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Evaluating Native Cyanobacterial Communities as Indicators of Ecological Disturbance in Freshwater Reservoirs

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Abstract: Freshwater reservoirs provide drinking water, irrigation and fisheries but regular monitoring does not show biological change as early signs of ecological disturbance. Cyanobacteria are sensitive to nutrient enrichment and hydrologic and temperature changes, and thus are useful indicators of ecosystem health. The present study aimed to determine if the composition in terms of structure, functional groups and toxigenic taxa of the native cyanobacterial community could differentiate reservoirs along an anthropogenic disturbance gradient in tropical southern India. Three reservoirs were grouped into low (R1), moderate (R2) and high (R3) disturbance and sampled monthly for a one-year period, according to an Anthropogenic Disturbance Score, which was created on the basis of the catchment. Quantification of water quality variables, phytoplankton biovolume, and cyanobacterial taxonomic and functional group composition and calculation of total microcystin and a Cyanobacterial Disturbance Index were performed. The total phosphorus concentration rose from 18.6 to 187.5 micrograms per litre over the gradient, while the trophic state index (TSI) rose from 46.2 to 73.6. The contribution of cyanobacteria to phytoplankton biovolume increased from 14.6% in R1 to 78.9% in R3 and the Shannon diversity of cyanobacteria decreased from 2.61 to 0.98. Small colonial and picocyanobacterial groups were the most abundant in the least disturbed reservoir while Microcystis and Raphidiopsis groups were most abundant in the most disturbed reservoir where microcystin was present in excess of the drinking-water guideline in most months. The disturbance index correlated more strongly with trophic state, and also discriminated eutrophic samples with greater accuracy than the abundance-based indices. However, the disturbance signal was temporarily weakened during the monsoon but not completely eliminated. Native cyanobacterial communities are useful sensitive, interpretable and cost-effective indicators, which can enhance reservoir bioassessment and help with ecosystem-based management in tropical reservoirs.

Keywords: Bioassessment; Eutrophication; Functional Groups; Microcystin; Trophic State; Tropical Limnology.

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I. Introduction

Reservoirs are one of the most heavily utilized aquatic ecosystems in the tropics. They provide water supplies for human consumption, for irrigation, hydropower, fisheries and are now being used for a growing range of recreational and cultural purposes. Reservoirs are manmade systems that are situated within catchments occupied by humans, and therefore nutrients, sediments and contaminants from agriculture, cities, and urban non-point sources are deposited in them, and their water levels are manipulated based on demand rather than a natural hydrological cycle. These pressures change food web, oxygen regime, and water quality, and in many areas have created a new "nutrient-rich" turbid reservoir that is susceptible to HABs.

Early identification of such degradation, before ecosystem services are degraded or public health is threatened, is one of the key problems in ecosystem-based management of freshwater resources. Cyanobacteria are in a special place in this challenge. They are primitive, cosmopolitan photosynthetic prokaryotes, and are a natural part of reservoir phytoplankton and can be diverse; however, some taxa grow under enrichment conditions to dominate the surface of the water, which leads to a decrease in the other primary producers due to shading, a lack of oxygen during decay, and production of potent toxins (e.g., microcystins and cylindrospermopsins) (Huisman et al., 2018). Global trends of increased cyanobacterial bloom frequency, intensity, and duration have been related to nutrient over-enrichment, hydrological alteration and temperature rise, and cyanobacteria are one of the most prevalent indicators of impaired

freshwater ecosystems (Paerl & Otten, 2013). In addition to the above, climate warming produces more intense and prolonged thermal stratification, lower viscosity of the water, higher water growth optima generally than for eukaryotic algae Paerl & Paul, (2012) which all favour cyanobacteria. Specific taxa take advantage of the environment created by the human disturbance with a variety of eco-physiological characteristics, for example buoyancy regulation by gas vesicles, nitrogen fixation, and efficient storage and tolerance of low light (Carey et al., 2012). The same characteristics make cyanobacteria good candidates as bioindicators. Early limnological syntheses established a predictable, progressive relationship between cyanobacterial dominance and nutrient loading and lake transparency Dokulil & Teubner, (2000) while empirical models revealed a sharp increase in the likelihood of cyanobacterial dominance as total phosphorus, total nitrogen and algal biomass increased (Downing et al., 2001). Primary production in many inland waters may be co-limited by N and P, and therefore the ratio of N to P may also influence which cyanobacterial taxa thrive; diazotrophic taxa thrive at low N:P ratios, whereas N:P ratios that are high or within the neutral range favour non-diazotrophic colonial taxa (Conley et al., 2009). Cyanobacteria consequently provide more of a temporally integrated signal than individual water chemistry measurements, as they incorporate several stressors in a single measurement on a day to week timescale. The vast majority of monitoring programmes, however, rely on the very limited definition of cyanobacteria: the number of cyanobacteria cells or the concentration of chlorophyll a is compared with thresholds for alerts that are established in relation to drinking water and recreational use. These abundance-based metrics provide information on the potential risk at the time of sampling but are not informative about the ecological condition of the system; a low biomass of non-toxic picocyanobacteria in a clear oligotrophic reservoir and a low biomass of toxic Microcystis in a deteriorating one have very different ecological implications. There are ways around this, however, with trait-based approaches. The subsequent development of the functional classification of freshwater phytoplankton groups was the establishment of the habitat templates and tolerances by species Reynolds et al., (2002) and the later development that explained how these codons should be used in reservoirs and other water bodies (Padisák et al., 2009). Functional groups based on morphology have also been seen to explain much of the ecological diversity in phytoplankton in climate zones (Kruk et al., 2010).

