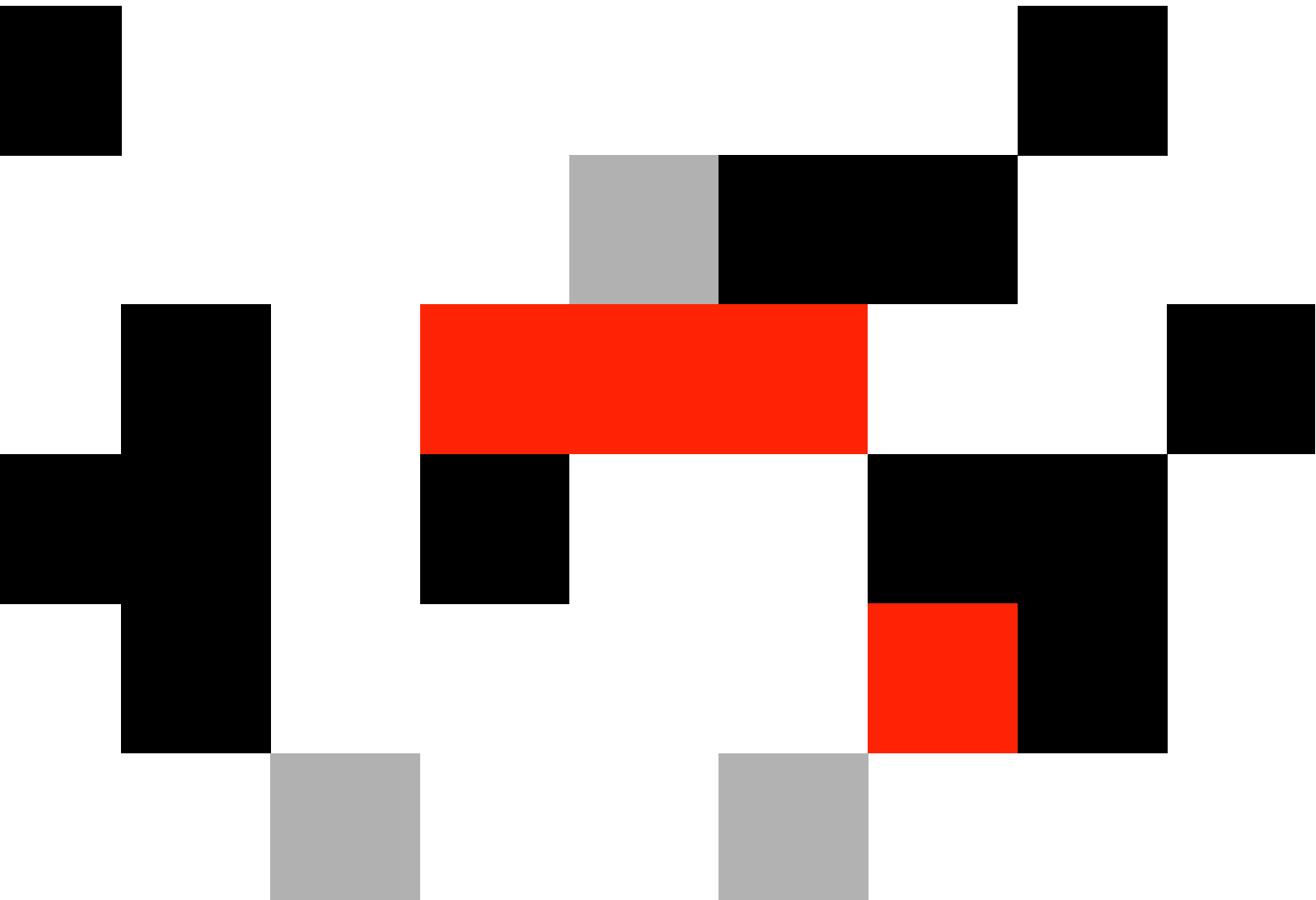
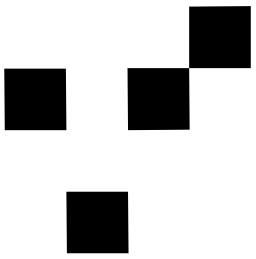
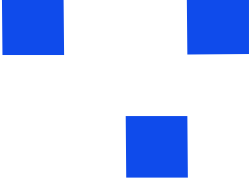




Faculty of Science,
University of Technology Sydney

The Darling Baaka River health project: Phytoplankton as indicators





REPORT PREPARED BY:



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1.0 Introduction

Background to project

In response to the 2023 flooding event, a river health monitoring program (the Darling Baaka River Health Project 'DBRHP') was established to develop and implement a monitoring program that adapts the River Condition Index (RCI) (DCCEEW, 2023) to the unique characteristics of the lower Darling Baaka River system to measure the rivers health and recovery post flood. The RCI combines data on hydrological stress, water quality, biodiversity condition, landscape disturbance, riparian vegetation condition and geomorphic condition. However, the RCI does not currently incorporate data on phytoplankton, free-floating algae, which are an important overall indicator of river health and water quality.

The work presented in this report is part of the DBRHP, which has been designed and delivered by the DCCEEW Science and Insights Division. The DBRHP is delivered under the EPA's Recovery Program for Water Quality Monitoring in the Darling Baaka and is funded as a Category D recovery measure under the joint Commonwealth and NSW Government Disaster Recovery Funding Arrangements. The University of Technology Sydney, were engaged by DCCEEW to provide an assessment of phytoplankton communities in the lower Darling Baaka river, and to provide a potential new algal indicator of river health.

A primary goal of the DBRHMP is to deliver ecological monitoring of a range of river health indicators to inform community understanding of event recovery, and future research and water management of the system. The analyses conducted by UTS as part of this project aims to deliver new information on the basal biotic processes governing riverine ecological function and has the potential to significantly contribute to future report card on river health.

Introduction

Phytoplankton, the free-floating algae, are important organisms in rivers and lakes. They are primary producers that fix carbon and produce oxygen through photosynthesis and an important basal energy source that fuels the food web. They are a diverse group of autotrophic organisms and can be indicative of the health of aquatic ecosystems as they are susceptible to changes in water quality and river flows (Mitrovic and Bowling 2013). Some phytoplankton can be considered harmful to humans and other animals due to the production of toxins. In freshwaters these are predominately the cyanobacteria, or blue-green algae, which are a group of photosynthetic bacteria.

Lowland rivers such as the Darling Baaka River have a distinct composition of phytoplankton which are adapted to different flow conditions. In the lower Darling Baaka River, flow varies considerably with prolonged periods of very low or no flow interspersed with moderate flows and some large overbank flood events (Sheldon 2017). These flow conditions also influence the light available to phytoplankton growth in the water column, with higher flows greatly restricting light availability due to increased turbidity while low flow periods lead to greater light availability as the water column clears up as fine particles fall out of suspension (Oliver et al. 2010). Further, flow can also affect the physical and chemical environment within a river, for example, by influencing the development of thermal stratification (Mitrovic et al. 2011). Some phytoplankton are adapted to this more stable water column and extended periods of thermal stratification can commonly lead to blooms of toxic cyanobacteria such as the saxitoxin producing *Dolichospermum circinale* (Mitrovic et al. 2011; Davis et al. in review).

The Darling Baaka River supports a diverse phytoplankton community and over a 20-year period (2002-2022) the most frequently occurring genera of cyanobacteria were *Aphanocapsa*, *Cyanocatena*, *Planktolyngbya*, *Pseudanabaena*, *Merismopedia*, *Anabaenopsis* and *Dolichospermum*, each being recorded over 150 times (Mitrovic et al. 2025). For the non-cyanobacterial taxa, 102 genera have been recorded over the last 20 years in the Barwon-Darling Baaka River dominated by *Ankistrodesmus*, *Scenedesmus*, *Chroomonas*, *Kirchneriella*, *Nitzschia* and *Cryptomonas*, each occurring over 350 times (Mitrovic et al. 2025). These phytoplankton are typical of shallow mesotrophic to eutrophic environments (Padisák et al. 2009) which may reflect the higher nutrient concentrations found in the Darling Baaka River relative to other rivers (Mitrovic et al. 2003).

Phytoplankton have been previously used as indicators of water quality in several different ways. Guidelines for the presence of cyanobacteria have been a primary concern for governments to ensure water is safe for recreation or drinking water purposes. In NSW the adoption of the National Health and Medical Research Council (NHMRC) guidelines for cyanobacteria is used to determine if water is safe for primary recreation such as swimming. The Q index and Ecological Quality Ratio (EQR) have been developed to characterise the quality of waters based on the composition of the phytoplankton community (Padisák et al., 2006). Other guidelines have been developed for lake and river water quality based on chlorophyll-a (Chl-a) concentration. For example, Carlson's Trophic State Index (TSI) (Carlson, 1977) uses Chl-a as a measure of aquatic ecosystem productivity and classes the system from oligotrophic (low phytoplankton productivity) to hypereutrophic (extreme phytoplankton

productivity). Many other guidelines exist for different purposes such as drinking water safety and livestock safety.

The aims of this project were to (i) assess the condition of the algae communities between March 2024 and November 2025 and (ii) develop a water quality health index, based on phytoplankton, for the lower Darling Baaka River. To achieve this, an integrated approach is needed that considers different guidelines, their purposes and overall use of the indicator. In this study samples collected on the lower Darling Baaka River were examined for patterns in the phytoplankton community, including occurrence of cyanobacterial blooms and diversity metrics. Also a new river health indicator was developed that integrates several existing approaches to phytoplankton health.

2.0 Methodology

2.1 Sampling and site locations

To support NSW DCCEE's Darling Baaka River Health Monitoring Project (DBRHMP), UTS performed analysis of river water samples collected by DCCEE for characterisation of phytoplankton community composition and biovolume. Microbial communities are key determinants and indicators of river system function, and this analysis will help to inform DCCEE's comprehensive river health monitoring program.

Grab samples were collected over a nine-month period (April 2024- December 2024), with intensive sampling campaigns in May and November of 2024. At each site, sampling containers were submerged to a depth of 0.2 m and inverted to collect approximately 250 mL of river water. At sites where algal surface scums were identified, samples were taken from those locations. Lugol's solution was used to preserve the samples, which were stored in cool, dark conditions until delivery to UTS for identification and analysis. Algae samples were collected alongside other biotic and abiotic parameters, as detailed in the DBRHMP technical report (DCCEE 2025).

2.2 Phytoplankton Identification

Phytoplankton samples were received from DCCEEW and were identified using an Olympus BX41 compound light microscope and a Sedgewick-Rafter counting chamber at 200 – 400 x magnification. Enumeration followed methods described by Hötzel & Croome (1999). All taxa were identified to genus level except for potentially toxic cyanobacteria that were identified to species level. Phytoplankton counts were converted to biovolume for comparison against national cyanobacterial guidelines. Chl-a data collected and analysed using the APHA 10200H method (APHA, 2012), were made available to UTS by DCCEEW and this was used to compare with biovolume data and in developing guidelines.

