; no threat to recreation, stock, or domestic use

EMERGING TECHNIQUES TO MONITOR BGA BLOOMS

As mentioned above blooms are often reported due to the appearance of water (scum and discolouration) and the collection of water samples whereby algal cells per mL are counted, measured and biomass calculated. There are a number of emerging techniques that can be used to monitor algal blooms. These include the following:

1. Remote sensing: remote sensing technologies such as satellites, drones, and planes can monitor blooms from a distance. Optical remote sensing works by measuring water spectral properties and targeting certain wavelengths representative of algal pigments such as chlorophyll a, and phycocyanin which are indicators of algal abundance. A number of remote sensing sensors have been recommended for use including radar, LiDAR, Multi-spectral and Hyper-spectral (Rolim et al. 2023).
2. Real-time Fluorescence sensors: sensors that use fluorescence to measure algal pigments to estimate algal abundance
3. Real-time cell counts using flow cytometry: flow cytometry count cells by running a sample of water past lasers causing them to fluoresce allowing cells to be counted and separated based on size, and fluorescence of key algal pigments. More recently cameras have been added to flow cytometers to allow for accurate identification of algal species such as the FlowCam Cyano (https://www.fluidimaging.com/products/flowcam-cyano-differentiate-cyanobacteria-and-other-algae) and the FlowCytobot (https://mclanelabs.com/imaging-flowcytobot/#:~:text=The%20Imaging%20FlowCytobot%20(IFCB)%20is,taken%20from%20the%20aquatic%20environment).
4. Molecular Methods: advances in molecular methods have led to the development of numerous methods which can be used to detect cyanobacterial species including fluorescence in situ hybridisation (FISH), sandwich hybridization assay (SHA), quantitative polymerase chain reaction (PCR), multiplex PCR, isothermal amplification technology (IAT), and gene chips (GC) (Liu et al. 2022). Ecogenomics combines ecology and genomics to explore genetic diversity and functional capabilities of taxa within a bloom and might help elucidate drivers of bloom and toxin production.
5. Omic approaches: combination of genomics, transcriptomics, proteomics, and metabolomics to determine species in blooms but also enhance understanding of mechanisms behind bloom formation, toxin production and drivers (Wood, Kelly et al. 2020). The combination of proteomics, genomics, and transcriptomics in a proteogenomic study of a toxic benthic bloom was able to identify not only the species that made up the mats and the main dominant species, but also the drivers of the proliferation (Tee, Waite et al. 2020).
6. Artificial Intelligence and Machine Learning: can be used to analyse large datasets and predict occurrence of algal blooms by identifying patterns and developing models. Artificial intelligence models can also be used for rapid classification of species (Li, Liao et al. 2017) (Gaur, Pant et al. 2022), to improve the classification accuracy and identify an ensemble method of software for accurate BGA classification.

CURRENT MANAGEMENT OPTIONS AND LIMITATIONS

A number of methods are employed globally to manage algal blooms (Fentie 2020). These include the following:

1. **Physical controls** such as aeration, hydrologic manipulations, mechanical mixing, reservoir drawdown, surface skimming and ultrasound have been used in the management of blooms. Aeration disrupts migration of cyanobacteria in the water column and has been shown to work in small ponds and waterbodies, however, is more efficient in deep water and depends on degree of stratification and air flow rate. Manipulation of water into and out of a system to disrupt stratification has also been shown to work in controlled systems such as dams and reservoirs, however, may be expensive as this requires sufficient water and the ability to control flow. Mechanical mixing can also disrupt cyanobacteria migration in the water column and can disrupt stratification and bloom formation, however devices tend to have limited range. The drawdown of reservoirs to cause desiccation of blooms and is easy to implement, however is likely to have cascading effects on downstream aquatic environments. The removal of surface scums via the use of oil-skimmers or flocculants can be successful in later stages of bloom development, however the adverse impacts of the bloom are likely to have already started and this technique is also expensive due to the use of equipment etc. Ultrasonic waves can disrupt the cellular structure of cyanobacteria, and has been successful in small ponds and waterbodies, however, is likely to also disrupt non target organisms such as green algae and could cause the release of toxins.
2. **Chemical controls** include the use of algaecides, coagulation, and flocculation. The use of algaecides is a rapid and effective treatment option, however, can cause cells to lyse and release toxins, increasing the toxic effects. This was the case on Palm Island when 148 people become ill after use of Copper-based algaecide. This can also have direct toxic effects on aquatic organisms. Coagulation and flocculants cause the sedimentation of algal cells, has been successfully implemented in large waterbodies, however, may cause the release of toxins via cell lysis and may impose direct effects on other aquatic organisms.
3. **Biological controls** such as the use of viruses, bacteria, fungi, and enzymes such as cellulases and proteases have been proposed, however are still in their infancy. The use of zooplankton and fish have also been proposed. The use of floating macrophyte beds or aquaponics have additionally been shown to be successful in reducing algal blooms. Barley straw can be used

to treat algal blooms due to the release of natural compounds that inhibit algal growth as it decomposes. This has been shown to be especially effective against Microcystis sp. however may take 2-8 weeks before taking effect. This can lead to hypoxic fish deaths due to the increase in bacterial growth.

4. **Environmental mitigation controls** include strategies to modify the environment to reduce cyanobacteria. These include reduction of nutrient inputs through a decrease in addition of fertilisers, riparian planting and implementation of policies relating to controlling nutrient pollution in freshwaters.

METHODS

DATA SOURCES

MDBA PROVIDED DATA

Phytoplankton cell counts for the Murray-Darling Basin monitoring sites were provided by the MDBA. The dataset includes microscopic ID and enumeration of algal species performed by ALS Environmental for all sites between 1979-2016, and Australian Water Quality Centre (AWQC) for all sites between 1997-2022. The records include counts of cyanobacteria (or blue-green algae), other planktonic protists and microalgae (e.g., diatoms and zooflagellates). The historical long-term monitoring records of blue-green algae in the MDB were originally collated for 14 sites from 1979 to 2022. However, not all sites had BGA data collected for the whole time period. Though generalised linear models were derived for all sites, a linear trend analysis was compiled only for sites which contained data for the whole time period, thus excluding Goolwa and Capels. The collated dataset included 268,707 algal count records. The dataset required curation and filtering prior to use in the trends analysis. For example, eight percent of the raw data were removed due to having units in measurements other than cells per mL. These included records where units were not provided or ambiguous, for example as 'filaments' or 'colonies per mL'. Samples with units recorded as 'per L' were assumed to be cells per litre and were converted to cells per mL by dividing by 10^3. Biovolume data ($mm^3\ L^{-1}$) was not originally determined for cell counts and not provided in the raw dataset. Instead, biovolume was calculated from cell counts using estimates of cell volume (in μm^3) for each species of potentially toxic cyanobacteria (Appendix A-1).

Station numbers in the BGA dataset were matched to those used in the River Murray Water Quality Monitoring Program (RMWQMP) data trends analysis project (Silvester et al 2022). The raw algal data contained multiple columns that could potentially match the RMWQT station numbers (AWRCID, PROVIDERSITEID, SamplingPointDescription). These columns were not consistent within sites. For instance, records from Goolwa included an AWRCID code of "4261034", a PROVIDERSITEID code of "3127" and a SamplingPointDescription of "MDBA Balranald 41010901". A new station identifier column, called "site", was created using the following rules: if a PROVIDERSITEID was provided then this was used otherwise, if an AWRCID was provided this was used, else the numeric portion of the SamplingPointDescription was used. In most cases this provided suitable matches to the RMWQT dataset, but some manual manipulation was required to resolve some discrepancies. For instance, a station with the site name CAPELS had an AWRCID code of 407252. In the RMWQT data, the code for

this site was 407252A. Also, sites that occurred close to each other were assigned the same site identification number but retained their original latitudes and longitudes. The final dataset included 21 unique locations that were consolidated to 14 sites along the River Murray System across NSW, Victoria, and South Australia (Figure 1).

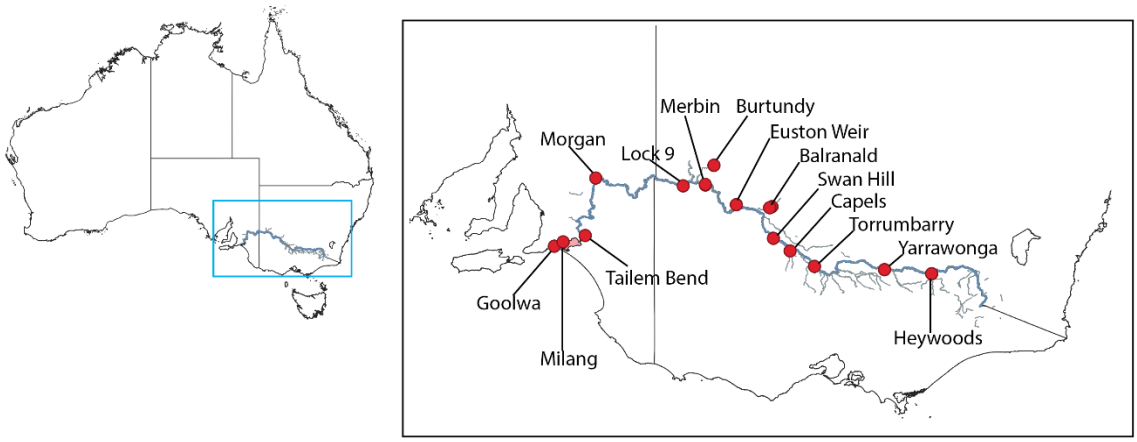


Figure 1. Map of 14 sites included in the algal dataset provided by MDBA. Note Goolwa, Milang and Capels were removed for trend analysis due to discontinuation of monitoring.

Each cell count record in the dataset included a taxon identifier based on the lowest level of taxonomic classification possible at the time of ID. Full taxonomic hierarchical information was retrieved for each taxon ID using the reference taxonomy database of the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/). Where a taxon could not be found on the NCBI database, taxonomic classifications were retrieved through searching on the Atlas of Living Australia (https://www.ala.org.au/) and AlgaeBase (https://www.algaebase.org/). During the period that this dataset spans (1979-2019) several key toxin-producing cyanobacteria have been reclassified. Notably in 2009 several *Anabaena* species were reclassified as belonging to the genus *Dolichospermum*, including *Anabaena circinalis*, *Anabaena crassa*, *Anabaena planktonica*, *Anabaena smithii*, and *Anabaena flos-aquae.* Most of the potentially toxic *Anabaena* species identified in Australian freshwaters were reclassified as *Dolichospermum* (Wacklin et al. 2009), therefore all *Anabaena* cell counts were reclassified to *Dolichospermum* without species-level ID in the dataset. Otherwise, without reclassification of *Anabaena* to *Dolichospermum* there was an artifact in the trends analysis for these genera pre and post 2009 when lab methods changed, which reflected differences in taxonomic level identified at various sites. The taxonomic revision in 2012 of two abundant BGA species *Aphanizomenon ovalisporum* and *Anabaena bergii* to the genus *Chrysosporum* also impacted the dataset (Komárek 2012). When species-level ID was available, *Aphanizomenon ovalisporum* and

Anabaena bergii were reclassified as *Chrysosporum* in the dataset. Since *Aphanizomenon ovalisporum* was the only species of *Aphanizomenon* identified in the dataset, the genus *Aphanizomenon* was reclassified as *Chrysosporum* for the entire period of the dataset. It is notable that *Chrysosporum* may still be artificially underrepresented in the dataset pre 2012 if *Anabaena bergii* were not identified to species-level during enumeration.

Another challenge faced when collating the long-term data from multiple sources was the treatment of replicate records. Since identification of individual samples was not consistently recorded, unique combinations of site and date were used to denote samples. The average and sum of the number of cells per mL were calculated for each taxon in each sample. Where the average and sum were not equivalent, multiple samples were assumed to have been collected and these were removed from the dataset due to potential issues for downstream analyses. Records removed from the dataset represented only 8% of the data. The final MDBA dataset included 216 taxa over 301,998 records. Only potentially toxic cyanobacteria taxa (Appendix B.1) identified to the genus level were used for trends analysis. These same taxonomic standards and data filtering strategies were applied to each of the state specific BGA datasets prior to trends analysis.

VICTORIA: GOULBURN-MURRAY WATER DATA

Cyanobacteria biovolume (mm^3 L^{-1}) and cell count (cells mL^{-1}) data were obtained for storages in northern Victoria from Goulburn-Murray Water (GMW, https://www.g-mwater.com.au/). Goulburn-Murray Water (GMW) is Australia's largest rural water corporation and manages, stores, and delivers water through approximately 10,000 km of infrastructure to northern Victoria. The GMW blue-green algae dataset includes 15 monitoring sites (Figure 2) being Cairn Curran Reservoir, Dartmouth Dam, Lake Eildon, Lake Nagambie (Goulburn Weir), Greens Lake, Hepburns Lagoon, Laanecoorie Reservoir, Lake Buffalo, Lake Eppalock, Lake Nillahcootie, Lake William Hovell, Newlyn Reservoir, Tullaroop Reservoir, Waranga Basin, and Yarrawonga Weir. The dataset includes cell count and biovolume measurements for a sampling period between 2004 (Tullaroop), 2007 (Yarrawonga, Lake Eildon, Goulburn Weir), or 2009 (other sites) to 2022. A second separate dataset which contains historical biovolume data for the period between 1998 to 2019 was also analysed. For the trends analysis only the potentially toxic cyanobacteria (Appendix B) identified to the genus level were included in the final dataset.

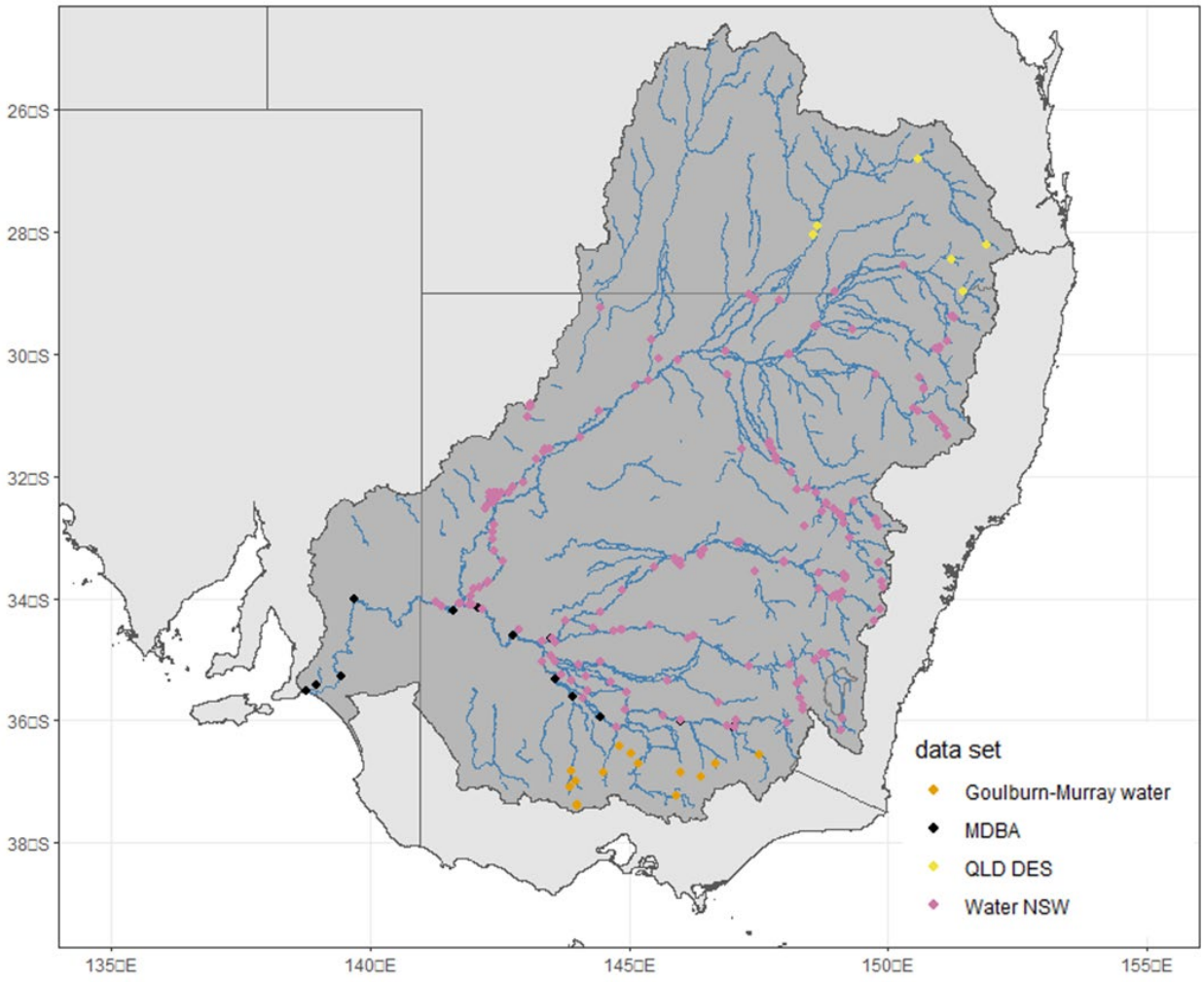


Figure 2. Map of all sites included in the algal dataset for the River Murray provided by MDBA (dark blue), Queensland sites provided by QLD Department Environment and Science/Sunwater (yellow), New South Wales sites provided by WaterNSW (pink), and Victorian Sites provided by Goulburn-Murray Water (orange).

NEW SOUTH WALES DATA

Cyanobacteria biovolume ($mm^3\ L^{-1}$) and cell count ($cells\ mL^{-1}$) data were obtained for New South Wales monitoring sites within the MDB from WaterNSW (https://www.waternsw.com.au/). WaterNSW is a state-owned corporation whose responsibilities include supplying water in compliance with appropriate standards of quality in the Sydney catchment area. WaterNSW undertakes extensive monitoring of blue-green algae within its catchments, lakes, and raw water supply system and in rivers downstream of storages to meet this objective. The WaterNSW dataset includes BGA counts for 237 sites from 2015 to 2022. The monitoring sites are in various catchments, lakes, and rivers across the state (Figure 2), including the Menindee Lakes, Copeton Dam, Lake Inverell, Wyanagala Dam, Murrumbidgee River, Darling River, River Murray, and Wakool River.

QUEENSLAND DATA

Cyanobacteria biovolume (mm^3 L^{-1}) and cell count (cells mL^{-1}) data were obtained for Queensland Murray Darling Basin (QMDB) monitoring sites from Sunwater (https://www.sunwater.com.au/) and the State of Queensland Department of Environment and Science (DES). The dataset includes six storage sites in the QMDB: Beardmore Dam, Chinchilla Weir, Coolmunda Dam, Glenlyon Dam, Jack Taylor Weir, and Leslie Dam (Figure 2). The dataset includes species level identification by cell concentration (cells mL^{-1}) and cell biovolume (mm^3 L^{-1}) for the storage sites for a sampling period between 2000 to 2022. For trend analysis, cell counts and biovolume were summed at the genus-level with only potentially toxic cyanobacteria included in the dataset (Appendix B).

WATER QUALITY

Data collated and analysed as part of the RMWQMP data trends analysis project was used to compare to the 14 matching sites within the MDB (Figure 1). A summary of the data analysis methods is provided in Silvester et al. (2022).

TREND ANALYSIS

To assess whether there had been a shift in the frequency and magnitude of blue-green algae blooms in the Murray-Darling Basin over the last decade, several statistical analytical methods were applied. The steps involved in the trends analysis and the subsequent development of a community model for water quality and BGA taxa are summarised in Table 2 and outlined in more detail below.

Table 2. Key analysis steps in the trend analysis and development of a community model

STEP	ANALYSIS GOAL	ANALYSIS STEP	OUTCOME
1	Replace missing data	Use Kalman filters for interpolation	Regular monthly time series without missing data points
2	Determine annual seasonality in parameters and account for moving average/autocorrelation	S/ARIMA models and extract the seasonal components of the models	Identification of seasonal or other regularly recurring patterns in the data – allowing the data to be 'de-trended' according to these patterns
3	Imputation of missing data across all sites and all seasonally-detrended cell density and biovolume data	Data imputation by Random Forest (RF) models	Regular, de-trended monthly time series of measured plus imputed values
4	Model the temporal trends of the cell density and biovolume at genus and phyla level for potentially toxic cyanobacteria after accounting for site specific seasonality	GAM spline fits to determine trends over the data that were interpolated	Spline fits to datasets allowing trend coefficients to be extracted and identification of change periods
5	Determine trends in BGA cell density and biovolume from year to year for monitoring sites during set periods	Fitting GLM to imputed data from ARIMA and RF models for BGA cell counts and biovolume	Linear change in cell density and biovolume for several time periods, including those that match RMWQMP report, and for looking at BGA trends in relation to Millennium Drought
6	Community model analysis of BGA taxa with water quality parameters	Bayesian ordination and regression analysis of BGA taxa abundance (as Genus cell counts) and abiotic parameters	Determine significant correlations of BGA taxa occurrence with abiotic water quality parameters for River Murray System sites for period between 1979-2019

S/ARIMA=Seasonal/ AutoRegressive Integrated Moving Average; GAM = Generalised additive model; GLM=generalised linear model

DATA INTERPOLATION

The most commonly occurring sampling frequency, for cyanobacteria was monthly. The original measurement data were therefore first consolidated into monthly means for each specific site and taxa combination. Any missing data in these monthly time series were then imputed using a Kalman filter and smoothing (an algorithm which identifies the most probable values based on the measured data). This process filled in any gaps between the minimum and maximum measurement dates, for each site and cyanobacteria taxa.

SEASONAL AND/OR AUTOCORRELATIVE DE-TRENDING

Once a continuous, monthly time series was produced, an automatic selection (via stepwise minimisation of model space) of the best-fitting Auto-Regressive Integrated Moving Average Model (ARIMA) for each specific time series was conducted. These models predict the time series values based on stationary (i.e., with long-term trends removed), linear regressions of past values, which potentially include both: (i) the auto-correlative function of the current and past values and (ii) a seasonal component (a.k.a., seasonal-ARIMA or SARIMA). This model selection was used to determine whether there were seasonal components present (i.e., whether the model selection chose an ARIMA or SARIMA model as the best fit). If seasonality was detected, the seasonal component of the corresponding SARIMA model was removed (i.e., the amount of variability in the data attributable to regular, seasonal variation in values) for the appropriate month from the original time series, resulting in a seasonally-detrended (where appropriate) series of values specific to each site and taxa.

DATA IMPUTATION

Missing time series data was imputed using random forest modelling. To do this, a monthly mean was calculated for the complete set of seasonally detrended time series for each taxa (i.e., all sites at which a particular taxa was measured). The seasonally detrended monthly mean was then fed into a chained random forest model (using data at all sites as predictors) in order to impute data across all sites, from the minimum to maximum dates at which the taxa was measured at any site. The result was a series of continuous, seasonally-detrended (where appropriate) series of monthly mean values for each site, across the entire measured period.

GENERALISED ADDITIVE MODELS

Generalised additive models (GAM; a smoothed prediction of trends in the parameter values based on date) were used to visualise and assess trends over time in cell density and biovolume of potentially toxic cyanobacteria. GAMs produce a prediction of the long-term trends in the data and the error

around these predictions. Change point analysis was included in the GAM for each dataset, based on the first derivative of the non-linear GAM component. In GAMs, these derivatives are estimated using the method of finite differences i.e., the difference between estimated values for two time points, separated by a very small time-shift, approximates the true first derivative of the trend (Simpson 2018). Significant periods of change (i.e., times at which monthly means were changing) were thus inferred from periods in which the 95% confidence interval for the estimated first derivative did not include zero. An example is provided in Figure 3 where significant periods of change are indicated by the red lines.

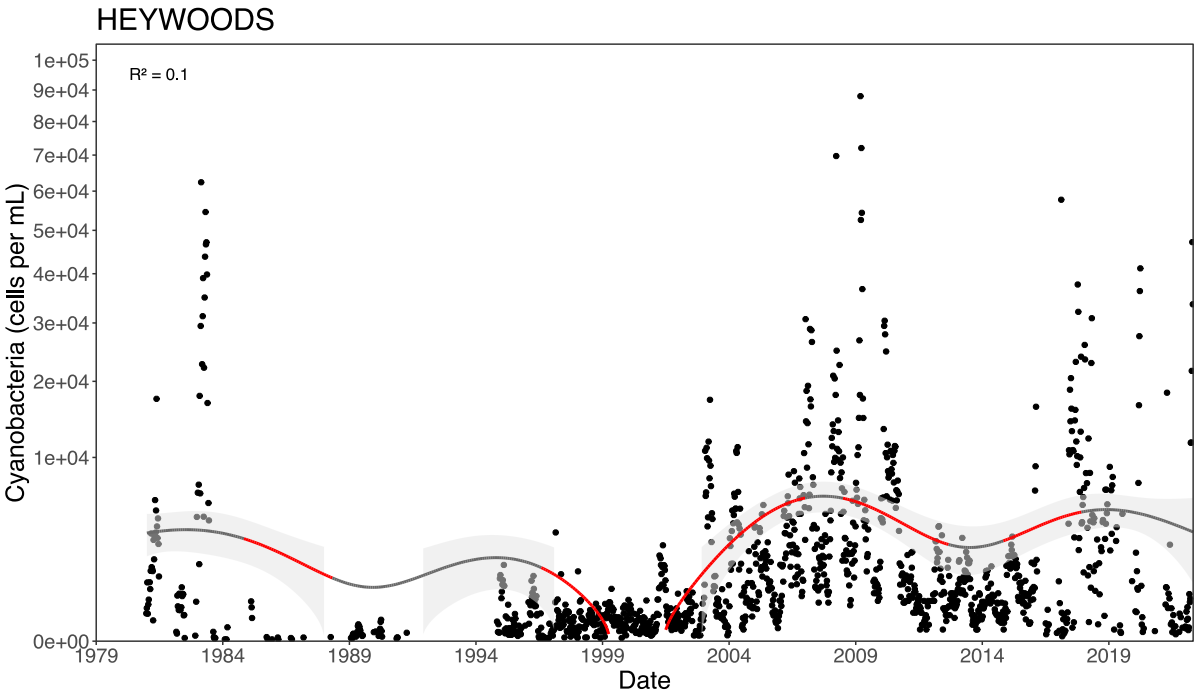


Figure 3. Example of generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in cell counts of total toxin producing Cyanobacteria (Heywoods site). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

GENERALISED LINEAR MODELLING

The linear change in cell density and biovolume from year to year was determined using generalised linear models (GLMs) as applied previously in Silvester et al 2022 to assess changes in water quality. Linear trends in BGA cell density and biovolume were determined from the estimated slopes (trend coefficients) of GLMs applied to the imputed data output of the random forest modelling of seasonally-detrended cell density and biovolume data. GLMs of imputed data were performed in R using packages 'mgcv', 'imputeTS', 'missRanger', and 'forecast'. Linear trends were assessed by performing Generalised linear models (GLMs) on MDBA BGA data for periods before, during, and

following the Millennium Drought (1997 to 2009). An assessment of the trends in BGA cell counts and biomass within the context of the Millennium Drought were undertaken because of the significant impact that the drought had both on water quality and on water management practices within the Murray-Darling system. For this analysis, linear trends were assessed over (i) the entire dataset from 1979 to 2022, (ii) the period before the drought, 1979 to 1996, (iii) the Millennium Drought from 1997 to 2009, and (iii) post drought, 2010 to 2022.

Blue-green algae data from other State and catchment management authorities represent a more recent period of monitoring, and do not go as far back in time as the MDBA dataset. When possible, BGA trends were assessed in context of the Millennium Drought. For Victorian sites in the Goulburn-Murray catchment, trends were assessed for both datasets (i) the entire dataset, (ii) during the Millennium Drought, and (iii) post drought 2010 to 2022. For Queensland MDB sites, trends were assessed for (i) the entire dataset from 2000 to 2022, (ii) during the Millennium Drought, 2000 to 2009, and (iii) post drought, 2010 to 2022. For WaterNSW, trends were only assessed for the entire dataset period from 2015 to 2022.

