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Indexed in
Scopus Compendex and Geobase Elsevier, Chemical Abstract Services-USA, Geo-Ref Information Services-USA, List B of Scientific Journals, Poland, Directory of Research Journals

ISSN 0974-5904, Volume 08, No. 02

International Journal of Earth Sciences and Engineering

April 2015, P.P.485-490

Selection of Cladocera Species for Applications in Biomanipulation

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Abstract: Lakes and reservoirs are lentic systems and most of them are suffering from eutrophication which is a common problem throughout the world today. The generalist feeding behavior of most Cladocerans has been applied in biomanipulation for controlling cyanobacterial densities and to accelerate the recovery of culturally eutrophic lakes. Similar experiment was carried out to compare grazing of five different Cladocera, Daphnia lumholtzi, Ceriodaphnia reticulata, Moina sp., Macrothrix sp., and Oxyurella sp. under laboratory conditions to select which grazer is better suited for the application in biomanipulation for Sri Lankan reservoirs. The five types of algae species, used in the experiment included, Scenedesmus sp., Chlorella sp., Cosmarium sp., Navicula sp., and Monoraphidium sp. Filtering of each algae by each species of Cladocera was assessed by counting algal cells in 50 µL of the culture media. The results were analyzed using the statistical software SYSTAT (v9) and Microsoft Excell (2007). The growth statistics showed that abundance of all the algae increase with time. Small limnetic Cladocera species C. reticulata showed a better control of the Chlorella sp. than other species. D.lumholtzi (limnetic Cladocera) and Oxyurella sp. (littoral Cladocera) was likely to be better grazers that control the growth of Scenedesmus sp. within a shorter period of time compared to other Cladocera used in the experiment. Both Moina sp. and D. lumholtzi, large limnetic Cladocera appeared to be better controllers of the Monoraphidium sp. Cosmarium sp. was also well controlled by the Cladocera species used in the experiment. The simple regression analysis of Cosmarium sp. growth along the temporal gradient showed a negative correlation with time in D. lumholtzi and Moina sp. dominating systems. Cladocera, Moina sp. appeared to be a better grazer that helps reduce the cell numbers of Cosmarium sp. with time. Navicula sp. was controlled by all the species of Cladocera. Thus our study shows that depending on the type of algae in the system the selection of zooplankton should be done if they are to be used in applications in biomanipulations. However large limnetic Cladocera such as D. lumholtzi and Moina sp. are better candidates for the applications in biomanipulation.

Keywords: *Biomanipulation, Cladocera, Cultural eutrophication*

1. Introduction

Eutrophication has been a major problem mainly in tropical water bodies all around the world. Excessive loading of nutrients (primarily phosphorus and nitrogen) in to lakes results in deterioration of water quality including increased turbidity and phytoplankton biomass predominance of cyanobacteria coupled with reduction of oxygenated habitat for organisms especially in the hypolimnion. In addition harmful algae growing in excessive rates forming blooms is also common to eutrophic waters. There are many factors known to affect the growth of algae. Especially enhance nutrients facilitate excessive growth of phytoplankton [7, 9].

Bloom-forming species may have one or both of two complementary strategies to form dense populations, higher growth rate (linked to bottom-up control or competition) and/or lower mortality rate (often associated with top-down control) than other phytoplanktonic species. Numerous adaptations have evolved in algal species that increase their competitive ability over other algal species, such as higher growth rate [15], production of allelopathic compounds that can reduce the growth of competitive species [11], higher nutrient uptake rates [15], diel vertical migration, allowing them to reach the nutrients in the deep layer at night and the light at the surface during the daytime [3]. Under such conditions water quality should be restored allowing the survival of aerobic organisms. However, in many cases the restoration of better water quality requires additional measures. Recovery is hampered by the structure of the present fish stock in the lakes and biomanipulation may help to get the recovery process started [7].With this approach, aquatic communities can be manipulated to maintain phytoplankton biomass at low levels. From a management point of view, biomanipulation is likely to be the long term successful method in controlling algal blooms in shallow eutrophic lakes and there is overwhelming evidence that biomanipulation is a successful management tool for

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shallow lakes [5,14], but the relevance to deep stratified lakes with a mean depth >5 m is less clear [2].

The generalist feeding behavior of most Cladocera has been applied in biomanipulation for controlling cyanobacterial densities, especially in temperate water bodies [6]. In several temperate water bodies, biomanipulation has been successful particularly when combining the use of large generalist filter feeders such as *Daphnia magna* and controlling the input of nutrients (particularly N and P) into the water body. Thus biomanipulation is now considered as a useful technique to accelerate the recovery of culturally eutrophic lakes. However regular monitoring, assessment and maintenance is required for long term sustainability [1, 12]. Therefore our laboratory experiment was designed to select the most suitable Cladocera species for applications in biomanipulation by comparing the grazing ability of the each selected Cladocera species.