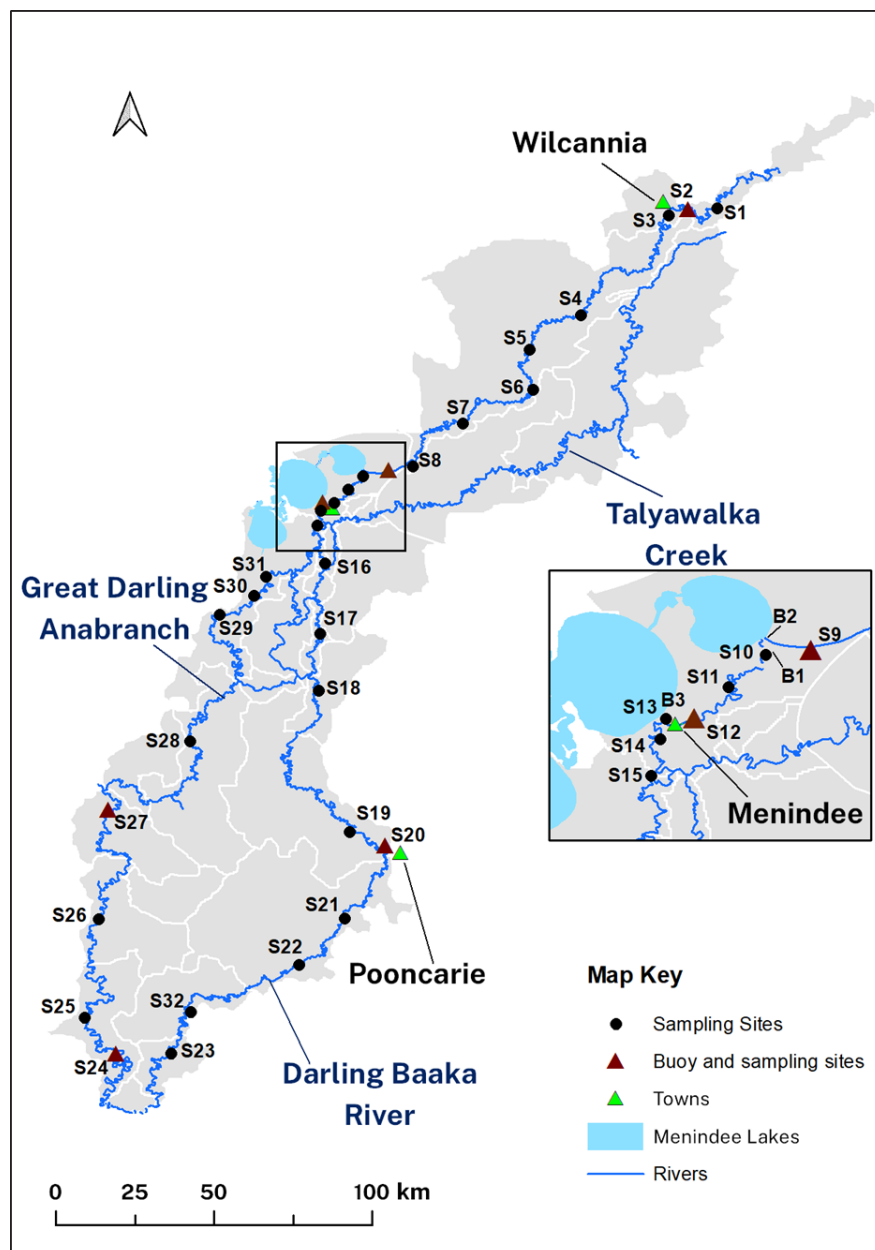


Figure 1. DCCEEW sampling sites used in the study (source: DCCEEW 2025)

2.3 Phytoplankton Diversity

Phytoplankton diversity was estimated using Simpson's diversity index (Simpson 1949) from the genus and biovolume data for each site on each sampling occasion.

Simpson's diversity index (SDI) is given as

$$SDI = 1 - \sum_{i=1}^K p_i^2$$

where K is the total number of phytoplankton genera, and p_i is the proportion of phytoplankton genus i in the total biovolume over all K genera. SDI ranges from 0 to 1, giving the probability of observing two individuals at random that belong to different taxa. SDI is free from any assumptions regarding the form of the frequency distribution of taxon abundance and gives weight to dominant taxa (Washington 1984; Krebs 1985). SDI was estimated using R package 'vegan' (Oksanen et al. 2024) in the statistical computing-and-graphics environment 'R' (ver. 4.4.0, R Foundation for Statistical Computing, Vienna, Austria, see <https://www.r-project.org/>).

2.4 Statistical Analysis

Chlorophyll-*a* and phytoplankton samples were $\log_{10}$ transformed to improve normality, and a regression scatter plot was generated to examine the relationship between these variables. Principal Component Analysis (PCA) was conducted on the major phytoplankton groups using PAST 4.11 software (Hammer et al. 2001).

2.5 Sub-Catchment Breakdown and Study Area

Algal sampling for this report was conducted by DCCEE staff across nine trips in 2024 (Table 1). Sites ranged from Wilcannia to Wentworth and included the lower Darling Baaka River and Great Darling Anabranch (Figure 1). Across these trips, 35 sites were visited, with a total of 101 phytoplankton samples and 97 chlorophyll-*a* collected. The 35 sites further represent five major sub-catchment zones of the lower Darling Baaka system (Table 2). Four of these five sub-catchment zones were situated on the Darling Baaka River and one along the Great Darling Anabranch. These zones were determined based on logical separation between river reaches, primarily defined by major weirs and associated flow regulation structures.

Site names ranged from 1 to 32, along with additional boundary sites (B2 and B4) and one legacy water quality sampling site (E1). Sites designated with a “B” prefix indicate boundary locations situated at outflows from adjacent lakes, while site E1 was part of earlier sampling efforts but was retired after Trip 25 (Table 1). Trip 26, did not include the collection of algal samples thus is excluded from Table 1 and this report.

Table 1. Table showing DCCEEW's field trips with trip reference, dates, number of sites visited and major month represented

Trip ID	DCCEEW Trip Reference	Date Range	Number of sites visited per trip	Major month represented per trip
T1	Trip 25	15/04/24 – 24/4/24	6	April
T2	Trip 27	20/05/24 – 06/06/24	24	May
T3	Trip 28	21/06/24 – 27/06/24	17	June
T4	Trip 29	30/7/24 – 01/08/24	3	July
T5	Trip 30	10/08/24 – 28/8/24	4	August
T6	Trip 31	09/09/24 – 24/09/24	5	September
T7	Trip 32	15/10/24 – 16/10/24	9	October
T8	Trip 33	11/11/24 – 20/11/24	23	November
T9	Trip 34	10/12/24 – 12/12/24	10	December

Table 2. River Reach Zones representing 'sub-catchments' of the study area

River Reach Zones	Sub-Catchment Names	Site Numbers	Replicates per Zone	Trips Samples
Zone 1	Willcannia to Lake Wetherell	S1 – S9, E1	35	T1, T2, T3, T4, T5, T6, T8, T9
Zone 2	Menindee Weir pool	S10 - S14, B2, B4	18	T1, T2, T3, T6, T8, T9
Zone 3	Pooncarie North	S15 – S20	18	T2, T3, T5, T6, T8, T9
Zone 4	Pooncarie South	S21 – S23, S32	9	T2, T3, T7
Zone 5	Great Darling Anabranh	S24 – S31	21	T2, T3, T4, T5, T6, T7, T8, T9

3.0 Results

3.1 Red Alert Toxic Cyanobacterial Blooms

The NHMRC guidelines for managing risks in recreational waters, provide guidelines for cyanobacteria in Australian waters. Cyanobacterial responses are divided into three alert categories, an alert of 'Green' is given to samples with a combined cyanobacterial biovolume between 0.04 and 0.4 mm³/L. An alert of 'Amber' is given to samples with a combined cyanobacterial biovolume between 0.4 and 10 mm³/L, or between 0.4 and 4 mm³/L of all cyanobacteria when a known toxin producer is dominant (> 75%). The 'Red' alert status is given to samples with a combined cyanobacterial content greater than 4 mm³/L and have a toxin producer dominant, or a combined cyanobacterial content greater than 10 mm³/L. Based on phytoplankton biovolumes and NHMRC guidelines, 4 of the 101 samples reached Red Alert status. Three of these samples were clustered around sites 1 to 3 in November 2024 and were dominated by the potentially toxic cyanobacteria *Dolichospermum circinale*. The non-toxic cyanobacteria species *Dolichospermum smithii* also comprised a large proportion of the cyanobacterial community at those sites in November. This meant that the cyanobacterial portion contributed approximately 45-70% of the total phytoplankton community during these Red alerts in November (Table 3).

The additional red alert sample was collected at site 9 in April 2024, and was heavily dominated ($>10 \text{ mm}^3/\text{L}$) by the non-toxic cyanobacteria *Prochlorothrix* (Table 3), although the sample contained the potentially toxic cyanobacteria *Dolichospermum circinale* in lower biovolume ($<0.2 \text{ mm}^3/\text{L}$ biovolume).

Table 3. Table of samples that triggered NHMRC Red alert

Site	Date	Zone	Total Biovolume (mm^3/L)	Cyanobacterial Biovolume (mm^3/L)	Dominant Cyanobacterial Species Present
1	12/11/24	Zone 1	11.44	4.10	<i>Dolichospermum circinale</i>
2	12/11/24	Zone 1	39.55	26.07	<i>Dolichospermum circinale</i>
3	12/11/24	Zone 1	19.65	14.76	<i>Dolichospermum circinale</i>
9	15/4/24	Zone 1	77.58	76.28	<i>Prochlorothrix</i>

Amber alerts were recorded for 44 of the 101 samples, and were largely clustered around April, May, October, November and December trips. Across the study, Amber alerts were found at 29 of the 35 sites, suggesting the lower Darling Baaka system can reach moderate cyanobacterial levels in both cool and warm periods.

Green alerts were recorded for 15 of the 101 samples, with the remaining 38 samples having no alert. Green alerts were largely around April, May, June, November and December 2024. The samples that recorded no alert based on NHMRC guidelines were found during all sampling trips except December (Appendix 2). These made up most samples from the colder months, June to September.

3.2 Chlorophyll-a and TSI

Chlorophyll-a (Chl-a) concentrations (an indicator of phytoplankton biomass) in the Darling Baaka River and the Great Darling Anabranch were generally high, with average concentrations at all sites exceeding guideline values ($5 \mu\text{g}/\text{L}$; ANZECC 2000). Individual samples revealed three replicates below the

ANZECC guideline value, all within Zone 1 (Wilcannia to Wetherell). Overall Chl-a values followed similar trends to those seen in the phytoplankton counts. Average Chl-a values were highest around the Pooncarie North and South zones, with the Wilcannia to Wetherell zone generally showing the lowest values across the study (Figure 2). Previous work looking at Chl-a on the Darling Baaka River has suggested the system is typically eutrophic across the year (Figure 3) (Oliver et al. 1999). To confirm this, the Trophic State Index (TSI) was determined using Chl-a values, showing 90 of the 97 samples as either eutrophic or hypereutrophic. The remaining 7 sites were found to be mesotrophic, and were only found from sites 2 to 9 in May and June 2024.