COMMUNITY MODELING OF WATER QUALITY AND BLUE-GREEN ALGAE

A community analysis of cell counts of measured BGA communities across the Murray River was constructed using the 'Bayesian Ordination and Regression Analysis' package (*boral*; Hui, 2017). There was a total of 31 taxa within the Cyanobacteria community and these were modelled against six abiotic parameters (i.e., dissolved organic carbon, nitrogen, nitrogen-oxides, phosphorous, dissolved silicon, and water temperature) to determine significant correlations with water quality and community co-occurrence at 11 sites for the period between 1979 to 2019. Three River Murray monitoring sites were excluded from the community model due to insufficient water quality data or BGA cell density measurements; these were Euston Weir, Goolwa, and Milang (Figure 1). Other water quality measurements also had to be removed as there was not a full dataset available for all 11 sites. The model included a random intercept for sites to allow for varying intercepts for each site to determine communities varied spatially. Coefficients were calculated with the 95% highest posterior density (HPD) of coefficient values in addition to mean and median values. Values that had 95% HPD intervals that did not overlap were considered significant drivers of a species abundance.

RESULTS AND DISCUSSION

TRENDS IN CYANOBACTERIA

RIVER MURRAY (MDBA DATASET)

Trend patterns to understand changes in cyanobacterial cell counts (cells mL^{-1}) and biomass (mm3 L^{-1}) across all sites are presented as linear trend coefficients in Figure 4, which give the direction and magnitude of the data trend derived over time via use of generalised linear models (GLMs). Negative coefficients mean that the trend is decreasing in regard to the mean over the entire dataset, whereas positive trends indicate an increase in that parameter (cell counts or biomass) during that period. Total cyanobacteria were shown to be increasing over time at all sites over the whole period (Figure 4). However, trends were dependant on the period chosen, with greatest increases in cell counts recorded over the 2010-2022 period, at sites Balranald upstream, Merbein, Burtundy, and Euston Weir, while Heywoods, Torrumbarry, Lock 9, Morgan, and Tailem Bend recording counts highest during the Millennium Drought (Figure 4). No significant difference in cell counts was shown between the last 12 years and the Millennium Drought at Yarrawonga and Swan Hill. All sites recorded higher cell counts since the period between 1979 to 1996. Merbein and Burtundy recorded the highest increase in cyanobacteria cell counts (cells mL^{-1}) over the last 12 years. On closer inspection of the last 12-year period it appears the greatest increase in total cyanobacteria has occurred during the period between 2016-2019, with a general decrease in cyanobacteria following the drought 2010 to 2015 and after 2019 (Figures 5 – 15).

Biomass followed a similar pattern to total cyanobacterial cell counts with generally higher biomass recorded over the last 12 years, or during the Millennium Drought depending on sites (Figure 4). Slight differences in trends between cell counts and biomass likely reflects a shift in the dominant taxa, which differ in cell size and thus biovolume. Our results support those of Croome et al. 2011 that also found that total cyanobacteria were increasing across the Basin.

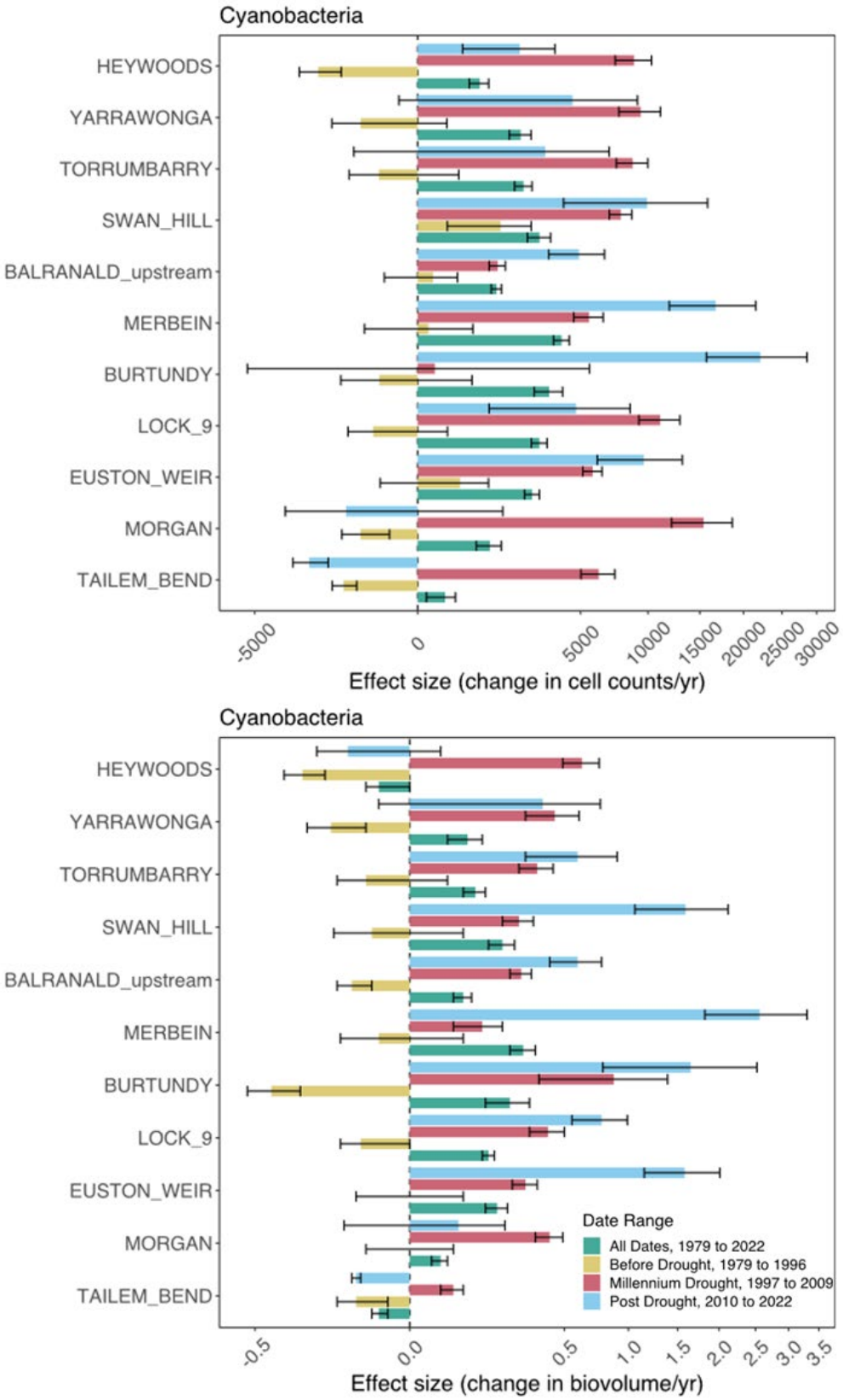


Figure 4. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in MDB River Murray sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset (1979 to 2019) and in context with the Millennium Drought (1997 to 2009). Sites are ordered from upstream to downstream on y-axis. Error bars are 1 standard deviation of the mean effect size.

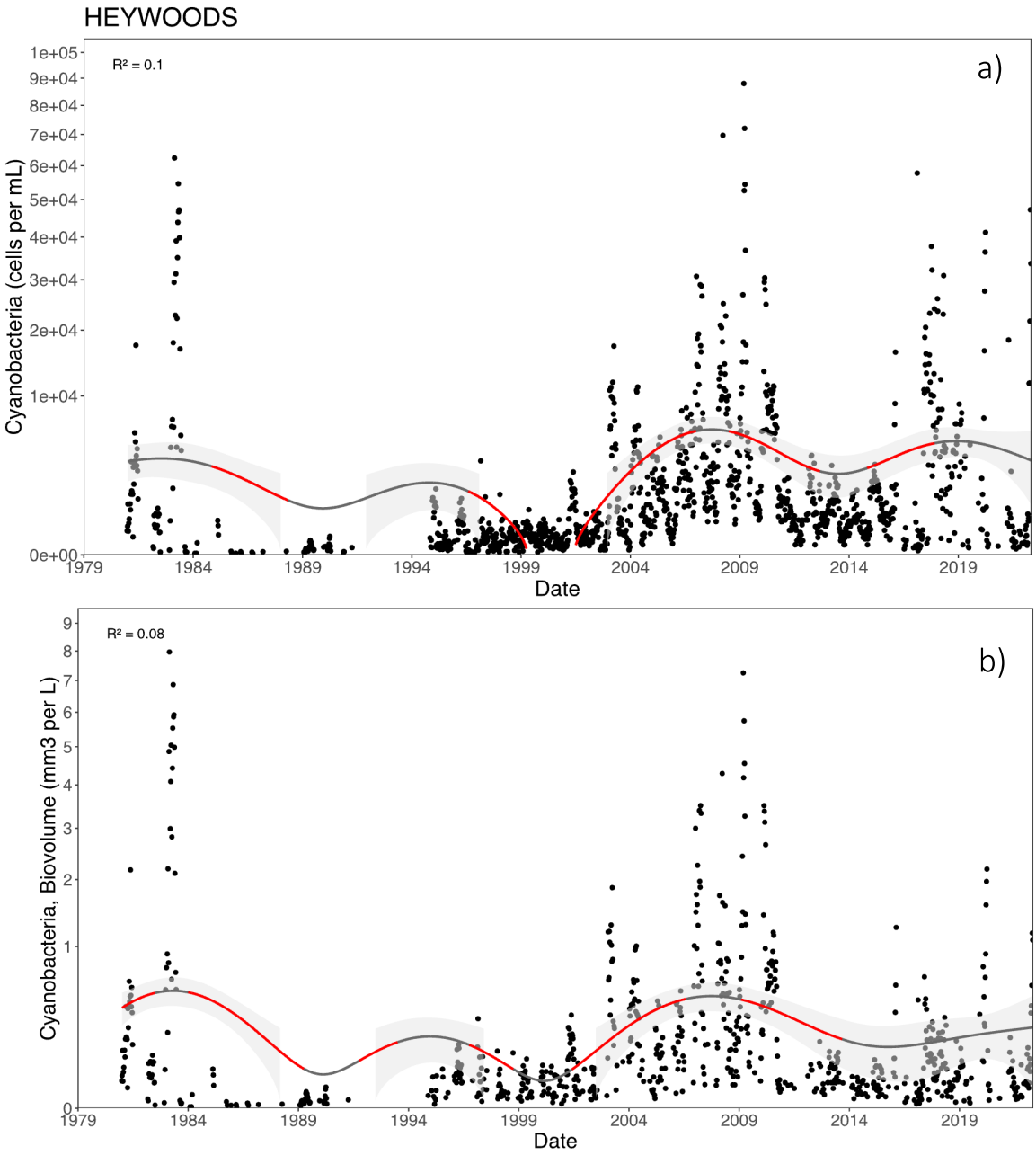


Figure 5. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass (mm^3 L^{-1}) data for Heywoods, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

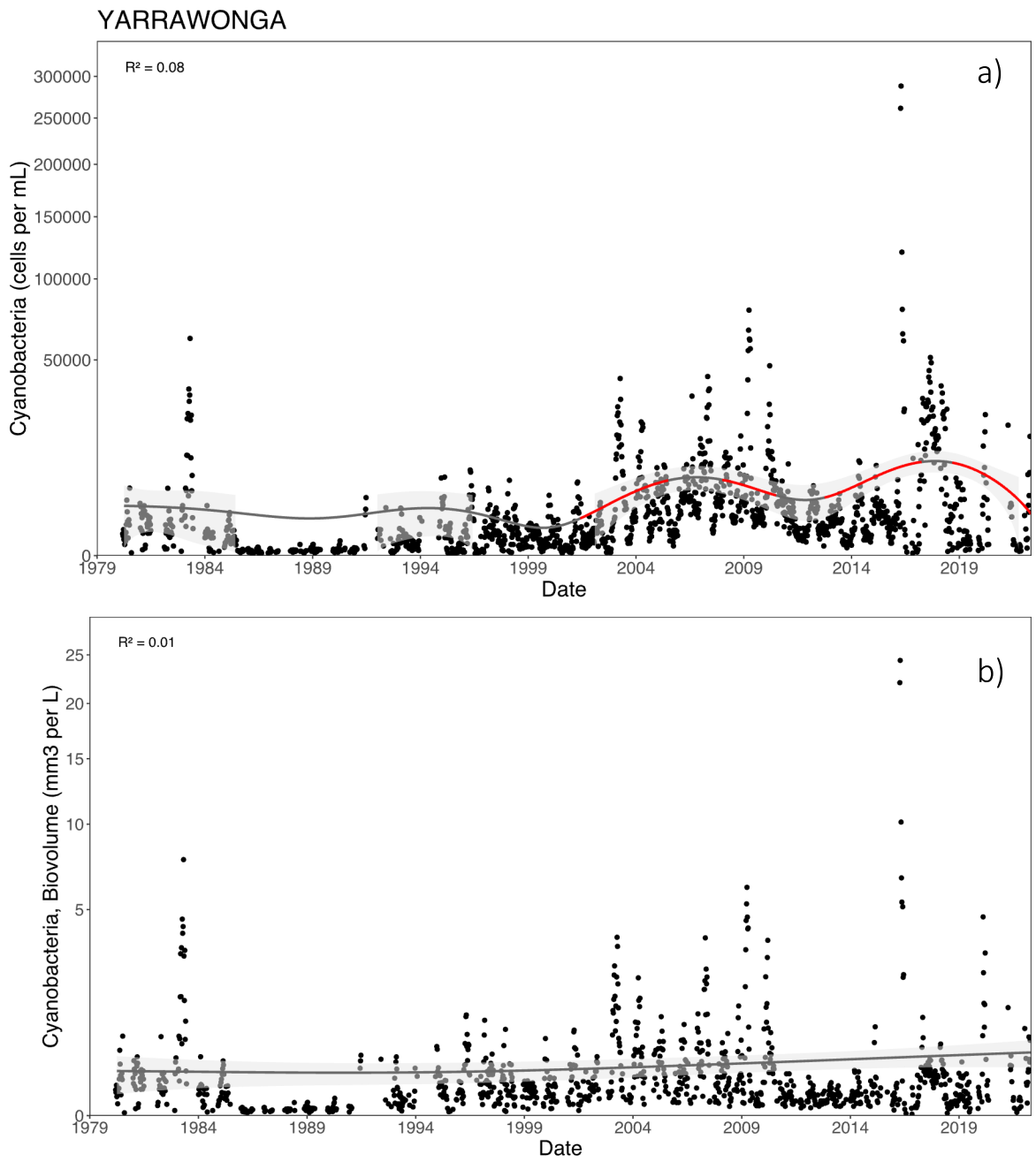


Figure 6. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass (mm^3 L^{-1}) data for Yarrawonga, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

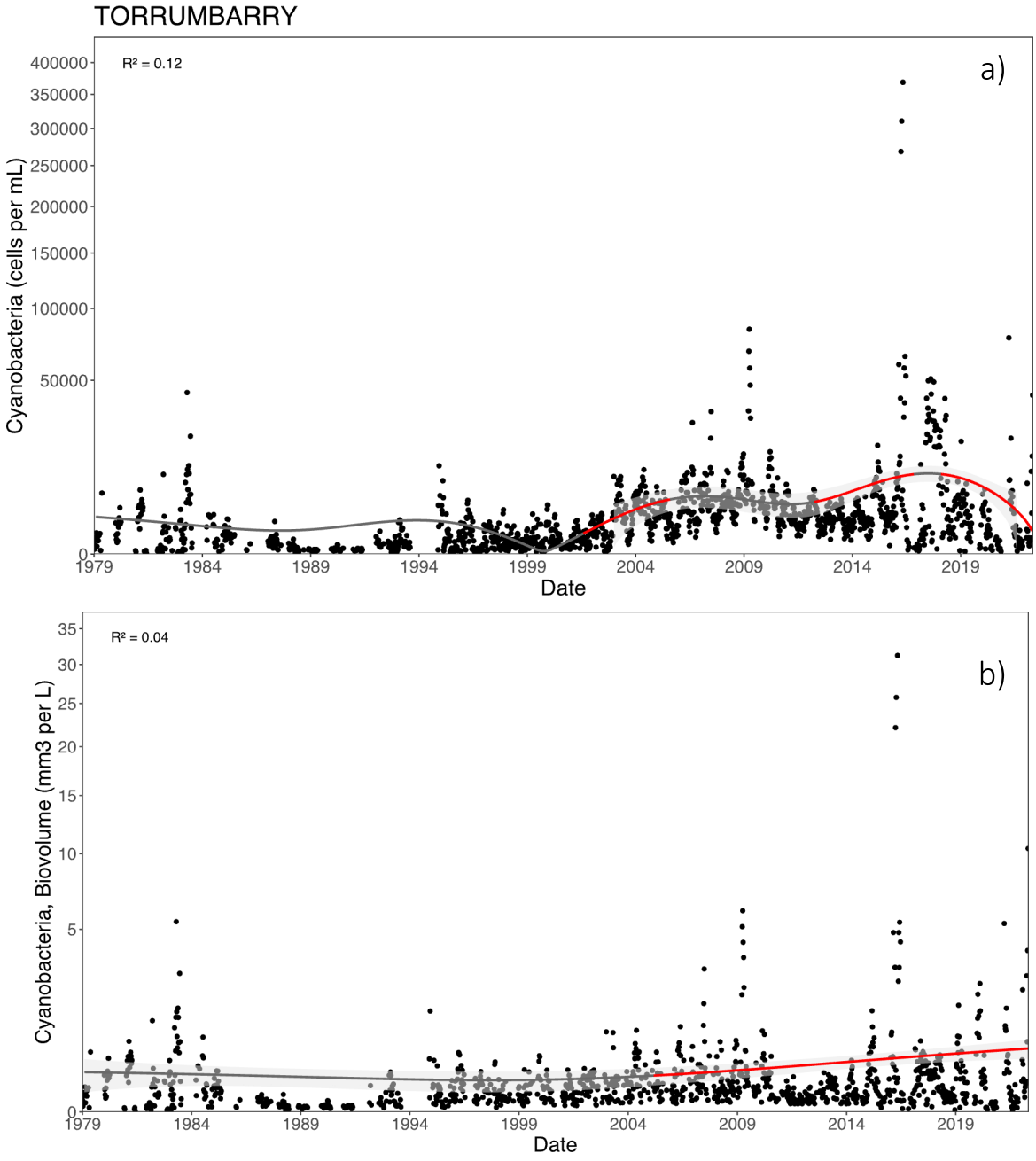


Figure 7. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass (mm^3 L^{-1}) data for Torrumbarry, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

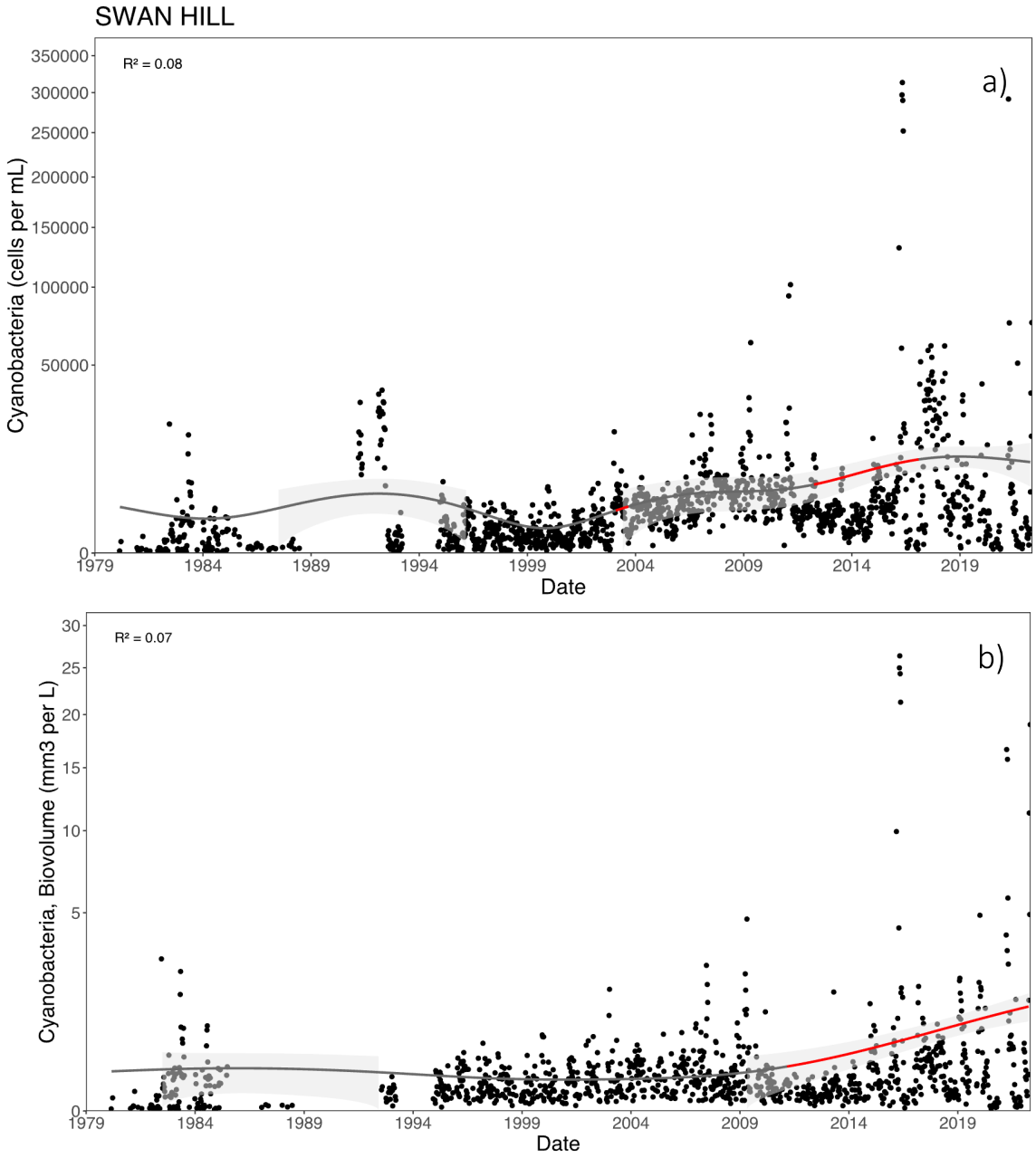


Figure 8. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass (mm^3 L^{-1}) data for Swan Hill, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

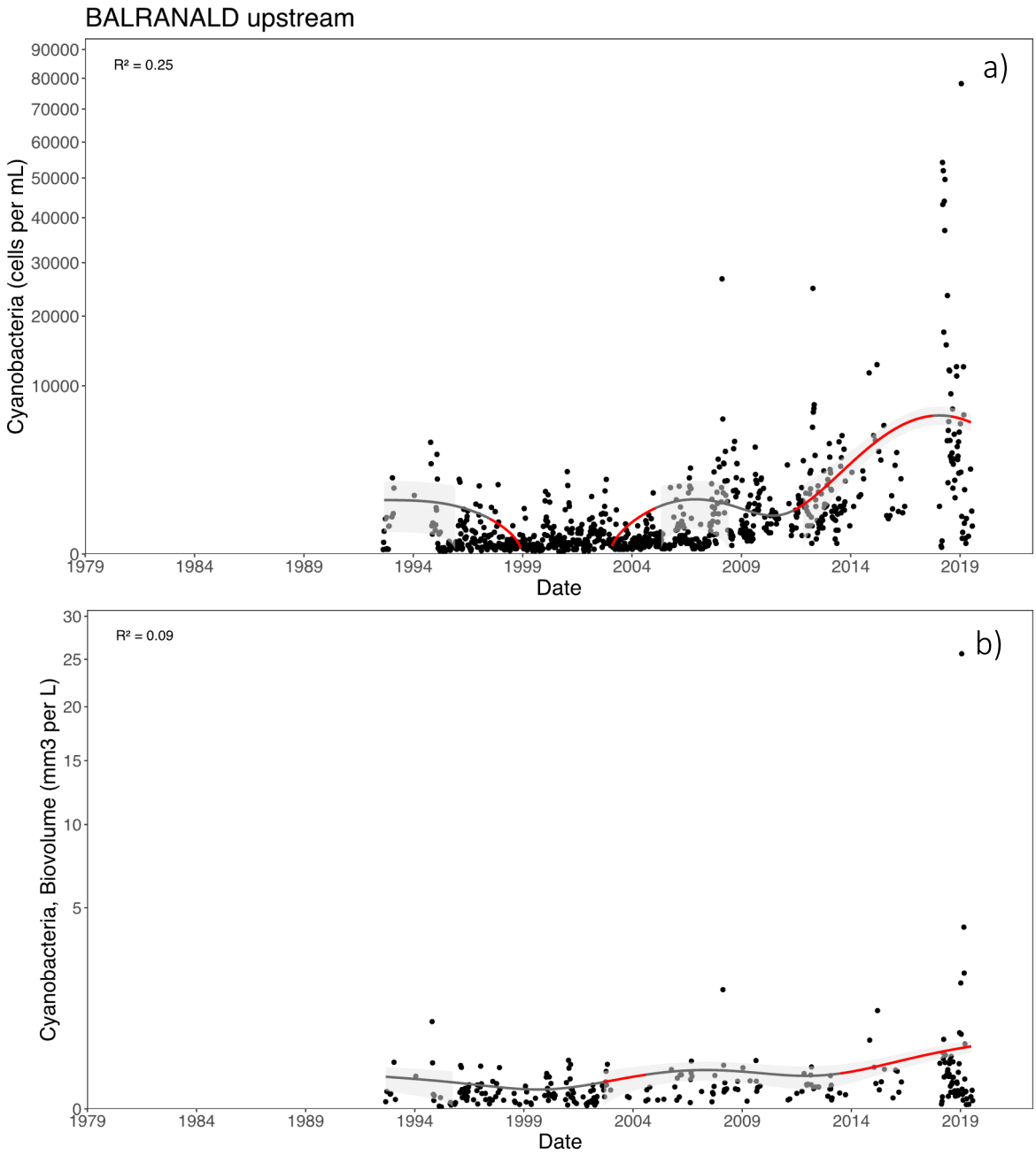


Figure 9. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass (mm^3 L^{-1}) data for Balranald, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

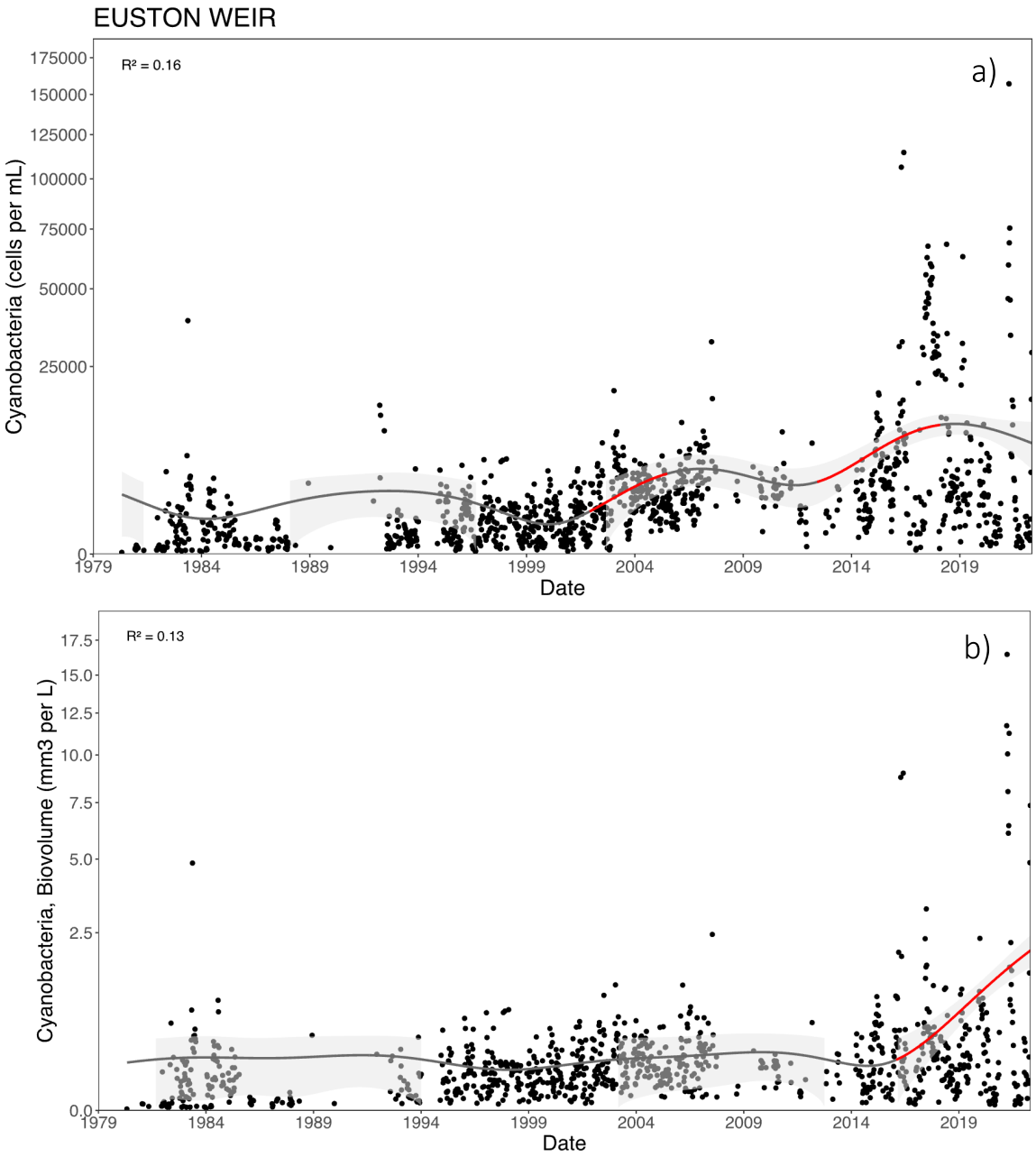


Figure 10. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass (mm^3 L^{-1}) data for Euston Weir, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