Such application, in particular to native cyanobacterial communities, could allow to translate taxonomic lists into meaningful indicators of disturbance. However, information on reservoirs from the tropics in South Asia is still limited, and there is a dearth of research testing if community attributes of cyanobacteria can distinguish between reservoirs under different levels of anthropogenic stressors in the same climatic and geological regions. Comparisons are needed since otherwise, the interpretation of biological indicators would be confounded by the effects of climate, basin morphometry and hydrology. Further, the hydrology of southern India, driven by the monsoon, is of a seasonally variable type, with strong flushing periods and extended dry season retaining periods, which imposes a further challenge, as a good hydrological indicator should be informative throughout the seasons. In view of this, the present study was carried out to assess the potential of the native cyanobacteria community to serve as an indicator of ecological disturbance in three tropical reservoirs located on an anthropogenic disturbance gradient.

The specific objectives were to: (i) describe the physicochemical gradient of each reservoir with respect to disturbance, (ii) characterize the taxonomic structure, seasonal dynamics and functional composition of cyanobacterial assemblages under different levels of disturbance, (iii) identify indicator taxa and functional groups for low and high disturbance, and (iv) develop and test a simple Cyanobacterial Disturbance Index potentially useful in routine reservoir bioassessment. We predicted that higher disturbance would increase the proportion of cyanobacteria in phytoplankton biovolume, decrease cyanobacterial diversity and result in a shift from small colonial and picoplanktonic cyanobacteria to larger-bloomed, buoyant and toxigenic cyanobacteria.

II. Materials and Methods

2.1 Study Area and Disturbance Gradient

The study area and disturbance gradient is 2.1. The study area and disturbance gradient is 2.1. The study was carried out in three impoundments in the rain shadow foothills of the Western Ghats, between 10.5° and 11.5° N and 76.5° and 77.5° E in southern India. The Tropical semi-arid climate, mean air temperature is about 26 °C, the region has two rainy seasons, southwest monsoon (June - September) and the northeast monsoon (October - December). The winter period (January-February) is a cool dry season while the pre-monsoon period (March-May) is a hot period with maximum evaporation and minimum inflow. The three reservoirs were warm polymictic and weakly stratifying and based on crystalline basement rocks, thereby reducing the differences between the reservoirs (geologically). Five pressures were scored for each reservoir based on the proportion of agricultural land, proportion of built-up land, annual water-level fluctuation, fishing and aquaculture intensity and recreational use, including bathing, and then given an Anthropogenic Disturbance Score (ADS) ranging from 0 (absent) to 3 (severe). The scores were assigned based on the land-cover maps, field verification of inflow channels and data from the local water resources authority. The surface area of Reservoir R1 is 4.8 km² and the maximum depth 22 m with an ADS of 3 (low disturbance) due to the predominantly forested catchment. The catchment of Reservoir R2 (7.2 km², 14 m) is a mixed agricultural catchment where coconut, banana and vegetable farming occur, and was classified as 8 (moderate disturbance). The growing peri-urban population drains to reservoir R3 (2.6 km², 9 m), which is partly supplied with partly treated sewage, and had a high disturbance score of 13.

2.2 Sampling Design

Samples were taken monthly from January to December 2022 between 08:00 and 11:00 h. Three stations were sampled at each reservoir, near the dam, mid-reservoir and near the main inflow. The water samples were taken at each station to twice the Secchi depth (maximum 5 m) with a flexible integrating tube sampler and the three station samples were averaged to one monthly composite sample per reservoir for a total of 36 samples. Subsamples for nutrients analysis were collected and kept on ice in acid-washed polyethylene bottles; subsamples for chlorophyll a and toxins were filtered within 6 h after the collection; the phytoplankton subsample (250 mL) was fixed immediately in acid Lugol's iodine solution.