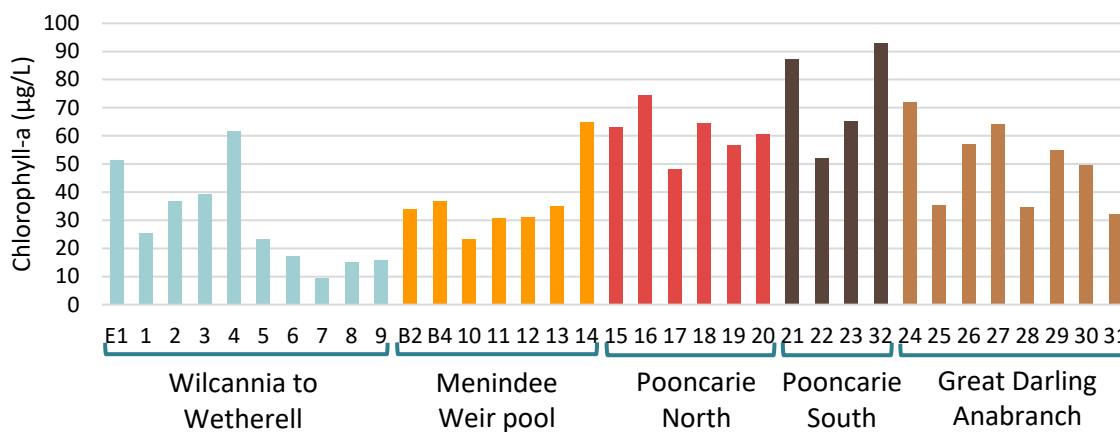


Figure 2. Average Chlorophyll-a values for all sites (n=1 to 7)

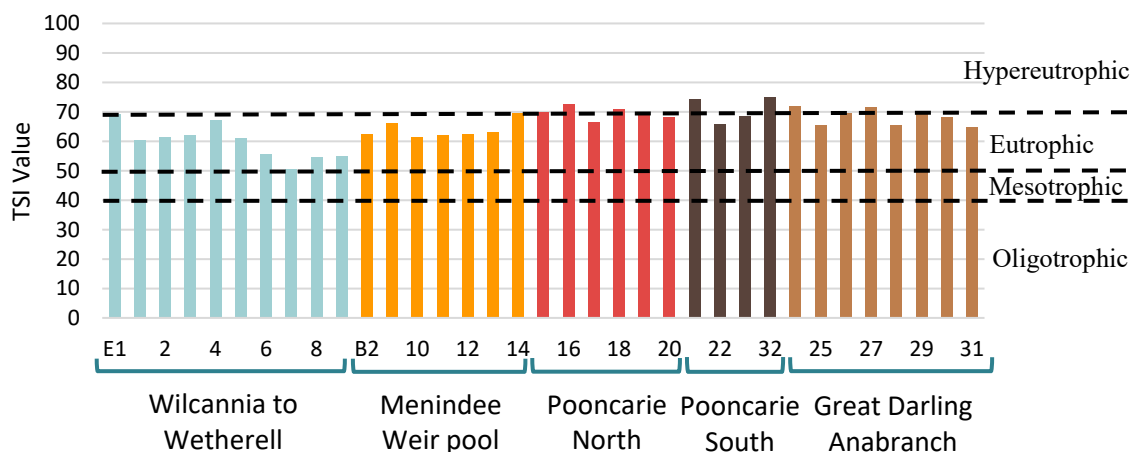


Figure 3. Average Trophic State Index (TSI) values for all sites (n=1 to 7)

3.3 EQR Values

The Ecological Quality ratio (EQR) was calculated using phytoplankton counts for all 101 samples collected (see Section 4.1 Q-index and Ecological Quality Ratio (EQR) for calculation details). Of these samples, 1 was considered Excellent, 6 were Good, 13 were Moderate, 28 were Poor and 53 were Bad. All Excellent and Good EQR values were found during the colder months of May, June and August, and only in Zone 1 and the most upstream site of Zone 2 (Figure 4). The 13 Moderate samples were found across all zones (except zone 2) in April, June, August, September and November, with the majority during June and November. The remaining 81 Poor and Bad samples covered all sites and zones. These EQR values suggest the whole system is in a Poor state, while from late June to August values show the system temporarily shifted to a better EQR state.

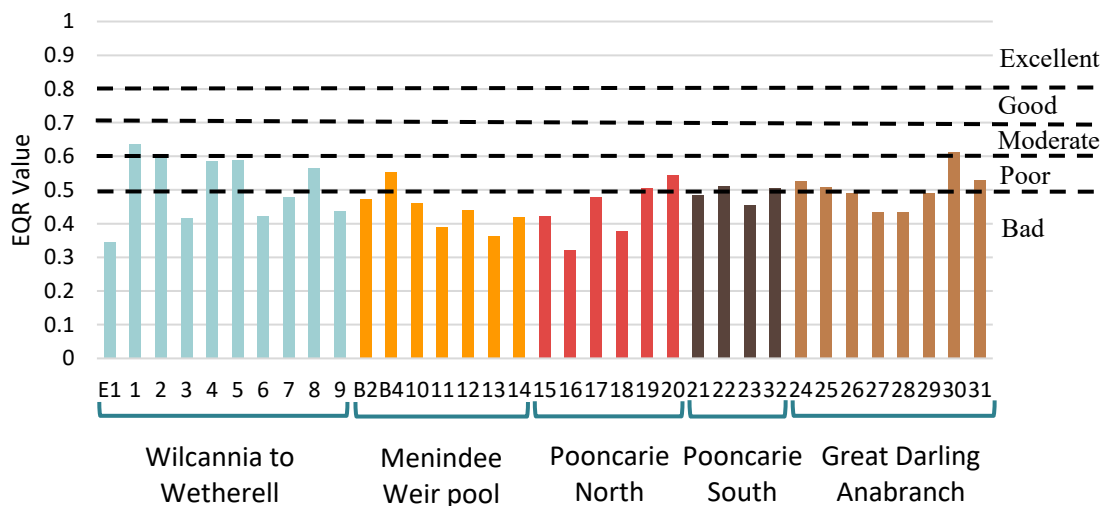


Figure 4. Average Ecological Quality ratio (EQR) values for all sites (n=1 to 7)

3.4 Phytoplankton trends

Phytoplankton biovolume was broken into six major groups, Cyanobacteria, Chlorophyta, Bacillariophyta, cryptomonads, euglenoids and others. Of these major groups, Cyanobacteria and Chlorophyta made up the largest proportion of the community at most sites (Figure 6). Between June and October, corresponding to the cooler months, phytoplankton biovolume was consistently low (Figure 5). Cyanobacterial relative biovolume was greatest in zones 1, 3 and 4 (Figure 5), suggesting these zones are the most susceptible to potential blooms. Chlorophyta was also high in these locations.

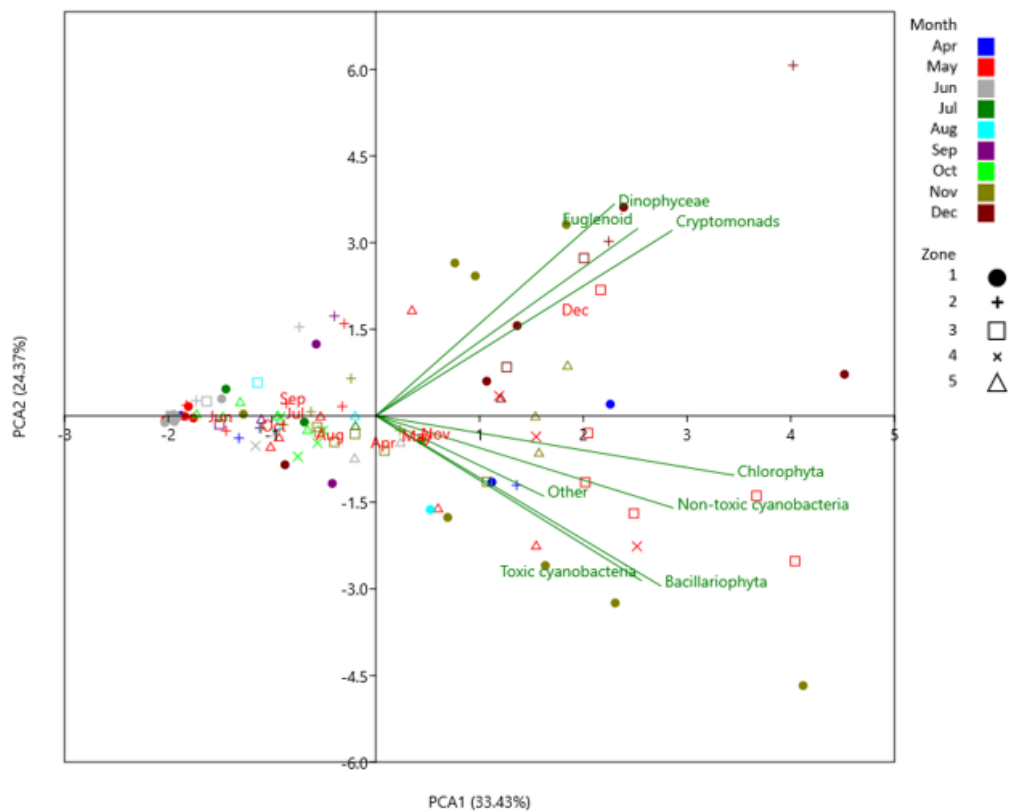


Figure 5. Principal component analysis (PCA) applied to major phytoplankton group community data over the study period. Sampling units are distinguished by sampling month (colour) and river reach zone (shape)

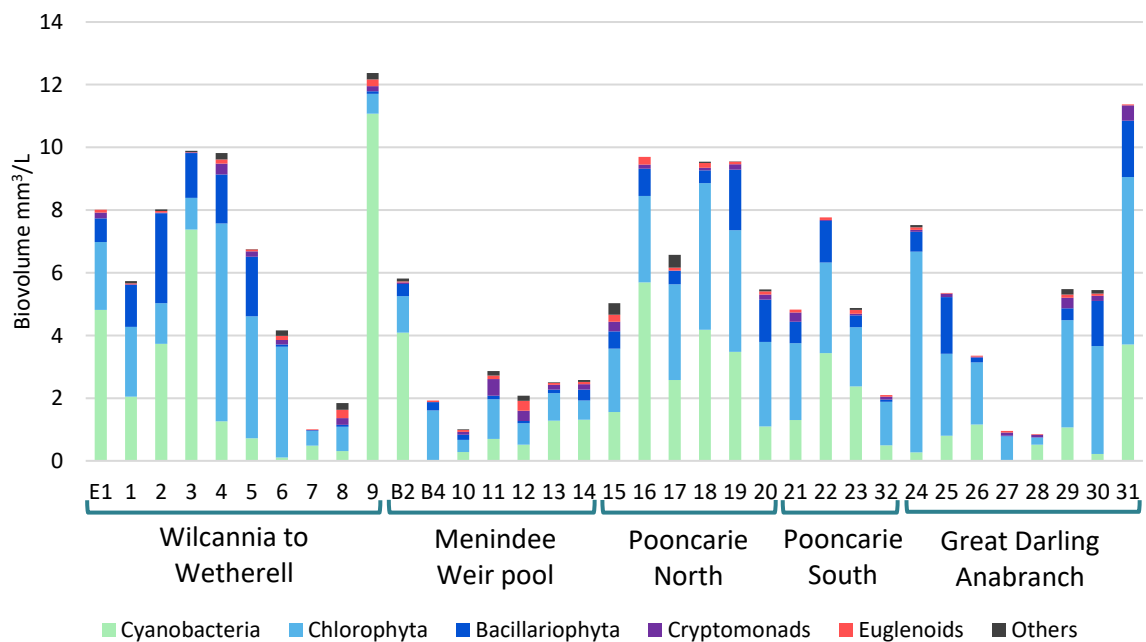


Figure 6. Average biovolume and proportions of different phytoplankton groups at sites in the study

A further breakdown of phytoplankton counts across zones was made to better understand how the community structure changed at all 35 sites and samples in the different zones. These were potentially toxic Cyanobacteria, non-toxic Cyanobacteria, Chlorophyta, Bacillariophyta, Dinophyceae, cryptomonads, euglenoids and others (Figure 7).

Of the 35 sites, 6 did not have potentially toxic cyanobacteria present (sites 8, 10, 27, 28, 32 and B4). With the remaining 30 sites showing evidence of potential toxin producers from at least one trip (Figure 7).

Zones 1 and 2 show a much more diverse phytoplankton community than other zones. Within Zone 1 (Wilcannia to Lake Wetherell), the phytoplankton community during the earlier and cooler months of April and June largely consisted of Chlorophyta, Bacillariophyta and euglenoids. In the warmer months of November and December, the community shifted to more Cyanobacteria and Chlorophyta (Figure 5, Figure 7), and away from Dinophyceae, cryptomonads and euglenoids (Figure 5, Figure 7). This is particularly evident at sites 1, 2 and 3, where cyanobacteria made up the largest proportion of the community during this time. The potentially toxic cyanobacteria *Dolichospermum circinale* was found to be the most abundant at these sites, and likely represented the start of a bloom. Zone 2 is characteristically a more lentic zone in the system, and did not follow trends in Zone 1. During both cooler and warmer periods of the year, potentially toxic and non-toxic cyanobacteria were consistently found. In the few samples where cyanobacteria were not detected, the community shifted to having a larger proportion of euglenoids. This potentially highlights the competition of resources by cyanobacteria and euglenoids, or the variability at sites within this more lentic zone.