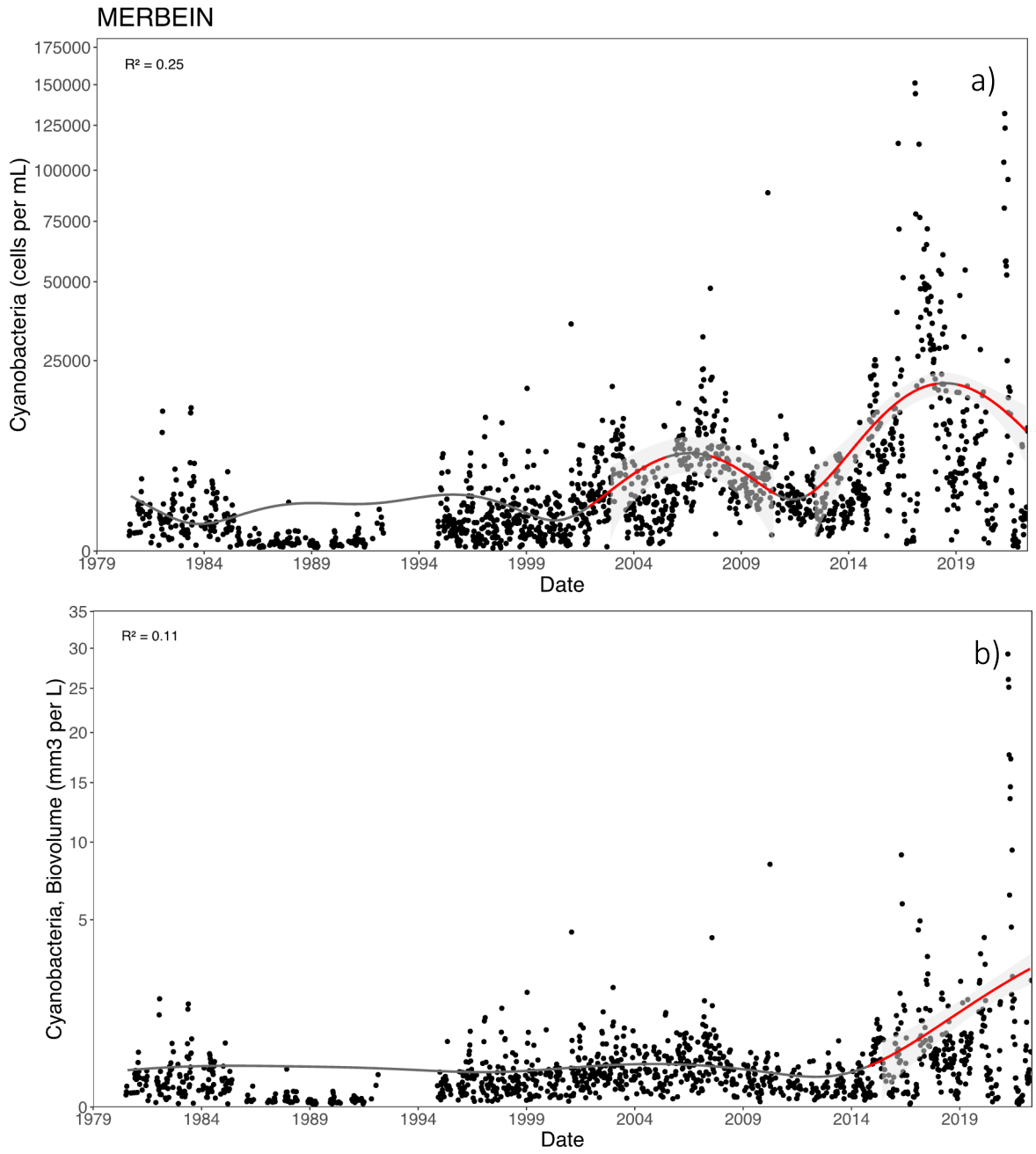


Figure 11. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass ($mm^3 L^{-}$) data for Merbein, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

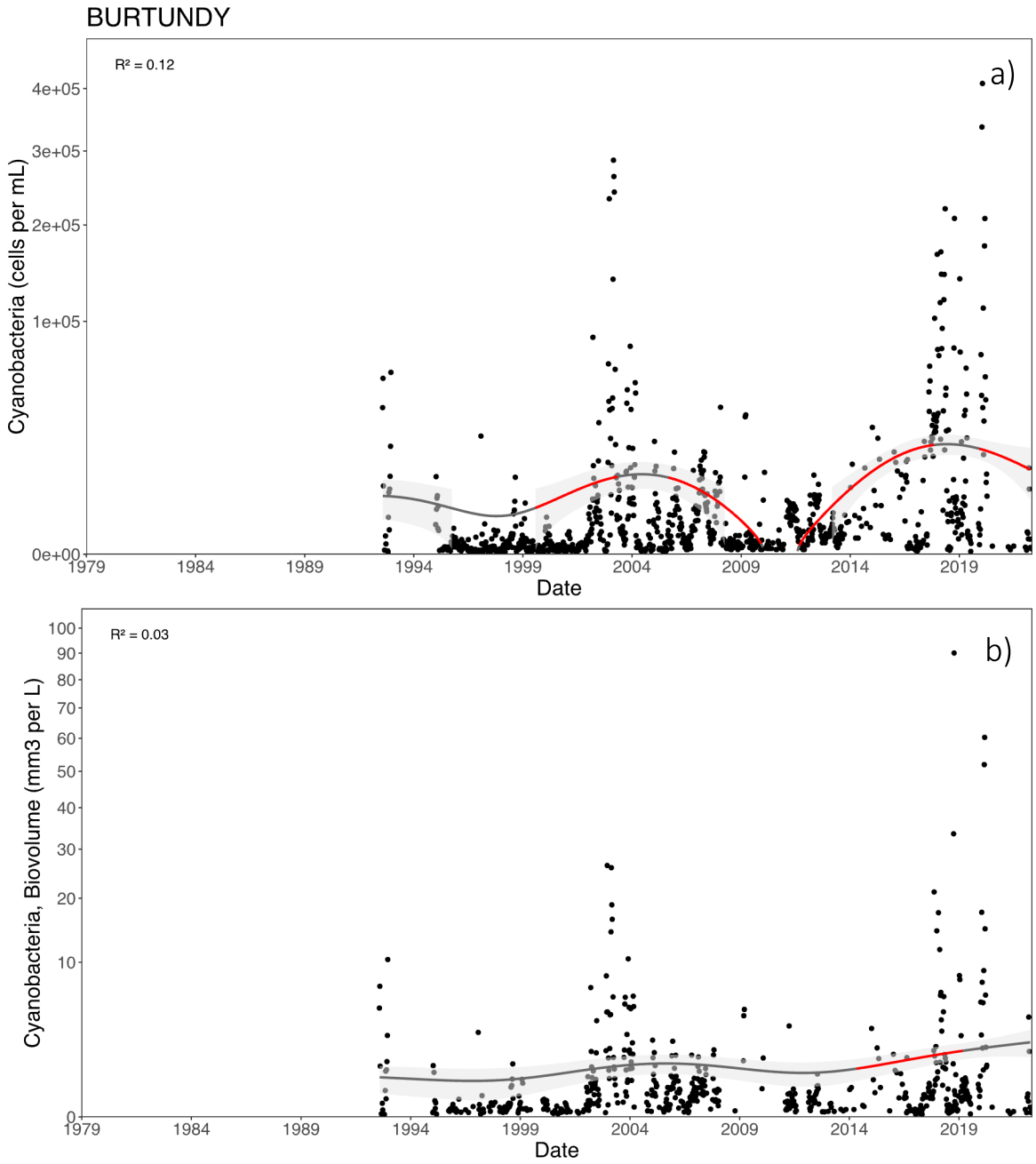


Figure 12. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass ($mm^3 L^{-1}$) data for Burtundy, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

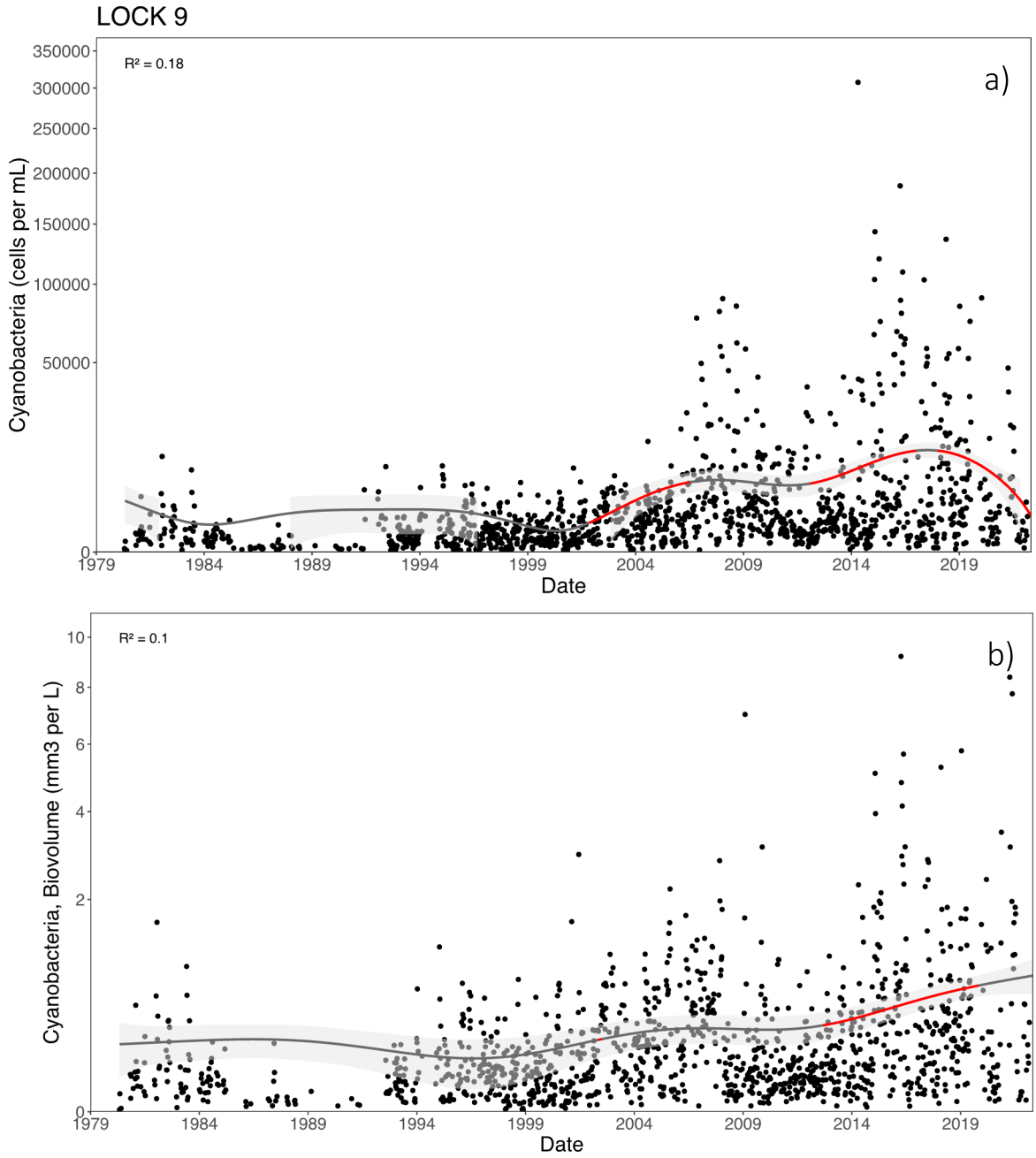


Figure 13. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass ($mm^3 L^{-1}$) data for Lock 9, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

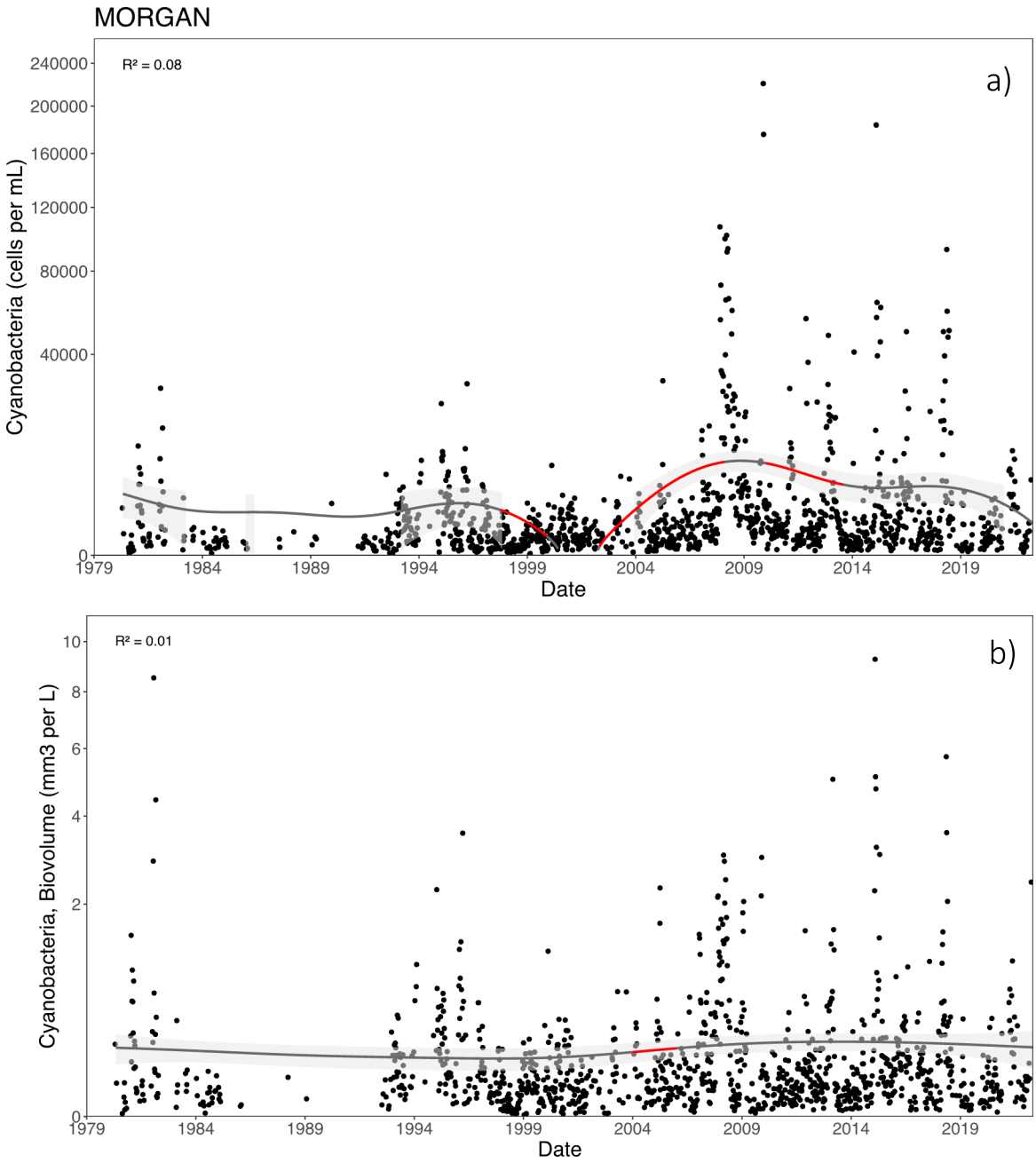


Figure 14. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL-1) and biomass (mm3 L-1) data for Morgan, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

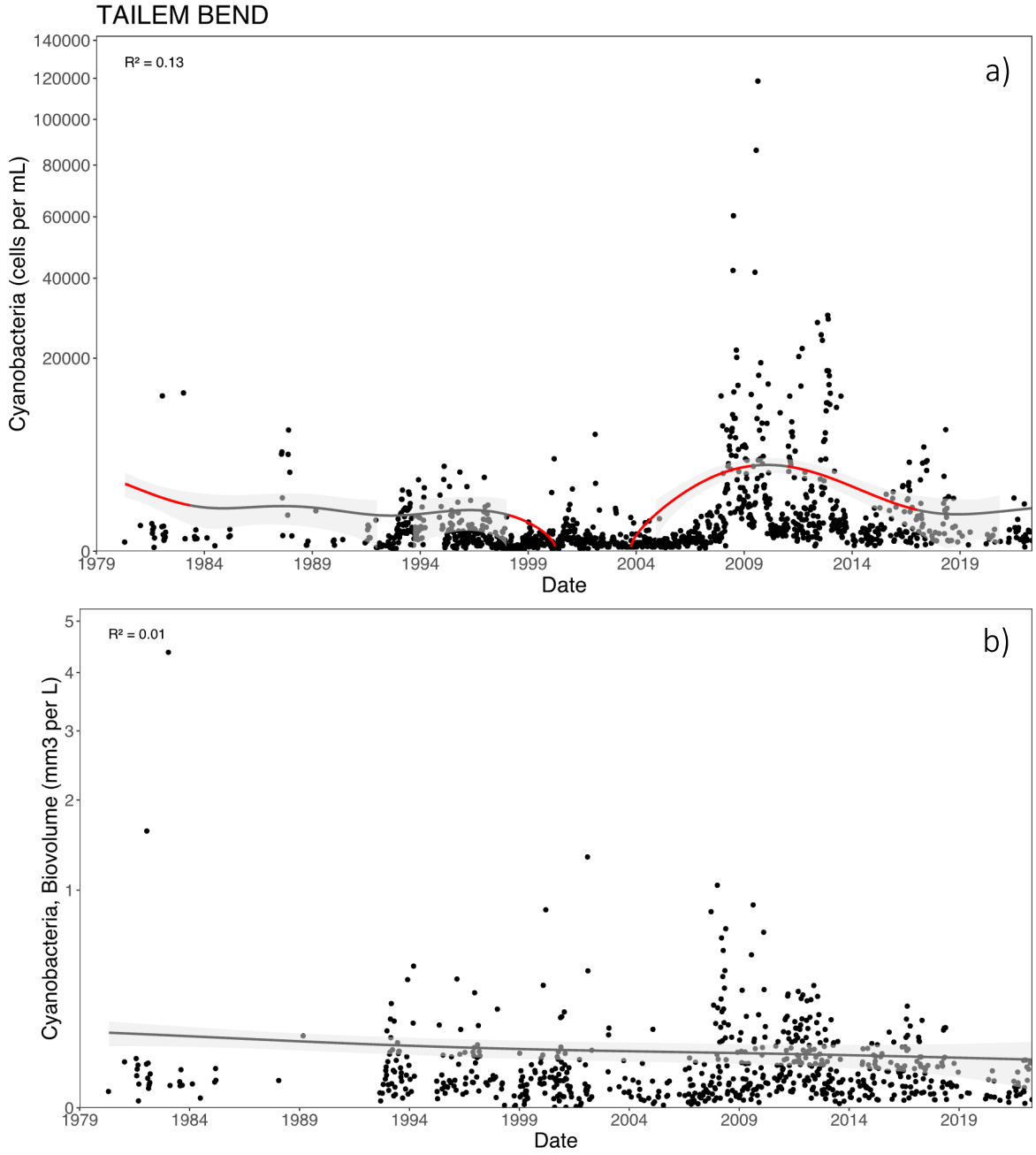


Figure 15. Generalised additive model (grey and red line; ± 95% confidence interval) predicting smoothed trends in the long-term, seasonally de-trended monthly average cyanobacteria cell count (cells mL^{-1}) and biomass (mm^3 L^{-1}) data for Tailem Bend, in comparison with the original, measured raw data (black dots). Colours for the model prediction line indicate periods of significant change (red sections) and no change (grey sections) in monthly mean concentrations, identified from analysis of additive model first derivatives. R^2 value indicates additive model fit to imputed monthly mean data.

QUEENSLAND SITES

Counts of cyanobacteria (cells mL^{-1}) and biomass ($mm^3 L^{-1}$) at Coolmunda Dam, and Leslie Dam in Queensland were generally shown to increase over the time period of data collected (2000-2022), however difference between time periods were not as strong as that shown for the River Murray sites (Figure 16) . Glenlyon Dam observed a decreasing trend in total cyanobacteria (cells mL^{-1}) over the whole study period, however, a significant increase after the Millennium Drought (Figure 16). Biomass ($mm^3 L^{-1}$) tended to follow patterns shown by cell counts, however during the Millennium drought counts were lower at Glenlyon, with a biomass increase indicating a shift in dominant species. Queensland sites tended to display more seasonal patterns as seen in the raw data (Appendix 1 & 2). Chinchilla weir and Beardmore Dam recorded overall decreases in cyanobacteria cell counts and biomass, however sample collection from these sites was sporadic (Figure 16 and Appendix 1 & 2).

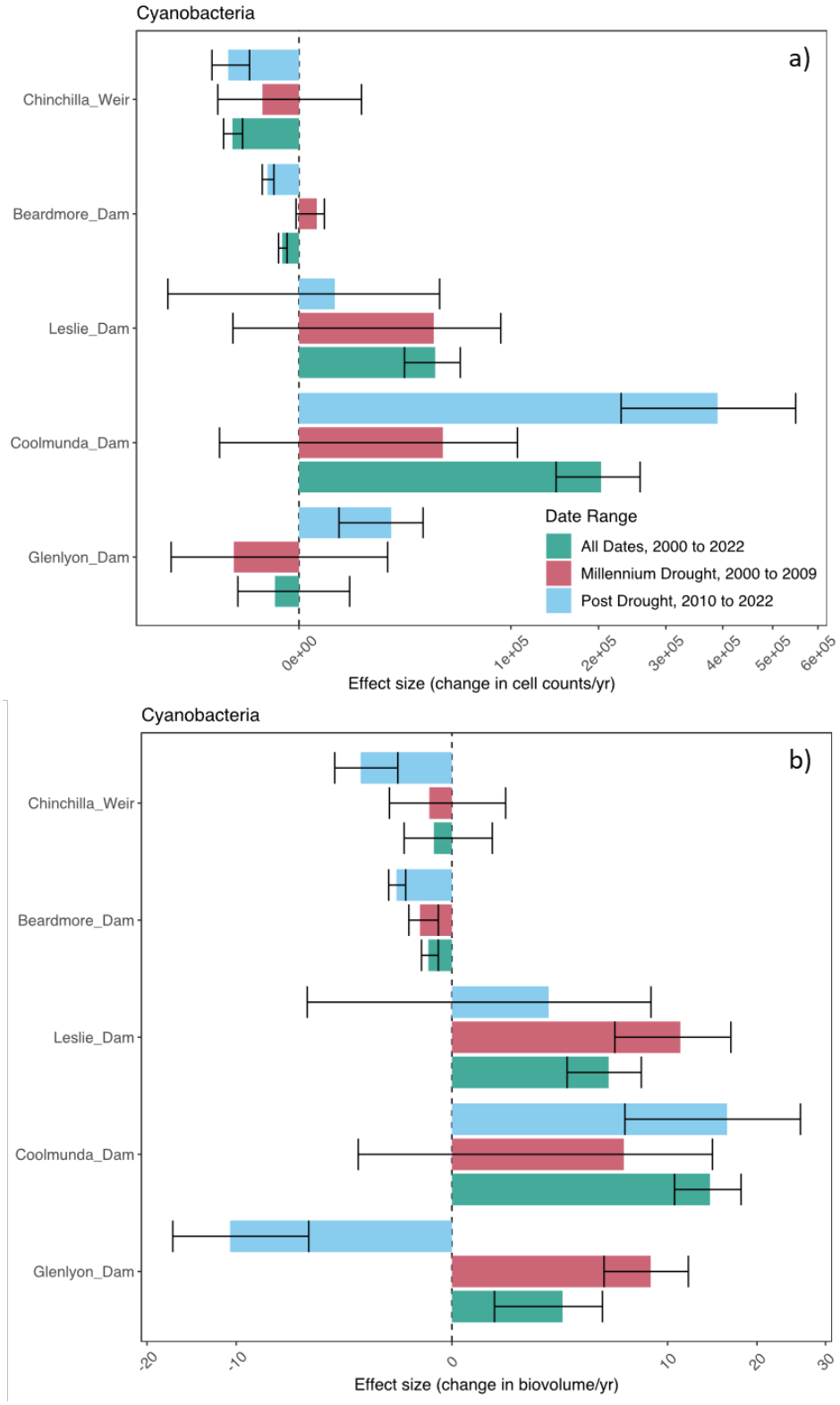


Figure 16. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Queensland sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset (2000 to 2022) and in context with the Millennium Drought (2000 to 2009). Sites are ordered from upstream to downstream on y-axis. Error bars are 1 standard deviation of the mean effect size.

VICTORIAN SITES

Victorian sites showed contrasting results depending on the dataset analysed, and the period selected (Figure 17-18). Regarding the dataset which contained biomass data from 1998-2019, biomass of cyanobacteria (mm^3 L^{-1}) were shown to increase overall at all but two sites (Newlyn Reservoir and Tullaroop Reservoir) (Figure 17). Biomass of potentially toxic cyanobacteria was significantly higher over the last 9-year period (2010-2019) at Cairn Curran Reservoir, Waranga Basin, Lake Nagambie/Goulburn Weir, and higher within Newlyn Reservoir during the Millennium Drought. Cairn Curran reservoir recorded a decrease in biomass during the Millennium Drought period, while Newlyn reservoir, and Lake Eildon have recorded a decrease over the post-drought period 2010-2019 (Figure 17). All other sites recorded no significant difference between the Millennium Drought and post drought periods (Figure 17).

When looking at the second dataset, which contained cell counts and biomass data, trends in cell counts of cyanobacteria (cells mL^{-1}) generally, showed decreases in cyanobacteria, with majority of sites showing a decrease over the period of data collection with the exception of Hepburns Lagoon, Waranga Basin, and Lake Nagambie (Figure 18). During the Millennium Drought however, cyanobacteria (cells mL^{-1}) were significantly increased at all, but three sites (Tullaroop Reservoir, Lake Eppalock and Greens Lake). Tullaroop Reservoir was the only site to show a significant increase over the last 12 years, post drought. Conversely, biomass showed contrasting results to abundance data (cells mL^{-1}) at most sites, with decreases during the Millennium Drought and increases post drought. This indicates a shift in dominance of species over the period had occurred, with genera having smaller cell volume dominant over the Millennium Drought period compared to the other period (Figure 18). Differences in trends between the two datasets and time periods analysed, highlights the influence period or the parameter (cell counts vs biomass) chosen for analysis have on overall trend results.