2.3 Physicochemical Analyses

In-situ water temperature, pH and DO measurements were taken using a calibrated multiparameter probe, and transparency was measured using a calibrated 20 cm secchi disc. Total phosphorus (TP) was measured using the molybdenum blue ascorbic acid method after digestion with persulfate and total nitrogen (TN) was measured by alkaline persulfate digestion by cadmium reduction method. The material collected on the glass-fibre filters was extracted in 90% acetone and analysed spectrophotometrically after correction for the presence of phaeopigments under the assumption that they are present in the samples. A molar ratio of TN:TP was determined for each sample. Reagent blank analysis, duplicate analysis of 10% of samples and certified reference standards were used to check analytical quality with recovery ranging from 92% to 106%.

2.4 Cyanobacterial Enumeration, Biovolume and Functional Classification

Phytoplankton counts were made using an inverted microscope following sedimentation of 10 to 50 mL subsamples depending on the cell density. The dominant taxa were observed at a magnification of 200× to 400× with at least 400 counting units (cells, colonies or filaments) counted per sample (counting error < 10%). Species identification was attempted by using the most recent and commonly used taxonomic monographs of tropical freshwater cyanobacteria, and the number of cells per colony and filaments, where possible, were estimated by counting cells in at least 30 random units per taxon. The cell biovolume has been calculated from geometric measurements taken from 25 individuals of each taxon and using appropriate geometric formulae based on cell shape Hillebrand et al., (1999) and assuming a specific density

of 1 g cm-3. The cyanobacterial taxa were allocated to the functional groups (codons) of the phytoplankton functional classification scheme Reynolds et al., (2002) with updated criteria for reservoirs Padisák et al., (2009) and compared with the morphological classification (Kruk et al., 2010). The groups encountered were Z - *picocyanobacteria*, K - small celled colonial forms without aerotopes, Lo - medium sized colonial forms of mesotrophic waters, S1 - shade tolerant thin filaments, H1 - nitrogen fixing Nostocales, SN - warm water nitrogen fixing *Rhaphidales* and related forms, and M - large mucilaginous colonies of Microcystis.

2.5 Cyanotoxin Analysis

Subsamples of whole water were taken for three cycles of freeze-thaw to release the microcystins from within the cells and into the dissolved water, before measuring the total microcystin (dissolved and intracellular). A congener-independent, Adda-specific enzyme-linked immunosorbent assay was used to measure concentrations, with calibration using microcystin-LR equivalents. The limit of detection was 0.10 $\mu g\ L^{-1}$ and samples below detection were set at half of the detection limit for statistical analysis. Values were compared with the provisional guideline of 1 $\mu g\ L^{-1}$ for lifetime exposure to microcystin-LR in drinking water recommended by the World Health Organization.

2.6 Trophic State and Cyanobacterial Disturbance Index

The trophic state of each sample was determined by Carlson trophic state index (TSI) Carlson, (1977) which is the arithmetic mean of the individual indices, Secchi depth, TP and chlorophyll a. A Cyanobacterial Disturbance Index (CDI) was defined as the percentage of cyanobacterial biovolume from functional groups associated in the literature with eutrophic, turbid and hydrologically stable conditions (M, SN, H1 and S1) to summaries community change in a single interpretable number. The range of the CDI is from 0 (sensitive cyanobacterial assemblage (only Z, K and Lo groups present)) to 1 (dominance of tolerant, bloom-forming, groups). The index was to be calculable based on standard microscope counts, rather than molecular testing or toxin analysis.

2.7 Statistical Analyses

To test differences among reservoirs, Kruskal–Wallis tests followed by Dunn's post hoc comparisons with Bonferroni correction were used due to violations of assumptions of normality and homogeneity of variance for several variables. The diversity of cyanobacteria was presented in terms of the Shannon index (H′) with respect to biovolume. Spearman rank correlations ($n = 36$) were used to assess any association between community metrics and environmental variables. Indicator taxa were determined using the indicator value (IndVal) method, followed by significance testing by 999 permutations. Redundancy analysis (RDA) with forward selection was used to look for relationships between cyanobacterial community composition (Hellinger-transformed biovolume) and the standardised environmental variables, with the significance of the retained variables evaluated by Monte Carlo permutation tests (999 permutations). The discriminatory power of the CDI and of abundance-based metrics (TSI > 60) from non-eutrophic ones were assessed by means of receiver operating characteristic (ROC) curves and the area under the curve (AUC). All analyses conducted in R version 4.2.1 (vegan, indicspecies and pROC packages; $p < 0.05$).