Zone 3 showed a consistent dominance by potentially toxic cyanobacteria, non-toxic Cyanobacteria and Chlorophyta, particularly around sites 15 to 19 across the study (Figure 7). During the cooler months, the potentially toxic *D. circinale* and *Aphanizomenon gracile*, and the non-toxic *Anabaenopsis* and *Prochlorothrix* dominated the Cyanobacteria, while *Pediastrum* was the largest contributor to the Chlorophyta. This community was very similar to the warmer months, with the same cyanobacteria and Chlorophyta species found in highest abundance, except for *Prochlorothrix*.

Zones 4 showed a similar breakdown of the community across most sites and dates. Potentially toxic cyanobacteria, non-toxic cyanobacteria and Chlorophytes again made up most of the community (Figure 7). The cyanobacterial portion saw a shift from the upstream Zone 3, and was mostly dominated by the non-toxic *Prochlorothrix*. The Chlorophyta *Pediastrum* again dominated in this Zone. The lack of potentially toxic cyanobacteria seen within

this Zone is likely due to sampling not being carried out at these sites in November and December 2024 as *D. circinale* was seen from sites 15 to 19 in November at Zone 3, as well as at site 20 in December 2024 in Zone 3.

The Phytoplankton community in Zone 5 was mostly dominated by Chlorophyta and non-toxic cyanobacteria. During cooler months, The Chlorophyta *Planctonema* and to a lesser extent, the non-toxic cyanobacteria *Aphanocapsa* were the major phytoplankton found at this time. DCCEE staff highlighted that Zone 5 (the Great Darling Anabranch), did not flow from late August to October 2024 (Figure 10). When the flows returned in October, the first response was from the cyanobacteria *Aphanocapsa*. However, a month later the community shifted back to a pre-cease to flow state, with *Planctonema* and *Aphanocapsa* abundant.

3.5 Identification of Potential Blooms

During T1 (April 2024), some sites in Zone 1 and 2 showed a *Dolichospermum circinale* and *Prochlorothrix* bloom, with spatial clustering around sites 9, 13 and 14. A month later during T2 (May 2024), the bloom of *D. circinale* and *Prochlorothrix* moved downstream to Zones 3 and 4, extending from sites 16 to 23. In these zones, the bloom also included *Anabaneopsis* and *Aphanizomenon gracile*.

The bloom observed during T8 (November 2024) spanned sites S1 to S3 (Zone 1), with a dense bloom of *D. circinale* ($>10\text{mm}^3/\text{L}$) at sites S2 and S3. This is likely the start of a *D. circinale* bloom, as downstream sites (S4 to S8) showed no presence of *D. circinale* in November, with only the non-toxic *Aphanocapsa* present.

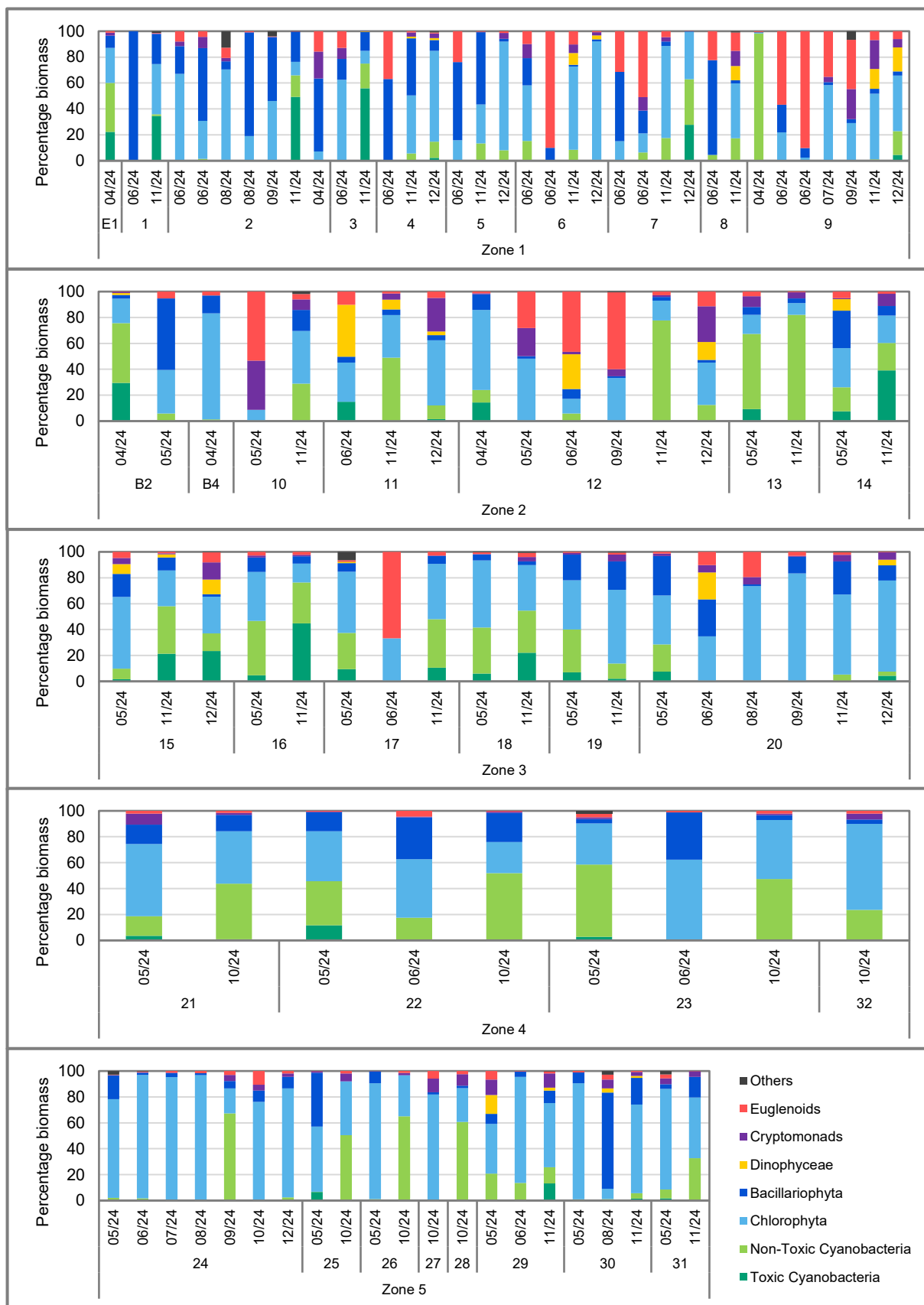


Figure 7. Phytoplankton communities across all sites and samples. Each figure represents samples collected per zone

3.6 Diversity Index

The site-average values of Simpson's diversity index (SDI) ranged from 0.48 to 0.90 (overall average: 0.75) in the studied section of the lower Darling Baaka River. The minimum SDI value was recorded at Site S13 in Zone 2 (Menindee Weir Pool) and the maximum SDI value was recorded at Site S15 in Zone 3 (Pooncarie North) (Figure 8).

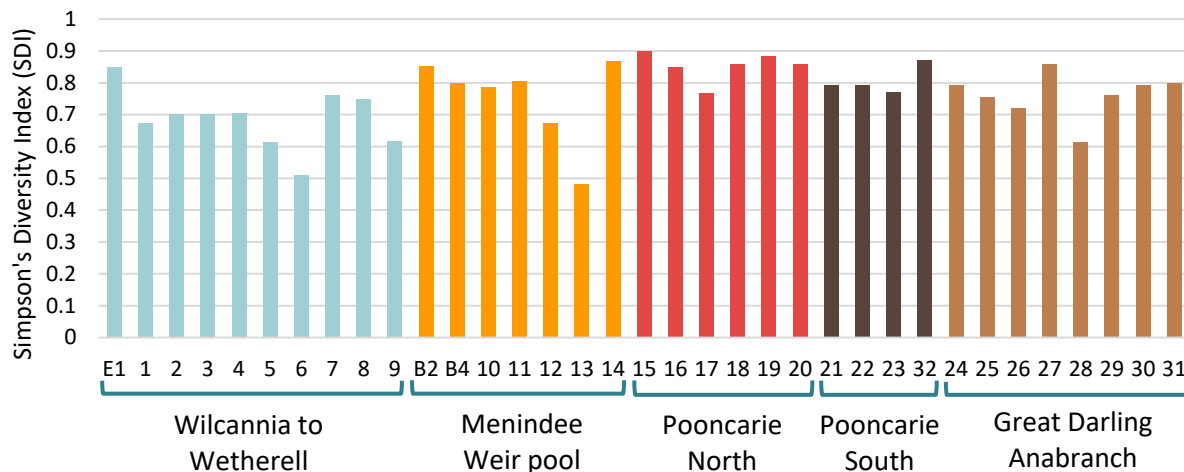


Figure 8. Average Simpson's diversity index values at all sites (n=1 to 7 at each site)

When the SDI values were averaged for each zone, Zone 1 (Wilcannia to Wetherell) had a minimum SDI value of 0.67 and Zone 3 (Pooncarie North) had a maximum SDI value of 0.85. Zone 3 also showed a less variability in SDI values than other zones (Figure 9).

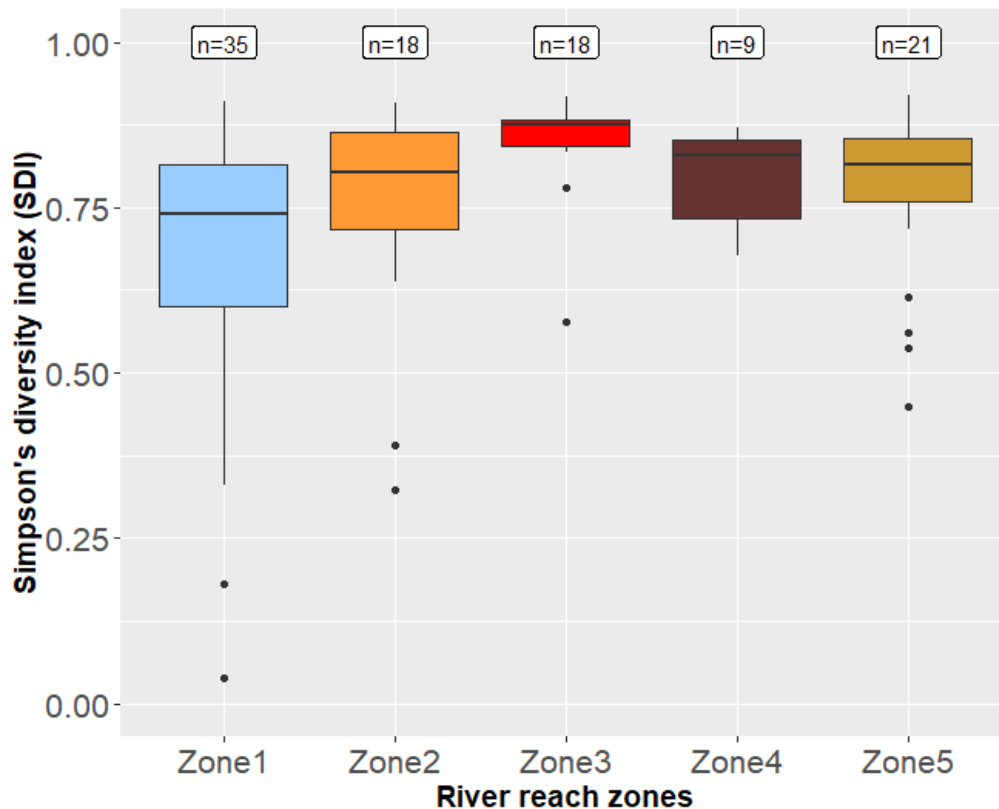


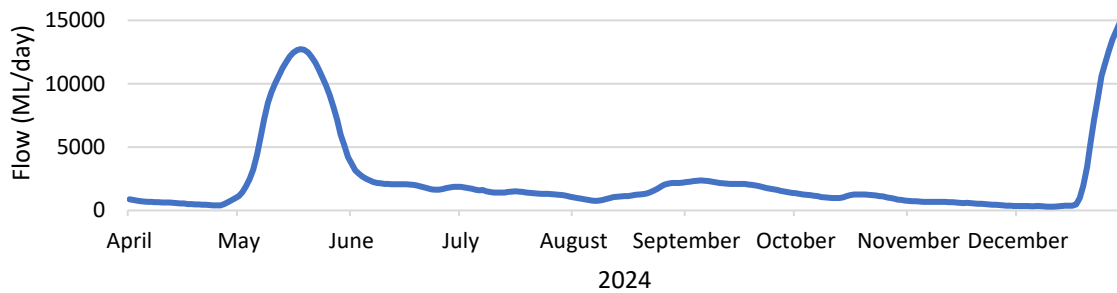
Figure 9. Boxplot of average Simpson's diversity index values at all zones (Zone 1: Wilcannia to Wetherell, Zone 2: Menindee Weir pool, Zone 3: Pooncarie North, Zone 4: Pooncarie South, Zone 5: Great Darling Anabranch, see Table 2 for details)

