Observations on Feeding Responses of Bivalve clam Villorita cyprinoides for control of toxic micro algae Microcystis aerugenosa

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Abstract

Harmful algal blooms (HABs) are a regular occurrence in eutrophic water bodies and their occurrence has been increasing in freshwaters due to climate change. Microcystins are among the most prevalent and potent of the cyanobacterial toxins. As physico-chemical methods such as often employed for its control have been observed to be only with limited success as the use of copper algicides, though effective in managing HABs, often results in negative impacts such as copper toxicity and release of microcystins into surrounding water after cyanobacterial lysis. One alternative approach to the control of algal blooms involves the use of biological control agents.. The study has demonstrated the possible use of biological agent *Villorita cyprinoides* for control of toxic algae Microcystis aeruginosa in laboratory based investigations.

Keywords:Cyanobacteria, Microcystin, *Villorita cyprinoides*, eutrophic

Introduction

Algal blooms have increased in frequency and intensity, worldwide, causing not only major environmental but also severe economic losses (Hallegraeff 1993; Rodríguez et al. 2011). Incidence of algal blooms is also largely a direct result of human induced pollution of water bodies, or by leaching of fertilizer residues. The toxins and metabolites of some of such organisms, like microcystins from *Microcystis* affect water quality with very serious adverse effects on human health. Microcystins are also a major concern to drinking water supplies and have been linked to chronic liver damage. The World Health Organization (WHO) has given directives to adopt a guideline value for microcystin (Gumbo et Al., ,2008). Microcystins being very stable compounds are resistant to chemical breakdown and persist in natural waters for weeks to several months (Sivonen and Jones, 1999). Although cyanobacteria in potable water are normally removed during the water treatment processes, consumers may be exposed to sub-lethal dosages of toxins necessitating the use of more expensive removal processes (Haider et al., 2003).The most direct control method employed for microcystin removal from water bodies involves the use of chemical treatments such as algaecides, including copper compounds etc, which often result in more serious negative environmental impacts as physically ruptured or damaged cells may release intracellular toxins into the surrounding water. One alternative approach

for control of algal blooms is by use of biocontrol agents. A study was organized to investigate the possibility of utilization of bivalve, *Villorita cyprinoides*, an endemic black clam species inhabiting estuarine waters in lake Vembenad, Kerala for assessing the algal clearing efficiency through consumption and assimilation of *Microcystis aeruginosa* in experimental systems. .

Materials and Methods

The study was carried out utilising bloom waters rich in cyanophycean blue green algae ,n *Microcystis aeruginosa* from a highly eutrophied open temple pond at Sankara Narayana Moorthy temple located close to the T.D Medical College campus at Vandanam, Ambalappuzha, where a massive algal bloom was reported during January, February 2022. The pond is of 3500 sq.m, located close to the National Highway (NH-66) and has been a derelict water body very poorly maintained and located very close to drinking water well source functioning at Ambalappuzha North village. Water quality parameters and algal incidence was monitored by collecting water samples both from surface and mid depths during the outbreak of rapid bloom of the blue green algae.

Water samples collected from open water using a Vandorn sampler were preserved at 4^{o}C till analysis. The physico chemical parameters of the water viz., pH, Salinity, Alkalinity, Hardness, Free CO_2, Dissolved Oxygen, Phosphate, Nitrate etc. were analyzed after APHA, (2005). Temperature was measured using a Mercury Thermometer and estimation of turbidity of water by secchi disk method. pH of water was measured electrometrically by using a pH meter . Salinity of water sample was measured by salinity meter (Oakton SALT 6+) and the same also confirmed titrimetrically as per Mohr-Knudsen method. Dissolved Oxygen (DO) was determined after modified Winkler method. The alkalinity of the sample and free CO_2 was monitored titrimetrically. Phosphate was estimated after Fonselius and Carlberg (1972) and Nitrates vide procedures described by Mullin and Riley (1955) after APHA (2005).