III. Results

3.1 Physicochemical Gradient

The water quality of the three reservoirs was very different and the differences were in the order of the Anthropogenic Disturbance Score (Table 1). A Kruskal–Wallis test revealed that the mean water temperature did not vary significantly among reservoirs ($H = 4.12$, $p = 0.127$) and the reservoirs were thus considered to have a similar thermal regime. All the nutrient, optical and biological variables were significantly different, however, from one another ($p < 0.001$ in all cases). The mean TP was about ten times higher in R3 than in R1 and the mean TN about 5 times higher. The Secchi depth decreased from

2.35 m in R1 to 0.48 m in R3, while the mean value of chlorophyll a rose from 4.2 to 58.6 µg L^{-1}. The mean TSI resulted in R1 being in the mesotrophic zone, R2 being at the eutrophy/hypereutrophy boundary and R3 being in the hypereutrophy zone. Along the gradient, dissolved oxygen decreased and pH increased, as would be expected if intense photosynthesis and organic loading occurred in R3 as observed in the southwest monsoon with DO below 3 mg L^{-1} on three occasions. The molar TN:TP ratio decreased from 49.0 in R1 to 25.4 in R3 showing a change from phosphorus to a more phosphorus surplus situation.

Table 1: Physicochemical Characteristics and Cyanobacterial Community Attributes of the Three Study Reservoirs (Annual Mean ± Standard Deviation, n = 12 Per Reservoir, January–December 2022)

Variable	R1 (low)	R2 (moderate)	R3 (high)
Anthropogenic Disturbance Score (0–15)	3	8	13
Water temperature (°C)	26.4 ± 2.1	27.1 ± 2.3	28.0 ± 2.0
pH	7.4 ± 0.3	8.1 ± 0.4	8.7 ± 0.5
Dissolved oxygen (mg L^{-1})	7.6 ± 0.6	6.4 ± 0.9	4.9 ± 1.4
Secchi depth (m)	2.35 ± 0.31	1.12 ± 0.24	0.48 ± 0.13
Total phosphorus (µg L^{-1})	18.6 ± 5.2	64.3 ± 14.8	187.5 ± 42.6
Total nitrogen (µg L^{-1})	412 ± 96	986 ± 210	2154 ± 437
TN:TP (molar)	49.0	34.0	25.4
Chlorophyll *a* (µg L^{-1})	4.2 ± 1.6	17.8 ± 6.3	58.6 ± 19.4
Trophic state index (TSI)	46.2	60.5	73.6
Cyanobacterial density (×10^3 cells mL^{-1})	3.8 ± 1.9	46.5 ± 21.7	312.4 ± 128.6
Cyanobacteria in phytoplankton biovolume (%)	14.6 ± 5.2	41.3 ± 12.4	78.9 ± 11.7
Cyanobacterial taxa richness (annual)	24	19	11
Shannon diversity, H′ (cyanobacteria)	2.61 ± 0.18	1.94 ± 0.27	0.98 ± 0.31
Dominant functional groups	Lo, Z, K	H1, Lo, S1	M, SN, H1
Cyanobacterial Disturbance Index (CDI)	0.14 ± 0.06	0.58 ± 0.11	0.91 ± 0.04
Total microcystins (µg L^{-1})	< 0.10	0.62 ± 0.41	3.84 ± 2.17

Note: All variables except water temperature differed significantly among reservoirs (Kruskal–Wallis, $p < 0.001$). TN:TP and TSI were calculated from annual mean values. Functional group codes follow Reynolds et al., (2002) and Padisák et al., (2009).

3.2 Community Composition and Seasonal Dynamics

In total, 37 cyanobacterial taxa were recorded, which belong to the orders *Chroococcales* (15 taxa), *Synechococcales* (9), *Oscillatoriales* (7) and *Nostocales* (6). The annual richness increased with the number of taxa (R1 24 taxa, R2 19 taxa and R3 11 taxa), with a corresponding decrease in biovolume related Shannon diversity (R1 2.9, R2 2.3 and R3 1.8). The mean cyanobacterial cell density in R3 was over 80 times that of R1. Cyanobacterial biovolume contribution to total phytoplankton biovolume was well differentiated between reservoirs with a clear year round trend (Figure 1). In R1, the contribution of this group was between 7% and 25% with the rest of the phytoplankton consisting mainly of green algae and diatoms. In R2, the range was from 22% to 61% and in R3 it never dropped below 58% with the highest reading in April being 95%. All three reservoirs had a similar seasonal pattern. The share of cyanobacteria was higher in the pre-monsoon season when inflow was least, water temperature was highest and retention time was longest, and also the highest in April or May, which is also the maximum for the year. All reservoirs experienced a sharp drop with the onset of Southwest monsoon, attaining minima in August which corresponded to the highest inflows, more turbidity due to suspended sediment and the lowest water residence times. The proportion of cyanobacteria gradually increased from north-east monsoon. Importantly, the reduction was least for the most disturbed reservoir, as the August minimum in R3 (58%) was still more than double the annual maximum in R1 (25%), and the order of the three reservoirs was maintained for all months of the year.