2. Materials &Methods

2.1. Selection of Algae Types & Cladocera Species

A pilot study was carried out to select the most suitable Cladocera species and the most suitable algae species for the study. Fourteen species of Cladocera were cultured in the Environmental Research Laboratory of the Department of Zoology. Five Cladocera species were selected based on their filtering capacity, mean generation time and tolerance to various environmental conditions. The selected Cladocera species included *Daphnia lumholtzi*, *Ceriodaphnia reticulata*, *Moina* sp., *Macrothrix* sp., and *Oxyurella* sp. Six algae species that has the ability to grow under various environmental conditions were selected for the experiment and it included, *Scenedesmus* sp., *Chlorella* sp., *Cosmarium* sp., *Navicula* sp., *Monoraphidium* sp., and *Spirulina* sp.

2.2. Preparation of the Culture Media and Introduction of Cladocera

Cow dung was used as the main nutrient source for the medium. Six grams of cow dung was sterilized using boiling water (500ml) and added to Pyrex beakers of 3L capacity. Then 2500ml of distilled water was added and transferred to 3L containers and covered with mosquito nets. Each container was allowed to settle for one week before introducing algae. In total thirty six glass containers were prepared for the experiment and were divided into six series for the introduction of six selected algae, *Scenedesmus* sp., *Cosmarium* sp., *Navicula* sp., *Monoraphidium* sp., *Chlorella* sp., and *Spirulina* sp. Then all six series of culture media were kept aside for ten days for the algae to grow. *Spirulina* sp. was unable to form a monoculture hence it was removed from the experiment after several trials. In each series five species of Cladocera (25 individuals each) were introduced to five containers and the sixth container was maintained as the control. Hence each container of each series of algal culture included one species of algae and one species of Cladocera and the containers maintained as controls included a single species of algae without any Cladocera species.

2.3. Counting of Algae Cells

Glass containers were gently shaken to maintain equal distribution of algal cells through the medium before drawing out a sample. Sufficient amount of sample was taken out from the middle of the container to a small beaker. Using a micropipette, 50µL of the sample in the beaker was taken and placed on a microscope slide and one drop of glycerin was added to avoid desiccation during counting. Then the sample was covered with a cover slip that covers 400 µm of area. The number of cells was counted under medium power of a light microscope (Zeiss Primo Star Light Microscope). Counting of algal cells was done at each week for three months.

2.4. Data Analysis

Effect of Cladocera species on different types of algal species and effect of five Cladocera species on the number of cells in each series were analyzed using Microsoft Excel (2007) and SYSTAT (v9) statistical software.

3. Results

3.1. Growth Pattern of Algae used in the Experiment

Results revealed that the growth pattern of all the algae used in the experiment was similar. However the number of cells present in a unit volume of water is different and *Scenedesmus* sp. showed the highest growth, while *Navicula* sp. showed the slowest growth. Interestingly the growth of *Monoraphidium* sp. was slow at the beginning but after the 5th week the intensity of the growth increased remarkably. However the growth statistics indicated that all the algae demonstrated an increasing trend in growth with high R^2 values along the temporal gradient (Table1). This situation was common to all the species of algae used in the study.

Table 1: *R^2 values of the growth of algae in control*

Algae	R^2
Chlorella sp.	0.929
*Monoraphidium*sp.	0.993
*Scenedesmus*sp.	0.963
*Cosmarium*sp.	0.937
*Navicula*sp.	0.97

3.2. Control of *Chlorella* sp.

According to our experiment the growth of *Chlorella* sp. was gradual. However, small limnetic Cladocera species *Ceriodaphnia reticulata* showed a better control of the alga than the other species. Although the total controlling of *Chlorella* sp. by the other species of Cladocera except *Daphnia lumholtzi* exhibited a similar pattern of variation, disappearance of the algae started after the second week of the introduction of Cladocera. Although the growth of algae with *D. lumholtzi* was abrupt during the first two weeks, a sharp decline was noticed after the second week. However this pattern was not seen in other algae with *D. lumholtzi* as a grazer.