Samples for algal counts were collected from mid depths from the water body and immediately preserved in ‘Lugols solution’ as per methods described by Saraceni and Ruggiu (1969) and Throndsen (1978) and cell counts were enumerated by using a modified Sedgwick-Rafter cell as recommended by Lund et al., (1958) and Frontier (1972).

The potentials of utilization of bivalves for bio control of algae was investigated by conducting laboratory based experiments through *in vivo* exposure trials, by exposing the live clams to algal rich waters of known cell density to the indigenous filter feeding black clam *V. cyprinoides* . For this, bivalve clams were collected from the clam beds in Thottappally backwaters, Alappuzha and were utilized for algal feeding trial as per methodologies described (Smith etal.,2012).

The freshly collected live clams *Villorita cyprinoides* were cleaned off epiphytes and other encrusting organisms and were kept in the laboratory in a running water system for over 24 hours prior to their utilization in the in vivo feeding experiments. The bivalves were maintained at average temperature, 26-26.8^{0}C in 10L capacity in semi transparent PVC tanks filled with filtered sand bed substrates up to 12 cm depth. The experimental tanks were stocked with live baby clams of size 2.1- 3.9 cm were stocked in algal rich pond water of known algal density, with water filled up to 40 cm depth @10 liters per tank . Aeration was provided round the clock in the experimental tank by using an aquarium aerator and the filtration activity with reference to volume of water in the tank and clearance rate of the algae through consumption and assimilation by the stocked bivalve clam was monitored. For this, each morning, the filter feeding bivalves from the tanks were carefully transferred to a container with filtered water and the water samples with in the tank were drawn from the experimental tanks for enumeration of cell count and various water quality parameters.

The efficacy of clam, *V. cyprinoides* to consume algae by bio filtration was estimated by monitoring algal counts in exposure trials in two treatments, viz., 1. stocking of bivalve *V. cyprinoides* @ 3 nos /l,and a second system with clams @ 6 nos /l. Pond water containing algae of known cell concentration and clams were maintained in four replicates and two tanks as control with same quantum of pond water and algal density but without clams. The water quality parameters of the tank water were also monitored on a daily basis. The algal clearance rates by the bivalves were calculated as per procedures described by Riisgård (2001). This method measures the filtration activity with reference to bivalve biomass in the experimental system.

Results

The massive algal mat that struck the temple pond at Sankara Narayana Moorthy temple Ambalappuzha, was identified to be due to blooming of *Microcystis aeruginosa,* a single-celled blue green algae, cyanobacterium, that occur naturally in surface waters. It was observed to proliferate to a dense bloom of floating mat in the warm, turbid, and slow-moving water.

During the bloom period, the pond water looked turbid with transparency as low as 1 cm. pH of the pond water was 9 and free carbon dioxide was absent. The air and the water temperature in the pond system were 29°C and 28.5°C respectively. The Dissolved Oxygen (DO) was very low, 3.6 mg /l. The water hardness in the pond water was 35mg /l. The total alkalinity was 117.5 mg/l. The Phosphate and Nitrate concentrations were very low, observed only in traces and free Ammonia in the pond was measured as 1.0 mg/l. The algal concentration in the pond system was 3 x10^6 No/ l and was predominantly comprised of *Microcystis.*

In the *in vivo* trial, the water temperatures in the experimental tank were maintained at 26.0-27.0 ^{o}C. pH of water in the experimental tanks remained almost alkaline during the experimental period, and it varied from at 8.0 to 9. Dissolved Oxygen(DO) in the tanks stocked with clam in low density,(3 nos/L)fluctuated from 3.6 -11.5 mg/L, close to super saturation by continuous aeration initially, which gradually declined 3.6 mg/L on the 7 th day after stocking (7 DAS) .The total alkalinity of water varied from 50 to 125 mg/l in the low density