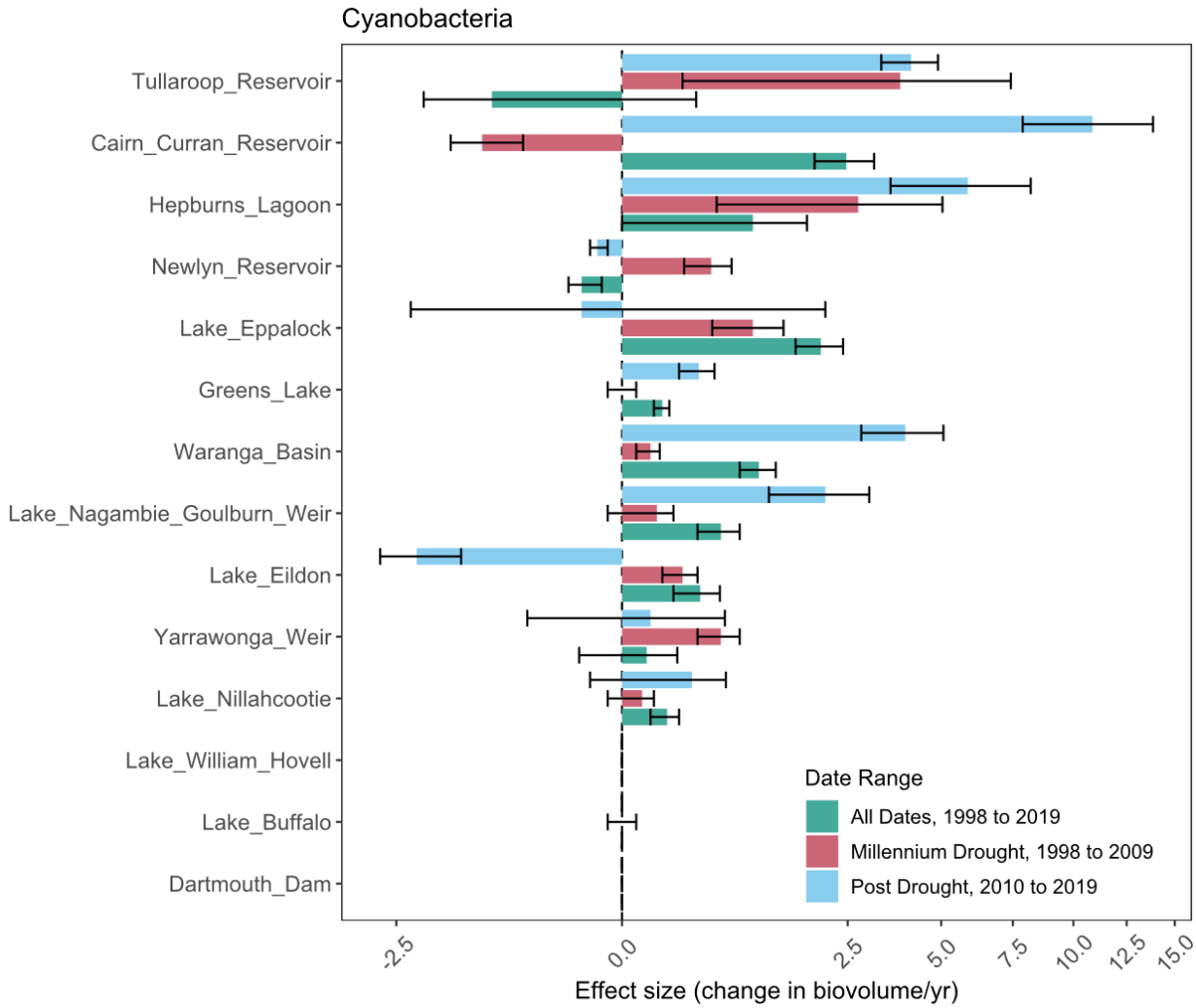


Figure 17. Linear trends in annual changes of biomass ($mm^3 L^{-1}$) of potentially toxic Cyanobacteria. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset (1998 to 2019) and in context with the Millennium Drought (1998 to 2009). Sites are ordered from upstream to downstream on y-axis. Error bars are 1 standard deviation of the mean effect size.

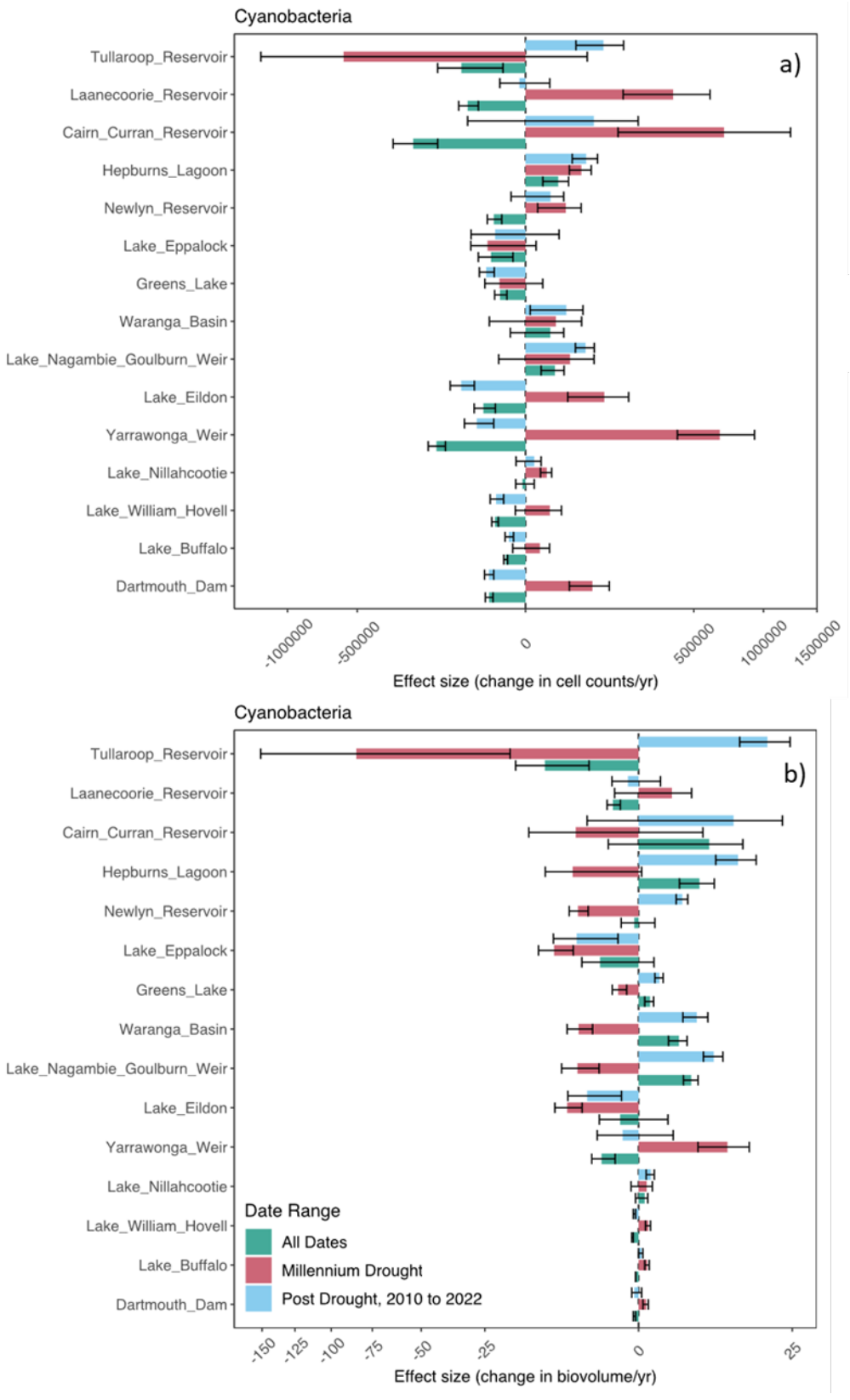


Figure 18. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Victorian sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset (2004 to 2019) and in context with the Millennium Drought (2004 to 2009). Sites are ordered from upstream to downstream on y-axis. Error bars are 1 standard deviation of the mean effect size.

NEW SOUTH WALES SITES

The New South Wales dataset contained data from the most sites however, from the least amount of time, with data only available from 2015-2022. Data is grouped by sites in similar area, catchment, or river system below. Sites within the Wyangala region and Wyangala Dam all recorded increases in cyanobacteria (cells mL^{-1}) except for Carcoar Dam (Figure 19). Within the Macquarie River catchment most sites showed no change (error bars overlapping 0) or a slight decrease over the 7-year period except for Burrendong Dam at the dam wall which recorded an increase (Figure 20). Within the Split Rock and Keepit catchments mixed results were shown (Figure 21). Chaffey Dam recorded increases at all three monitored sites (Figure 20). Copeton Dam consistently recorded increases at all four sites within the dam however no change was reported downstream from the dam (Figure 22). Pindar Dam and Lake Inverell all recorded inconclusive results (Figure 22). Overall, the Edward-Wakool system and the Murrumbidgee catchment recorded contrasting results depending on site (Figures 23 and 24). Sites with the Darling River catchment mostly tended to record decrease over time except for the Barwon River at Mungindi and at Tolarno (Figure 25). Sites along the River Murray also recorded contrasting results over this period in regard to cell counts but generally increases in biomass (Figure 26). Menindee Lakes also tended to record inconclusive results with error bars spanning both the positive and negative effect size areas (Figure 27). Lake Cargelligo and Lake Brewster tended to record increases in cyanobacteria however the Lachlan River tended to record decreases (Figure 28). Overall trends in cyanobacteria abundance (cells mL^{-1}) and biomass (mm^3 L^{-1}) tend to be site, river, or catchment specific with no clear pattern over the seven-year (2015-2022) period.

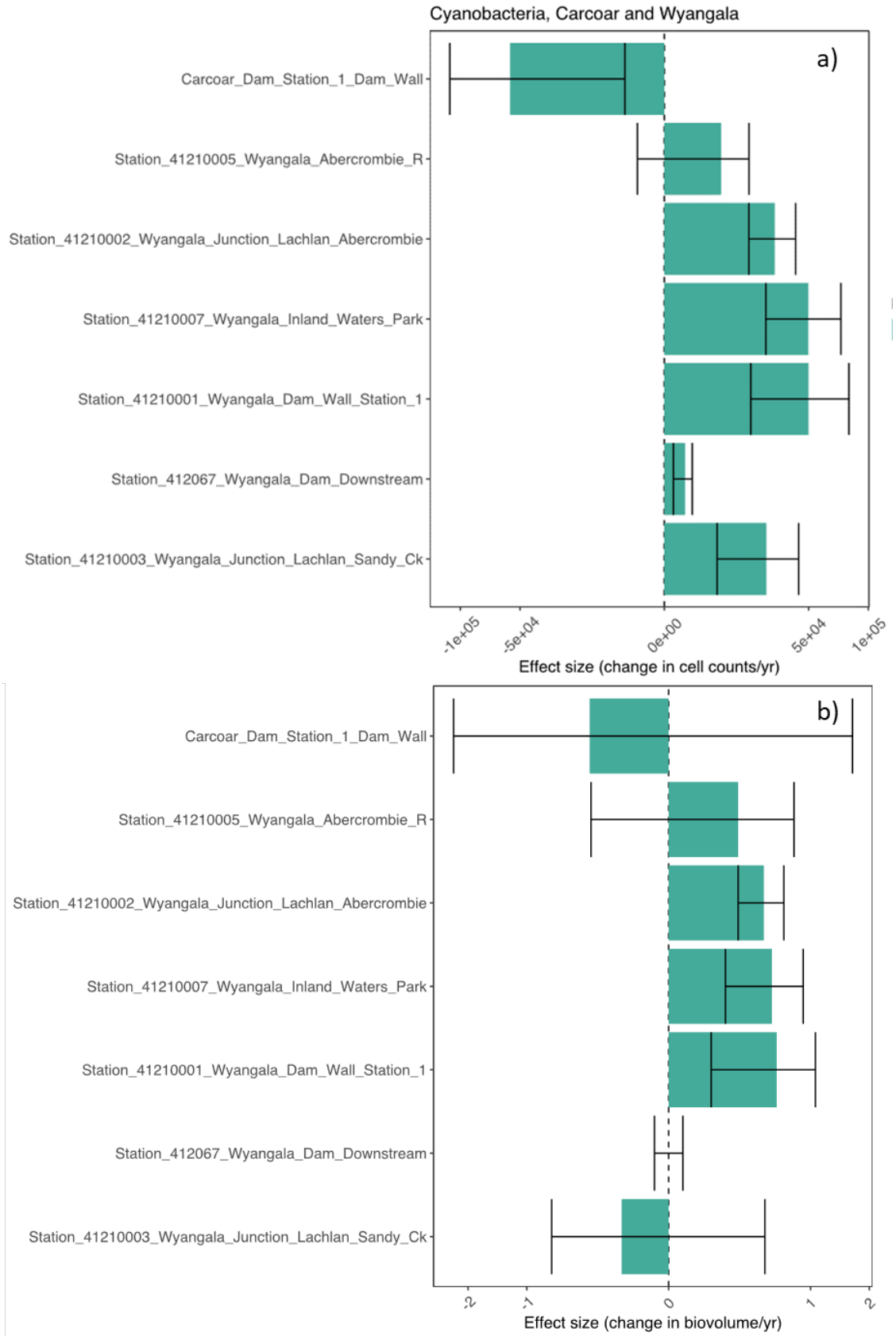


Figure 19. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Carcoar and Wyangala sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

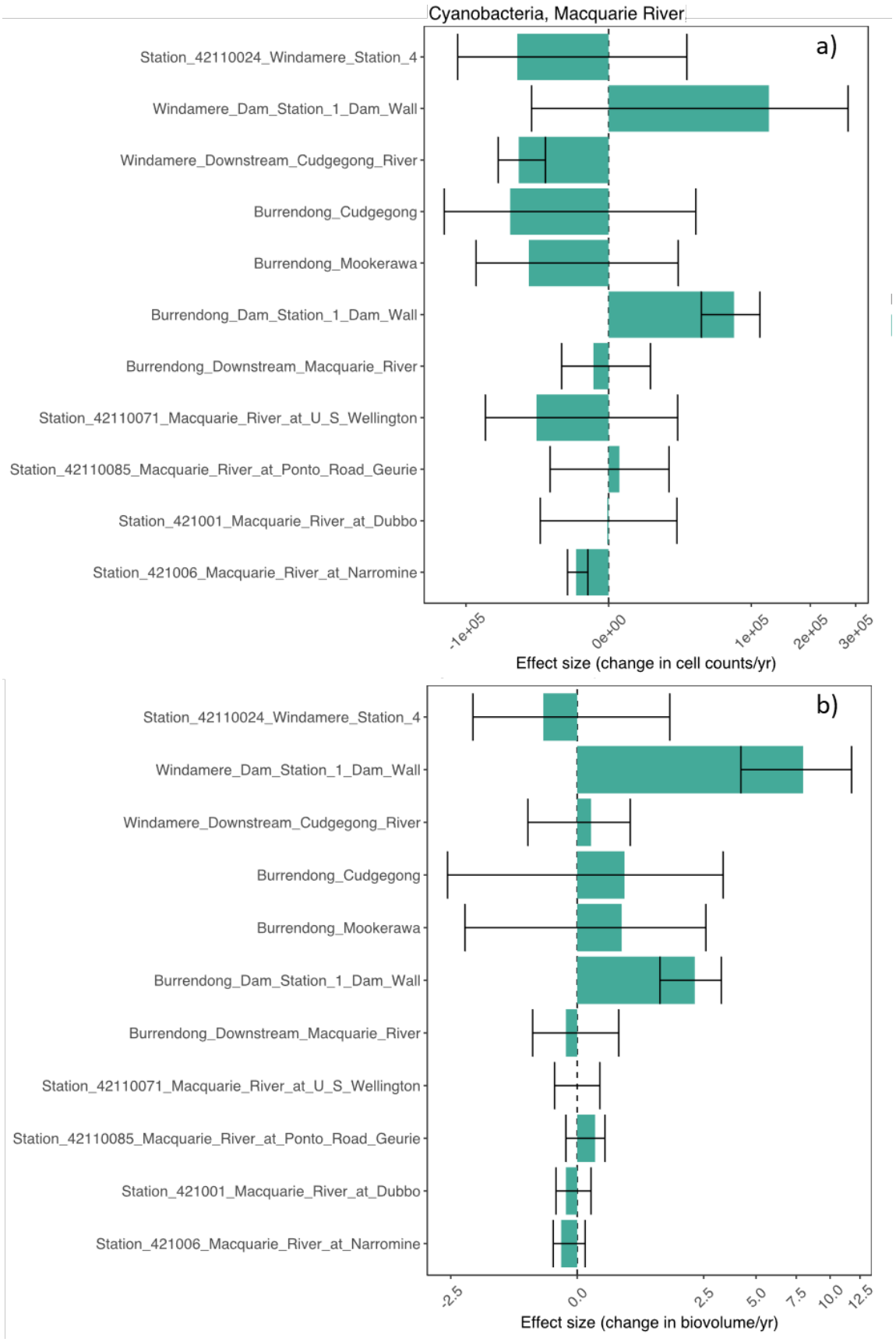


Figure 20. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Macquarie River sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

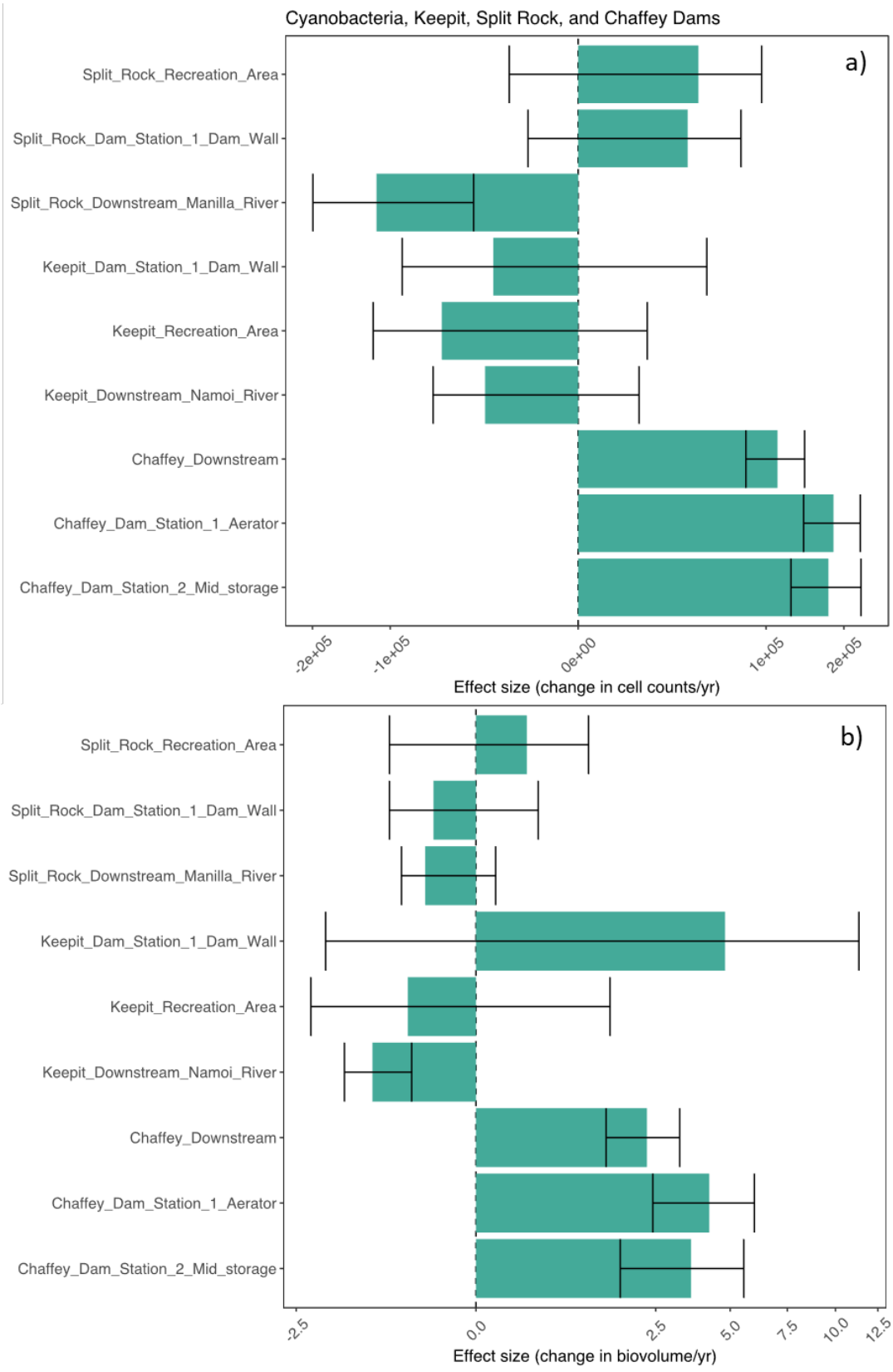


Figure 21. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Keepit, Split Rock and Chaffey Dam sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

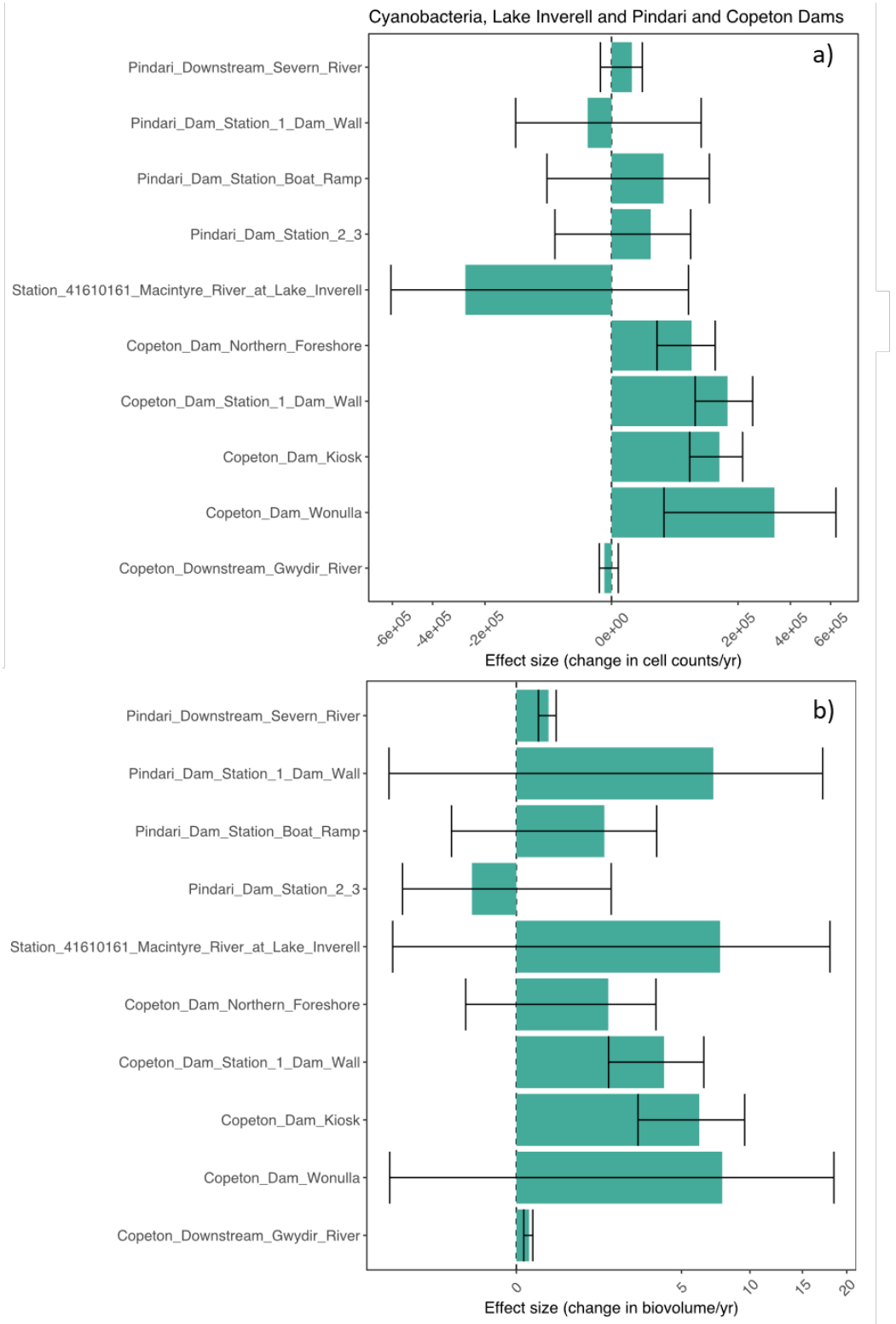


Figure 22. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Lake Inverell, and Pindari and Copeton Dam sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

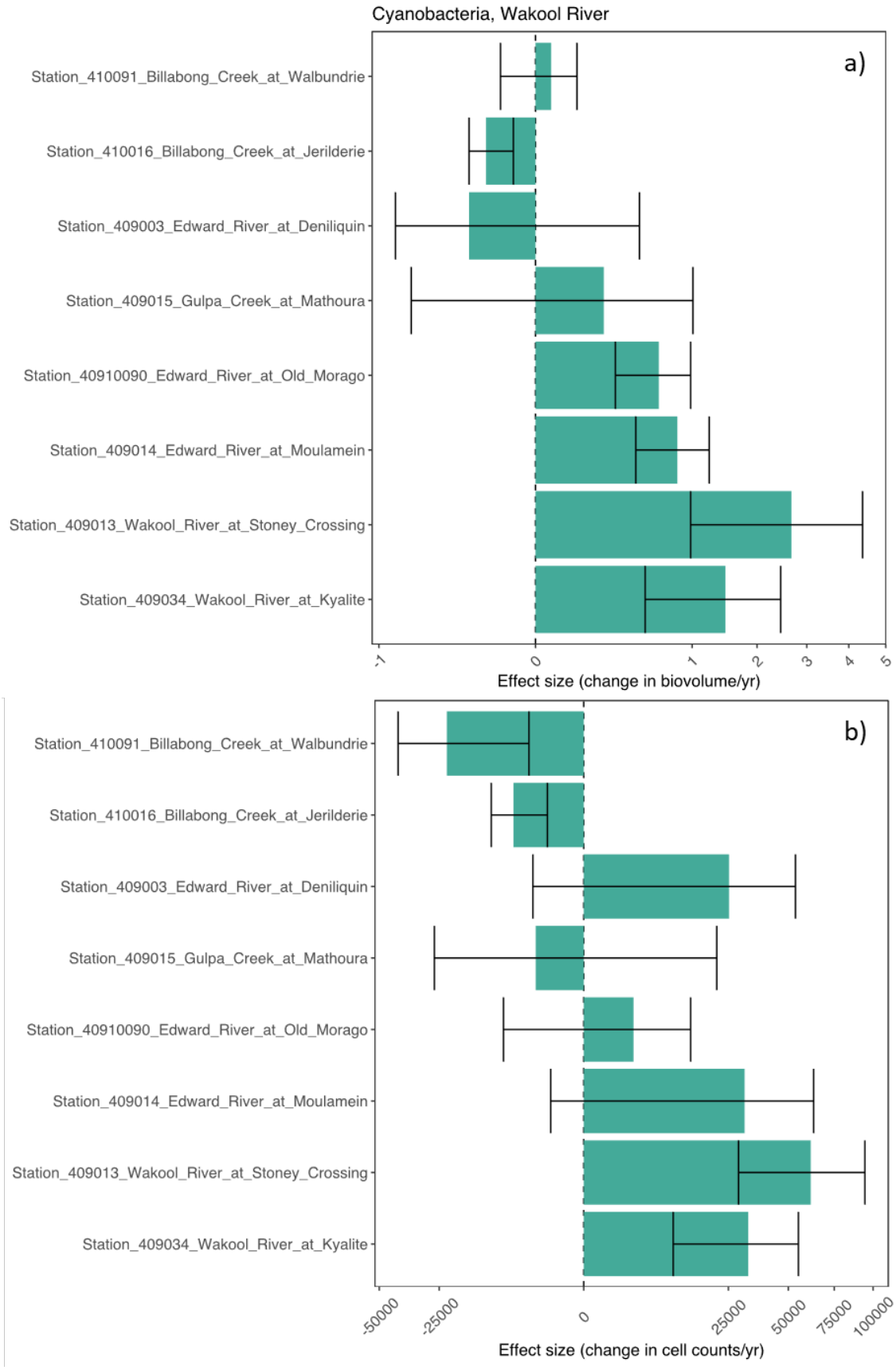


Figure 23. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Edward-Wakool sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

