Control and management of cynophycean (*Spirulina platensis*) bloom in Padmatheertham, Thiruvananthapuram, India

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The blue–green algal (BGA) bloom that appeared in Padmatheertham, the sacred pond in Sree Padmanabha Swamy Temple, Thiruvananthapuram, Kerala, India is associated with *Spirulina platensis*, a cyanobacterium rich in proteins, considered as a safe, functional food. Considering the unaesthetic appearance of the BGA bloom and its foul odour on open decomposition, various non-chemical methods were employed for its control. Several methods for nutrient remediation in the pond system were also explored. The efficacy of using decomposing rice straw to inhibit algal growth was studied. The possibility of control of BGA by stocking tilapia and filter-feeding bivalve, *Villorita cyprinoides* capable of ingesting and digesting the algae was analysed. Experimental assays carried out on *V. cyprinoides* revealed that it helped in the rapid utilization of BGA. The present study reinforces our understanding of the fundamental ecosystem services that filter-feeder communities provide to counter the invasive effects of eutrophication through consumption and assimilation.

Keywords: Bioremediation, blue–green algae, eutrophication, hypoxic, *Spirulina platensis*.

BLUE–GREEN Algae (BGA) associated harmful algal blooms (HABs) and their toxic effects are a growing concern worldwide[1–3]. There is a wealth of research on this topic[4–6]. A report of the United Nations Intergovernmental Panel on Climate Change (IPCC) directly links HABs to climate change[7]. BGA can grow in excess, causing BGA blooms when the water is warm and if there are sufficient nutrients, viz. phosphorus and nitrogen in the water body. Run-off from fertilized agricultural areas and sewage effluents are the major external sources of phosphorus and nitrogen in the waterways. Large-scale death and decomposition of blooming algae can deplete oxygen and promote the internal origin of nutrients from the lake/reservoir sediments.

The massive blooming of an unknown alga with an unpleasant odour was seen in Padmatheertham, the pond in Sree Padmanabhaswamy temple, Thiruvananthapuram, Kerala, India, in August 2019, after the renovation work of the pond by the Archaeology Department. Due to an unaesthetic appearance of the algal bloom that became intense during the summer months and its foul odour on open decomposition, the temple authorities sought nonchemical remedial measures to contain the menace before the ‘murajapam’ a 56-day traditional ceremony held every sixth year, when Jalajapam, chanting of the Vedas is performed by the pundits in the water body at the Padmatheertham pond.

Materials and methods

This study was carried out at the Padmatheertham (13,500 sq. m), one of the oldest ponds in Thiruvananthapuram situated in front of the East Nada of the Sree Padmanabhaswamy Temple. Water quality and algal abundance were monitored from August to November 2019, when the pond witnessed a rapid bloom of algae immediately after the reconstruction process (Figure 1).

Surface-water samples were collected using a Van Dorn sampler and analysed for physico-chemical parameters, viz. pH, salinity, alkalinity, hardness, free carbon dioxide (CO_2), dissolved oxygen (DO), biochemical oxygen demand (BOD), phosphate and nitrate in the waterbody. Sediment organic carbon, qualitative and quantitative assessment of algal count and chlorophyll was analysed after American Public Health Association (APHA)[8]. Transparency was measured with a Secchi disc and the pH of water was measured electrometrically using a pH meter. Salinity was measured with a salinity meter (Oakton SALT 6+) and the same was also confirmed titrimetrically according to the Mohr-Knudsen method. DO was determined by the modified Winkler method. The alkalinity of the sample and free CO_2 were estimated titrimetrically. Phosphate was estimated after Fonselius and Carlberg[9], and nitrites and nitrates were estimated as per methods described by Mullin and Riley[10] after APHA[8]. Total soil organic carbon was estimated using the Walkley and Black method[11], as described by Buchanan[12]. The light and dark bottle method was used for measuring the primary productivity of the water body[13].

Zooplanktons were collected using plankton net of bolting silk having a mesh aperture of 100 μm. The total count and numerical abundance of individual plankter species were

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*For correspondence. (e-mail: kgpadman@gmail.com) determined. Algal counts were monitored according to the methods described by Saraceni and Ruggiu[14]. Cell counts were enumerated using a modified Sedgwick–Rafter cell, as recommended by Lund *et al.*[15] and Frontier[16]. Chlorophyll analysis was done by acetone extraction method[8].