3.3. Control of *Scenedesmus* sp.

Unlike the other algae, the growth of *Scenedesmus* sp. was rapid. However the alga appears to be controlled by all the species of Cladocera, although small variations of the cell number were noticed during the 12 weeks of the study period. For this algae, *D. lumholtzi* (limnetic Cladocera) and *Oxyurella* sp. (littoral Cladocera) was likely to be better grazers that control the growth of *Scenedesmus* sp. within a shorter period of time compared to the other Cladocera used in the experiment. Although *Oxyurella* sp. was able to control *Scenedesmus* sp. and other algae in the same pattern, the growth of *Monoraphidium* sp. increased after one month. However, after the 11th week a clear decline was noticed. On the other hand *Moina* sp. was likely to be weak in controlling *Scenedesmus* sp. initially. This situation is different compared to the behavior of *Moina* sp. with other algae used in the experiment especially *Monoraphidium* sp. an algae that demonstrated delayed establishment in the system. Unlike other Cladocera species *Moina* sp. appeared to be a better controller for *Monoraphidium* sp. as the growth of the algae was suppressed throughout the study period.

3.4. Control of *Monoraphidium* sp.

Compared to the other algae *Monoraphidium* sp. colonies started to form after about one month of the beginning of experiment. However both *Moina* sp. and *D. lumholtzi* which are large limnetic Cladocera appeared to be better controllers of the algae. On the other hand *Monoraphidium* sp. appeared as large blooms with *Oxyurella* sp. and *C. reticulate* after the second month. However within a shorter period of one month *Monoraphidium* sp. also demonstrated a decline of growth and at the end of the 3 month experimental period the growth of the algae was controlled by all the species of Cladocera. Although *C. reticulata* is a small limnetic Cladocera, all the algae showed a decline after the 11th week except *Cosmarium* sp. Similar patterns were noticed with the large limnetic *D. lumholtzi* species.

3.5. Control of *Cosmarium* sp.

Cosmarium sp. was also well controlled by the Cladocera species used in the experiment. However the patterns of the growth of *Cosmarium* sp. with *D. lumholtzi* and *C. reticulata* were uncertain as an increasing trend was noticed after the 11th week.

3.6. Control of *Navicula* sp.

Although there were fluctuations of the growth of *Navicula* sp. after the 10th week, the algae was controlled by all the species of Cladocera. However the control of algae by *Macrothrix* sp. was not very certain. *Macrothrix* sp. appears to be a good grazer that control all the species of algae used in the experiment. Although there were variations of the growth of algae after the 11th week all the species of algae were reduced in number.

3.7. Statistical Analysis

3.7.1. Effect of Ceriodaphnia reticulata

The simple linear regression performed using the statistical software SYSTAT (v9) revealed that *C. reticulata* helped in controlling *Scenedesmus* sp. with high negative R^2value (-0.627). In addition *C. reticulata* showed a relatively weak negative correlation with *Chlorella* sp. and *Navicula* sp. however weakly. The algae *Cosmarium* sp. and *Monoraphidium* sp. positively correlate with *C. reticulata* (Table 2).

3.7.2. Effect of Macrothrix sp.

Unlike *C. reticulata*, *Macrothrix* sp. showed negative relationships with all the algae used in the experiment except with *Monoraphidium* sp. Especially *Scenedesmus* sp. and bottom dwelling *Navicula* sp. were better controlled indicated by the high values of negative correlation (Table 2).

3.7.3. Effect of Daphnia lumholtzi

According to the results, large limnetic Cladocera, *Daphnia lumholtzi* effectively controlled the growth of *Chlorella* sp., *Scenedesmus* sp., and *Navicula* sp. *Monoraphidium* sp. was also not able to colonize and weakly appeared in the system for a short period of time. However, the growth of large unicellular alga *Cosmarium* sp., positively correlated with *D. lumholtzi* (Table 2).

3.7.4. Effect of Oxyurella sp.

Oxyurella sp. which is a large littoral Cladocera showed a strong negative correlation with bottom dwelling micro algae, *Navicula* sp. and *Scenedesmus* sp. The surface dwelling *Monoraphidium* sp. and *Cosmarium* sp. positively correlate with time in the *Oxyurella* sp. system. Similar to the results with other Cladocera, *Chlorella* sp. showed a weak negative relationship along the temporal gradient (Table 2).

3.7.5. Effect of Moina sp.

Moina sp. which is an average sized limnetic Cladocera, likely to be a good candidate that helps filter all the microalgae used in the experiment. Interestingly *Monoraphidium* sp. was better filtered by *Moina* sp. and showed a strong negative correlation along the temporal scale. In addition large unicellular *Cosmarium* sp. also showed a strong negative correlation in the *Moina* sp. System (Table 2).