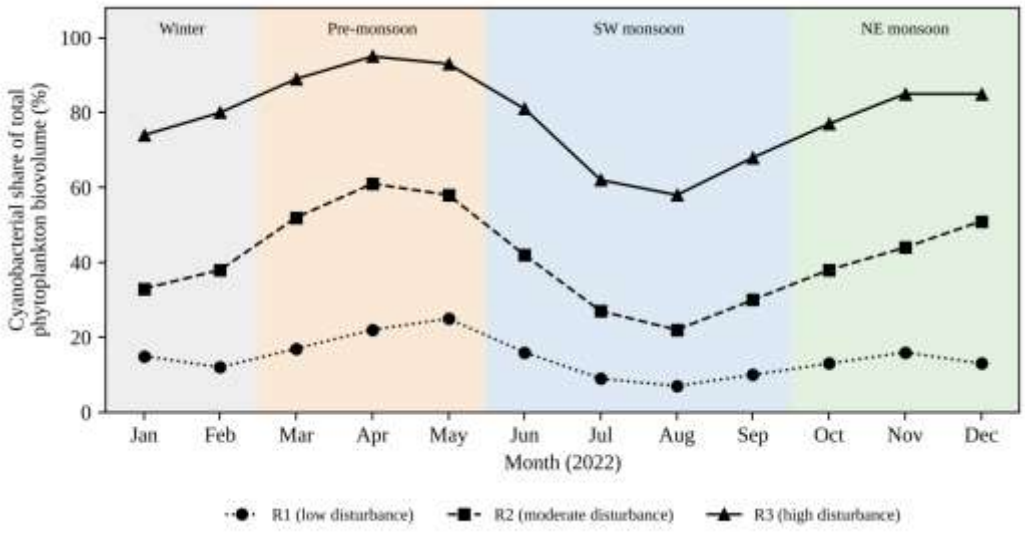


Figure 1: Monthly Contribution of Cyanobacteria to Total Phytoplankton Biovolume in Reservoirs R1 (Low Disturbance), R2 (Moderate Disturbance) and R3 (High Disturbance) During 2022. Shaded bands Indicate Winter (January–February), Pre-Monsoon (March–May), Southwest Monsoon (June–September) and Northeast Monsoon (October–December) Seasons

3.3 Indicator Taxa and Functional Groups

Indicator species analysis was used to recognise different groups of taxa representing different levels of disturbance. In R1, *Merismopedia tenuissima* (IndVal = 0.82, p = 0.001), *Aphanocapsa delicatissima* (0.76, p = 0.001) and Synechococcus sp. were significant indicators. (0.71, p = 0.002). The indicators in R2 were *Dolichospermum* circinale (0.73, p = 0.001) and *Pseudanabaena limnetica* (0.64, p = 0.004). In R3, the strongest indicators were Microcystis aeruginosa (0.91, p = 0.001), *Raphidiopsis raciborskii* (0.84, p = 0.001) and *Planktothrix agardhii* (0.69, p = 0.003). Taxonomic differences were reflected in functional group composition. Sensitive groups, represented by Lo (52%), Z (19%) and K (15%), dominated the cyanobacteria biovolume in R1, whereas tolerant groups (S1 and H1) accounted for only 14% of the cyanobacteria biovolume. In R2, the assemblage was mixed: Lo still contributed 28% and Z 8%, but H1 (34%), S1 (18%) and M (6%) together made up 58%. In R3, M alone contributed 58% of cyanobacterial biovolume, followed by SN (21%), H1 (8%) and S1 (5%) while the sensitive groups were reduced to less than 10%. Consequently, mean CDI values were 0.14 ± 0.06 in R1, 0.58 ± 0.11 in R2 and 0.91 ± 0.04 in R3, and all pairwise differences were significant (Dunn's test, $p < 0.01$). Microcystins were never detected in R1, were detected in 9 of 12 samples at low concentrations in R2, but were detected in all samples of R3, and exceeded the guideline value of 1 µg L^{-1} in 10 of 12 months, with a maximum concentration of 8.12 µg L^{-1} recorded in April. The total microcystin level was positively correlated with the biovolume of group M in all samples ($\rho = 0.74$, $p < 0.001$).