regime (3 nos/L) and from 50 to 100 mg/l in the high density tank system(6 nos/L). The water hardness in the experimental tank ranged from 12.5 to 25 mg/l in the high density system, and 25 to 43.7 mg/L in the low density regime. Nitrate concentration was close to zero in the low stocking regime while it increased to 10 mg/l on day 11(11DAS) in the high density regime. Free Ammonia in the low density regime ranged from 0.0- 0.5 ppm while in high density regime, ammonia concentration attained very high values at 0.30 - 2.00 ppm. Transparency of water was highly variable, and it fluctuated with reference to algal concentration and release of pseudo faeces by the experimental animals. No perceptible improvement in transparency was evident.

Bivalve, *Villorita cyprinoides* was found to voraciously feed on suspended particulate matter including micro algae *Microcystis aeruginosa,* initially and the stocked Clams apparently increased the transparency two fold in the experimental system till 6(DAS) . Thus, the clarity water due to filter feeding was found to increase gradually within a week after stocking of the bivalve . However the transparency in controls tanks without clams demonstrated only smaller changes over time.

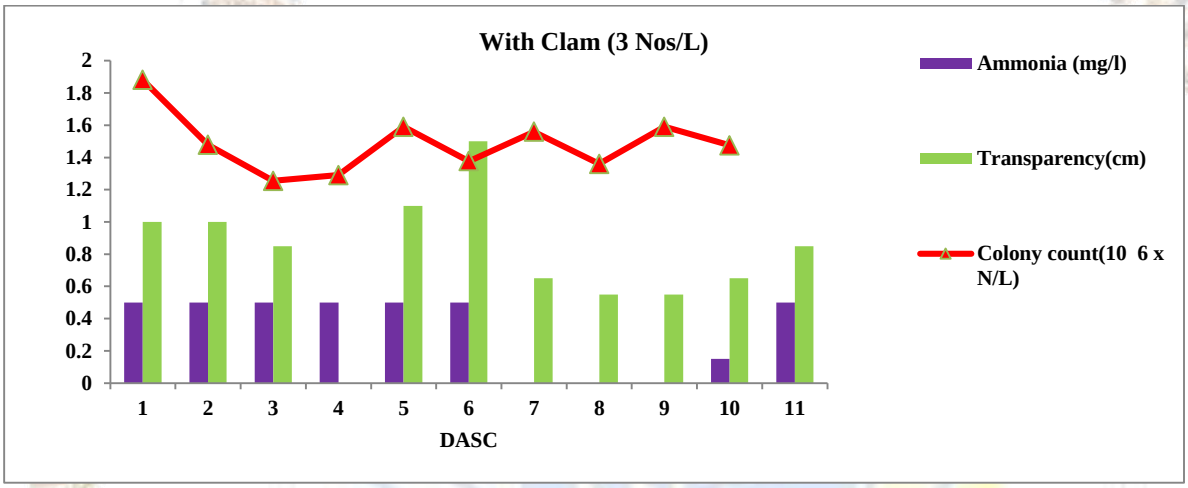
The bivalve feeding responses differed perceptibly in the two treatments. The microalgal count which was 2.4 $x10^6$ $/L^{-1}$ in the beginning at 1 DAS gradually got reduced to 1.45$x10^6$ L^{-1} from in the low density regime (3 nos/ L) while in high density regime (6nos/L) the concentration increased from 1.75 $x10^6$ to 2.15$x10^6$ $/L^{-1}$ on 11 DAS, with the enhanced liberation of pseudo faeces form 7th DAS. The algal clearance rate by clam which was 0.006 $X10^6$ L^{-1}cells initially at high density regime was observed to be reversed with no perceptible improvement at 11 DOS. The highest clearance rate was observed in the low density system where clams were stocked at low stocking density @ 3 Nos/L .