3.7.6. Comparison of growth statistics of different algae in different Cladocera

The R^2 values showed the correlation between the growths of microalgae along the temporal gradient, in different Cladocera dominated systems. *Scenedesmus* sp. was better controlled by *Ceriodaphnia reticulata* and *Macrothrix* sp. *Monoraphidium* sp. which thrived for a longer period of time than the other species used in the experiment was well controlled by the surface dwelling *Moina* sp. In addition, *Moina* sp. effectively filtered *Cosmarium* sp. showing a strong negative correlation with time. Small limnetic Cladocera species, *Ceriodaphnia reticulata* effectively filtered *Scenedesmus* sp. while large unicellular *Cosmarium* sp. was better filtered by large limnetic Cladocera, *Moina* sp. On the other hand a benthic microalga, *Navicula* sp., showed strong negative correlation along the temporal gradient with bottom feeding *Oxyurella* sp., and *Macrothrix* sp. (Table 2).

Table 2: *R^2 values of algae along a temporal gradient in different Cladocera systems*

Algae	*C. reticulata*	*Macrothrix sp.*	*D. lumholtzi*	*Oxyurella sp.*	*Moina sp.*
Chlorella sp.	**-0.09**	**-0.281**	**-0.432**	**-0.15**	**-0.084**
Monoraphidium sp.	0.3882*	0.3326*	**-0.037**	0.658*	**-0.444**
Scenedesmus sp.	**`-0.627**	**`-0.551**	**`-0.214**	**-0.341**	**-0.273**
Cosmarium sp.	**-0.117**	**-0.025**	0.4833*	0.2529*	**-0.754**
Navicula sp.	0.2211*	**-0.548**	**-0.447**	**-0.804**	**-0.289**

**Positive R^2 values*

Highlights-negative R^2 values showing strong negative correlation

4. Discussion and Conclusion

Our laboratory experiment of identifying better candidates for the applications of biomanipulation reveals that all the Cladocera species used have the capacity to filter different types of algae depending on the size, habitat, feeding habits and the size of the algae. When considering the growth of algae in the culture medium in the absence of Cladocera as a grazer, the growth and then the volume of all the algae increased showing the blooming ability of the species (Table1).When compared to the systems with Cladocera where the growth statistics mainly indicated negative R^2 values along the temporal gradient, all the species of algae showed positive and significant R^2 values of over 0.9 along the temporal gradient. However there were large variations of cell numbers noticed in different species of algae although the culturing media was same (Table1). Microalgae respond to various environmental conditions and thus the culture media also should be altered according to their preferred environmental optima. Especially nitrogen (N) and phosphorus (P) and the N: P ratio determine the growth of different algae and it varies from 8.2 to 45.0 under different light conditions [10]. This could be the reason for the variation of cell number we noticed in the artificial systems of our experiment.

When considering the growth of *Scenedesmus* sp. it is evident that there is early colonization of the algae compared to the other algae used in the experiment. However, *Scenedesmus* sp. was well controlled during the 12 weeks of observation period in all the Cladocera systems. *Scenedesmus* sp. is used as food for zooplankton especially for *Daphnia* sp. culture systems [17]. In addition *Scenedesmus* sp. is also used in other Cladocera growing systems. This is well evident from our regression analysis that the growth of *Scenedesmus* sp. reduced along the temporal gradient. All the Cladocera species used included both planktonic and littoral species and they all helped in controlling the algae. Interestingly large *Daphnia lumholtzi* appear to be a weak grazer of the alga when compared to the other Cladocera. In *D. lumholtzi* system R^2 value was low and in littoral Cladocera (*Ceriodaphnia reticulata*) and bottom feeding Cladocera (*Oxyurella* sp. and *Macrothrix* sp.) systems *Scenedesmus* sp. was better controlled (Table 2). This situation may be attributed to the development of *Scenedesmus* sp. in the water column and the bottom. Although *Scenedesmus* sp. is known to be an algae with wider tolerance to various environmental conditions including light [19]. In our experiment the initial development of the algae was recorded on the bottom surface although later the whole water column facilitated the growth of *Scenedesmus* sp., and as *Moina* sp. and *D. lumholtzi* are large limnetic zooplankton with less records ofbottom grazing, the low R^2 value may be related to the feeding habit. However, during the initial four weeks *Scenedesmus* sp. was well controlled by *D. lumholtzi* although *Moina* sp. started controlling the alga after the 4th week. According to our results small *Ceriodaphnia reticulata* has the ability to better control of *Scenedesmus* sp. with the gradual decrease of the alga throughout the 12 week period. *Chlorella* sp. is a single celled fresh water green microalgae with full of nutrients known as a super food that contain more nucleic acids (RNA/DNA) than any other food, known to provide energy producing potential. It has a wide variety of useful nutritional applications, which include supporting natural detoxification, digestive health, immune function, inflammation reduction, antioxidants, estrogen balance, cholesterol metabolism, and circulation in human. In addition *Chlorella* sp. is used in culturing of zooplankton especially in Cladocera cultures. In our experiment the growth of *Chlorella* sp. was exponential along the temporal gradient (Table1). However in different Cladocera cultures *Chlorella* sp. showed different growth patterns. The growth statistics reveal that the *Chlorella* sp. show negative correlation along the temporal gradient. However in the presence of *D. lumholtzi* the R^2 value was a higher negative value showing that *D. lumholtzi* is an effective grazer for *Chlorella* sp. that helps to control the algae within a shorter period of time. The growth of *Chlorella* sp. is limited by light intensity than any other environmental conditions [13]. As we provide the same light intensity for all the cultures of *Chlorella* sp. it is apparent that the growth was controlled by the zooplankton grazers.