3.4 Environment–Community Relationships and Index Performance

The first two RDA axes accounted for 52.7% and 15.7% of the variation in the composition of the cyanobacterial communities, respectively (68.4% in total). Forward selection retained TP ($F = 14.6$, $p = 0.001$), Secchi depth ($F = 6.8$, $p = 0.002$), water temperature ($F = 4.1$, $p = 0.008$) and the TN:TP ratio ($F = 2.9$, $p = 0.031$). The first axis was a trophic and transparency gradient, which differentiated R1 (groups Z, K and Lo) from R3 (groups M and SN). The second axis was mainly related to water temperature and was able to discriminate pre-monsoon from monsoon samples in each reservoir. The CDI was strongly correlated with TSI ($\rho = 0.88$, $p < 0.001$), TP ($\rho = 0.84$, $p < 0.001$) and Secchi depth ($\rho = -0.86$, $p < 0.001$).

The cyanobacterial Shannon diversity was negatively correlated to TSI ($\rho = -0.81$, $p < 0.001$). Additionally, there were weak, but significant, correlations between abundance-based metrics and trophic state; TSI and cyanobacterial share of biovolume were correlated at 0.82 and cyanobacterial cell density and TSI were correlated at 0.79. The ROC analysis showed that the CDI can discriminate eutrophic from non-eutrophic samples with an AUC of 0.94, giving a threshold value of 0.50 with a sensitivity of 0.89 and a specificity of 0.92. The AUC values were 0.87 for cyanobacterial share of biovolume and 0.83 for cyanobacterial cell density. CDI also had the lowest coefficient of variation within seasons in R3 (4.4%), suggesting that this measure was less impacted by monsoon flushing compared to the abundance-based measures.

IV. Discussion

4.1 Cyanobacterial Community Structure Tracks Ecological Disturbance

The findings are consistent with the hypothesis of a coherent and predictable response of native cyanobacterial communities to anthropogenic disturbances occurring in tropical reservoirs. Increased catchment pressure led to a ten-fold increase in phosphorus concentrations, five-fold decrease in transparency, a change from mesotrophic to hypereutrophic water quality and a corresponding shift in the cyanobacterial assemblage along a gradient of similar climate, geology and thermal regime. This trend is consistent with the well-documented trend of cyanobacterial dominance with increased nutrient loading and decreased light availability in lakes (5), and with models indicating that cyanobacterial dominance increases rapidly with increasing TP and TN (6). The present data also bring in these relationships for monsoonal reservoirs from southern India, and demonstrate that the relationships are valid for not only the biomass of cyanobacteria, but for the diversity and functional composition of the assemblage. It is important to highlight that the loss of cyanobacterial richness and diversity as a function of disturbance is often not considered in cyanobacterial monitoring, and monitoring to only include taxa that form blooms. A high diversity of small colonial and picoplanktonic cyanobacteria was observed in the least disturbed reservoir, which is an integral component of the phytoplankton and constitutes a natural and ecologically significant component of the phytoplankton, contributing to the primary production and as a food source for microzooplankton. Enrichment didn't just introduce cyanobacteria to the water; it took the place of the diverse native community, where a few competitively dominant taxa took up the space. This homogenisation is known to be associated with degraded ecosystems and associated loss of functional redundancy that could lower the resilience of food webs in reservoir ecosystems to additional stress (Huisman et al., 2018; Paerl & Otten, 2013).

4.2 Functional Groups as Diagnostic Indicators of Disturbance

The functional classification proved to be of great assistance in the interpretation of the observed changes. The small cells or colonies of groups Z, K and Lo that dominate R1 have high surface-to-volume ratios and they are good competitors for the limited amount of phosphorus and light-saturated cells in clear, well-mixed waters (Reynolds et al., 2002). They are associated with the indicator species *Merismopedia tenuissima* and *Aphanocapsa delicatissima* which are typical of mesotrophic water. In contrast, group M (Microcystis) and group SN (*Raphidiopsis raciborskii*) dominated R3. Microcystis also has gas vesicles which help it control its buoyancy and stay at the top of the water column in turbid, stable warm reservoirs, and the colonies are mucilaginous, which decreases grazing losses (Carey et al., 2012). *Raphidiopsis* is tolerant of low light, nitrogen-fixing and efficient in the uptake of phosphorus and its spread to tropical and subtropical waters has been associated with warming and eutrophication (Paerl & Paul, 2012; Carey et al., 2012). The intermediate reservoir is also used to show how functional groups can show the path of degradation before the system is completely degraded. In R2 the sensitive groups were still present, and were now supported by high biovolumes of the nitrogen-fixing H1 taxa and the shade-tolerant S1 filaments. This high relative proportion of *Dolichospermum* in R2 and the lower TN:TP ratio compared to R1 is in line with the notion that eutrophication control will need to focus on both nitrogen and phosphorus as the