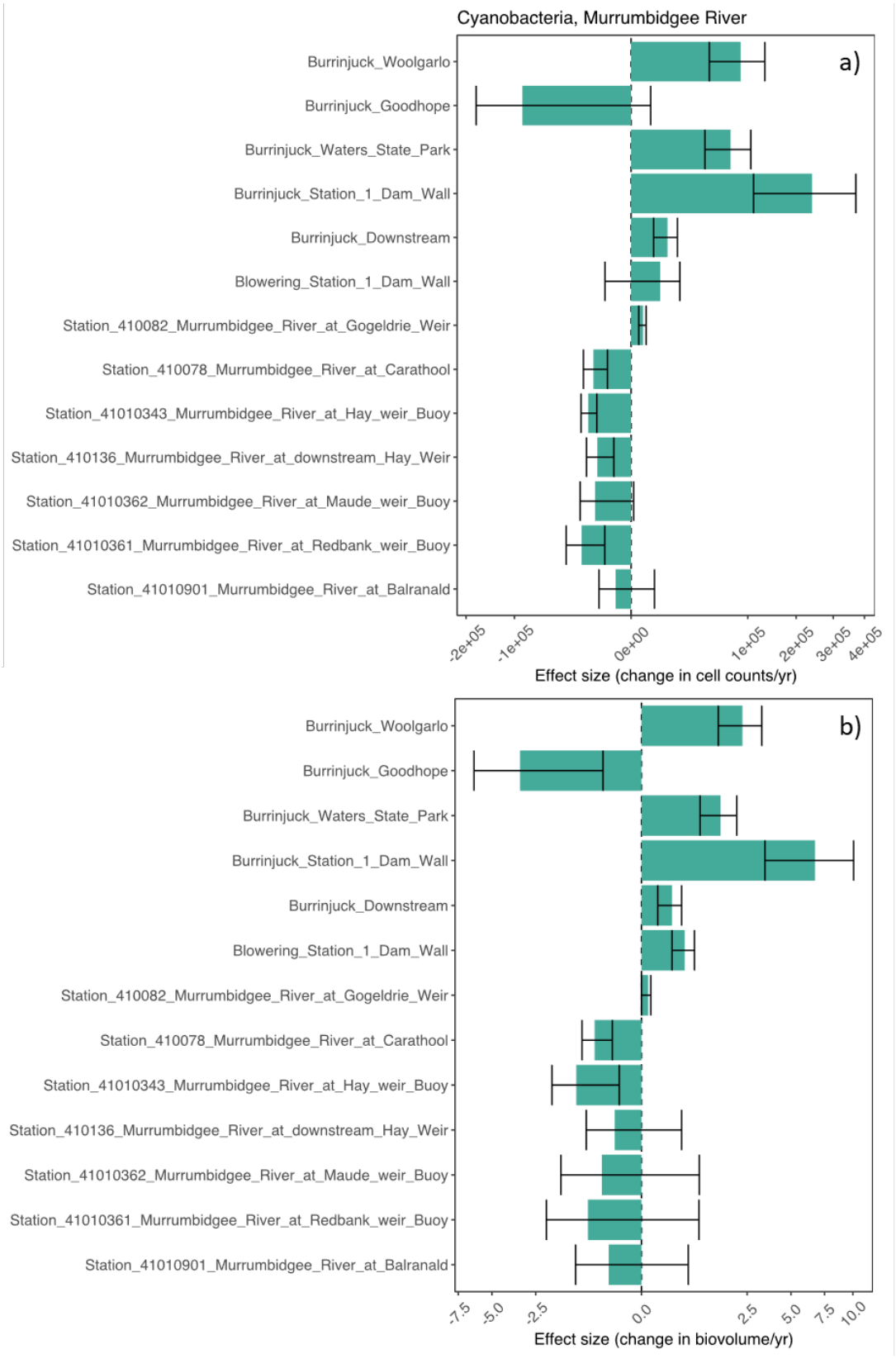


Figure 24. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Murrumbidgee sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

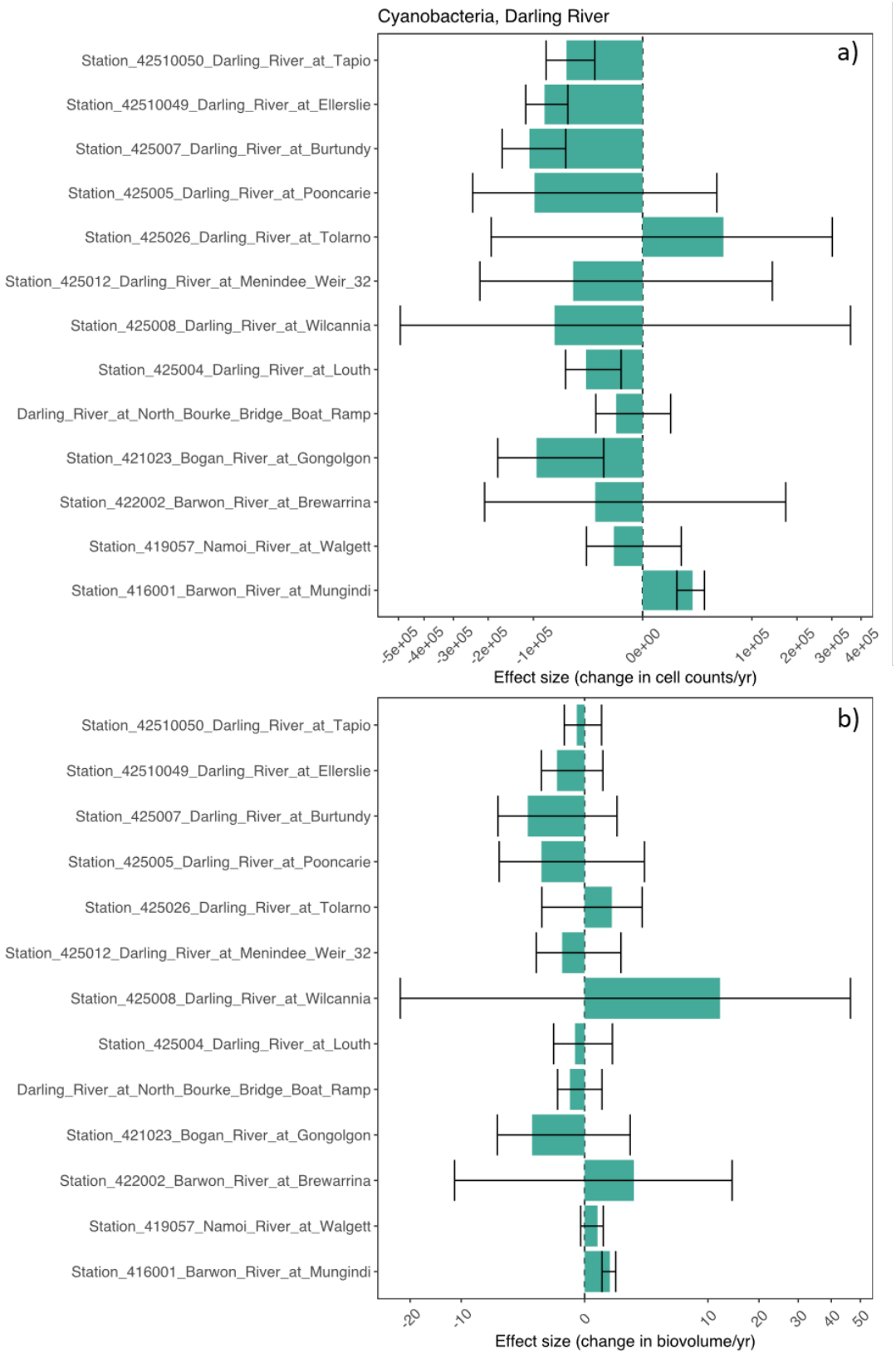


Figure 25. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass ($mm^3 L^{-1}$) in Darling River sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

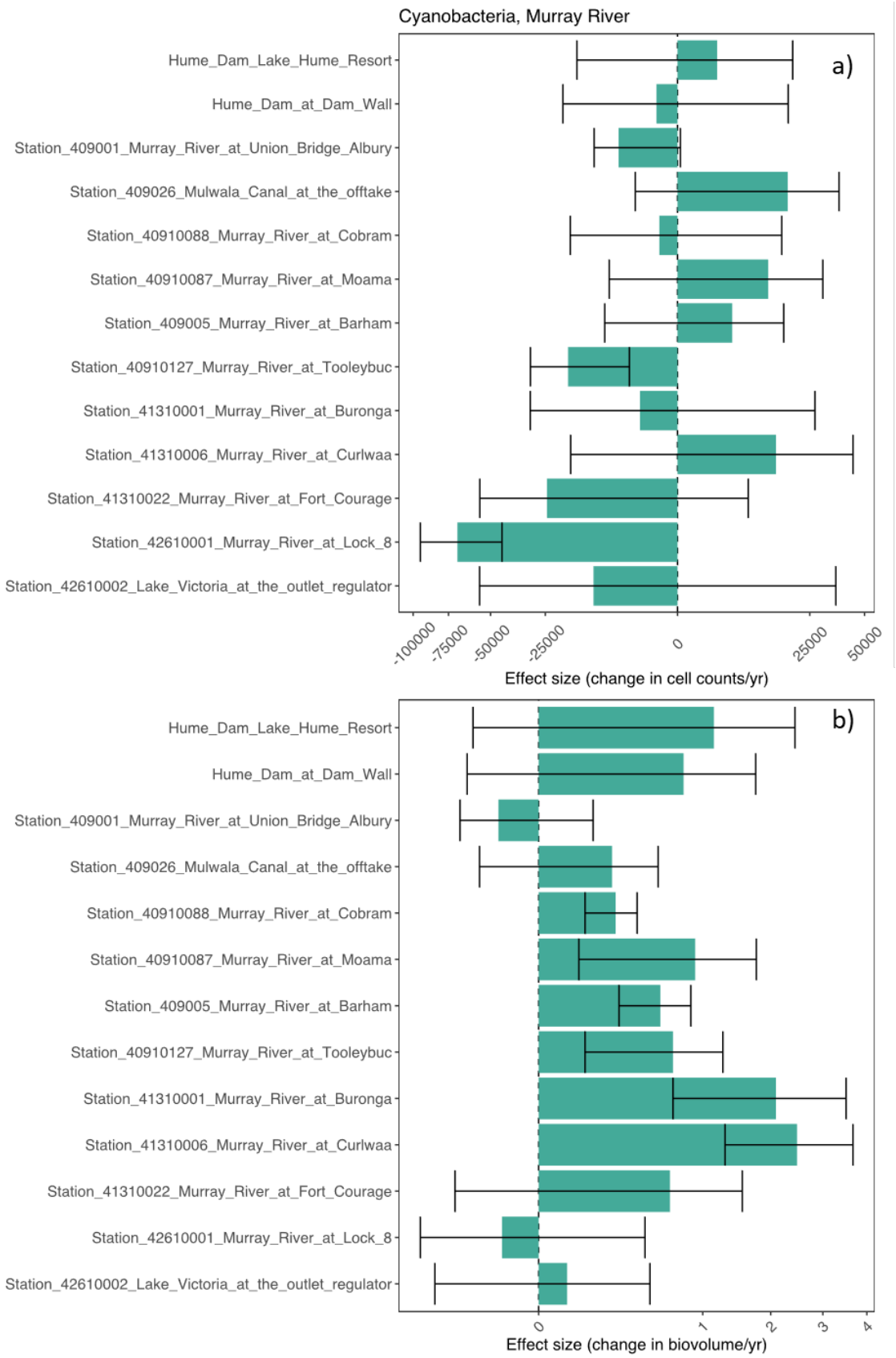


Figure 26. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in River Murray sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

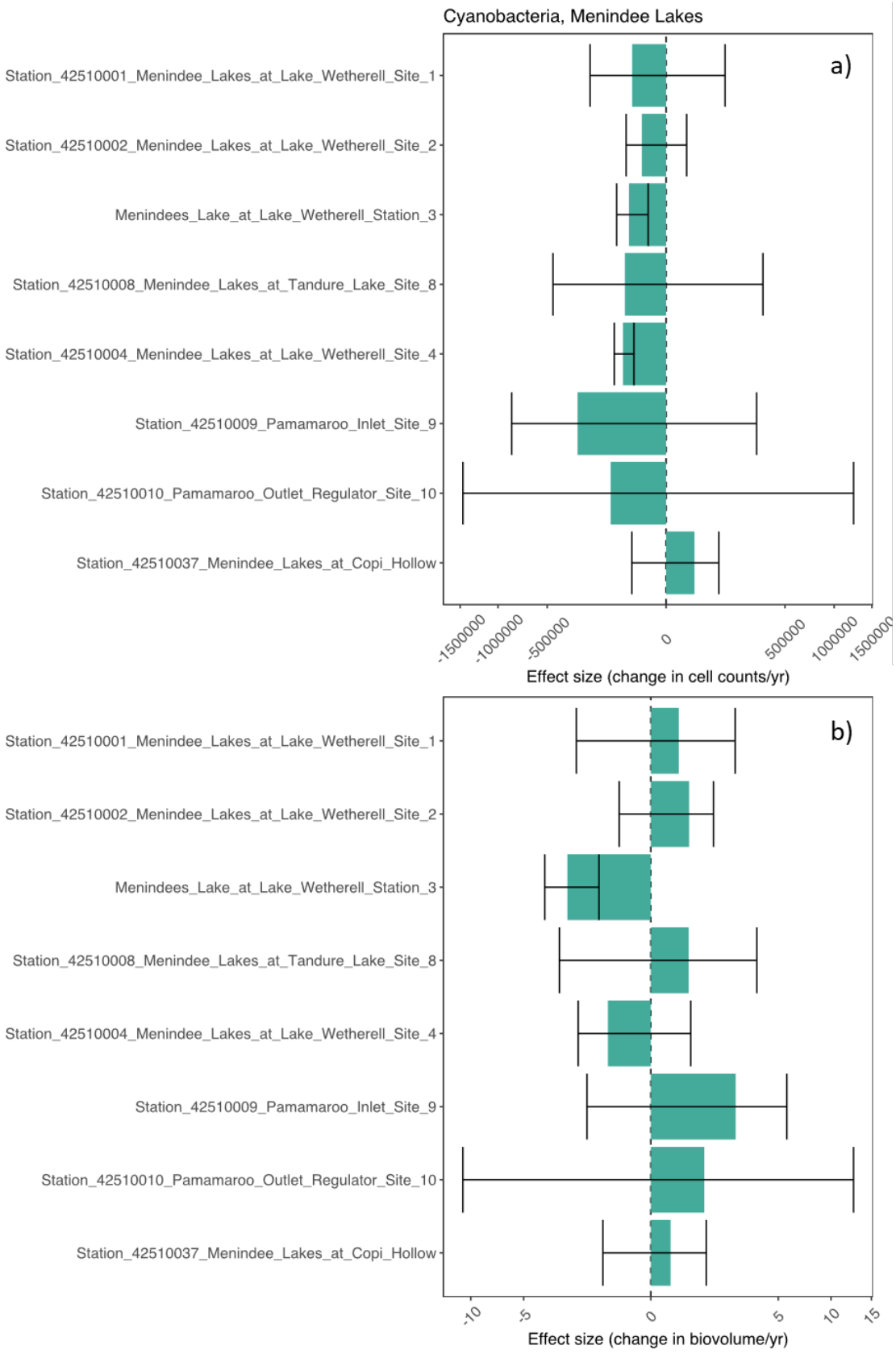


Figure 27. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass (mm^3 L^{-1}) in Menindee sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

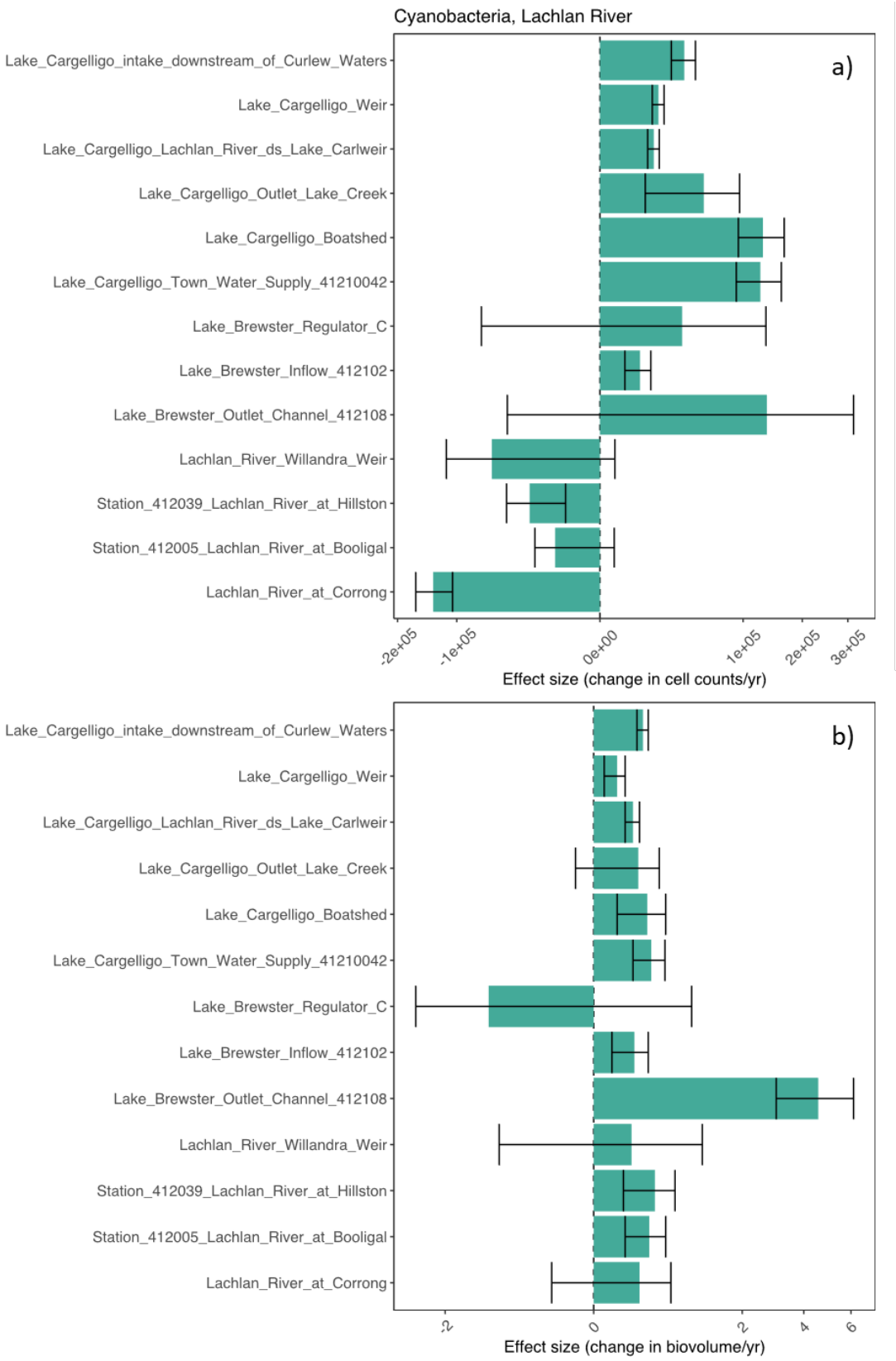


Figure 28. Linear trends in annual changes of potentially toxic Cyanobacteria (A) cell counts (cells mL^{-1}) and (B) biomass ($mm^3 L^{-1}$) in Lachlan River sites. Trends were determined for the entire period of the dataset from 2015 to 2022. Error bars are 1 standard deviation of the mean effect size.

CHANGES IN CELL COUNTS OF KEY CYANOBACTERIA OVER TIME

RIVER MURRAY

Chrysosporum was found to be increasing at all sites within the River Murray over time, with the highest counts recorded during the period 2010-2022 at most sites (Figure 29). In contrast, the abundance of *Dolichospermum* within the River Murray decreased at the top two sites (Heywoods, and Yarrawonga) and bottom sites (Morgan and Tailem Bend), with increases from Swan Hill through to Euston Weir recorded (Figure 29). *Raphidiopsis* previously *Cylindrospermopsis* increased significantly at all sites except Balranald upstream during the Millennium Drought and has also greatly increased in abundance at Burtundy over the last 12 years (2010-2022) (Figure 30). *Microcystis* was found to increase at the top four sites (Heywoods, Yarrawonga, Torrumbarry, Swan Hill), Merbein and Euston Weir during the Millennium Drought, with significant decreases at Euston Weir and Tailem Bend over the last 12 years (2010-2022) (Figure 30) suggesting drought conditions favour these taxa (Bowling et al. 2016). *Chrysosporum* is more adaptable across a variety of different flow regimes and has been known to bloom under relatively high flow conditions such as in 2016, 2020-2021 and 2021-22. Generalised additive models of these key genera are provided for each taxa in Appendix 3.

QUEENSLAND SITES

At the three most monitored Queensland sites (Coolmunda Dam, Leslie Dam and Glenlyon Dam) *Chrysosporum* and *Dolichospermum* was found to increase over the Millennium Drought, with decreases in these taxa generally reported post drought (except *Chrysosporum* in Glenlyon) (Figure 31). Trends in regard to *Microcystis* and *Raphidiopsis* seem to be site specific with Glenlyon generally recording an increase in *Microcystis* and Coolmunda *Raphidiopsis* over all periods, while Coolmunda Dam recorded increases in *Microcystis* over the post drought period and Leslie Dam during the drought (Figure 32).

VICTORIAN SITES

Increases in four main cyanobacteria taxa of interest in Australian waters could not explain the increases in cyanobacterial abundance (cells mL^{-1}) recorded during the Millennium Drought in Victorian waterways. *Chrysosporum* was only recorded from 5 out of the 15 Victorian sites with increases present at all sites over the period with decreases generally recorded during the Millennium Drought (Figure 33). Decreases in *Dolichospermum* were generally recorded at all sites over the period 2004-2022 except for Hepburns Lagoon and Lake Nagambie. Five sites recorded increase in *Dolichospermum* post-drought (Figure 33). Similar to *Chrysosporum, Raphidiopsis* was only detected at five sites, but cell counts were generally low for this taxa. This is not surprising given it is said to be a tropical species. Microcystis also generally decreased over the whole period except at Cairn Curran Reservoir and Lake Nillahcootie. Tullaroop, Cairn Curran Reservoir and Hepburns Lagoon all recorded increases post-drought (Figure 34). Taxa that were shown to significantly increase at all or majority sites during the drought included *Synechococcus, Aphanocapsa,* and *Cyanodictyon* (Appendix 3).

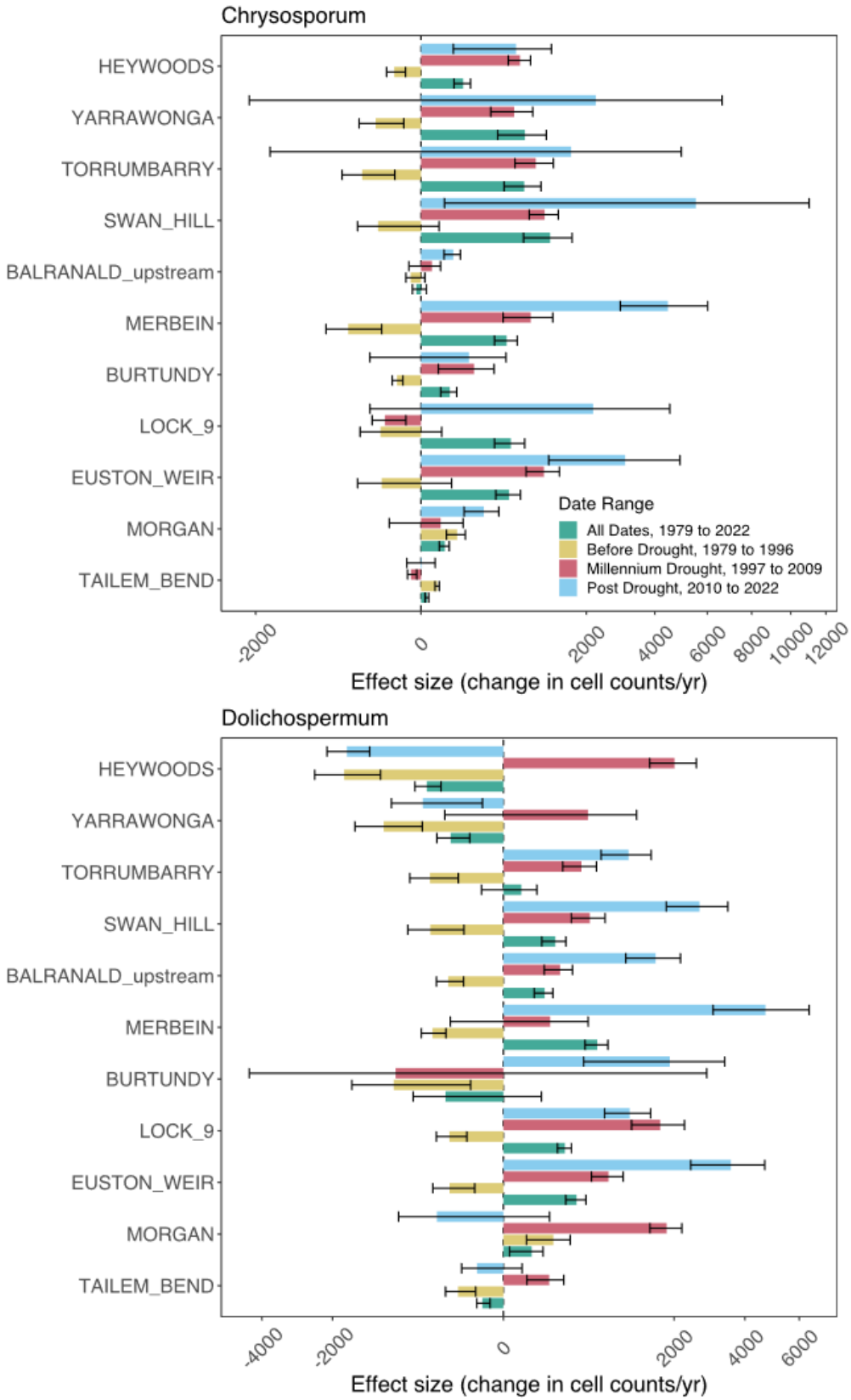


Figure 29. Linear trends in cell counts (cells mL^{-1}) of Chrysosporum and Dolichospermum. These bar graphs show the linear trends in cell counts per year for MDBA River Murray monitoring sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset (1979 to 2022) and in context with the Millennium Drought (1997 to 2009). Error bars are 1 standard deviation of the mean effect size.