As the temple authorities were keen on tackling the menace as soon as possible, before the traditional ceremony and wanted to adopt only non-chemical control methods, a combination of bloom control methods (BCMs) were employed for integrated control and management of the algal bloom[17]. Biological option of stocking fishes, viz. *Oreochromis niloticus*, GIFT breed and bivalve *Villorita cyprinoides*, capable of ingesting BGA, were also employed. GIFT breed tilapia was procured from the Marine Product Export Development Authority (MPEDA) hatchery at Kochi, Kerala and was stocked @ 5180/ha in the pond.

As part of the algal control plan, decomposing rice straw known to release algicidal exudates was utilized according to the procedure described by Islami and Filizadeh[18]. For this, 42 bales of paddy straw weighing 1050 kg were chopped into small pieces and were placed loosely in onion net bags in the pond at 20 cm below the surface at 10 g straw per sq. m (ref. 19). The straw bales were, safely anchored on to the side steps of the pond. Straw bundles were allowed to rot for the entire study period so that the exudates released during the process could be utilized to inhibit the growth of algae. In order to compensate for the reduction of oxygen and limit the release of sediment-bound phosphorus that promotes sustained eutrophication, violent aeration of water was ensured by employing two 5 HP pumps fitted with a sprinkler system.

Figure 1. ***a***, Padmatheertham pond reconstruction work. ***b***, Blue–green algal bloom. ***c***, Pond after removal of algal bloom.

Filter-feeding black clams, *V. cyprinoides* of size 2.3 cm (7.4 g) capable of ingesting algae were stocked according to the methodology described by Smith *et al.*[20]. For this, baby clams collected from the clam beds in Thottappally backwaters, Alappuzha, Kerala, were carefully transported live and stocked in the pond. They were deposited at 9000/ha, along the pond's submerged steps and sandy bottom slopes.

In order to assess the rate of utilization of algae and their zooremediation by the bivalve, *V. cyprinoides* feeding assays were undertaken through laboratory trials by stocking black clams in 40-litre capacity experimental tanks filled with filtered sand bed up to 20 cm depth (Figure 2). The tanks were filled up to 40 cm depth with algal-rich water collected from the temple pond with stocking density at 6 nos/l. Moderate aeration was provided in the experimental tanks and the biocontrol efficacy of the clams to consume algae by biofiltration was estimated by continually monitoring algal counts in the tanks. Replicated trials were carried out and the results were compared with control tanks without animals, both under continuous lighting and aeration. The water quality parameters and algal counts were evaluated.

Results

The algal bloom that appeared in the temple pond in August 2019 was identified as *Spirulina platensis*. With the

Figure 2. Results after treatment with clam.

Table 1. Environmental parameters in Padmatheertham pond during the algal bloom period

Month (2019)	August	September	October	November
Temperature (°C)	27.5	30	29.5	31
pH	9	8.9	9	8.9
Free CO_2 (mg/l)	–	–	–	–
Dissolved oxygen (Bottom; mg/l)	5.5 (1.2)	6.5 (1.2)	4.2 (1.0)	4.0 (0.0)
Biochemical oxygen demand (mg/l)	1.8	0	0	0
Productivity (mg $C/m^{-1}/h^{-1}$)	468.5	–	–	–
Alkalinity (mg/l)	307.3	200	240	200
PO_4–P (mg/l)	1.65	0	0	0
NO_3–N (mg/l)	0.15	0.05	0	0
Ammonia (mg/l)	0.86	0.05	0.5	0
Transparency (cm)	5	20	2	–
Organic carbon (%)	0.47	0.28	0.26	–
Spirulina platensis (10^{10}/ml)	18.56	0.01	1.08	0.13