3.7 Lower Darling Baaka river flows

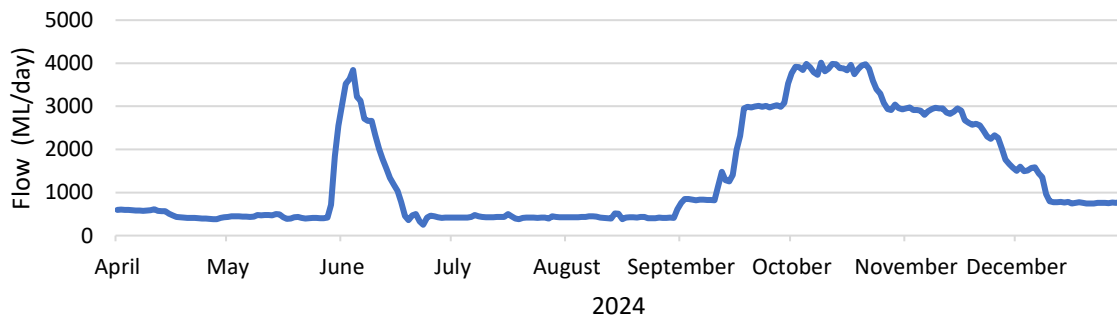
Two major flow events occurred during the sampling duration for this study, as well as one drying event on the Great Darling Anabranch. At Wilcannia, there was a flow event during the month of May, peaking at 12,000 ML/d. The flow then dropped to 3000 to 4000 ML/d as it moved past Weir 32 and Pooncarie in June, which are both found in Zone 3 and 4 (Figure 10). This flow event likely flushed the May bloom of *D. circinale*, *A. gracile*, *Anabaenopsis* and *Prochlorothrix* from these zones downstream, potentially reaching the Murray river. Similarly, flows at Wilcannia in late December reached 14,000 ML/d, which likely flushed the emerging November bloom of *D. circinale* at sites 1 to 3.

The Great Darling Anabranch ceased flow from the 21/8/24 till the 28/10/24 (Figure 10). Prior to this period, the phytoplankton community was dominated by the chlorophyte *Planctonema*, with the non-toxic Cyanobacteria *Aphanocapsa* also abundant. As the flows returned in late October, *Aphanocapsa* responded first. Then by November the community had shifted back to a pre-cess to flow state, with *Planctonema* the most abundant taxa.

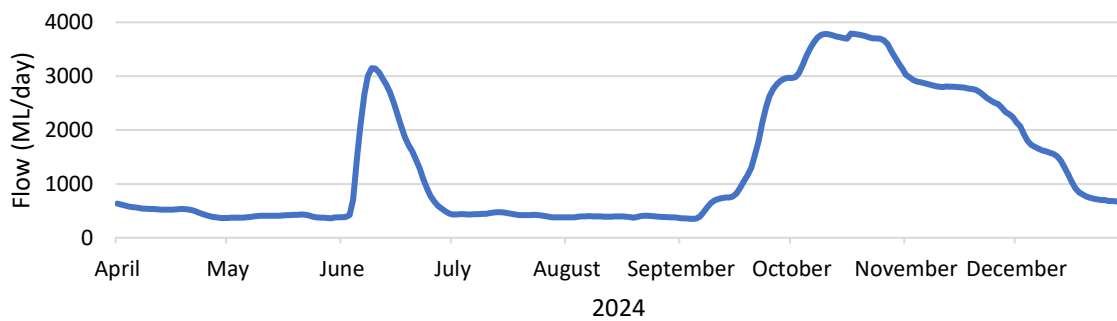
Wilcannia



Weir 32



Pooncarie



Great Darling Anabranh (Wycot)

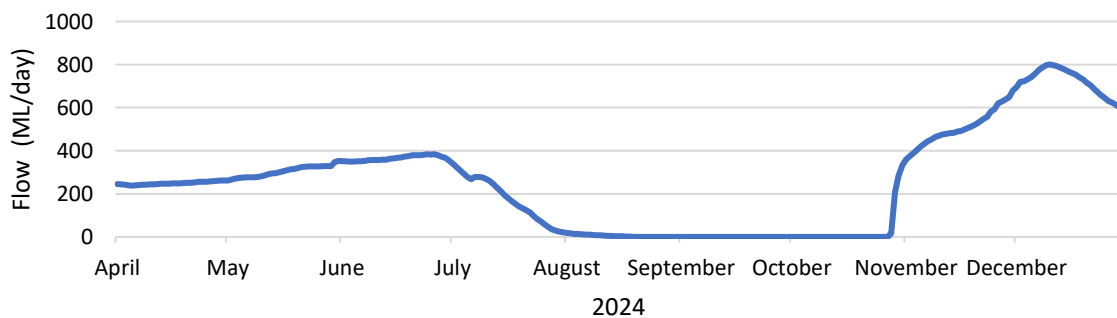


Figure 10. Flow data for 4 main sites across the Darling-Baaka River and Great Darling Anabranh

4.0 Review of current guideline values for algal indicators

In developing a new comprehensive Phytoplankton health index (CPHI) for the lower Darling Baaka River area, several existing guidelines were examined to establish the most suitable for use in the CPHI. Table 4 shows some of the existing guidelines based on phytoplankton and Chl-a data.

4.1 Algal biovolume or cell concentration trigger values

There are three commonly used guidelines based on biovolume or cell concentration values for determining algal risks in freshwaters (Table 4). These are

- (i) The NHMRC Australian Guidelines for managing risks in recreational water have established guidelines for cyanobacteria in freshwater. These guidelines were adapted to species and concentrations of cyanobacteria commonly encountered in Australian waters. Cyanobacterial biovolume is then categorised into three categories, these being Green, Amber and Red alert categories. See table 4 for details.
- (ii) NRMCC – drinking water guidelines have alerts triggered for biovolume of various potentially toxic cyanobacteria including *Raphidiopsis raciborskii* at 0.6 mm³/L, *Microcystis* at 0.6 mm³/L, and *Dolichospermum* at 5 mm³/L.
- (iii) The Australian and New Zealand Environment and Conservation Council (ANZECC) has established a trigger value for livestock water. Cyanobacteria are at an increased risk to livestock health when *Microcystis* concentration is above 11,500 cells/mL.

Table 4. Guidelines used in determination of a new Darling River Phytoplankton health indicator and suggested categories.

Guideline/index	Levels				
NHMRC (Cyanobacteria Community biovolume)	Green >500 to <5000 cells/mL M. aeruginosa or >0.04 to <0.4 mm³/L for the combined total of all cyanobacteria.	Amber ≥5000 to <50 000 cells/mL M. aeruginosa or biovolume equivalent of ≥0.4 to <4 mm³/L for the combined total of all cyanobacteria where a known toxin producer is dominant in the total biovolume or ≥0.4 to <10 mm³/L for the combined total of all cyanobacteria where known toxin producers are not present.	Red ≥50 000 cells/mL toxic M. aeruginosa or biovolume equivalent of ≥4 mm³L for the combined total of all cyanobacteria where a known toxin producer is dominant in the total biovolume.		
TSI (Carlson, 1977) (All Community) (Chlorophyll-a value)	<40 oligotrophic	>40-50 mesotrophic	>50-70 eutrophic	>70 hypereutrophic)	
ANZECC (Livestock)	An increasing risk to livestock health is likely when cell counts of Microcystis exceed 11 500 cells/mL				
ANZECC (Chl-a – South East Australia)	5ug/L				
NRMMC (drinking water guidelines)	C. raciborskii 15,000-20,000 cells/mL, which is equivalent to a biovolume of 0.6- 0.8 mm³/L of M. aeruginosa a cell density of approximately 6,500 cells/mL (biovolume of 0.6 mm³/L)				
Q-index / EQR (ecological quality ratio) (Community)	Excellent, 0.8 - 1	Good, 0.7 - 0.8	Moderate 0.6-0.7	Poor 0.2 – 0.4	Bad 0.00 - 0.2



4.2 Chlorophyll-a and Trophic state index (TSI)

Trigger values for stressors in lowland rivers of South Eastern Australia are 5 µg/L of chlorophyll-a based on ANZECC (2000) guidelines for the protection of aquatic ecosystems. Trigger values for chlorophyll-a are data deficient within the South-central Australia – low rainfall area so no values are given (ANZECC and ARMCANZ, 2000). Given this the relationship of chlorophyll-a to phytoplankton biovolume was examined from the data collected to determine if a relationship existed and if new categories could be developed based on this data.

The trophic state index (TSI) is a measure of biological productivity within a system (Carlson, 1977) based on chlorophyll-a data. The TSI value is calculated according to this equation by Carlson (1977):

$$TSI(Chl) = 10 \left(6 - \frac{2.04 - 0.68 \ln Chl}{\ln 2} \right)$$

TSI values will range from 0 – 100, with values ranging from oligotrophic (low production) to hypereutrophic (extreme production) (See Table 4).

4.3 Q-index and Ecological Quality Ratio (EQR)

The assemblage index (Q index) and Ecological Quality Ratio (EQR) are tools used to assess the health of aquatic ecosystems such as lakes and rivers (Padisák et al., 2006; Borics et al., 2007). Q index calculates the relative share (p_i , where $p_i = n_i/N$; n_i : biomass of the i -th functional group; N : total biomass) of functional groups in total biomass and a factor number (F) determined for the i -th functional group in the given aquatic system.

Phytoplankton taxa are grouped into functional groups, based on adaptations to specific environmental niches (Reynolds et al., 2002; Borics et al., 2007; Padisák et al., 2009). Factor F was assigned to each functional group based on the pristine status of the natural ecosystem and potential algal assemblages. Higher F values indicate groups found in pristine conditions, while lower values represent undesirable assemblages. In this study, we assigned factor F specific to rivers following Borics et al. (2007). The Q index value can range from 0 – 5 and from this, water quality can be classified into five categories: 0-1: very poor; 1-2: tolerable; 2-3: medium; 3-4: good; 4-5: excellent. Using this score, an EQR number can be calculated ($Q \text{ index}/5$) (Borics et al., 2007). The EQR value ranges from 0 to 1, and since the study site is characterized as a 'Large river' (Borics et al., 2006), water quality is classified into five categories: <0.5: bad; 0.5-0.6: poor; 0.6-0.7: moderate; 0.7-0.8: good; >0.8: excellent.

5.0 Development of a new Comprehensive Phytoplankton Health index (CPHI)

5.1 Comparison of results to existing guideline values

101 samples were analysed for phytoplankton identification and enumeration and biovolume determination across the Lower Darling Baaka River area spanning from April 2024 till December 2024 across 33 sites. 93 genera of phytoplankton were identified across the samples including 17 cyanobacteria. The potentially toxic species *Dolichospermum circinale*, *Raphidiopsis raciborskii*, *Microcystis flos-aquae*, *Chrysosporum ovalisporum*, *Aphanizomenon gracile* were present across the study.