ratios of these nutrients will affect which cyanobacteria thrive (Conley et al., 2009). The early signs of group M in low but measurable biovolume coupled with the occurrence of microcystins in majority of the R2 samples are early indicators that the reservoir is heading towards the situation in R3 from a management perspective. The applicability of the morphological and trait-based groups in different climatic zones Kruk et al., (2010) and the clear instructions for the use of codons in reservoirs Padisák et al., (2009) warrants the transferability of this interpretation to other tropical reservoirs.

4.3 Seasonal Hydrology Modulates, But Does not Erase, the Disturbance Signal

One of the practical challenges with any type of biological indicator is the need for the signal to not be lost in natural variation. Hydrological fluctuations are also very high in monsoonal reservoirs – the southwest monsoon brings short residence time, high turbidity due to inorganic sediment and low concentration of phytoplankton, while the pre-monsoon period brings warm and stable conditions and high nutrient concentration. In all reservoirs the cyanobacteria percentage of phytoplankton was maximum in pre-monsoon season and minimum in monsoon season, as expected because cyanobacteria prefer warm and stable water column (Paerl & Paul, 2012; Carey et al., 2012). The ranking of reservoirs remained constant during the year while the CDI fluctuated less than the abundance-based measures. This robustness comes as this is a proportional measure of the CDI that shows who is the dominant species in the cyanobacterial assemblage, not the amount of cyanobacterial biomass. Although flushing decreases biomass, it is not fast enough to quickly clear a seed population of Microcystis and replace it with a seed population of groups more sensitive to the treatment. The CDI is thus more representative of the history of disturbances than a single biomass sample, which is an additional benefit for low-frequency sampled reservoirs.

4.4 Implications for Reservoir Bioassessment and Management

The results have several implications on practice. The functional composition of cyanobacteria, as captured in the CDI, can add an ecological dimension to physicochemical monitoring and abundance-based alert levels, to provide a better sense of the current state of the reservoir ecosystem and not just the current public health threat. The CDI threshold was around 0.50, closely related to the eutrophication threshold indicated by the trophic state index Carlson, (1977) and is therefore a provisional threshold that can be tested in other locations. Secondly, the index relies solely on microscope counts and biovolume estimation Hillebrand et al., (1999) which are routinely performed in many water quality laboratories in developing areas of the world and can be applied without molecular or toxin testing. Third, there is a high linkage between the concentration of microcystin and group M, so functional monitoring can be applied in order to prioritise the more costly sampling for microcystin testing in samples where the risk is highest. From a management perspective, the results suggest that R3 needs to urgently be treated and diverted and that the abstraction of untreated drinking-water in the pre-monsoon months (when microcystin concentrations peaked) be curtailed. R2 is a system in risk where nutrient management of the catchment area, especially reducing the amount of fertiliser and manure runoff, may be capable of avoiding a shift to persistent Microcystis dominance. A cyanobacterial community is present in R1 that can be used as a reference condition for restoration targets in the region. If such biological reference conditions could be incorporated into national water quality programmes, this would enhance the management of reservoirs as engineered water stores to better meet the needs of the ecosystems (Huisman et al., 2018; Paerl & Otten, 2013).

4.5 Limitations and Future Directions

There are a few factors to consider. The results are limited by the number of reservoirs (n = 3) and annual cycles (n = 1) in the study, which prevents distinguishing the impact of disturbance from that of the reservoir-specific character (morphometry and depth). A larger number of reservoirs and annual cycles would allow calibration of the CDI threshold statistically. The Anthropogenic Disturbance Score was semi-quantitative and potentially could be improved with direct nutrient loadings estimates. Toxigenic and non-toxigenic strains of the same species cannot be differentiated by microscopy and molecular methods targeting the toxin synthesis genes would provide additional precision. Lastly, zooplankton and fish data would enable the cascading effects of cyanobacterial dominance on the food web to be quantified. However,

the uniformity of the patterns within taxonomic, functional and toxicological measures does make better use of the native cyanobacterial communities as indicators of ecological disturbance.