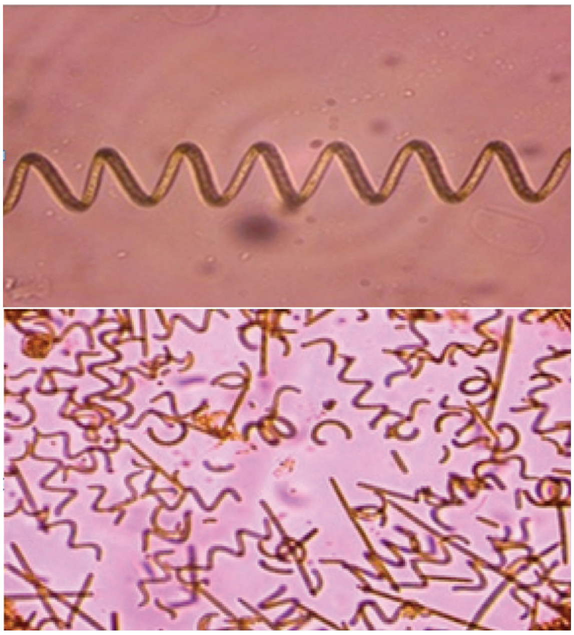

Figure 3. *Spirulina platensis*.

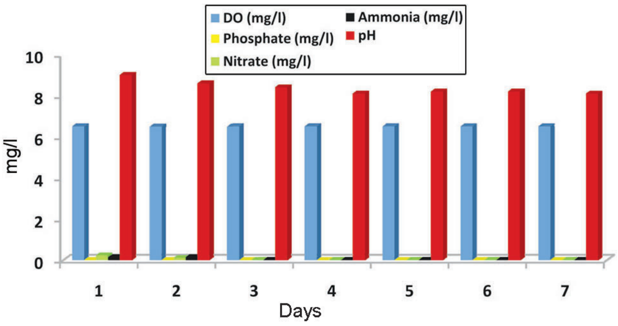

Figure 4. Water quality changes *in vivo* tank experiments using *Villorita* cyprinoides.

overgrowth of algae, the pond surface was covered with a scum mat having an unpleasant odour (Figure 3). Thick blooms block light from reaching the ponds bottom and dead cells decompose and consume oxygen, resulting in hypoxic 'dead zones' in the pond.

The moderately warm temperature (26.25°–31°C) and the highly alkaline pond water conditions at pH 9 and high nutrient concentration favoured the growth and proliferation of *S. platensis*. Table 1 shows the water quality parameters in the pond before and during the treatment period. The air temperature varied from 26.25°C to 31°C and water temperature ranged from 27.5°C to 31°C. The water was almost turbid throughout, with low transparency ranging from 2 to 20 cm, while the pH ranged from 8.9 to 9. Free CO_2 was not detected at any time. DO in the surface water was observed to vary from 4.2 to 6.5 mg/l, while the bottom water was hypoxic with DO ranging from 1.00 to 1.2 mg/l. The BOD of surface water during the peak of bloom was 1.80 mg/l (Figure 4). The total alkalinity of the pond water varied from 200 to 307.30 mg/l (Figure 5). The gross primary productivity was very high, i.e. 468.5 $mg/cm^{-1}/h^{-1}$. The phosphate and nitrate concentrations were very low, ranging from nil to 1.65 and 0.05 to 0.15 mg/l respectively. Free ammonia in the pond varied from 0.05 to 0.86 mg/l, with the highest value during August when the bloom was almost at its peak. The organic carbon percentage in the pond sediments ranged from 0.26% to 0.47% by employing control measures, there was a marginal reduction in pH to 8.9 by September and the algal concentration was subsequently reduced to 0.13×10^{10} cells/ml.

The algal count of *S. platensis* during the peak bloom period was 18.56×10^{10} cells/ml, which could be gradually brought down to 0.13×10^{10} cells/ml by November 2019, with the adoption of different BCMs. The BGA was represented predominantly by *Spirulina* and occasionally by *Oscillatoria* sp. With low pH, *Oscilatoria* sp. was encountered in higher numbers. In the initial phase, no algal elements other than *Spirulina* could be observed till the subsidence of the bloom in November 2019.