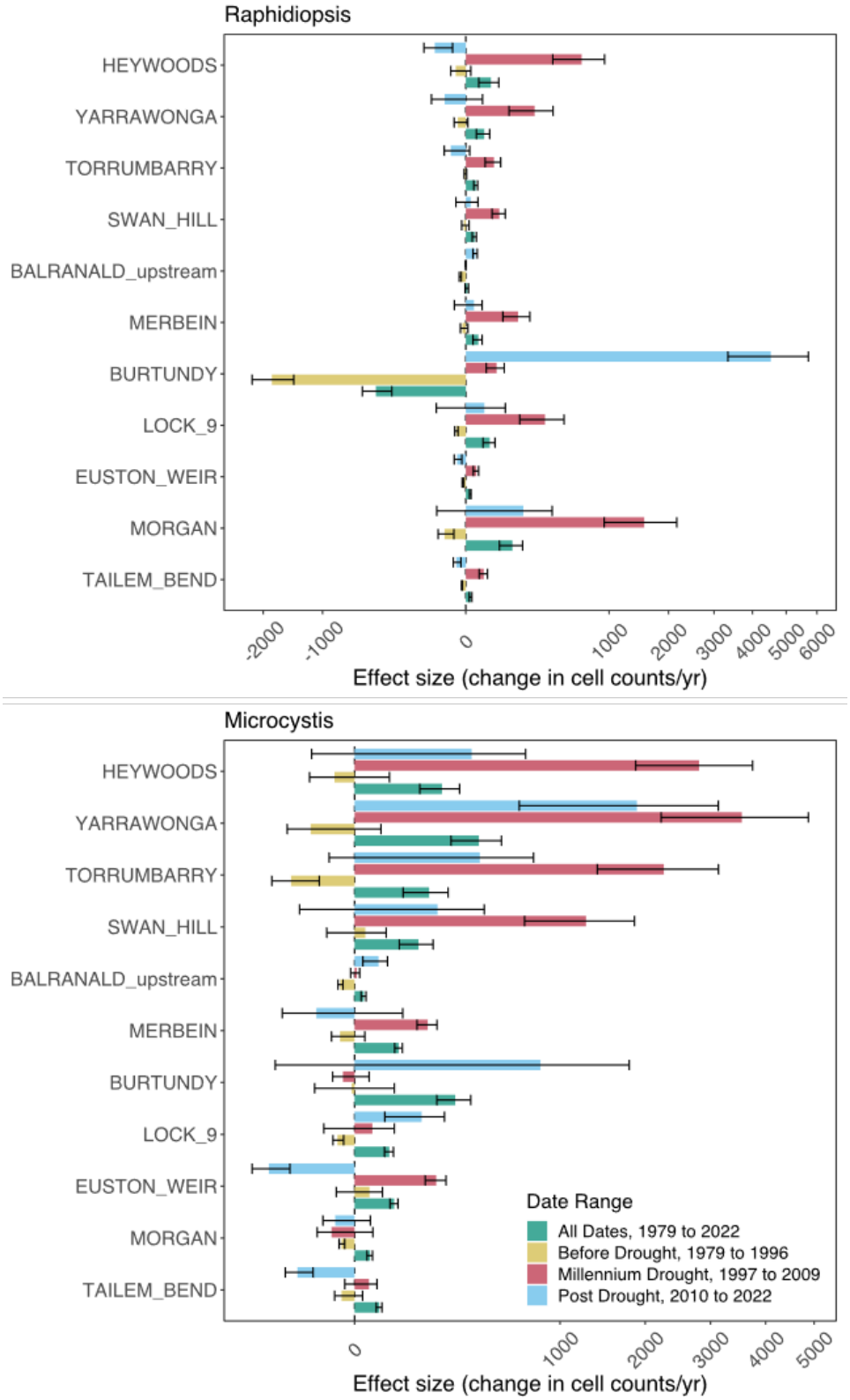


Figure 30. Linear trends in cell counts (cells mL^{-1}) of Raphidiopsis (Cylindrospermopsis) and Microcystis. These bar graphs show the linear trends in cell counts per year for MDBA River Murray monitoring sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset (1979 to 2022) and in context with the Millennium Drought (1997 to 2009). Error bars are 1 standard deviation of the mean effect size.

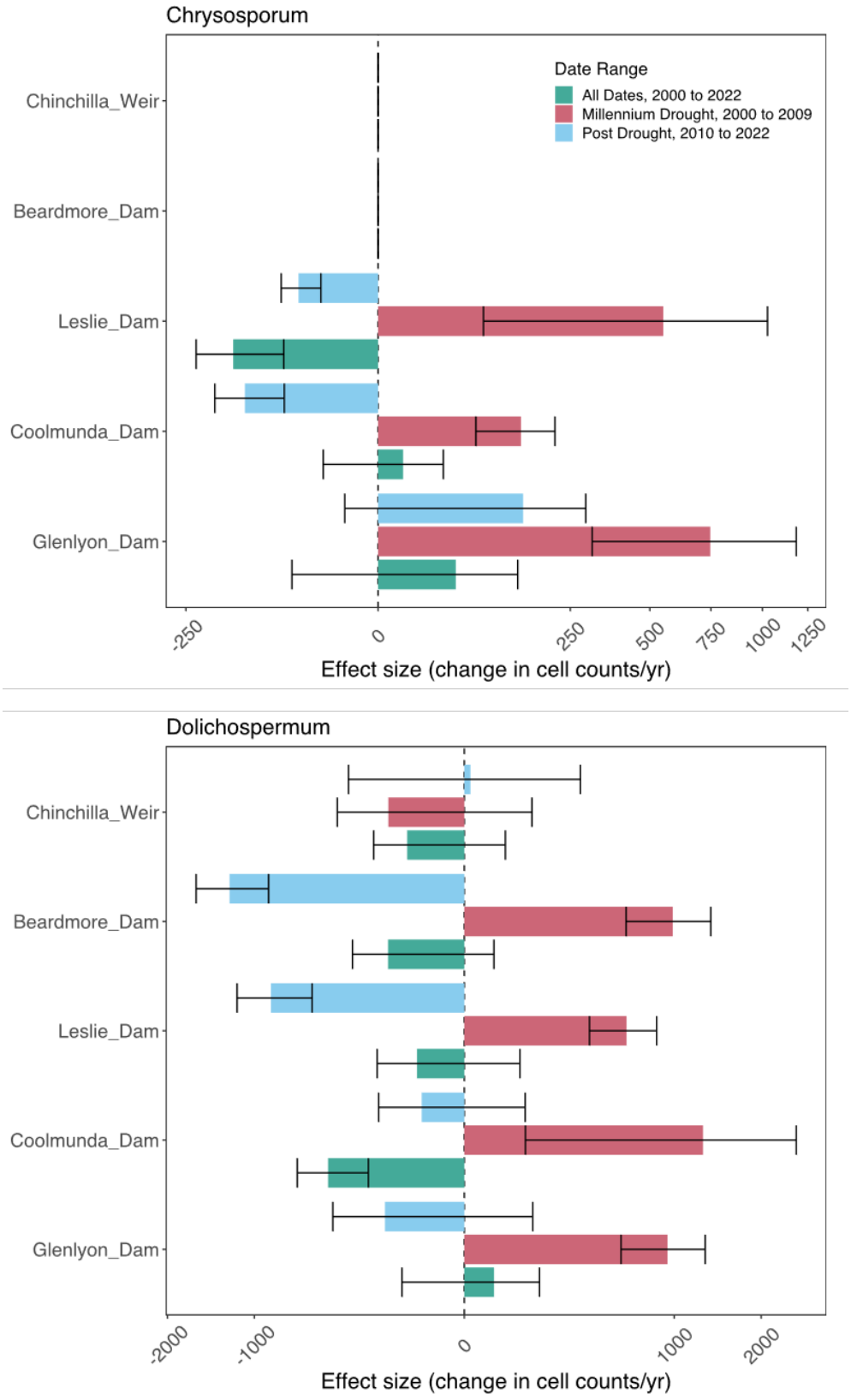


Figure 31. Linear trends in in cell counts (cells mL^{-1}) of Chrysosporum and Dolichospermum in Queensland Murray-Darling Basin monitoring sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset (2000 to 2022) and in context with the Millennium Drought (2000 to 2009). Error bars are 1 standard deviation of the mean effect size.

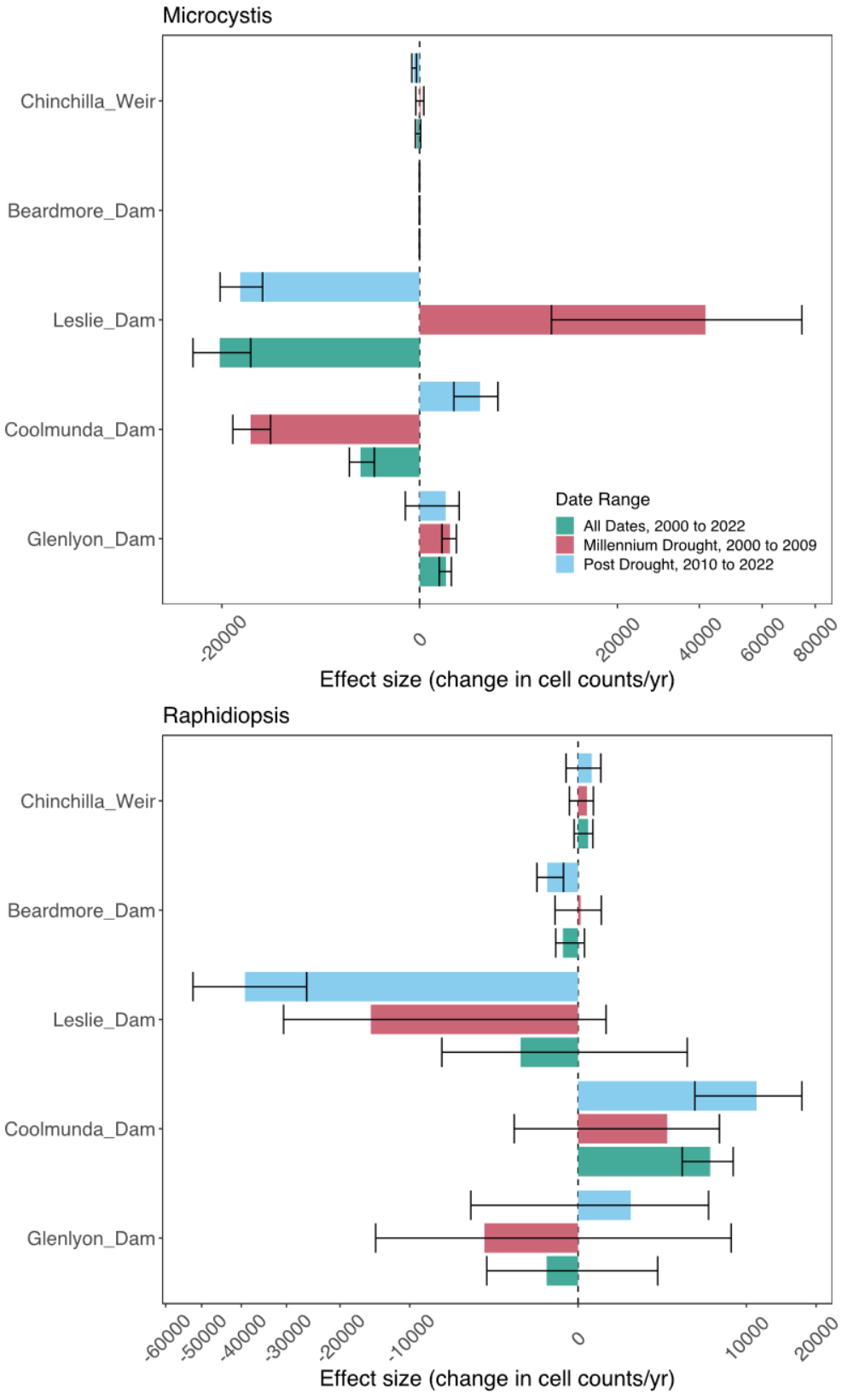


Figure 32. Linear trends in cell counts (cells mL^{-1}) of Raphidiopsis (Cylindrospermopsis) and Microcystis in Queensland Murray-Darling Basin monitoring sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset (2000 to 2022) and in context with the Millennium Drought (2000 to 2009). Error bars are 1 standard deviation of the mean effect size.

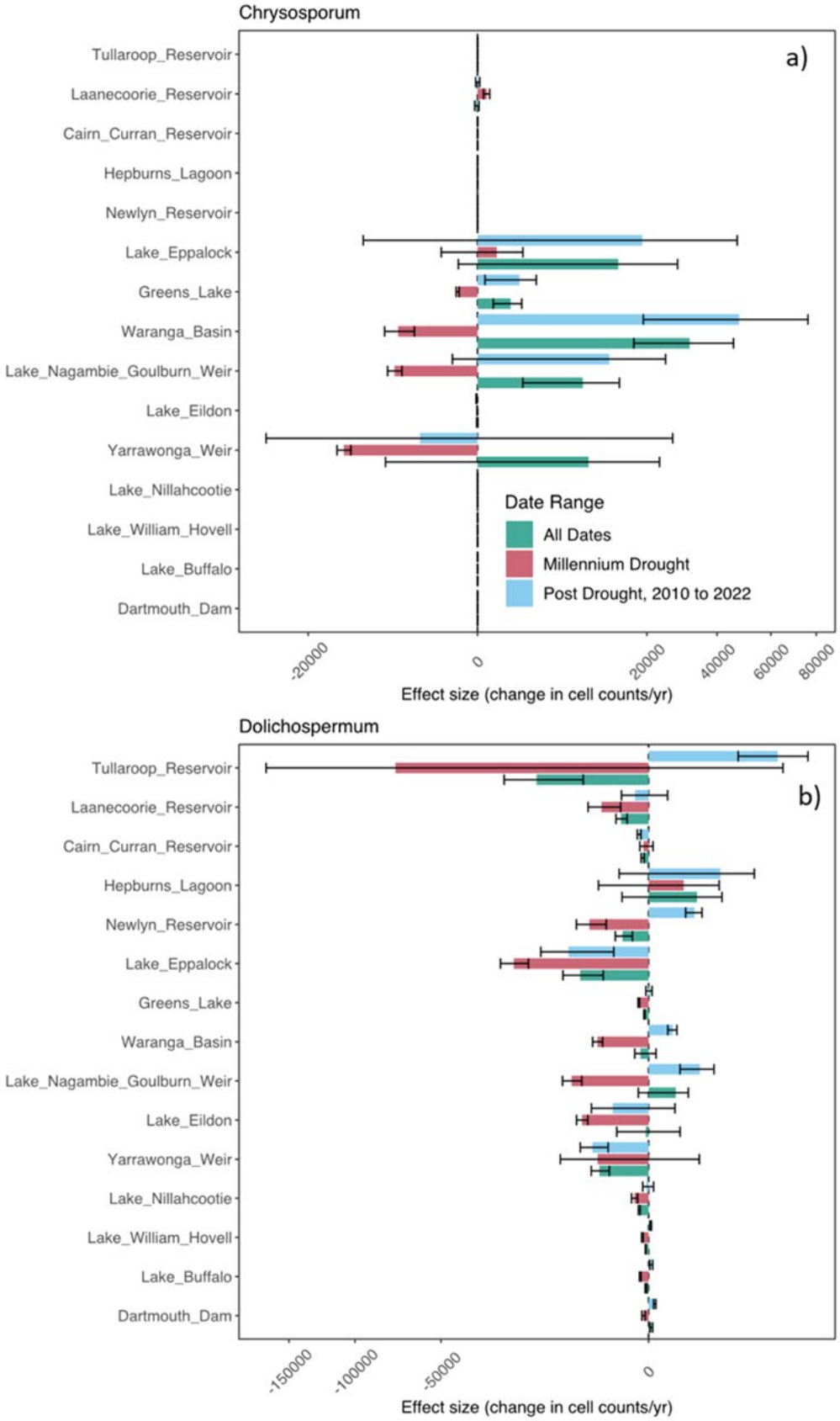


Figure 33. Linear trends in in cell counts (cells mL^{-1}) of Chrysosporum and Dolichospermum in Victorian Murray-Darling Basin monitoring sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset and in context with the Millennium Drought. Error bars are 1 standard deviation of the mean effect size.

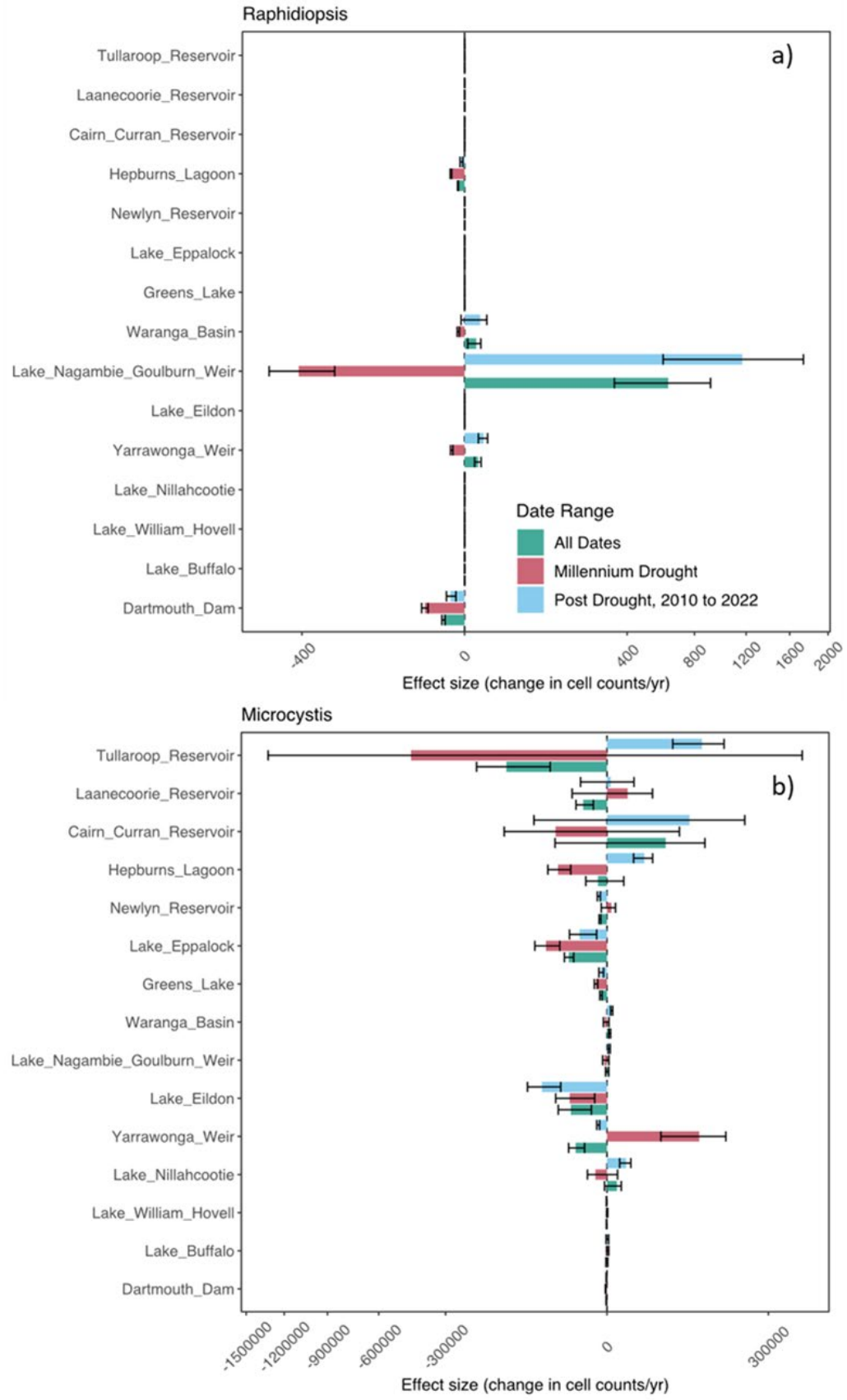


Figure 34. Linear trends in cell counts (cells mL^{-1}) of Raphidiopsis (Cylindrospermopsis) and Microcystis in Victorian Murray-Darling Basin monitoring sites. Colours of the bars indicate times when linear trends were estimated for the entire period of the dataset and in context with the Millennium Drought. Error bars are 1 standard deviation of the mean effect size.

DRIVERS OF CYANOBACTERIA IN RIVER MURRAY SITES

Different cyanobacteria taxa respond differently to different water quality variables. In this study we explored links between cyanobacteria cell counts and key water quality parameters for the MDBA dataset. Six parameters that were monitored across all sites across the period that matched the cyanobacteria data were chosen. We also explored associations between cyanobacteria taxa and the final model consisting of both the response to the environmental variable in question and community-related responses (co-occurrence) at that time. Most genera significantly responded to at least one variable except for 5 genera including *Anathece, Calothrix, Gomphosphaeria, Nostoc* and *Tychonema* that reported no significant response to any of the six water quality variables tested (Figure 35). Total Kjeldahl Nitrogen (TKN) (reflecting organic nitrogen bound in organic substances, ammonia, and ammonium in the water) reported the most significant responses with 13 taxa significantly related to increases in TKN including *Dolichospermum,* and six related to decreases including *Chrysosporum* and *Raphidiopsis (Cylindrospermopsis)* (Figure 35). Relationships with increases in other nutrients such as nitrogen oxides (NOx) and soluble reactive phosphorous (SRP) were not as obvious, being responses of 6 and 3 cyanobacteria genera respectively, with a greater number of genera showing negative responses (7 for NOx and 12 for SRP) (Figure 35). This is surprising given that increased algal blooms have been related with increased nutrients. The modelling used in this study, however, might not be sensitive enough to pull apart the relationship of increased nutrients at the start of the bloom, and decreases during the bloom due to nutrient uptake. This may also reflect the relationship with TKN, as TKN includes bound organic nitrogen and, given the sample is not filtered, still contains algal cells. Increases in this parameter might not reflect dissolved organic nitrogen/ammonia in the water but nitrogen bound in algal biomass.

Besides TKN, water temperature recorded the second highest number of positive responses with the abundance of 10 taxa increasing with rising temperature, including the genera *Dolichospermum* (Figure 35). Surprisingly, five taxa recorded decreases with increasing temperature, including the genera *Chrysosporum*. Sixteen taxa also did not significantly respond, making it difficult to predict the effects changes in water temperature under climate change scenarios will have on cyanobacteria (Figure 35).

The four main cyanobacteria of interest (Mi*crocystis, Dolichospermum, Raphidiopsis* and *Chyrsosporum)* responded differently to different water quality parameters (Figure 35). *Chrysosporum and Microcystis* did not respond positively to any increases in any parameter; instead, increased abundance of these genera was associated with decreases in DOC, TKN, SRP and

temperature for *Chrysosporum,* and silica (Si) and temperature for *Microcystis*. *Raphidiopsis* responded to increases in DOC and NOx concentrations and was negatively associated with TKN and SRP. *Dolichospermum* responded to increased DOC but instead of NOx it preferred the organic nitrogen as shown by positive response to TKN (Figure 34). *Dolichospermum* also responded positively to Si concentrations and temperature.

Table 3 Responses of four main cyanobacteria genera to the six key water quality parameters, noting '–' indicates a significant negative response, '+' indicates a significant positive response, and '0' indicates no significant response.

Genera	DOC	TKN	NOx	SRP	Si	Temp
Chrysosporum	-	-	0	-	0	-
Raphidiopsis	+	-	+	-	0	0
Dolichospermum	+	+	-	-	+	+
Microcystis	0	0	0	0	-	-

Succession in phytoplankton communities have been reported to follow patterns of diatoms during winter/spring, followed by green algae (spring) and then cyanobacteria (summer-autumn) (Rocha et al. 2002). The shift in dominant taxa from diatoms to cyanobacteria generally reflects shifts in nutrient dynamics and temperature. Decreases in Si have been associated with increases in cyanobacteria such as *Microcystis* (Rocha et al. 2002) and this response of *Microcystis* to decreased Si is also shown in this study. Previous reports showed that *Dolichospermum*, *Microcystis*, *Raphidiopsis* and *Chrysospermum* can co-occur together within River Murray sites, and shifts in succession likely reflect responses to changes in environmental variables. The co-occurrence of *Dolichospermum*, *Raphidiopsis* and *Chrysospermum* was also determined in this study by the significant positive correlation (Figure 35). Previous research has shown that a shift in dominance from *Dolichospermum* to *Microcystis* occurred within the River Murray during the 2009 bloom over the summer to autumn period (Al-tebrineh et al. 2012). This succession pattern may be explained by each genera response to key water quality parameters, with *Dolichospermum* responding to increased DOC, TKN, Si and temperature, and *Microcystis* responding to decreases in Si and temperature. During the initial summer months, water temperatures are increasing and there are higher Si and DOC concentrations due to increased flows, leading to rapid growth of *Dolichospermum* due to Si and temperature decreases over the transition of summer to autumn resulting in *Microcystis* sp. becoming more prevalent. Shifts in dominant taxa from *Dolichospermum* to *Chrysosporum* and *Microcystis* have also been reported for Lake Hume (Holland pers comm). The negative response of *Chrysosporum* to temperature and nutrients indicated in this study is interesting. Previous work exploring correlations between *Chrysosporum* with environmental variables during the 2016 bloom reported strong positive correlations between TN and *Chrysosporum*, and a weak correlation with temperature only at the Albury site (Bowling et al. 2018).

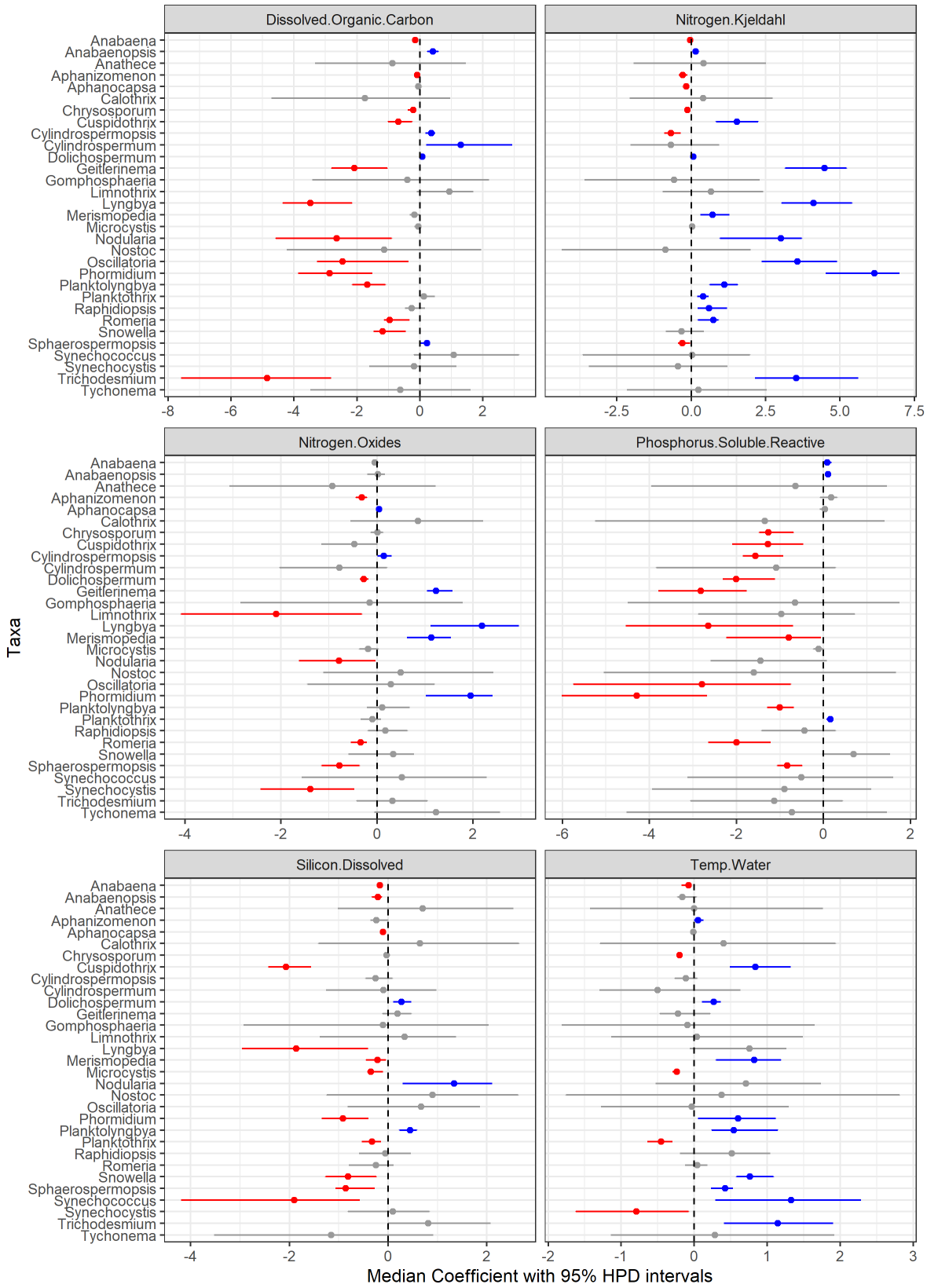


Figure 35. Median taxa responses to environmental covariates. Median response value is shown as a point with 95% Highest posterior density limits. The responses coloured in red represent significantly negative responses of the associated taxa/parameter combination, while those in blue represent significantly positive responses and those in grey had HPD intervals that overlapped 0 indicating a non-significant effect of the parameter on taxa abundances.