In order to assess the most appropriate guidelines to use in the CPHI, existing data was compared to the guidelines identified in Table 4.

(i) NHMRC guideline comparison

Based on NHMRC cyanobacterial guidelines 38 samples had no alert, 15 samples were green alert, 44 were amber alert and 4 were Red Alert (Figure 11). Of the red alert samples, the dominant cyanobacteria was *Dolichospermum circinale*.

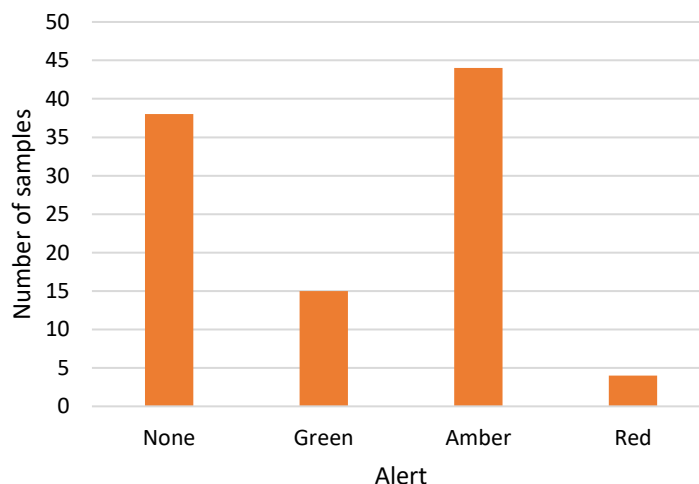


Figure 11. Sample number falling into the NHMRC guidelines categories for cyanobacteria and recreational water use

(ii) Q index and EQR comparison

The Q index and Ecological Quality Ratio (EQR) were determined for the samples. The Q index showed that 2 samples were rated as bad, 20 were

tolerable, 59 were medium, 19 were good and 1 was excellent. The EQR is a function of the Q index ($Q/5$) and using this 53 samples were rated as bad, 28 poor, 13 moderate, 6 good and 1 excellent (Figure 12).

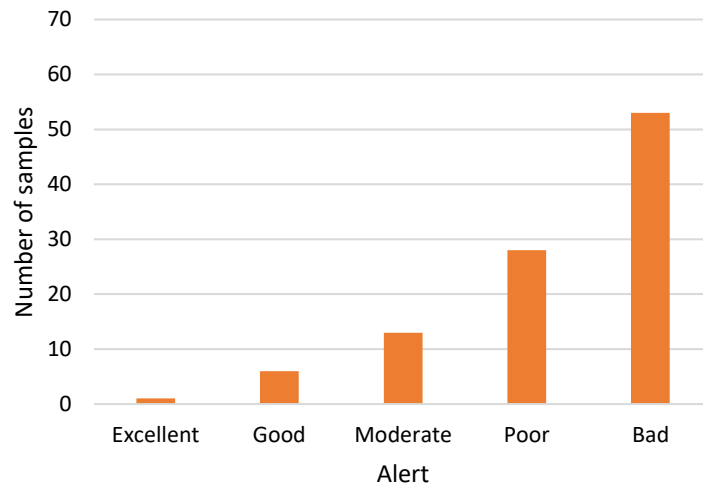


Figure 12. Sample number falling into the determined EQR categories

(iii) Chl-a and TSI comparison

The TSI index was applied to the Chl-a data for the sites. No sites were classed oligotrophic, 7 sites were classed as mesotrophic, 60 sites were classed as eutrophic and 30 sites were classed as hypereutrophic (Figure 13). ANZECC (2000) guidelines for protection of ecosystems for inland rivers guideline value of 5 $\mu\text{g/L}$ was exceeded in every sample.

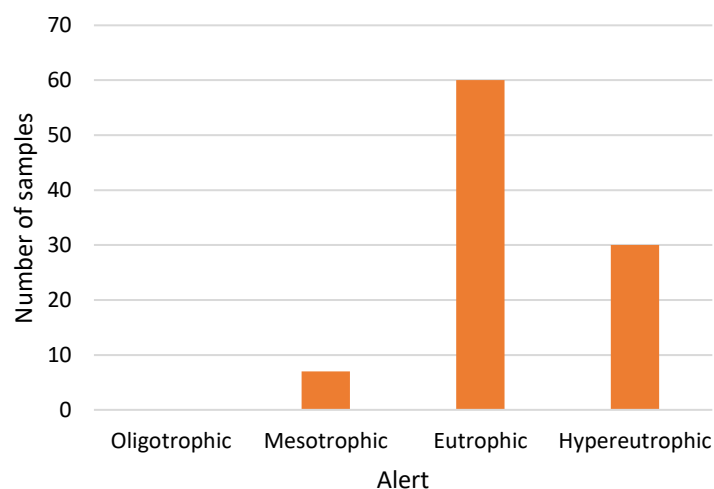


Figure 13. Sample number falling into the TSI classifications

Total algal biovolume from samples was correlated with Chl-a data. Total phytoplankton biovolumes and Chl-a values were $\log_{10}$ transformed and plotted (Figure 14). A significant positive log- log relationship was found between Chl-a and phytoplankton biovolume (adjusted $r^2 = 0.41$, $P < 0.01$, $n = 101$), indicating that phytoplankton biovolumes can be predicted from Chl-a concentrations in the studied section of the Darling Baaka River between Wilcannia to Wentworth including Great Darling Anabranch.

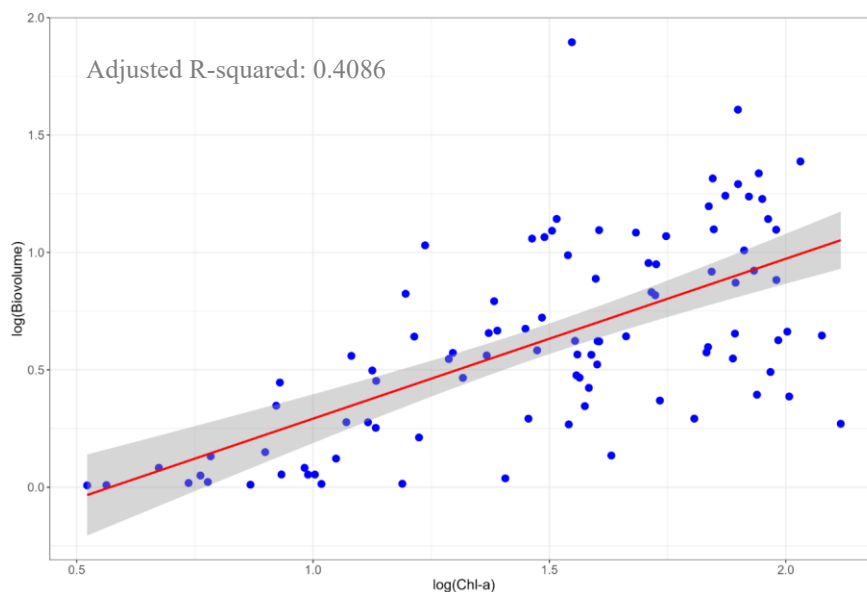


Figure 14. Log-log relationship of chlorophyll-a (Chl-a) and phytoplankton biovolume (Biovolume) in the studied section of the Darling Baaka River. 95% confidence interval for the fitted regression line is shown by grey-coloured area

5.2 Conclusion for existing guideline values

It was apparent from applying the currently available phytoplankton and chlorophyll a guidelines and indices that no individual one was appropriate for giving an overall assessment of river health based on phytoplankton. This is likely due to each being developed for a different purpose such as for safe recreation from cyanobacterial toxins (NHMRC guidelines), or for estimating the trophic state of a water system such as the TSI. Some guidelines such as the ANZECC (2000) for protection of aquatic ecosystems were not useful at differentiating sites as all samples were above the guideline.

To comprehensively assess the health of a system based on phytoplankton criteria, it is recommended that several of the indices need to be combined and applied in a useful and meaningful way to characterise sampling site data. This combination of indicators resulted in the development of the CPHI

6.0 The Comprehensive phytoplankton health index (CPHI)

6.1 The CPHI assessment framework

From the data collected in this study it is suggested that 3 indicators should be combined to give a holistic view of the river health at any point in time. These indicators are (i) the NHMRC cyanobacterial recreation guidelines, (ii) TSI and (iii) the EQR.

By combining these three indicators, we are encompassing aspects of human and aquatic organism safety (NHMRC guidelines), measuring eutrophication and nutrient over enrichment (TSI) and the phytoplankton community health (Q index/EQR). We suggest our **Comprehensive Phytoplankton Health index (CPHI)** have 5 levels, being **Very Good, Good, Moderate, Poor and Very Poor**. Figure 15 shows how the existing guidelines broadly integrate to create the new guideline.

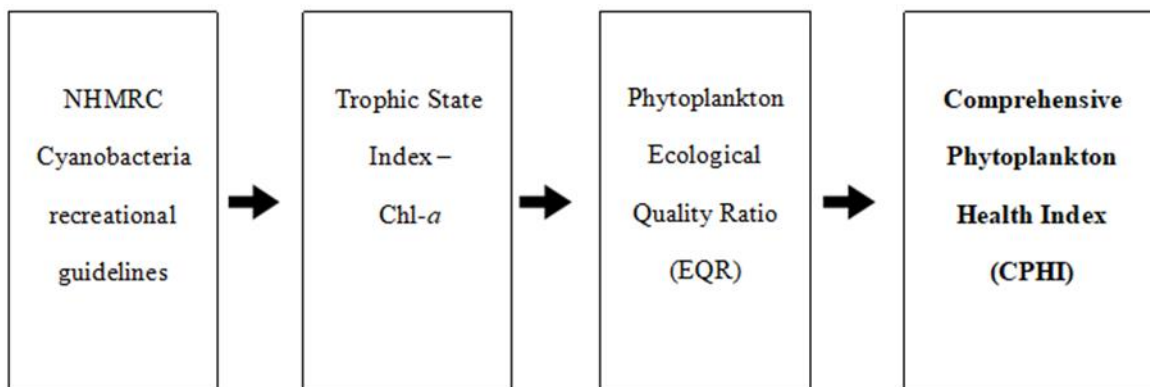


Figure 15. General process of developing the Comprehensive Phytoplankton Health Index (CPHI).

Due to the importance of safety for human recreational users of the water, we suggest a three stepped approach to the CPHI assessment process

Step one:

In this initial analysis of data, it is proposed that the NHMRC guidelines should be revised and used as a first assessment tier (Figure 17). Based on the biovolume and proportion of potentially toxic to non-toxic cyanobacteria the sample/site can be rated as Red Alert, Amber Alert, Green Alert or no Alert.

The adjustment of the NHMRC guidelines is to accommodate for a very broad range of cyanobacteria in the Amber alert category. As the levels for Amber alert are quite broad, 0.4 – 4 mm³/L (for potentially toxic dominant) or 0.4 – 10 mm³/L

(for all cyanobacteria), we propose a new category of 2 - 4 mm³/L (for potentially toxic dominant) or 2 – 10 mm³/L for all cyanobacteria (Figure 17). Under these adjusted NHMRC guidelines, 4 samples were at Red alert, 16 were at Amber alert and 81 showed no alert (Figure 16).

No alert and Green alert samples are automatically shifted to step 2 (Figure 17). While Red Alert samples automatically generate a **Very Poor** rating on the CPHI due the high biovolume of potentially toxic cyanobacteria (>4 mm³/L with dominant potentially toxic species) or very high overall cyanobacterial biovolume (>10 mm³/L).