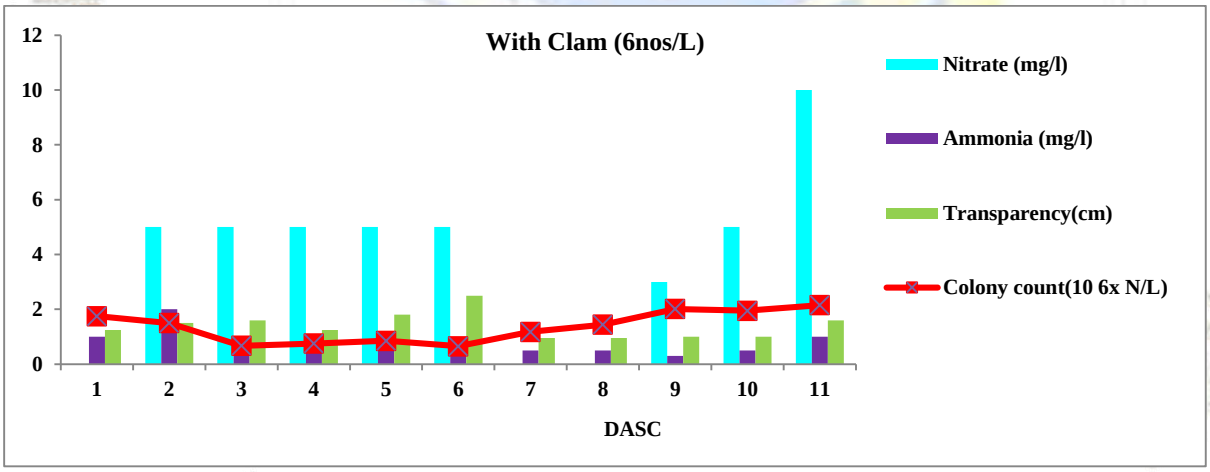
Table 1: Treatment with Black Clams *V.cyprionoides(*3 nos/ L)

	With clam (3 nos/ L**.)**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Water Temperature(°C)	**27**	**27**	**26.75**	**26.75**	**26.5**	**26.5**	**26.5**	**26.5**	**27**	**26.5**	**26.25**
pH	**9**	**9**	**8.5**	**9**	**9**	**8**	**9**	**9**	**9**	**9**	**9**
Dissolved Oxygen(mg/l)	**11.1**	**11.5**	**9.7**	**6.5**	**4.1**	**7.8**	**3.6**	**5.8**	**5.8**	**6.2**	**6.7**
Alkalinity(mg/l)	**125**	**100**	**100**	**50**	**50**	**50**	**50**	**50**	**100**	**100**	**100**
Hardness(mg/l)	**43.7**	**25**	**31.25**	**25**	**25**	**25**	**37.5**	**25**	**25**	**25**	**25**
Nitrate (mg/l)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Ammonia (mg/l)	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0**	**0**	**0**	**0.15**	**0.5**
Transparency(cm)	**1**	**1**	**0.85**		**1.1**	**1.5**	**0.65**	**0.55**	**0.55**	**0.65**	**0.85**
Colony count(10 6 x N/L)	**2.41**	**2.07**	**1.165**	**1.2**	**1.4**	**1.27**	**2.1**	**0.76**	**1.82**	**1.5**	**1.45**

With Clam (6 nos/ L)											
	1	2	3	4	5	6	7	8	9	10	11
Water Temperature(°C)	26.75	26.75	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26
pH	9	9	9	9	9	9	9	9	9	9	9
Dissolved Oxygen(mg/l)	15.8	22.4	17.8	13.4	12.6	7.6	11	8.52	12.2	12.3	12.2
Alkalinity(mg/l)	100	100	100	50	50	50	50	50	100	100	100
Hardness(mg/l)	25	25	18.8	18.8	12.5	12.5	25	25	25	25	25
Nitrate (mg/l)	0	5	5	5	5	5	0	0	3	5	10
Ammonia (mg/l)	1	2	0.75	0.5	0.5	0.5	0.5	0.5	0.3	0.5	1
Transparency(cm)	1.25	1.5	1.6	1.25	1.8	2.5	0.95	0.95	1	1	1.6
Colony count(10 6x N/L)	1.75	1.5	0.67	0.75	0.85	0.65	1.18	1.44	2.01	1.95	2.15