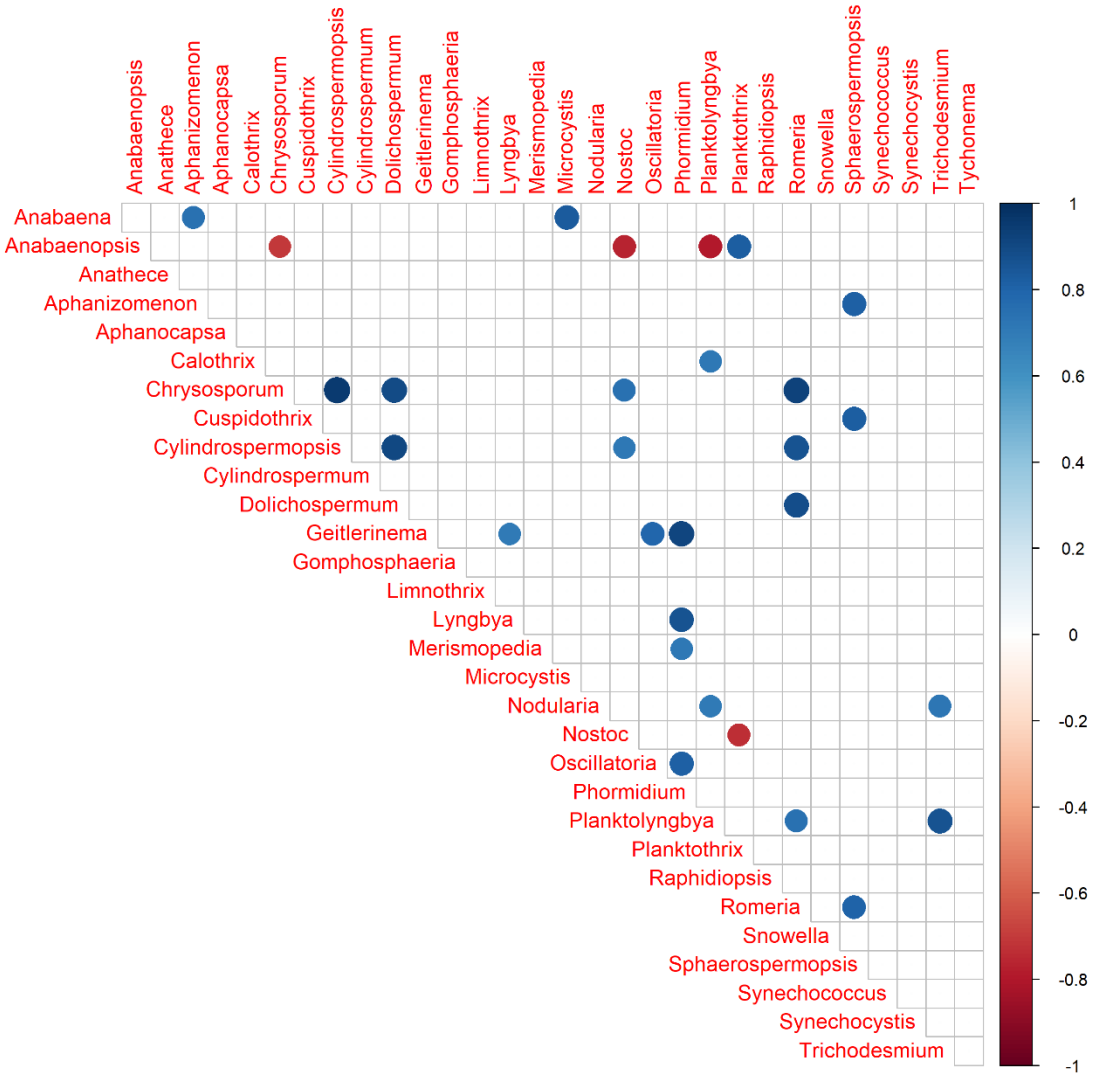


Figure 36. Environmental co-occurance of taxa within the community. Co-occurance represents similar responses to environmental factors between species. Significant responses that had an absolute correlation of >0.7 are presented here, with the darker blues or reds representing stronger positive or negative correlations, respectively (shown in scale bar on right hand side of plot).

KEY FINDINGS

Total cyanobacteria (cells mL^{-1}) within the River Murray (MDBA dataset) were shown to be increasing over time at all sites over the whole period. However, trends were dependant on period chosen, with greatest increases in cell counts recorded over the last 12-year period 2010-2022 at sites Balranald upstream, Merbein, Burtundy, and Euston Weir, while Heywoods, Torrumbarry, Lock 9, Morgan, and Tailem Bend recorded counts highest during the Millennium Drought. No significant difference was shown between the last 12 years and the Millennium Drought at Yarrawonga and Swan Hill. Merbein and Burtundy recorded the highest increase in cyanobacteria cell counts (cells mL^{-1}) over the last 12 years. Trends in cyanobacteria (cells mL^{-1}) within Queensland sites did not show as strong patterns, with two sites shown to increase over the period of data collected (2000-2022), with another three sites recording decreasing trends. Queensland sites tended to display more seasonal patterns as seen in the raw data (Appendix 1 & 2), however statistical analyses used in this study could not pull apart these patterns. This suggests that seasonal changes rather than annual trends may be more reflective of changes in cyanobacteria within these sites. Victorian sites in contrast to the River Murray sites generally showed decreases in cyanobacteria (cells mL^{-1}), with most sites showing decreases over the period of data collection (2004-2022). During the Millennium Drought, however, cyanobacteria (cells mL^{-1}) were significantly increased at all but three sites, with decreases shown post drought. New South Wales sites generally showed inconclusive trends, with trends in cyanobacteria abundance (cells mL^{-1}) and biomass ($mm^3\ L^{-1}$) tending to be site, river or catchment specific, with no clear overall pattern over the seven-year (2015-2022) period.

The increase in cyanobacteria during drought periods is not surprising with blooms being reported globally during such periods. The increased temperatures, decreased windspeed, low flows, and sporadic high intensity rainfalls which wash nutrients into smaller volumes of water, all contribute to increased cyanobacteria during periods of drought (Bowling 2013). Similarly, periods of flood also can contribute to bloom formation by increasing availability of key nutrients, especially if periods of low flows or high temperatures soon follow.

Biomass generally followed a similar pattern to total cyanobacterial cell counts in the River Murray sites, with generally higher biomass recorded over the last 12 years, or during the Millennium Drought depending on site. Biomass ($mm^3\ L^{-1}$) at Queensland sites also tended to follow the same patterns shown by cell counts however, as during the Millennium Drought counts were lower at Glenlyon but biomass increased, indicating a shift in dominant species. Victorian sites showed contrasting results depending on the dataset analysed, and the period selected, highlighting the need to consider as much

data as possible when making inferences to overall trends. Therefore, the results from the biomass dataset from 1998-2019 will be used to summarise overall trends. From 1998-2019, biomass of cyanobacteria (mm^3 L^{-1}) was shown to increase overall at all but two sites (Newlyn Reservoir and Tullaroop Reservoir). Biomass was significantly higher over the last 9-year period (2010-2019) at Cairn Curran Reservoir, Waranga Basin, Lake Nagambie/Goulburn Weir, and lower in Newlyn Reservoir and Lake Eildon. Higher biomass during the Millennium Drought was only recorded in Newlyn Reservoir, with a decrease in Cairn Curran Reservoir. Differences between biomass and patterns shown for abundance of cyanobacteria (cells mL^{-1}) in the second Victorian dataset (ranged from 2004 to 2022) indicate a shift in dominance of species over the period, for example in Victorian waters genera with smaller cell volume dominate the community during the Millennium Drought period as compared to the other periods. This is support by changes in smaller single celled taxa such as *Synechococcus* recorded.

Changes in the dominant cyanobacteria genera of interest in the River Murray also occurred. *Chrysosporum* was found to be increasing at all sites, with highest counts recorded during the period 2010-2022. In contrast, no overall patterns in increases or decreases were shown for the other three taxa with the effect size changes in cell counts being site/region specific. The abundance of *Dolichospermum* within the River Murray decreased at the top two sites (Heywoods, and Yarrawonga) and bottom sites (Morgan and Tailem Bend) over the last 12-year period, with increases associated with the Millennium Drought at these sites. Swan Hill through to Euston Weir recorded, in contrast recorded increases in *Dolichospermum o*ver the last 12 years. *Raphidiopsis* increased significantly at all sites except Balranald upstream during the Millennium Drought and has also greatly increased in abundance at Burtundy over the last 12 years, while decreased at Heywoods, Yarrawonga, Torrumbarry, and Tailem Bend. Microcystis increased during the Millennium Drought at the top four sites (Heywoods, Yarrawonga, Torrumbarry, Swan Hill), Merbein and Euston Weir, with significant decreases at Euston Weir and Tailem Bend over the last 12 years (2010-2022) suggesting drought conditions favour these taxa. Within Queensland sites, changes tend to be site specific, with no key patterns shown in regard to key cyanobacteria genera.

Cyanobacteria were reported to respond more positively to increases in Total Kjeldahl Nitrogen (TKN) (reflecting organic nitrogen bound in organic substances, ammonia, and ammonium in the water) followed by temperature. However, responses were genera specific, with some positively responding and some negatively responding to the same variable. In regard to the four main potentially toxic cyanobacteria of interest (*Microcystis, Dolichospermum, Raphidiopsis* and *Chyrsosporum)*, all taxa

responded differently to the different water quality parameters. *Chrysosporum and Microcystis* did not respond positively to any increases in any parameter; instead, increased abundance of these genera was associated with decreases in DOC, TKN, SRP and temperature for *Chrysosporum,* and silica (Si) and temperature for *Microcystis*. Conversely, *Raphidiopsis* responded to increases in DOC and NOx concentrations and was negatively associated with TKN and SRP. *Dolichospermum* also responded to increased DOC but, instead of NOx, preferred the organic nitrogen as shown by positive response to TKN. *Dolichospermum* was shown to respond positively to Si concentrations and temperature. *Dolichospermum*, *Raphidiopsis* and *Chrysospermum* were found to co-occur at the same sites, however, they likely respond differently to different water quality parameters as outlined above, leading to shifts in dominance over the year(s).

General increases in water temperature from 1978-2021 has also been recorded across the River Murray (Silvester et al. 2022). However, a cooling trend was recorded over the period 2012-2021. As mentioned above, changes in the dominant cyanobacteria genera of interest in the River Murray also occurred over the last 12 years, with *Chrysosporum* found to be increasing. *Chrysoporum* was shown to respond negatively to increase in temperature, suggesting this taxon in the River Murray prefers cooler water temperatures. The cooler water temperatures recorded over the period 2012-2021 might be associated with increased abundance of this taxon. This taxon tends to bloom during higher flow periods such as those in 2016 and 2020-2022, which are also generally associated with decreases in water temperature due to atmospheric changes leading to decreased air temperature and increased rainfall.

TKN has been reported to be decreasing across the Basin since 1978 to 2021 (Silvester et al. 2022), with values only reported to increase during the Millennium Drought. The decrease of *Dolichospermum* at some sites in the 12-year period post drought might reflect decreases in this parameter as indicated by the positive response of this taxon to increases in TKN shown in this study. TKN is a measure of organic nitrogen compounds (ammonium + organically bound N). Most unpolluted surface waters will contain very low concentrations of ammonium, with the majority of TKN consisting of dissolved organic nitrogen (DON). The role of DON in driving cyanobacteria blooms has mostly been overlooked, however recent evidence is showing DON may play an important role in driving algal blooms. Overall decreases in Si across the Basin especially at Burtundy over the last 12 years also likely has led to increases in cyanobacteria due to an increased competitive advantage over diatoms. Further research needs to explore the effect of key environmental drivers on algal blooms within the River Murray by measuring key water quality parameters at the same time as algal abundance and biomass.

Laboratory trials including mesocosms, exploring changes in algal community structure in response to key environment variables such as Si, temperature and TKN, would also improve understanding and risk assessment of future blooms.

COMMENTS ON SAMPLE COLLECTION, METHODS, AND IDENTIFICATION

Sample collection for determination of cyanobacteria presence/absence, cell counts, and biomass were often sporadic in nature and was not consistent across datasets or sites. Methods in regard to sample collection, preservation and taxonomic resolution required, tend to differ depending on which state or jurisdiction is collecting the samples. This caused issues especially in regard to nomenclature changes, such as several *Anabaena* species being reclassified as belonging to the genus *Dolichospermum*. However, not all *Anabaena* were reclassified, so when samples were only identified to genus in the years before the reclassification, it was difficult to determine whether these samples reflected current *Anabaena* species or *Dolichospermum*. This proved even more difficult when difference in identification level was present across a dataset, for example within the MDBA dataset for the River Murray the upper River Murray sites were identified generally to genus, with lower sites (Lock 9, Morgan, and Tailem Bend) often to species. Water quality parameters or collection of water samples for determination of nutrients/DOC/SI etc. and measurement of flow rate/discharge, are not routinely measured/collected at the same time as algal samples. This makes it nearly impossible to accurately determine key drivers of algal blooms within the River Murray. Data regarding concentrations of algal toxins also seems to be missing, indicating that samples are not routinely analysed for toxins.

COMMENTS ON DATA ANALYSIS AND MODELLING USED IN THIS STUDY

Generalised additive models were conducted on seasonally de-trended data to examine overall trends in cyanobacteria across sites. However, cyanobacteria blooms naturally have a seasonal pattern and future work should explore whether the frequency and occurrence of blooms across seasons such as summer vs winter etc. are changing, and whether blooms are becoming more frequent during cooler months. The period analysed also influences overall trend patterns and effect sizes; this is highlighted by the difference responses in biomass shown across the two Victorian datasets (one of which matches cell counts, but only consists of data from 2004 from one site, and 2009 for most others to 2022, and one that contained biomass from 1998-2019). This likely reflects the effect of the model either starting at a year with either high or low biomass.

Community modelling and associations with key water quality variables in this study just used cyanobacterial communities and did not include other taxonomic groups such as diatoms and green algae. Future work should therefore build on this model by including other taxa. Associations with key water quality parameters were only conducted using six variables measured across sites over all periods. Imputed data could also be used to fill in missing periods and to extend the suite of water quality variables available for modelling in the future.

KEY RECOMMENDATIONS

We make the following recommendations:

- A consistent methodology for sample collection, preservation, identification, and monitoring frequency be developed for deployment across the Basin to assist in future analyses and to enhance the general utility of the datasets
- Algal monitoring be maintained at the River Murray sites and sites currently frequently monitored within the other three datasets, with the inclusion of other parameters. Specifically, we recommend that data on water quality and nutrients be collected at the same time as algal samples to improve ability to determine linkages between key parameters and increases in cyanobacteria to determine environmental drivers of blooms.
- Samples be collected to determine algal toxins, as cyanobacteria abundance and biomass is not always reflective of risk, and different toxins have different levels of risk
- Further studies be conducted into:
  (i) the drivers of blooms in the River Murray especially regarding TKN and dissolved organic nitrogen its major constituent, temperature and silica by undertaking fieldwork that combines both analysis of algal abundance and environmental drivers supported by appropriate laboratory experiments

  (ii) succession of cyanobacteria species across the Basin

  (iii) risks that dominant taxa pose to human, livestock and aquatic organism health through measurement of algal toxins and/or use of molecular approaches to determine whether taxa contain genes for toxin production

  (iv) combined with laboratory bioassays into key drivers of toxin production and toxicity.
- The MDBA investigate new emerging techniques that will allow for monitoring of blooms in real time, and key environmental drivers such as remote sensing or deployment of analytical in-situ equipment to count algal cells, identify key taxa such as the FlowCam and measure key drivers such as nutrients

- The MDBA continue to reduce nutrient loads in the Basin and explore cost effective ways to minimise bloom formation that also provide additional benefits to aquatic biota such as floating macrophyte beds or riparian planting.

OVERALL CONCLUSIONS

Trends in cyanobacteria across the whole Basin did not show overall increasing trends or follow the same patterns. Increasing trends were only consistently shown for all River Murray sites, with increases in cyanobacterial abundance (cells mL^{-1}) and biomass (mm^3 L^{-1}) shown over the whole monitoring period especially since 1997. However, other areas of the Basin tend to show site specific responses. The understanding of factors that drive increases in cyanobacteria remains limited and improvements to current monitoring approaches are needed to better elucidate drivers within the MDB.

Under climate change scenarios the MDB is predicted to see increased temperatures, less rainfall with more intense and extreme storm events, longer and more frequent droughts, and low flows. Under these conditions it is likely that cyanobacteria abundances will increase across many Basin sites as shown across both the Millennium Drought and more recent high flow conditions. The MDBA should invest in emerging techniques that provide real-time in-situ monitoring of cyanobacteria and drivers such as nutrients and be proactive in managing and controlling BGA. The MDBA should consider environmentally friendly cost-effective options such as floating macrophytes beds and riparian planting, which will sustainably help in managing BGA, manage nutrient loads, mitigate clearing effects on temperature, and (depending on species chosen for planting) may release phytotoxic natural compounds that inhibit cyanobacterial growth. These options may also offer improved ecosystem services.

References

Akçaalan, R., L. Köker, A. Oğuz, L. Spoof, J. Meriluoto and M. Albay (2014). "First Report of Cylindrospermopsin Production by Two Cyanobacteria (Dolichospermum mendotae and Chrysosporum ovalisporum) in Lake Iznik, Turkey." Toxins **6**: 3173-3186.

Anderson, B., J. Voorhees, B. Phillips, R. Fadness, R. Stancheva, J. Nichols, D. Orr and S. A. Wood (2018). "Extracts from benthic anatoxin-producing Phormidium are toxic to 3 macroinvertebrate taxa at environmentally relevant concentrations." Environmental Toxicology and Chemistry **37**(11): 2851-2859.

Aráoz, R., J. Molgó and N. Tandeau de Marsac (2010). "Neurotoxic cyanobacterial toxins." Toxicon **56**(5): 813-828.

Bai, L., C. Cao, C. Wang, H. Xu, H. Zhang, V. I. Slaveykova, and H. Jiang. 2017. Toward Quantitative Understanding of the Bioavailability of Dissolved Organic Matter in Freshwater Lake during Cyanobacteria Blooming. Environmental Science & Technology **51**:6018-6026.

Baldwin, D. S., H. Gigney, J. S. Wilson, G. Watson, and A. N. Boulding. 2008. Drivers of water quality in a large water storage reservoir during a period of extreme drawdown. Water research **42**:4711-4724.

Benavides, M., H. Berthelot, S. Duhamel, P. Raimbault, and S. Bonnet. 2017. Dissolved organic matter uptake by Trichodesmium in the Southwest Pacific. Scientific Reports **7**:41315.

Bernard, C., S. Froscio, R. Campbell, P. Monis, A. Humpage and L. Fabbro (2011). "Novel toxic effects associated with a tropical Limnothrix/Geitlerinema-like cyanobacterium." Environmental Toxicology **26**(3): 260-270.

Berry, J. P., P. D. L. Gibbs, M. C. Schmale and M. L. Saker (2009). "Toxicity of cylindrospermopsin, and other apparent metabolites from Cylindrospermopsis raciborskii and Aphanizomenon ovalisporum, to the zebrafish (Danio rerio) embryo." Toxicon **53**(2): 289-299.

Beversdorf, L. J., T. R. Miller and K. D. McMahon (2013). "The Role of Nitrogen Fixation in Cyanobacterial Bloom Toxicity in a Temperate, Eutrophic Lake." PLOS ONE **8**(2): e56103.

Beversdorf, L. J., T. R. Miller and K. D. McMahon (2015). "Long-term monitoring reveals carbon–nitrogen metabolism key to microcystin production in eutrophic lakes." Frontiers in Microbiology **6**.

Bocaniov, S. A., C. Ullmann, K. Rinke, K. G. Lamb, and B. Boehrer. 2014. Internal waves and mixing in a stratified reservoir: Insights from three-dimensional modeling. Limnologica **49**:52-67.

Bowling, L. C., C. Merrick, J. Swann, D. Green, G. Smith, and B. A. Neilan. 2013. Effects of hydrology and river management on the distribution, abundance and persistence of cyanobacterial blooms in the Murray River, Australia. Harmful Algae **30**:27-36.

Bowling, L., D. Baldwin, C. Merrick, J. Brayan, and J. Panther. 2018. Possible drivers of a *Chrysosporum ovalisporum* bloom in the Murray River, Australia, in 2016. Marine and Freshwater Research **69**:1649-1662.

Brooks, J. R., J. J. Gibson, S. J. Birks, M. H. Weber, K. D. Rodecap, and J. L. Stoddard. 2014. Stable isotope estimates of evaporation: inflow and water residence time for lakes across the United States as a tool for national lake water quality assessments. Limnology and Oceanography **59**:2150-2165.

Burford, M. A., C. C. Carey, D. P. Hamilton, J. Huisman, H. W. Paerl, S. A. Wood, and A. Wulff. 2020. Perspective: Advancing the research agenda for improving understanding of cyanobacteria in a future of global change. Harmful Algae **91**:101601.

Campos, A. and V. Vasconcelos (2010). "Molecular mechanisms of microcystin toxicity in animal cells." Int J Mol Sci **11**(1): 268-287.

Carey, C. C., B. W. Ibelings, E. P. Hoffmann, D. P. Hamilton, and J. D. Brookes. 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water research **46**:1394-1407.

Chaffin, J. D., and T. B. Bridgeman. 2014. Organic and inorganic nitrogen utilization by nitrogen-stressed cyanobacteria during bloom conditions. Journal of Applied Phycology **26**:299-309.

Chia, M. A., J. G. Jankowiak, B. J. Kramer, J. A. Goleski, I. S. Huang, P. V. Zimba, M. do Carmo Bittencourt-Oliveira and C. J. Gobler (2018). "Succession and toxicity of Microcystis and Anabaena (Dolichospermum) blooms are controlled by nutrient-dependent allelopathic interactions." Harmful Algae **74**: 67-77.

Chorus, I., J. Fastner and M. Welker (2021). "Cyanobacteria and Cyanotoxins in a Changing Environment: Concepts, Controversies, Challenges." Water **13**(18): 2463.

Christensen, V. G. and E. Khan (2020). "Freshwater neurotoxins and concerns for human, animal, and ecosystem health: A review of anatoxin-a and saxitoxin." Science of The Total Environment **736**: 139515.

Clearwater, S. J., S. A. Wood, N. R. Phillips, S. M. Parkyn, R. Van Ginkel and K. J. Thompson (2014). "Toxicity thresholds for juvenile freshwater mussels Echyridella menziesii and crayfish Paranephrops planifrons, after acute or chronic exposure to Microcystis sp." Environmental Toxicology **29**(5): 487-502.

Cottingham, K. L., H. A. Ewing, M. L. Greer, C. C. Carey and K. C. Weathers (2015). "Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling." Ecosphere **6**(1): art1.

Cottingham, K. L., H. A. Ewing, M. L. Greer, C. C. Carey, and K. C. Weathers. 2015. Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling. Ecosphere **6**:art1.

Cottingham, K. L., K. C. Weathers, H. A. Ewing, M. L. Greer, and C. C. Carey. 2020. Predicting the effects of climate change on freshwater cyanobacterial blooms requires consideration of the complete cyanobacterial life cycle. Journal of Plankton Research **43**:10-19.

Cox, P. A., S. A. Banack, S. J. Murch, U. Rasmussen, G. Tien, R. R. Bidigare, J. S. Metcalf, L. F. Morrison, G. A. Codd and B. Bergman (2005). "Diverse taxa of cyanobacteria produce β-N-methylamino-l-alanine, a neurotoxic amino acid." Proceedings of the National Academy of Sciences **102**(14): 5074-5078.

Crawford, A., J. Holliday, C. Merrick, J. Brayan, M. van Asten, and L. Bowling. 2017. Use of three monitoring approaches to manage a major Chrysosporum ovalisporum bloom in the Murray River, Australia, 2016. Environmental Monitoring and Assessment **189**:202.

Davis, T. W., D. L. Berry, G. L. Boyer and C. J. Gobler (2009). "The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of Microcystis during cyanobacteria blooms." Harmful Algae **8**(5): 715-725.

Davis, T. W., G. S. Bullerjahn, T. Tuttle, R. M. McKay and S. B. Watson (2015). "Effects of Increasing Nitrogen and Phosphorus Concentrations on Phytoplankton Community Growth and Toxicity During Planktothrix Blooms in Sandusky Bay, Lake Erie." Environmental Science & Technology **49**(12): 7197-7207.

Deutsch, E. S., I. Alameddine and S. S. Qian (2020). "Using structural equation modeling to better understand microcystis biovolume dynamics in a mediterranean hypereutrophic reservoir." Ecological Modelling **435**: 109282.

dos Santos Silva, R. D., M. A. Chia, V. V. Barbosa, J. dos Santos Severiano and J. E. de Lucena Barbosa (2022). "Synergistic effects of temperature and nutrients on growth and saxitoxin content of the cyanobacterium Raphidiopsis raciborskii." Journal of Applied Phycology **34**(2): 941-952.

Dunlop RA, Cox PA, Banack SA, Rodgers KJ (2013) The Non-Protein Amino Acid BMAA Is Misincorporated into Human Proteins in Place of l-Serine Causing Protein Misfolding and Aggregation. PLoS ONE 8(9): e75376.

EPA Aguilera, A., E. B. Gómez, J. Kaštovský, R. O. Echenique and G. L. Salerno (2018). "The polyphasic analysis of two native Raphidiopsis isolates supports the unification of the genera Raphidiopsis and Cylindrospermopsis (Nostocales, Cyanobacteria)." Phycologia **57**(2): 130-146.

Erratt, K. J., I. F. Creed and C. G. Trick (2022). "Harmonizing science and management options to reduce risks of cyanobacteria." Harmful Algae **116**: 102264.

Faassen, E. J., L. Harkema, L. Begeman and M. Lurling (2012). "First report of (homo)anatoxin-a and dog neurotoxicosis after ingestion of benthic cyanobacteria in The Netherlands." Toxicon **60**(3): 378-384.

Facey, J. A., L. E. Michie, J. J. King, J. N. Hitchcock, S. C. Apte, and S. M. Mitrovic. 2022. Severe cyanobacterial blooms in an Australian lake; causes and factors controlling succession patterns. Harmful Algae **117**:102284.

Facey, J. A., S. C. Apte, and S. M. Mitrovic. 2019. A Review of the Effect of Trace Metals on Freshwater Cyanobacterial Growth and Toxin Production. Toxins **11**:643.

Fentie D (2020) Methods of Algal Control: A review Paper, IJESC

Fiore, M. F., S. T. de Lima, W. W. Carmichael, S. M. K. McKinnie, J. R. Chekan and B. S. Moore (2020). "Guanitoxin, re-naming a cyanobacterial organophosphate toxin." Harmful Algae **92**: 101737.

Fukushima, M., N. Tomioka, T. Jutagate, M. Hiroki, T. Murata, C. Preecha, P. Avakul, P. Phomikong and A. Imai (2017). "The dynamics of pico-sized and bloom-forming cyanobacteria in large water bodies in the Mekong River Basin." PLoS One **12**(12): e0189609.

Funari, E. and E. Testai (2008). "Human Health Risk Assessment Related to Cyanotoxins Exposure." Critical Reviews in Toxicology **38**(2): 97-125.

Furtado, A. L. F. F., M. d. C. Calijuri, A. S. Lorenzi, R. Y. Honda, D. B. Genuário and M. F. Fiore (2009). "Morphological and molecular characterization of cyanobacteria from a Brazilian facultative wastewater stabilization pond and evaluation of microcystin production." Hydrobiologia **627**(1): 195-209.

Gaur, A., G. Pant and A. S. Jalal (2022). "Computer-aided cyanobacterial harmful algae blooms (CyanoHABs) studies based on fused artificial intelligence (AI) models." Algal Research **67**: 102842.

Gin, K. Y.-H., Z. Y. Sim, K. C. Goh, J. W. K. Kok, S. H. Te, N. H. Tran, W. Li and Y. He (2021). "Novel cyanotoxin-producing Synechococcus in tropical lakes." Water Research **192**: 116828.

Glibert, P., C. A. Heil, D. Hollander, M. Revilla, A. Hoare, J. Alexander, and S. Murasko. 2004. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Marine Ecology Progress Series **280**:73-83.

Gobler, C. J., J. M. Burkholder, T. W. Davis, M. J. Harke, T. Johengen, C. A. Stow and D. B. Van de Waal (2016). "The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms." Harmful Algae **54**: 87-97.

Gobler, C. J., J. M. Burkholder, T. W. Davis, M. J. Harke, T. Johengen, C. A. Stow, and D. B. Van de Waal. 2016. The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms. Harmful Algae **54**:87-97.

Hamilton, D. P., N. Salmaso, and H. W. Paerl. 2016. Mitigating harmful cyanobacterial blooms: strategies for control of nitrogen and phosphorus loads. Aquatic Ecology **50**:351-366.

Harke, M. J., T. W. Davis, S. B. Watson and C. J. Gobler (2016). "Nutrient-Controlled Niche Differentiation of Western Lake Erie Cyanobacterial Populations Revealed via Metatranscriptomic Surveys." Environmental Science & Technology **50**(2): 604-615.