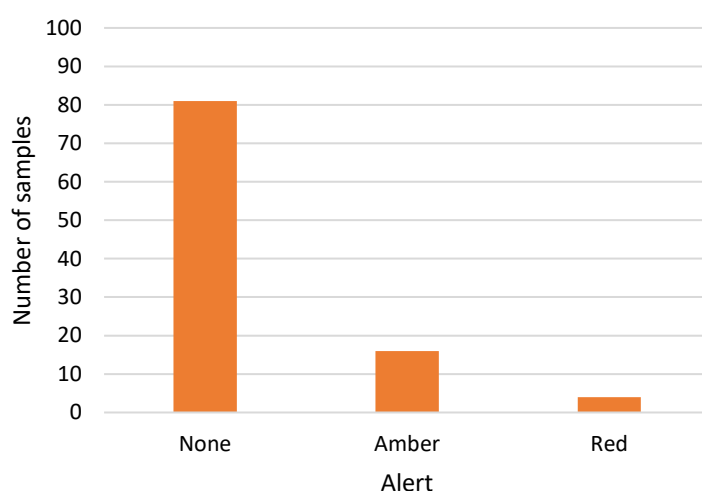


Figure 16. Sample number for the new adapted categories deviating from the NHMRC cyanobacteria guidelines

Step 2:

Samples that fall into the new 'Amber Alert' group are further assessed using the overall trophic state measure using chlorophyll a data and turning this into the Trophic State Index, a measure of the productivity of the system in Step 2.

If cyanobacteria are below 2 mm³/L, then the TSI is used in Step 2. This is important as high Chl-a or TSI can indicate a stressed and poor health system even if no cyanobacteria are present such as blooms of other algal groups (such as green algae). The TSI takes the chl-a data (either direct measurement if available or conversion from biovolume) and categorises the sample into oligotrophic (low productivity), mesotrophic (moderate productivity), eutrophic (high productivity) or hypereutrophic (extreme productivity).

If a sample is categorised as hypereutrophic it automatically bi-passes Step 3 and is allocated a CPHI rating of **Very Poor** (Figure 17). All other TSI ratings allocated in Step 2 progress to Step 3 of the CPHI process.

Step 3

The final step in the CPHI assessment process is to use the Ecosystem Quality Ratio to examine the overall health of the phytoplankton community.

If samples are Eutrophic on the TSI, and have a 'Bad' EQR score, samples are allocated a CPHI rating of **Very Poor**. If it has an EQR of Poor, Moderate, Good or Excellent a CPHI of **Poor** is returned regardless (Figure 17).

If samples are Mesotrophic on the TSI, and have an EQR score of Bad or Poor, it is allocated a CPHI of **Poor**. If the EQR score is Moderate, Good or Excellent a CPHI of **Moderate** is returned regardless.

Samples with a TSI classification of 'Oligotrophic' are also assessed in Step 3 using the EQR rating. When samples have an EQR of Moderate, Poor or Bad, they are allocated a CPHI of **Medium**. If it has an EQR of Good it gets a CPHI of **Good**. And if it has an EQR of Excellent, it gets a CPHI of **Very Good** regardless.

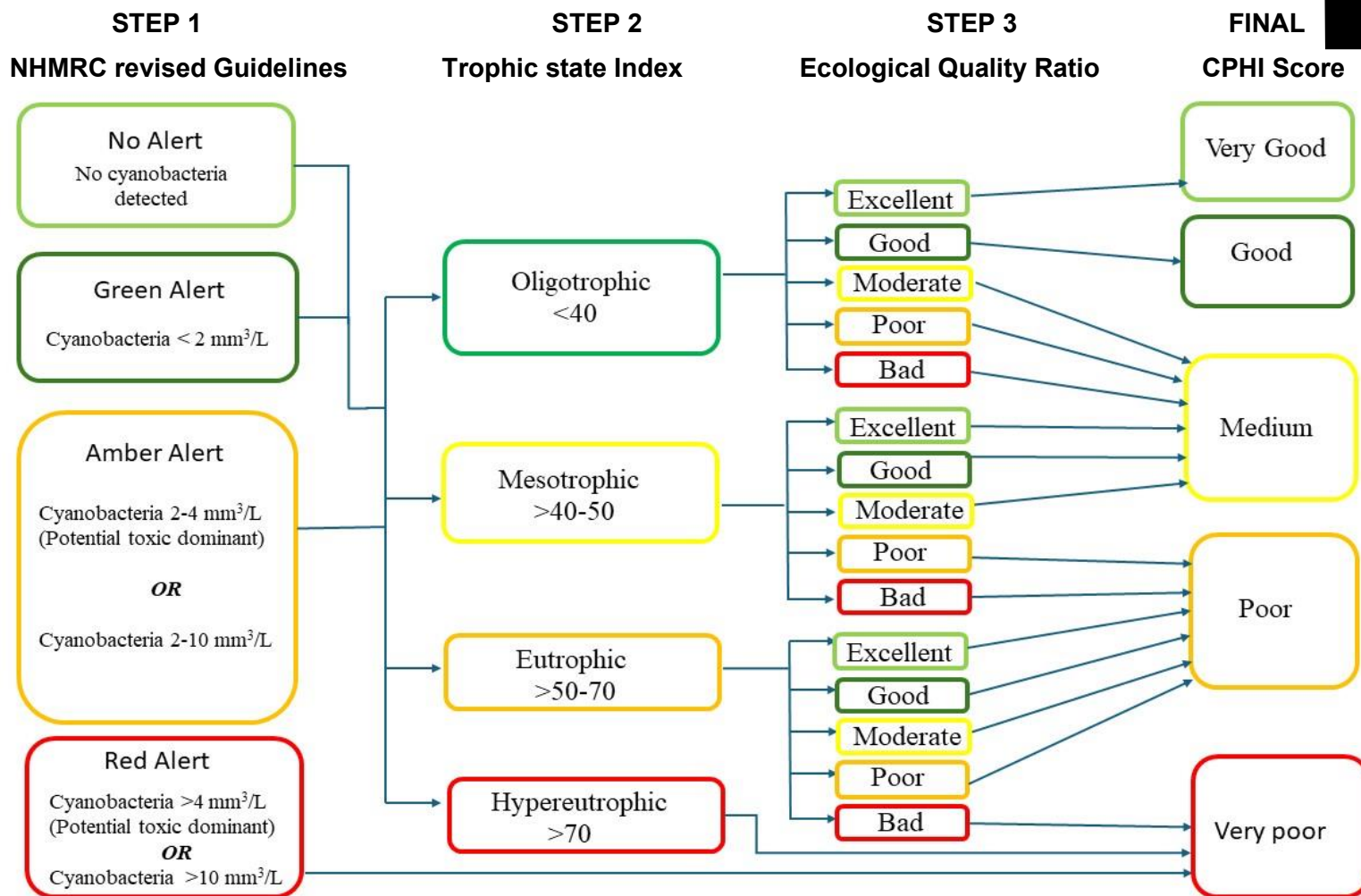


Figure 17: Comprehensive Phytoplankton Health Index (CPHI) assessment process

6.2 Applying the CPHI to the lower Darling Baaka river project

The Comprehensive Phytoplankton Health Index (CPHI) has been determined for each of the samples collected as part of NSW DCCEE's Darling-Baaka River Health Monitoring Project (DBRHMP) (Appendix 2). Using this new framework 58 samples were rated Very Poor, 36 Poor, and 3 Medium. Appendix 2 shows the individual sample Comprehensive Phytoplankton Health Index (CPHI) ratings. Sampling periods with the most sites visited were around May/June and November/December, with nearly all sites listed as Poor or Very Poor (Table 5).

Table 5 CPHI ratings for sites during two periods across the study

Site	CPHI rating	
	May/June	November/December
S1	Poor	Very Poor
S2	Poor	Very Poor
S3	Poor	Very Poor
S4	NA	Very Poor
S5	NA	Very Poor
S6	Poor	Very Poor
S7	Medium	Very Poor
S8	Medium	Very Poor
S9	Poor	Very Poor
S10	Very Poor	Very Poor
S11	Very Poor	Very Poor
S12	Very Poor	Very Poor
S13	Very Poor	Very Poor
S14	Very Poor	Poor
S15	Very Poor	Poor
S16	Very Poor	Poor
S17	Very Poor	Very Poor
S18	Very Poor	Very Poor
S19	Very Poor	Poor
S20	Very Poor	Poor
S21	Very Poor	Very Poor
S22	Very Poor	Very Poor
S23	Very Poor	Very Poor
S24	Very Poor	Very Poor
S25	Poor	Very Poor
S26	Poor	Very Poor
S27	NA	Very Poor
S28	NA	Very Poor
S29	Very Poor	Very Poor
S30	Poor	Poor
S31	Very Poor	Poor
S32	NA	Very Poor

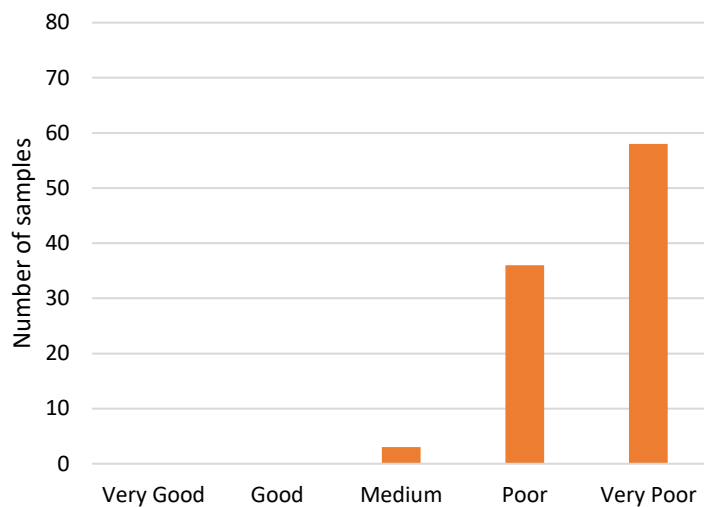


Figure 18. Sample number and ratings for the new Comprehensive Phytoplankton Health Index (CPHI) classifications

The **Comprehensive Phytoplankton Health Index (CPHI)** has been developed on limited data from the Darling-Baaka River area over a relatively short period of time and after some major fish kills have occurred in the 2019-20 and 2023 years (Vertessy et al. 2019; Sheldon et al. 2021; Williams and Schulz 2023). This Comprehensive Phytoplankton Health Index (CPHI) should be tested against a longer time period of samples and including those prior to the fish kill events. Further it should be tested in other impacted (i.e. Murray River) and less impacted rivers if data is available. This will allow fine tuning of the process and allow adjustments if required such as introducing weightings on particular elements.

7.0 Key findings and Recommendations

Overall, 85 different phytoplankton taxa were identified across the study. Of these taxa, 19 Cyanobacteria were identified, with 5 potentially toxic species found (*Dolichospermum circinale*, *Aphanizomenon gracile*, *Microcystis flos-aquae*, *Raphidiopsis raciborskii* and *Chrysosprum ovalisporum*). The most prolific of these, *Dolichospermum circinale*, was found to be blooming during April and May from the lake Wetherell area down to Wentworth (Murray River and Darling River confluence). Blooms also occurred in November, at the most upstream sites of Zone 1 (Wilcannia to Wetherell). NHMRC cyanobacterial alerts for recreational water use showed 4 Red alerts and 44 Amber alerts. During April/May (T1 and T2) a bloom of *D. circinale* and *Prochlorothrix* moved from zone 1 and 2 to zone 3 and 4. As it moved, the phytoplankton community shifted to also include *Anabaenopsis* and *Aphanizomenon gracile*. Similarly, in November a bloom of *D. circinale* started around sites 1 to 3 in the upstream section of Zone 1. NHMRC Red alerts fell into both bloom periods, with Amber alerts largely clustered around the bloom event times and sites.

The lowest cyanobacterial counts occurred during Spring times, particularly around September and October. During this period, the community had very little Cyanobacteria, with the major Phytoplankton groups Chlorophyta, Bacillariophyta and euglenoids dominating.