Table 2: Treatment with Black Clams *V.cyprionoides*(6 nos/ L)





DISCUSSION

Cyanobacteria-associated Harmful Algal Blooms (HABs) and their toxins are a growing concern worldwide *(*Moore et al 2009, Anderson et al 2012, Glibert,2013, Gobler et al ,2017*).*It has direct implications for the use of water bodies for recreation and drinking, and for the overall degradation of aquatic resources. Climate change confronts natural ecosystems with increased temperature, enhanced surface stratification, stimulation of photosynthesis by elevated CO2, and micronutrient availability. Numerous researchers have noted that cyanobacteria are also well adapted to high CO_2 concentrations and cyanobacterial carbonic anhydrase gives *Microcystis* another advantage to utilize bicarbonate as a carbon source. It should be noted that Blue green algae hence are capable to utilize organic compounds either in dissolved or particulate form (Taylor &Pollingher 1987, Berg et al. 1996).

Microcystis aeruginosa is the most common toxic algae that thrives in eutrophic waters.(Tanabe etal,2018) *Microcystis* is a toxic blue green algae and the blooms of this species may sometimes produce toxins called microcy*stin.* These toxins are generally very stable compounds, resistant to chemical breakdown and are persistent in natural waters for weeks to several months (Sivonen *and Jones, 1999).*

It is hypothesized that microcystis community contain genes needed to synthesize the potent hepatotoxin microcystin, a compound originally known as "fast death factor(Bishop etal,1959).They needs excess nitrogen to produce toxins. Producing these toxins is reportedy a big energy burden on the organisms , as the organism need almost double the nitrogen concentration than their bodily nitrogen biomass. A critical factor in bloom development is hence nutrient supply. This includes the major nutrients such as nitrogen, and phosphorus. In the present investigation, the observed nutrient concentrations especially phosphate has been negligible apparently due to its utilization for the blooming of the algal species .

The predominance of the blue green algae is explained to be due to the success of *Microcystis* in raising the pH conditions, that are generally unfavorable for other phytoplankton, for e.g., the siliceous frustules of diatoms become soluble, and Si is likely incorporated at lower rates under such alkaline pH conditions. Increasing temperatures during climate change provide another condition that favors some cyanobacteria like *Microcystis* populations that grow faster at warmer temperatures (Paerl and Huisman , 2008).

The most direct control method for *Microcystis* removal is chemical treatment by use of algicides and copper compounds. which are considered disastrous as these chemicals induces cyanobacterial cell lysis, which result in the release of toxins, microcystin into surrounding waters. (Jabulani et al , 2008). It is established that microcystin can move through the aquatic food web, exposing fish and shellfish, and humans. The bloom event commonly increase surface water pH to well above 9 as observed in the study, as the cyanobacterium rapidly

consumes available inorganic carbon. Generally, biological control of algae are facilitated by use of algicidal bacteria, plant bioactive enzymes, herbivorous fish, and filter feeding bivalves.