Harland, F., S. A. Wood, P. Broady, W. Williamson and S. Gaw (2015). "Changes in saxitoxin-production through growth phases in the metaphytic cyanobacterium Scytonema cf. crispum." Toxicon **103**: 74-79.

Heath, M. W., S. A. Wood and K. G. Ryan (2010). "Polyphasic assessment of fresh-water benthic mat-forming cyanobacteria isolated from New Zealand." FEMS Microbiology Ecology **73**(1): 95-109.

Heath, M. W., S. A. Wood, R. F. Barbieri, R. G. Young and K. G. Ryan (2014). "Effects of nitrogen and phosphorus on anatoxin-a, homoanatoxin-a, dihydroanatoxin-a and dihydrohomoanatoxin-a production by Phormidium autumnale." Toxicon **92**: 179-185.

Hellweger, F. L., R. M. Martin, F. Eigemann, D. J. Smith, G. J. Dick and S. W. Wilhelm (2022). "Models predict planned phosphorus load reduction will make Lake Erie more toxic." Science **376**(6596): 1001-1005.

Henderson, B., Y. Liu and D. Baldwin (2013). Trends in physical and chemical aspects of water quality in the Murray-Darling basin 1978-2012, CSIRO Water for a Healthy Country Flagship, Australia.

Henriksen, P., W. W. Carmichael, J. An and Ø. Moestrup (1997). "Detection of an anatoxin-a(s)-like anticholinesterase in natural blooms and cultures of cyanobacteria/blue-green algae from danish lakes and in the stomach contents of poisoned birds." Toxicon **35**(6): 901-913.

Hoeger, S. J., G. Shaw, B. C. Hitzfeld and D. R. Dietrich (2004). "Occurrence and elimination of cyanobacterial toxins in two Australian drinking water treatment plants." Toxicon **43**(6): 639-649.

Holland, A. and S. Kinnear (2013). "Interpreting the Possible Ecological Role(s) of Cyanotoxins: Compounds for Competitive Advantage and/or Physiological Aide?" Marine Drugs **11**(7): 2239-2258.

Holland, A., and S. Kinnear. 2013. Interpreting the possible ecological role (s) of cyanotoxins: compounds for competitive advantage and/or physiological aide? Marine drugs **11**:2239-2258.

Hui, F.K., 2016. boral–Bayesian ordination and regression analysis of multivariate abundance data in R. Methods in Ecology and Evolution, 7(6), pp.744-750.

Huisman, J., G. A. Codd, H. W. Paerl, B. W. Ibelings, J. M. Verspagen, and P. M. Visser. 2018. Cyanobacterial blooms. Nature Reviews Microbiology **16**:471-483.

Huisman, J., J. Sharples, J. M. Stroom, P. M. Visser, W. E. A. Kardinaal, J. M. H. Verspagen, and B. Sommeijer. 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology **85**:2960-2970.

Humpage, A. R., F. Fontaine, S. Froscio, P. Burcham and I. R. Falconer (2005). "Cylindrospermopsin Genotoxicity and Cytotoxicity: Role Of Cytochrome P-450 and Oxidative Stress." Journal of Toxicology and Environmental Health, Part A **68**(9): 739-753.

Humpage, A., I. Falconer, C. Bernard, S. Froscio and L. Fabbro (2012). "Toxicity of the cyanobacterium Limnothrix AC0243 to male Balb/c mice." Water Research **46**(5): 1576-1583.

Ibelings, B. W., and S. C. Maberly. 1998. Photoinhibition and the availability of inorganic carbon restrict photosynthesis by surface blooms of cyanobacteria. Limnology and Oceanography **43**:408-419.

Ibelings, B. W., L. C. Backer, W. E. A. Kardinaal and I. Chorus (2014). "Current approaches to cyanotoxin risk assessment and risk management around the globe." Harmful Algae **40**: 63-74.

Jackson, S., Woods, R. and Hooper, F., 2021. Empowering First Nations in the governance and management of the Murray–Darling Basin. In Murray-Darling Basin, Australia (pp. 313-338). Elsevier. https://doi.org/10.1016/B978-0-12-818152-2.00015-2

Jake P. Violi, Simon M. Mitrovic, Anne Colville, Brendan J. Main, Kenneth J. Rodgers, Prevalence of β-methylamino-L-alanine (BMAA) and its isomers in freshwater cyanobacteria isolated from eastern Australia, Ecotoxicology and Environmental Safety, 172, 72-81

Jöhnk, K. D., J. Huisman, J. Sharples, B. Sommeijer, P. M. Visser, and J. M. Stroom. 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Global Change Biology **14**:495-512.

Kaebernick, M. and B. A. Neilan (2001). "Ecological and molecular investigations of cyanotoxin production." FEMS Microbiology Ecology **35**(1): 1-9.

Kahru, M., J. M. Leppanen and O. E. Rud (1993). "Cyanobacterial blooms cause heating of the sea surface." Marine Ecology Progress Series **101**: 1-7.

Kahru, M., O. Savchuk and R. Elmgren (2007). "Satellite measurements of cyanobacterial bloom frequency in the Baltic Sea: Interannual and spatial variability." Marine Ecology-progress Series - MAR ECOL-PROGR SER **343**: 15-23.

Kearns, K. D. and M. D. Hunter (2000). "Green algal extracellular products regulate antialgal toxin production in a cyanobacterium." Environmental Microbiology **2**(3): 291-297.

Kelly, L. T., J. Puddick, K. G. Ryan, O. Champeau and S. A. Wood (2020). "An ecotoxicological assessment of the acute toxicity of anatoxin congeners on New Zealand Deleatidium species (mayflies)." Inland Waters **10**(1): 101-108.

Kinnear, S. H. W., L. J. Duivenvoorden and L. D. Fabbro (2007). "Sublethal responses in Melanoides tuberculata following exposure to Cylindrospermopsis raciborskii containing cylindrospermopsin." Harmful Algae **6**(5): 642-650.

Komárek, J. (2012). Nomenclatural changes in heterocytous Cyanoprokaryotes (Cyanobacteria, Cyanophytes). Fottea 12(1): 141-148.

Kosten, S., V. L. Huszar, E. Bécares, L. S. Costa, E. van Donk, L. A. Hansson, E. Jeppesen, C. Kruk, G. Lacerot, and N. Mazzeo. 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biology **18**:118-126.

Kragh, T., and K. Sand-Jensen. 2018. Carbon limitation of lake productivity. Proc Biol Sci **285**.

Kurmayer, R., E. Dittmann, J. Fastner and I. Chorus (2002). "Diversity of microcystin genes within a population of the toxic cyanobacterium Microcystis spp. in Lake Wannsee (Berlin, Germany)." Microbial Ecology: 107-118.

Li, J., L.-A. Hansson and K. M. Persson (2018). "Nutrient Control to Prevent the Occurrence of Cyanobacterial Blooms in a Eutrophic Lake in Southern Sweden, Used for Drinking Water Supply." Water **10**(7): 919.

Li, X., R. Liao, J. Zhou, P. T. Y. Leung, M. Yan and H. Ma (2017). "Classification of morphologically similar algae and cyanobacteria using Mueller matrix imaging and convolutional neural networks." Applied Optics **56**(23): 6520-6530.

Lima, S. T., T. R. Fallon, J. L. Cordoza, J. R. Chekan, E. Delbaje, A. R. Hopiavuori, D. O. Alvarenga, S. M. Wood, H. Luhavaya, J. T. Baumgartner, F. A. Dörr, A. Etchegaray, E. Pinto, S. M. K. McKinnie, M. F. Fiore and B. S. Moore (2022). "Biosynthesis of Guanitoxin Enables Global Environmental Detection in Freshwater Cyanobacteria." Journal of the American Chemical Society **144**(21): 9372-9379.

Liu, F, Chunyun Zhang, Yuanyuan Wang, Guofu Chen,2022 A review of the current and emerging detection methods of marine harmful microalgae, Science of The Total Environment, 815

Lürling, M., F. Eshetu, E. J. Faassen, S. Kosten, and V. L. M. Huszar. 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshwater Biology **58**:552-559.

Lürling, M., M. M. e. Mello, F. van Oosterhout, L. de Senerpont Domis and M. M. Marinho (2018). "Response of Natural Cyanobacteria and Algae Assemblages to a Nutrient Pulse and Elevated Temperature." Frontiers in Microbiology **9**.

Ma, H., Y. Wu, N. Gan, L. Zheng, T. Li and L. Song (2015). "Growth inhibitory effect of Microcystis on Aphanizomenon flos-aquae isolated from cyanobacteria bloom in Lake Dianchi, China." Harmful Algae **42**: 43-51.

Maavara, T., Q. Chen, K. Van Meter, L. E. Brown, J. Zhang, J. Ni, and C. Zarfl. 2020. River dam impacts on biogeochemical cycling. Nature Reviews Earth & Environment **1**:103-116.

MacIntyre, S., and R. Jellison. 2001. Nutrient fluxes from upwelling and enhanced turbulence at the top of the pycnocline in Mono Lake, California. Pages 13-29 Saline Lakes. Springer.

Main, B. Lee C. Bowling, Matthew P. Padula, David P. Bishop, Simon M. Mitrovic, Gilles J. Guillemin, Kenneth J. Rodgers (2018) Detection of the suspected neurotoxin β-methylamino-l-alanine (BMAA) in cyanobacterial blooms from multiple water bodies in Eastern Australia, Harmful Algae,74,10-18

Matantseva, O. V., and S. O. Skarlato. 2013. Mixotrophy in microorganisms: Ecological and cytophysiological aspects. Journal of Evolutionary Biochemistry and Physiology **49**:377-388.

Matsunaga, S., R. E. Moore, W. P. Niemczura and W. W. Carmichael (1989). "Anatoxin-a(s), a potent anticholinesterase from Anabaena flos-aquae." Journal of the American Chemical Society **111**(20): 8021-8023.

McBARRON, E.J., WALKER, R.I., GARDNER, I. and WALKER, K.H. (1975), Toxicity to livestock of the blue-green alga *Anabaena Circinalis*. Australian Veterinary Journal, 51: 587-588.

Méjean, A., S. Mann, T. Maldiney, G. Vassiliadis, O. Lequin and O. Ploux (2009). "Evidence that Biosynthesis of the Neurotoxic Alkaloids Anatoxin-a and Homoanatoxin-a in the Cyanobacterium Oscillatoria PCC 6506 Occurs on a Modular Polyketide Synthase Initiated by l-Proline." Journal of the American Chemical Society **131**(22): 7512-7513.

Mello, F. D., N. Braidy, H. Marçal, G. Guillemin, S. M. Nabavi and B. A. Neilan (2018). "Mechanisms and effects posed by neurotoxic products of cyanobacteria/microbial eukaryotes/dinoflagellates in algae blooms: A review." Neurotoxicity Research **33**(1): 153-167.

Mesquita, M. C. B., M. Lürling, F. Dorr, E. Pinto and M. M. Marinho (2019). "Combined Effect of Light and Temperature on the Production of Saxitoxins in Cylindrospermopsis raciborskii Strains." Toxins **11**(1): 38.

Mitrovic, S. M., L. Hardwick, and F. Dorani. 2010. Use of flow management to mitigate cyanobacterial blooms in the Lower Darling River, Australia. Journal of Plankton Research **33**:229-241.

Molot, L. A., S. B. Watson, I. F. Creed, C. G. Trick, S. K. McCabe, M. J. Verschoor, R. J. Sorichetti, C. Powe, J. J. Venkiteswaran, and S. L. Schiff. 2014. A novel model for cyanobacteria bloom formation: the critical role of anoxia and ferrous iron. Freshwater Biology **59**:1323-1340.

Müller, S., and S. M. Mitrovic. 2015. Phytoplankton co-limitation by nitrogen and phosphorus in a shallow reservoir: progressing from the phosphorus limitation paradigm. Hydrobiologia **744**:255-269.

Negri AP, Jones GJ, Hindmarsh M. Sheep mortality associated with paralytic shellfish poisons from the cyanobacterium Anabaena circinalis. Toxicon. 1995 Oct;33(10):1321-9.

Niemeyer, R. J., Y. Cheng, Y. Mao, J. R. Yearsley, and B. Nijssen. 2018. A Thermally Stratified Reservoir Module for Large-Scale Distributed Stream Temperature Models With Application in the Tennessee River Basin. Water Resources Research **54**:8103-8119.

NRMMC (2005) National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Fresh Waters

O'Neil, J. M., T. W. Davis, M. A. Burford and C. J. Gobler (2012). "The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change." Harmful Algae **14**: 313-334.

Ohtani, I., R. E. Moore and M. T. C. Runnegar (1992). "Cylindrospermopsin: a potent hepatotoxin from the blue-green alga Cylindrospermopsis raciborskii." Journal of the American Chemical Society **114**(20): 7941-7942.

Paerl, H. W. and J. Huisman (2009). "Climate change: a catalyst for global expansion of harmful cyanobacterial blooms." Environmental microbiology reports **1**(1): 27-37.

Paerl, H. W. and T. G. Otten (2016). "Duelling 'CyanoHABs': unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N2-fixing harmful cyanobacteria." Environmental Microbiology **18**(2): 316-324.

Paerl, H. W., and T. G. Otten. 2013. Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. Microbial Ecology **65**:995-1010.

Paerl, H. W., W. S. Gardner, K. E. Havens, A. R. Joyner, M. J. McCarthy, S. E. Newell, B. Qin, and J. T. Scott. 2016. Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. Harmful Algae **54**:213-222.

Palikova, M., S. Navratil, B. Maršálek and L. Bláha (2003). "Toxicity of crude extracts of cyanobacteria for embryos and larvae of carp (Cyprinus carpio L.)." Acta Veterinaria Brno **72**(3): 437-443.

Peng, G., R. M. Martin, S. P. Dearth, X. Sun, G. L. Boyer, S. R. Campagna, S. Lin, and S. W. Wilhelm. 2018. Seasonally relevant cool temperatures interact with N chemistry to increase microcystins produced in lab cultures of Microcystis aeruginosa NIES-843. Environmental Science & Technology **52**:4127-4136.

Pérez-Morales, A., S. S. S. Sarma and S. Nandini (2015). "Microcystins production in Microcystis induced by Daphnia pulex (Cladocera) and Brachionus calyciflorus (Rotifera)." Hidrobiologica **25**: 411-415.

Preußel, K., A. Stüken, C. Wiedner, I. Chorus and J. Fastner (2006). "First report on cylindrospermopsin producing Aphanizomenon flos-aquae (Cyanobacteria) isolated from two German lakes." Toxicon **47**(2): 156-162.

Puschner, B., B. Hoff and E. R. Tor (2008). "Diagnosis of anatoxin-a poisoning in dogs from North America." J Vet Diagn Invest **20**(1): 89-92.

Quiblier, C., S. Wood, I. Echenique-Subiabre, M. Heath, A. Villeneuve, and J.-F. Humbert. 2013. A review of current knowledge on toxic benthic freshwater cyanobacteria – Ecology, toxin production and risk management. Water research **47**:5464-5479.

Reinl, K. L., J. D. Brookes, C. C. Carey, T. D. Harris, B. W. Ibelings, A. M. Morales-Williams, L. N. De Senerpont Domis, K. S. Atkins, P. D. F. Isles, J. P. Mesman, R. L. North, L. G. Rudstam, J. A. A. Stelzer, J. J. Venkiteswaran, K. Yokota, and Q. Zhan. 2021. Cyanobacterial blooms in oligotrophic lakes: Shifting the high-nutrient paradigm. Freshwater Biology **66**:1846-1859.

Reinl, K. L., T. D. Harris, I. Elfferich, A. Coker, Q. Zhan, L. N. De Senerpont Domis, A. M. Morales-Williams, R. Bhattacharya, H.-P. Grossart, R. L. North, and J. N. Sweetman. 2022. The role of organic nutrients in structuring freshwater phytoplankton communities in a rapidly changing world. Water research **219**:118573.

Reynolds, C. S. 2006. The ecology of phytoplankton. Cambridge University Press.

Rigosi, A., C. C. Carey, B. W. Ibelings and J. D. Brookes (2014). "The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa." Limnology and Oceanography **59**(1): 99-114.

Rigosi, A., C. C. Carey, B. W. Ibelings, and J. D. Brookes. 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. Limnology and Oceanography **59**:99-114.

Rocha, C., H. Galvão, and A. Barbosa. 2002. Role of transient silicon limitation in the development of cyanobacteria blooms in the Guadiana estuary, south-western Iberia. Marine Ecology Progress Series **228**:35-45.

Rolim, S.B.A., Veettil, B.K., Vieiro, A.P. *et al.* 2023 Remote sensing for mapping algal blooms in freshwater lakes: a review. *Environ Sci Pollut Res* **30**, 19602–19616

Rutkowska, M., J. Płotka-Wasylka, T. Majchrzak, W. Wojnowski, H. Mazur-Marzec and J. Namieśnik (2019). "Recent trends in determination of neurotoxins in aquatic environmental samples." TrAC Trends in Analytical Chemistry **112**: 112-122.

Salmaso, N. 2000. Factors affecting the seasonality and distribution of cyanobacteria and chlorophytes: a case study from the large lakes south of the Alps, with special reference to Lake Garda. Hydrobiologia **438**:43-63.

Sandrini, G., X. Ji, J. M. H. Verspagen, R. P. Tann, P. C. Slot, V. M. Luimstra, J. M. Schuurmans, H. C. P. Matthijs, and J. Huisman. 2016. Rapid adaptation of harmful cyanobacteria to rising CO₂. Proceedings of the National Academy of Sciences **113**:9315-9320.

Scarlett, K. R., S. Kim, L. M. Lovin, S. Chatterjee, J. T. Scott and B. W. Brooks (2020). "Global scanning of cylindrospermopsin: Critical review and analysis of aquatic occurrence, bioaccumulation, toxicity and health hazards." Science of The Total Environment **738**: 139807.

Seifert, M., G. McGregor, G. Eaglesham, W. Wickramasinghe and G. Shaw (2007). "First evidence for the production of cylindrospermopsin and deoxy-cylindrospermopsin by the freshwater benthic cyanobacterium, Lyngbya wollei (Farlow ex Gomont) Speziale and Dyck." Harmful Algae **6**(1): 73-80.

Shan, K., L. Song, W. Chen, L. Li, L. Liu, Y. Wu, Y. Jia, Q. Zhou and L. Peng (2019). "Analysis of environmental drivers influencing interspecific variations and associations among bloom-forming cyanobacteria in large, shallow eutrophic lakes." Harmful Algae **84**: 84-94.

Shapiro, J. 1997. The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes. Freshwater Biology **37**:307-323.

Silvester, E, Andre Siebers, Luke McPhan, Michael Shackleton, Julia Mynott, Nick Bond, Alison King, Aleicia Holland, 2021 River Murray Water Quality Monitoring Program (RMWQMP) Data Trends Analysis, CFE Publication No. 276.

Simpson, G. L. (2018). "Modelling Palaeoecological Time Series Using Generalised Additive Models." Frontiers in Ecology and Evolution **6**.

Spoof L, Catherine A (2017) Tables of microcystins and nodularins. In: Meriluoto J, Spoof L, Codd GA (eds) Handbook of cyanobacterial monitoring and cyanotoxin analysis. Wiley, Chichester, pp 526–537

Spoof, L., K. A. Berg, J. Rapala, K. Lahti, L. Lepistö, J. S. Metcalf, G. A. Codd and J. Meriluoto (2006). "First observation of cylindrospermopsin in Anabaena lapponica isolated from the boreal environment (Finland)." Environmental Toxicology **21**(6): 552-560.

Staal, M., F. J. R. Meysman, and L. J. Stal. 2003. Temperature excludes N2-fixing heterocystous cyanobacteria in the tropical oceans. Nature **425**:504-507.

Steffensen, D. A. 2008. Economic cost of cyanobacterial blooms. Pages 855-865 *in* H. K. Hudnell, editor. Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer New York, New York, NY.

Stockenreiter, M., J. Isanta Navarro, F. Buchberger, and H. Stibor. 2021. Community shifts from eukaryote to cyanobacteria dominated phytoplankton: The role of mixing depth and light quality. Freshwater Biology **66**:2145-2157.

Stuart, R. K., X. Mayali, J. Z. Lee, R. Craig Everroad, M. Hwang, B. M. Bebout, P. K. Weber, J. Pett-Ridge, and M. P. Thelen. 2016. Cyanobacterial reuse of extracellular organic carbon in microbial mats. The ISME Journal **10**:1240-1251.

Tee, H. S., D. Waite, L. Payne, M. Middleditch, S. Wood and K. M. Handley (2020). "Tools for successful proliferation: diverse strategies of nutrient acquisition by a benthic cyanobacterium." Isme j **14**(8): 2164-2178.

Terao, K., S. Ohmori, K. Igarashi, I. Ohtani, M. F. Watanabe, K. I. Harada, E. Ito and M. Watanabe (1994). "Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga Umezakia natans." Toxicon **32**(7): 833-843.

Toporowska, M., B. Pawlik-Skowronska and R. Kalinowska (2014). "Accumulation and effects of cyanobacterial microcystins and anatoxin-a on benthic larvae of Chironomus spp.(Diptera: Chironomidae)." European Journal of Entomology **111**(1): 83.

Verspagen, J. M. H., D. B. Van de Waal, J. F. Finke, P. M. Visser, E. Van Donk, and J. Huisman. 2014. Rising CO2 Levels Will Intensify Phytoplankton Blooms in Eutrophic and Hypertrophic Lakes. PLOS ONE **9**:e104325.

Vézie, C., J. Rapala, J. Vaitomaa, J. Seitsonen and K. Sivonen (2002). "Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations." Microb Ecol **43**(4): 443-454.

Vico, P., S. Bonilla, B. Cremella, L. Aubriot, A. Iriarte and C. Piccini (2020). "Biogeography of the cyanobacterium Raphidiopsis (Cylindrospermopsis) raciborskii: Integrating genomics, phylogenetic and toxicity data." Mol Phylogenet Evol **148**: 106824.

Vieira, J. G., A. G. da Silva Manetti, E. Jacob-Lopes, and M. I. Queiroz. 2012. Uptake of phosphorus from dairy wastewater by heterotrophic cultures of cyanobacteria. Desalination and Water Treatment **40**:224-230.

Wacklin, P., Hoffmann, L. & Komárek, J. (2009). Nomenclatural validation of the genetically revised cyanobacterial genus Dolichospermum (Ralfs ex Bornet et Flahault) comb. nova. Fottea 9(1): 59-64

Wan, L., X. Chen, Q. Deng, L. Yang, X. Li, J. Zhang, C. Song, Y. Zhou and X. Cao (2019). "Phosphorus strategy in bloom-forming cyanobacteria (Dolichospermum and Microcystis) and its role in their succession." Harmful Algae **84**: 46-55.

Wang, K., X. Mou, H. Cao, I. Struewing, J. Allen, and J. Lu. 2021a. Co-occurring microorganisms regulate the succession of cyanobacterial harmful algal blooms. Environmental Pollution **288**:117682.

Wang, Z., S. Akbar, Y. Sun, L. Gu, L. Zhang, K. Lyu, Y. Huang and Z. Yang (2021). "Cyanobacterial dominance and succession: Factors, mechanisms, predictions, and managements." Journal of Environmental Management **297**: 113281.

Wang, Z., S. Akbar, Y. Sun, L. Gu, L. Zhang, K. Lyu, Y. Huang, and Z. Yang. 2021b. Cyanobacterial dominance and succession: Factors, mechanisms, predictions, and managements. Journal of Environmental Management **297**:113281.

Wells, M. L., V. L. Trainer, T. J. Smayda, B. S. O. Karlson, C. G. Trick, R. M. Kudela, A. Ishikawa, S. Bernard, A. Wulff, D. M. Anderson, and W. P. Cochlan. 2015. Harmful algal blooms and climate change: Learning from the past and present to forecast the future. Harmful Algae **49**:68-93.

Wiese, M., P. M. D'Agostino, T. K. Mihali, M. C. Moffitt and B. A. Neilan (2010). "Neurotoxic Alkaloids: Saxitoxin and Its Analogs." Marine Drugs **8**(7): 2185-2211.

Wilhelm, C., C. Büchel, J. Fisahn, R. Goss, T. Jakob, J. LaRoche, J. Lavaud, M. Lohr, U. Riebesell, K. Stehfest, K. Valentin, and P. G. Kroth. 2006. The Regulation of Carbon and Nutrient Assimilation in Diatoms is Significantly Different from Green Algae. Protist **157**:91-124.

Wilhelm, S. W., G. S. Bullerjahn, and R. M. L. McKay. 2020. The Complicated and Confusing Ecology of *Microcystis* Blooms. mBio **11**:e00529-00520.

Wood, S. A., A. I. Selwood, A. Rueckert, P. T. Holland, J. R. Milne, K. F. Smith, B. Smits, L. F. Watts and C. S. Cary (2007). "First report of homoanatoxin-a and associated dog neurotoxicosis in New Zealand." Toxicon **50**(2): 292-301.

Wood, S. A., D. P. Hamilton, W. J. Paul, K. A. Safi and W. M. Williamson (2009). "New Zealand Guidelines for cyanobacteria in recreational fresh waters: interim guidelines."

Wood, S. A., L. Biessy and J. Puddick (2018). "Anatoxins are consistently released into the water of streams with Microcoleus autumnalis-dominated (cyanobacteria) proliferations." Harmful Algae **80**: 88-95.

Wood, S. A., L. T. Kelly, K. Bouma-Gregson, J.-F. Humbert, H. D. Laughinghouse IV, J. Lazorchak, T. G. McAllister, A. McQueen, K. Pokrzywinski, J. Puddick, C. Quiblier, L. A. Reitz, K. G. Ryan, Y. Vadeboncoeur, A. Zastepa and T. W. Davis (2020). "Toxic benthic freshwater cyanobacterial proliferations: Challenges and solutions for enhancing knowledge and improving monitoring and mitigation." Freshwater Biology **65**(10): 1824-1842.

Wood, S. A., M. W. Heath, P. T. Holland, R. Munday, G. B. McGregor and K. G. Ryan (2010). "Identification of a benthic microcystin-producing filamentous cyanobacterium (Oscillatoriales) associated with a dog poisoning in New Zealand." Toxicon **55**(4): 897-903.

Wood, S. A., N. R. Phillips, M. de Winton and M. Gibbs (2012). "Consumption of benthic cyanobacterial mats and nodularin-R accumulation in freshwater crayfish (Paranephrops planifrons) in Lake Tikitapu (Rotorua, New Zealand)." Harmful Algae **20**: 175-179.

World Health Organization (2020). Cyanobacterial toxins: anatoxin-a and analogues, World Health Organization.

World Health Organization (2020). Cyanobacterial toxins: cylindrospermopsins, World Health Organization.

World Health Organization (2020). Cyanobacterial toxins: microcystins, World Health Organization.

World Health Organization (2020). Cyanobacterial toxins: saxitoxins, World Health Organization.

Wu, Y., L. Li, L. Zheng, G. Dai, H. Ma, K. Shan, H. Wu, Q. Zhou and L. Song (2016). "Patterns of succession between bloom-forming cyanobacteria Aphanizomenon flos-aquae and Microcystis and related environmental factors in large, shallow Dianchi Lake, China." Hydrobiologia **765**(1): 1-13.

Xia, X., W. Guo, S. Tan and H. Liu (2017). "Synechococcus Assemblages across the Salinity Gradient in a Salt Wedge Estuary." Frontiers in Microbiology **8**.

Xu, H., and H. Jiang. 2013. UV-induced photochemical heterogeneity of dissolved and attached organic matter associated with cyanobacterial blooms in a eutrophic freshwater lake. Water research **47**:6506-6515.

Yang, Y., G. Yu, Y. Chen, N. Jia and R. Li (2021). "Four decades of progress in cylindrospermopsin research: The ins and outs of a potent cyanotoxin." Journal of Hazardous Materials **406**: 124653.

Zhang, Z., X. Fan, W. J. G. M. Peijnenburg, M. Zhang, L. Sun, Y. Zhai, Q. Yu, J. Wu, T. Lu and H. Qian (2021). "Alteration of dominant cyanobacteria in different bloom periods caused by abiotic factors and species interactions." Journal of Environmental Sciences **99**: 1-9.

Zubkov, M. V., B. M. Fuchs, G. A. Tarran, P. H. Burkill, and R. Amann. 2003. High rate of uptake of organic nitrogen compounds by Prochlorococcus cyanobacteria as a key to their dominance in oligotrophic oceanic waters. Appl Environ Microbiol **69**:1299-1304.