The internal generation of nutrients at anaerobic conditions was limited through aeration and oxygenation and by blocking all below-ground seepage connections to the pond. As part of the integrated approaches to control the bloom, paddy straw bales were deposited, which underwent active decomposition from around 1–2 weeks. At water temperatures from 27.5°C to 30°C, the decomposition of straw was almost complete in three months. With a gradual lowering of pH, the abundance of *S. platensis* had also drastically reduced. The biological control facilitated by stocking of fish, especially *Oreochromis mossambicus* and filter-feeding bivalve *V. cyprinoides*, also helped control the algal elements, as the stocked fish and shell-fish voraciously consumed the algal mats. The integrated approaches adopted helped control the BGA menace from the third month with a rapid reduction in algal cell concentration.

The phosphate and nitrate concentration in the pond water was found to be low, although the bloom was seemingly sustained by internal regeneration of nutrients with the decomposition of algal biomass and near hypoxic conditions at the bottom sediments. DO was very low during the entire bloom period and hypoxic concentrations (1.0–1.2 mg/l) persisted throughout the bottom waters.

In order to assess the biocontrol properties of black clam, *V. cyprinoide*, on blue-green algae *S. platensis*, laboratory based tank trial assays were carried out concurrently. These studies indicated a rapid reduction of the algal cell count from 0.12×10^{10} cells/ml to nil in seven days in the first tank trial and from 1.08×10^{10} cells/ml to nil in 4 day after stocking (DAS) in subsequent trials (Figure 6). In the control system, without clams but with aeration, there was a marginal reduction of algal density. High DO was maintained close to 6.5 mg through aeration in the laboratory assay. The algal feeding capacity of bivalve clam was perceptible in all the replicated trials, as evident from reduced algal counts.

With the adoption of integrated measures, including deposition of paddy straw and stocking of tilapia and *V. cyprinoides*, and aeration, water transparency gradually improved and the bloom almost subsided by November. The pond could be restored to a near-normal situation prior to the conduct of the traditional ceremony.

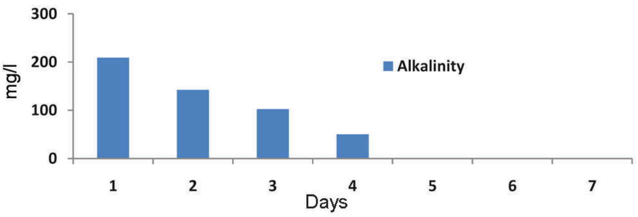

Figure 5. Alkalinity changes in temple pond.

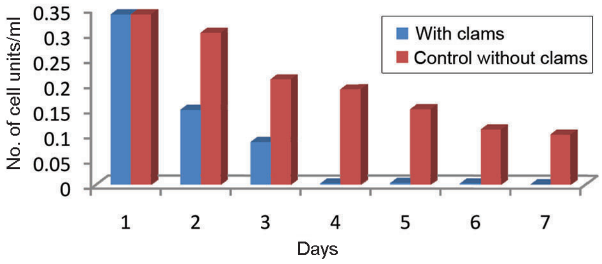

Figure 6. Bivalve feeding assay using *V. cyprinoides*.

Discussion

Chemical remediation of algal blooms is generally performed using different algaecides[21]. However, a biological option was preferred by the temple authorities in the sacred pond. The *S. platensis* bloom in the pond was apparently an outcome of elevated pH and alkalinity of water due to the use of highly alkaline lime mortar mix used for the pond reconstruction work following the Ancient and Historical Monuments and Archaeologic Conservation Procedure (AHMACP).

As observed in this study, during the peak bloom, bicarbonate ion (HCO_3^-) and carbonate ion (CO_3^{2-}) increased the pH of surface water to around pH 9–10. Such a highly alkaline environment is inimical to most other algae that rely on dissolved CO_2 compared to BGA that consume HCO_3. *S. platensis* thrives well in highly alkaline waters where no other microorganisms can grow[22]. The highest biomass and algal abundance were also noticed at pH 9, which is considered as the optimum value for survival of *S. platensis*[23,24].

The bloom was also contributed by high nutrient conditions due to the influence of sewage influx and urban run-off coupled with stagnant water conditions consequent to blockage of the traditional flushing system in the temple pond. The pond reconstruction works for restoration by desiltation served little on the levels of reactive phosphorus, as during the renovation work, the bottom soils that were excavated and bailed out were not removed but were utilized to rebuild the pond dykes[25].