Chlorophyll-a (Chl-a) values show that Zones 3 and 4 (Pooncarie North and South) were the highest across the study area, with Zone 1 (Wilcannia to Wetherell) being the lowest. Despite this, nearly all Chlorophyll-a values were above 10 µg/L. Carson's trophic state index (TSI) was applied to Chl-a data and revealed 90 of 97 samples across the system were Eutrophic or Hypereutrophic. With the remaining 7 samples being Mesotrophic. This suggests the system is generally in a poor state, irrespective of month and location.

The Ecological Quality Ratio (EQR) values were determined based on phytoplankton counts, which revealed 1 sample as Excellent, 6 as Good, 13 as Moderate, 28 as Poor and 53 as Bad. The Excellent and Good categories only accounted for 7 of the 101 samples which were found mostly during the cooler months of May, June and August. Whereas Poor and Bad categories accounted for 81 of the 101 samples and were found across all sites and dates in the study. This suggests the system is largely in a poor state, although from July to August the system temporarily shifted to a better EQR state.

Simpson's Diversity Index (SDI) values when averaged across zones show that the community was most diverse in Zone 3 (Pooncarie North) and least diverse in Zone 1 (Wilcannia to Wetherell). These trends loosely follow the Chl-a

findings and suggest Zone 3 provides the river environment conducive to highly diverse and abundant phytoplankton.

The Comprehensive Phytoplankton Health Index (CPHI) showed generally poor or very poor ratings for the Darling-Baaka River area. However, this is over a relatively short period of time and after some major fish kills have occurred. The proposed CPHI should be tested against a longer time period, including the time period prior to the fish kill events and should be tested in other impacted (i.e., Murray River) as well as less impacted rivers.

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Appendix

Appendix 1 – List of phytoplankton from samples collected.

Cyanobacteria	Chlorophyta (Greens)	Bacillariophyta (Diatoms)
Dolichospermum circinale	Ankistrodesmus	Cyclotella/Coscinodiscus
Dolichospermum other	Actinastrum	Cymbella
Raphidiopsis raciborkii	Botryococcus	Navicula
Anabaenopsis	Chlamydomonas	Nitzschia
Microcystis flos-aquae	Chlorella	Uroselenia/Acanthoceras
Chrysosporum ovalisporum	Chodatella	Synedra/Fragilaria
Planktothrix	Closterium	Aulacosiera
Prochlorothrix	Coelastrum	Gyrosigma
Pseudoanabaena	Cosmarium	Gomphonema
Aphanocapsa	Chlorococcus	Brebissonia
Chroococcus	Crucigenia	Amphipleura
Radiocystis	Dicanthos	Astrionella
Romeria	Dictyosphaerium	Melosira
Spirulina	Dysmorphococcus	Amphora
Dolichospermum affinis	Elakatothrix	Ceratoneis
Aphanizomenon gracile	Hyalotheca	Dinophyceae
Glaucoispira	Kirchierella	Ceratium
Dolichospermum smithii	Nephrocystium	Peridinium
Dolichospermum flos-aqua	Microspora	Gymnodinium
Cuspidothrix issatschenkoi	Mougeotia	Cryptomonads
	Monoraphidium	Euglena
	Oocystis	Phacus
	Pediastrum	Euglenoids
	Pseudosphaerocystis	Cryptomonas

	Selenastrum	Chromonas
	Scenedesmus	Trachelomonas
	Snowlla	Others
	Sphaerocystis	Dinobryon
	Staurostrum	Mallomonas
	Staurodesmus	Synura
	Tetraspora	Chlorobotrys
	Tetradon	
	Tetrasporopsis	
	Planctonema	
	Chlorogonium	
	Dichotomococcus	
	Desmodesmus	
	Golenkinia	
	Willea	
	Ankyra	

Appendix 2 – Samples examined and the Comprehensive Phytoplankton Health Index (CPHI) rating

Site	Date	EQR	TSI	NHMRC	CPHI
B4	24/04/2024	Poor	Eutrophic	None	Poor
S2	16/04/2024	Moderate	Eutrophic	None	Poor
S12	17/04/2024	Bad	Eutrophic	None	Very Poor
B2	17/04/2024	Bad	Hypereutrophic	Amber	Very Poor
E1	17/04/2024	Bad	Eutrophic	Amber	Poor
S9	15/04/2024	Bad	Eutrophic	Red	Very Poor
S15	22/05/2024	Bad	Hypereutrophic	None	Very Poor
S21	28/05/2024	Bad	Hypereutrophic	None	Very Poor
S12	23/05/2024	Bad	Eutrophic	None	Very Poor
S14	22/05/2024	Poor	Hypereutrophic	None	Very Poor
S9	5/06/2024	Poor	Mesotrophic	None	Poor
S10	23/05/2024	Bad	Eutrophic	None	Very Poor
S7	6/06/2024	Good	Mesotrophic	None	Medium
S13	22/05/2024	Bad	Hypereutrophic	None	Very Poor
S29	20/05/2024	Bad	Eutrophic	None	Very Poor
S6	6/06/2024	Bad	Mesotrophic	None	Poor
S31	20/05/2024	Bad	Eutrophic	None	Very Poor
S23	27/05/2024	Bad	Hypereutrophic	Amber	Very Poor
S25	29/05/2024	Poor	Eutrophic	None	Poor
S30	20/05/2024	Poor	Eutrophic	None	Poor
S24	29/05/2024	Poor	Hypereutrophic	None	Very Poor
S26	29/05/2024	Poor	Eutrophic	None	Poor
S2	4/06/2024	Poor	Mesotrophic	None	Poor
B2	23/05/2024	Good	Eutrophic	None	Poor

S16	21/05/2024	Bad	Hypereutrophic	Amber	Very Poor
S22	28/05/2024	Bad	Hypereutrophic	Amber	Very Poor
S19	27/05/2024	Bad	Hypereutrophic	Amber	Very Poor
S20	21/05/2024	Poor	Hypereutrophic	Amber	Very Poor
S17	27/05/2024	Bad	Hypereutrophic	Amber	Very Poor
S18	21/05/2024	Bad	Hypereutrophic	Amber	Very Poor
S20	25/06/2024	Poor	Eutrophic	None	Poor
S12	21/06/2024	Bad	Eutrophic	None	Very Poor
S11	21/06/2024	Bad	Eutrophic	None	Very Poor
S8	26/06/2024	Good	Mesotrophic	None	Medium
S5	27/06/2024	Good	NA	None	NA
S6	27/06/2024	Moderate	Eutrophic	None	Poor
S2	24/06/2024	Moderate	Eutrophic	None	Poor
S4	27/06/2024	Good	NA	None	NA
S17	25/06/2024	Moderate	Eutrophic	None	Poor
S9	25/06/2024	Moderate	Mesotrophic	None	Medium
S7	26/06/2024	Poor	Mesotrophic	None	Poor
S1	24/06/2024	Excellent	Eutrophic	None	Poor
S3	24/06/2024	Poor	Eutrophic	None	Poor
S22	25/06/2024	Moderate	Eutrophic	None	Poor
S23	25/06/2024	Moderate	Eutrophic	None	Poor
S24	25/06/2024	Bad	Hypereutrophic	None	Very Poor
S29	25/06/2024	Poor	Hypereutrophic	None	Very Poor
S9	30/07/2024	Poor	Eutrophic	None	Poor
S2	1/08/2024	Poor	Hypereutrophic	None	Very Poor
S24	31/07/2024	Poor	NA	None	NA
S30	10/08/2024	Moderate	Hypereutrophic	None	Very Poor

S24	11/08/2024	Poor	Hypereutrophic	None	Very Poor
S20	13/08/2024	Poor	Hypereutrophic	None	Very Poor
S2	28/08/2024	Good	Eutrophic	None	Poor
S9	11/09/2024	Poor	Eutrophic	None	Poor
S12	17/09/2024	Poor	Eutrophic	None	Poor
S20	19/09/2024	Poor	Eutrophic	None	Poor
S2	9/09/2024	Moderate	Eutrophic	None	Poor
S24	24/09/2024	Bad	Eutrophic	None	Very Poor
S21	15/10/2024	Poor	Hypereutrophic	None	Very Poor
S22	15/10/2024	Poor	Hypereutrophic	None	Very Poor
S23	16/10/2024	Bad	Hypereutrophic	None	Very Poor
S24	16/10/2024	Poor	Eutrophic	None	Poor
S25	16/10/2024	Bad	Eutrophic	None	Very Poor
S26	16/10/2024	Bad	Hypereutrophic	Amber	Very Poor
S27	16/10/2024	Bad	Hypereutrophic	None	Very Poor
S28	16/10/2024	Bad	Eutrophic	None	Very Poor
S32	16/10/2024	Poor	Hypereutrophic	None	Very Poor
S1	12/11/2024	Bad	Eutrophic	Red	Very Poor
S2	12/11/2024	Bad	Hypereutrophic	Red	Very Poor
S9	16/11/2024	Bad	Eutrophic	None	Very Poor
S3	12/11/2024	Bad	Hypereutrophic	Red	Very Poor
S4	14/11/2024	Moderate	Eutrophic	None	Poor
S5	14/11/2024	Moderate	Eutrophic	None	Poor
S6	14/11/2024	Bad	Eutrophic	None	Very Poor
S7	14/11/2024	Bad	Eutrophic	None	Very Poor
S8	16/11/2024	Bad	Eutrophic	None	Very Poor
S10	16/11/2024	Bad	Eutrophic	None	Very Poor

S11	20/11/2024	Bad	Eutrophic	None	Very Poor
S12	15/11/2024	Bad	Eutrophic	Amber	Poor
S13	15/11/2024	Bad	Eutrophic	None	Very Poor
S14	17/11/2024	Bad	Eutrophic	Amber	Poor
S15	16/11/2024	Bad	Eutrophic	Amber	Poor
S16	15/11/2024	Bad	Eutrophic	Amber	Poor
S17	13/11/2024	Bad	Eutrophic	None	Very Poor
S18	13/11/2024	Bad	Eutrophic	None	Very Poor
S19	13/11/2024	Poor	Eutrophic	None	Poor
S20	13/11/2024	Moderate	Eutrophic	None	Poor
S29	11/11/2024	Bad	Eutrophic	None	Very Poor
S30	11/11/2024	Moderate	Eutrophic	None	Poor
S31	11/11/2024	Poor	Eutrophic	Amber	Poor
S4	12/12/2024	Bad	Hypereutrophic	Amber	Very Poor
S5	12/12/2024	Bad	Eutrophic	None	Very Poor
S6	12/12/2024	Bad	Eutrophic	None	Very Poor
S7	12/12/2024	Bad	Eutrophic	None	Very Poor
S9	10/12/2024	Bad	Eutrophic	None	Very Poor
S11	11/12/2024	Bad	Eutrophic	None	Very Poor
S12	11/12/2024	Bad	Eutrophic	None	Very Poor
S15	11/12/2024	Bad	NA	None	NA
S20	10/12/2024	Bad	Hypereutrophic	None	Very Poor
S24	11/12/2024	Poor	Hypereutrophic	None	Very Poor

Appendix 3 – Table and values for EQR ranges based on river type.

Table 4. Proposed river types and Ecological Quality Ratio (EQR = Q/5) values for different water quality classes.

River type	Code of the type	Stream order ¹	Residence time (day)	EQR				
				excellent	good	moderate	poor	bad
Brooks and small streams	1	1–5	<2	1	0.99	0.97	0.95	<0.05
Streams	2	3–6	2–4	0.99	0.97	0.95	0.90	<0.90
Small rivers (lowland streams)	3	4–7	4–8	0.95	0.9	0.8	0.7	<0.7
Rivers	4	6–9	8–12	0.9	0.8	0.7	0.6	<0.6
Large rivers	5	7–1	12–16	0.8	0.7	0.6	0.5	<0.5
Very large rivers	6	>10	16<	0.7	0.6	0.5	0.4	<0.4

¹ Depending on local conditions