Laboratory assays on bio control *Microcystis aerugenosa* by using black clam *V.cyprinoides*, point to an important connection between biodiversity and ecosystem services, as this filter feeding bivalve is known to feed and utilize the microalgal blooms. The bivalves apparently cleared the microalgae at high efficiency in the high density regime, 6 Nos/L from the initial days of the trial emphasizing that bivalve density and cell concentration is important for clearance of microalgae. Interestingly, the feeding responses were observed to vary with reference to algal densities and animal densities. The filtering rate behavior of bivalve species differ when exposed to diverse stocking destines of filtering species and at varying cell concentrations .Apparently, the algal feeding capacity of bivalve clam was perceptible in all the replicated trials. This is evident from reduced algal counts. Among the free-living aquatic invertebrates, suspension-feeding is a widespread and successful trophic strategy. In suspension-feeding bivalves, both of these processes culminate also in rejection, which results in the formation of pre - ingestive rejection particles, termed pseudofaeces' comprising low nutrition and toxic particulate matter (Sierszen & Frost 1992). Pseudofeces elimination is the final rejection step in the cascade of particle processing event. The mode of pseudofeces rejection, is stated to be determined by the gill type (Beninger et al., 1993). The present study explicitly illustrates that native bivalve species can be of help in mitigation and prevention of harmful algal menace by clearing and assimilating bloom-forming microalgae. However, it is said that they in effect cleans excess nutrients in the water body by clearing the algal blooms .(ref) However, the clearing rates of bivalves is likely to decrease with increasing concentrations of algl biomass as the animals must circulate more material through their gills (Montagna et al. 1993; Bacon et al. 1998). All these observations signify the role of epifaunal and benthic filter feeders like bivalves for restoring the environment and mitigation of harmful algae blooms in coastal waters.

The present findings however, suggest that as compared to other microalgae, cyanobacteria like *Microcystis* are not preferred food to fin fish and shell fish. Shellfish exposed to such toxic algae are (e.g., oysters, clams, mussels) a potential source of exposure to concentrated cyanotoxins. Some suspension-feeding bivalves filter algal particles from the water column egests this particles as mucus permeated as fragile masses called pseudo feces. The rate of ingestion of algal biomass and clearance by the bivalves gradually slows down with the increased appearance of pseudo feces. The production of pseudofaeces *per se* does not, by itself, signify *selective* rejection. Some authors believe that pseudofaeces simply prevent the further ingestion of algae into the stomach ("ingestion volume regulation": Foster-Smith, 1978).

In the present study, the low clearance rates of algae by black clam *V. cyprinoides* indicate that physical or chemical surface properties of this cyanobacterium determine preference by the bivalves (Rosa et al.2017). When bivalves are fed with algae the clearance rate declined with increasing algal concentration. In experiments with naturally occurring toxic *M. aeruginosa* experimented mussels exhibited lowered or normal filtering rates with rejection of *M. aeruginosa* as pseudofaeces.

This selective rejection was attributed to 'unpalatable' nature of toxic strains of *M. aeruginosa* that occur as large colonies and were rejected spontaneously .Small algal biomass, were however, ingested. This explains the marginal reduction in numerical counts of the toxic algae in the experimental system as *M. aeruginosa* are known to cause blockage of feeding apparatus as observed in some zooplankton(Henry et al ,2001)

Contamination of drinking water sources by toxins from cyanobacterial blooms in is a serious concern which is known to damage internal organs and disrupt nervous system functions. The study indicates that bloom-forming algal species are cleared and assimilated by resident bivalves. This might help mitigation of algal blooms, which is invariably related to algal species and their cell concentrations. However more detailed studies are needed to enquire into the residual toxicity, and the best fit bivalve species, and mechanism of pseudo faeces voidance by the bivalve species.

In the context that HABs have direct implications for the use of water bodies for recreation and drinking, water source etc. all these treatment methods are only short-term fixes to larger issue of nutrient loading. Rather than treating the symptoms, there is a dire need to adopt more comprehensive nutrient management techniques to contain such menaces as the root cause of bloom is loading of nitrogen and phosphorous in excess. Proactive approaches to controlling blooms calls for long-term management strategies, with emphasis more on mitigation of nutrient inputs.

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Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by AFM and SMS. The first draft of the manuscript was written by FM and SMS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest

Author contribution

All authors contributed to the study conception and design. The manuscript was written by CC. All authors read and approved the final manuscript.

Data Availability

The materials used and datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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