V. Conclusion

This study shows that native cyanobacterial communities can be sensitive and informative bioindicators of ecological disturbance in tropical freshwater reservoirs. Increased disturbance in three reservoirs with a common climate and geology, but different catchment pressures resulted in a ten-fold increase in TP and appeared to drive a steady change from mesotrophic to hypereutrophic conditions accompanied by a significant reduction in transparency. The cyanobacterial assemblage responded together: the proportion of biovolume (7% to 42%) and the abundance of non-bloom-forming small colonial and picoplanktonic groups (26% to 19%) decreased while that of large, buoyant, bloom-forming and toxigenic groups, such as Microcystis and *Raphidiopsis*, increased (3% to 32%). The proportion of cyanobacteria in the total phytoplankton biovolume increased from around one-seventh to nearly four-fifths, while the number of cyanobacteria taxa and the diversity of the cyanobacterial assemblage decreased. These changes were accompanied by the appearance of microcystins and in the most disturbed reservoir, their concentration surpassed the guideline for drinking water in most months. The proposed Cyanobacterial Disturbance Index based on routine microscope counts was highly correlated with trophic state, was highly accurate in distinguishing eutrophic samples and was more stable to monsoon flushing than abundance-based metrics. The threshold between reservoirs in acceptable condition and reservoirs in eutrophication state is about 0.50. The intermediate reservoir demonstrated the usefulness of detecting low levels of toxin and functional shifts as early warnings before persistent bloom dominance occurs, allowing managers to take action. A cyanobacterial functional composition assessment would be valuable to integrate into monitoring programmes for reservoirs to provide an ecological dimension to water quality assessment, to prioritise testing for toxins, and to support regional restoration goals. The index should be further validated with additional reservoirs and years and molecular detection of toxigenic genotypes is suggested to further refine the index for broader use.

Declarations

Conflict of Interest: All authors have seen and disclose no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability: The data used in this study are available from the corresponding author upon reasonable request.

References

[1] Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M., & Visser, P. M. (2018). Cyanobacterial blooms. *Nature reviews microbiology*, *16*(8), 471-483. https://doi.org/10.1038/s41579-018-0040-1

[2] Paerl, H. W., & Otten, T. G. (2013). Harmful cyanobacterial blooms: causes, consequences, and controls. *Microbial ecology*, *65*(4), 995-1010. https://doi.org/10.1007/s00248-012-0159-y

[3] Paerl, H. W., & Paul, V. J. (2012). Climate change: links to global expansion of harmful cyanobacteria. *Water research*, *46*(5), 1349-1363. https://doi.org/10.1016/j.watres.2011.08.002

[4] Carey, C. C., Ibelings, B. W., Hoffmann, E. P., Hamilton, D. P., & Brookes, J. D. (2012). Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water research*, *46*(5), 1394-1407. https://doi.org/10.1016/j.watres.2011.12.016

[5] Dokulil, M. T., & Teubner, K. (2000). Cyanobacterial dominance in lakes. *Hydrobiologia*, *438*(1), 1-12. https://doi.org/10.1023/A:1004155810302

[6] Downing, J. A., Watson, S. B., & McCauley, E. (2001). Predicting cyanobacteria dominance in lakes. *Canadian journal of fisheries and aquatic sciences*, *58*(10), 1905-1908. https://doi.org/10.1139/f01-143

[7] Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., ... & Likens, G. E. (2009). Controlling eutrophication: nitrogen and phosphorus. *science*, *323*(5917), 1014-1015. https://doi.org/10.1126/science.1167755

[8] Reynolds, C. S., Huszar, V., Kruk, C., Naselli-Flores, L., & Melo, S. (2002). Towards a functional classification of the freshwater phytoplankton. *Journal of plankton research*, *24*(5), 417-428. https://doi.org/10.1093/plankt/24.5.417

[9] Padisák, J., Crossetti, L. O., & Naselli-Flores, L. (2009). Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. *Hydrobiologia*, *621*(1), 1-19. https://doi.org/10.1007/s10750-008-9645-0

[10] Kruk, C., Huszar, V. L., Peeters, E. T., Bonilla, S., Costa, L., Lürling, M., ... & Scheffer, M. (2010). A morphological classification capturing functional variation in phytoplankton. *Freshwater biology*, *55*(3), 614-627. https://doi.org/10.1111/j.1365-2427.2009.02298.x

[11] Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U., & Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of phycology*, *35*(2), 403-424. https://doi.org/10.1046/j.1529-8817.1999.3520403.x

[12] Carlson, R. E. (1977). A trophic state index for lakes 1. *Limnology and oceanography*, *22*(2), 361-369. https://doi.org/10.4319/lo.1977.22.2.0361