The breakdown of the century-old, self-flushing mechanism that brought in water from the Kochar, a tributary of the Killi River, constructed during the Travancore period also contributed to the algal bloom. However, the low concentrations of phosphate ion (PO_4–P) and inorganic nitrogen during the bloom are attributed to the rapid consumption of nutrients by the algae.

Evidently, with the hypoxic condition of the pond, during the algal bloom and its eventual decay, the release of sediment-bound phosphates sustained the algal growth. Aeration facilitated as part of the integrated management helped check the release of sediment-bound phosphorus to the water column[26]. The artificial destratification due to violent aeration also promoted water circulation between the shallower and deeper layers of the water body[27,28]. This not only helped enhance DO in the water body but also limited the availability of reactive phosphorus.

Diverse options were used to control the algal bloom in the pond. Stocking filter-feeding fish, *O. niloticus*, helped ingest and digest BGA in the experimental aquaculture system[25]. This is a highly evolved planktonic herbivore fish with an unusually low stomach acid pH of 1.4 that help lyse BGA cells and digest them[29]. Bivalves are a natural solution to remove particles from the water column and remediate nitrogen loads[30]. *O. niloticus* and bivalve, *V. cyprinoides* helped control the algal bloom. Positive effects of combining two native filter-feeders, bighead carp (*Aristichthys nobilis*) and Asian clam (*Corbicula fluminea*), to control cyanobacterial bloom have been reported earlier[31]. This is a typical case of aquatic zooremediation, wherein biotic communities are deployed to remediate eutrophied environments, similar to that reported for some species of green mussels *Perna viridis*[32,33].

The experimental assays on feeding responses by black clams indicate that bloom-forming algal species are foraged by *V. cyprinoides*. This indicates that healthy populations of this native filter-feeding species aid in the mitigation and prevention of HABs in estuarine systems. The present study calls for a more detailed examination on the role of indigenous epifaunal and benthic filtering communities that silently perform significant ecosystem functions[34,35]. *V. cyprinoides* with high filtration capacity, apparently aids in the bioremediation of algae.

Use of decomposing straw, especially barley straw, for the control of algae and cyanobacteria has been a subject of research since early 1990 (refs 36–38). In the present study, we used readily available paddy straw in place of barley straw. Paddy straw is also effective in inhibiting algal growth[39,40]. Thus, the agricultural straw application is an effective and eco-friendly method for the inhibition of algal blooms in eutrophic waters[41–43].

According to some researchers, as the straw decomposes and 'rots' under aerobic conditions, phenolic compounds such as lignin, especially oxidized phenolics are released and the inhibiting effects of straw extract are attributed to release of chemical compounds such as hydrogen peroxide[44]. Some authors, however, suggest that the decomposing straw provides a carbon source for microbial growth in the carbon-limited environment and this microbial community 'hinders' phosphorus uptake by the algae and limits algal growth. The straw application is a preventive measure, rather than a treatment for the existing blooms as it helped facilitate a form of 'nutrient stripping', which reduces conditions favourable for the growth of harmful algae. The identification of rice straw as an effective material for the inhibition of BGA implies that it may be used as an environment-friendly biomaterial for controlling algal blooms in eutrophic waters.

The observation supports the view that indigenous clam *V. cyprinoides*, have a carbon sequestration role as carbon from its environment is utilized for shell formation during their various growth stages[45]. The present study reveals that native filter-feeding fish and bivalve species are adapted for mitigation and prevention of harmful algal menace in estuarine systems.

Conclusion

The study indicates that *V. cyprinoides* with high filtration capacity can be employed as an agent for the bioremediation of cyanophycean algae. The positive effects of combining omnivorous fish tilapia and a filter-feeding bivalve species to control cyanobacterial bloom have been demonstrated. The identification that common paddy straw is an effective bio material for inhibition of BGA blooms implies that it can be used as an environment-friendly biomaterial for controlling algal menace in eutrophic waters. The present study reinforces our understanding of the fundamental ecosystem services that filter-feeder communities provide by countering the adverse effects of algal eutrophication.

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