The growth of *Monoraphidium* sp. is also controlled by various environmental factors including nutrient, light intensity, temperature and oxygen [4]. However when compared to the colonization of the algae it was noted that the colonies started to form one month after starting the experiment except in the *Moina* sp. dominating system . The growth statistics showed the positive correlation along the temporal gradient in *C. reticulata*, *Macrothrix* sp., and *Oxyurella* sp. systems with R^2values of 0.3882, 0.3326, and 0.658 respectively. However in *Moina* sp. dominating system the R^2 value was -0.444. When considering the growth patterns although a sharp decline was noted at the end of the experiment, during the 6 to 10 week period a maximum growth was noticed. In *D. lumholtzi* dominating system, the maximum number of cells was observed during the 7^{th} week but the cell density was very low. However, in *Moina* sp. system *Monoraphidium* sp. showed a rapid growth during the first month and thus the controlling of the algae may have occurred at the later stage. These conditions observed may attribute to the habitat specificity and behavior of the zooplankton used in the experiment. When considering the microhabitat of *Monoraphidium* sp., it is usually found in the surface water and this may be the reason why littoral Cladocera could not control the bloom when limnetic Cladocera (*Moina* sp. and *D. lumholtzi*) helped in controlling the bloom. Similar situation was clearly demonstrated by *Navicula* sp. the benthic micro algae used in the experiment. Correlation of *Navicula* sp. with time demonstrated positive R^2 values in *Macrothrix* sp., *D. lumholtzi* and *Oxyurella* sp. systems.

When consider the habitats of the microalgae *Navicula* sp., it is a surface and bottom dwelling species that has the ability to grow in micro movements with low light conditions. It was noted that the walls of *Navicula* sp. systems were covered with the microalgae during the mid-part of the experiment. Around the 7^{th} week *Navicula* sp. was well controlled in all the systems except in the systems of *C. reticulate* and *Macrothrix* sp. As *Macrothrix* sp. and *Oxyurella* sp. are well known bottom feeders it is no wonder why *Navicula* sp. population was reduced. However the large *D. lumholtzi* which has the ability to cling on the surfaces and feed, also have done a better role of removing *Navicula* sp. attached to the surface of the walls of containers.

Large unicellular microalgae, *Cosmarium* sp. was also well controlled by the zooplankton used in the experiment. However *Oxyurella* sp. dominating system demonstrated a positive correlation which may be a condition attributed to the feeding habit of the zooplankton. The simple regression analysis of *Cosmarium* sp. growth along the temporal gradient showed a strong negative correlation of the algae with the time in *Moina* sp. dominating systems. Therefore it is apparent that large limnetic Cladocera, *Moina* sp. was a better grazer that helped to reduce the cell numbers of *Cosmarium* sp. with the time. Thus our study shows that depending on the type of algae in the system the selection of zooplankton should be done if they are used in applications in biomanipulation. However, in general, large limnetic Cladocera such as *D. lumholtzi* and *Moina* sp. are better candidates for the applications in biomanipulation.

According to our study, depending on the types of algae, grazing behaviors of five selected Cladocera species differed from one another especially due their micro habitats. With the growth of *Scenedesmus* sp., interestingly large *D. lumholtzi* appear to be a weak grazer of the alga when compared to the other Cladocera. According to our results, small *Ceriodaphnia reticulata* has a better control of *Scenedesmus* sp. with the gradual decrease of the alga during observed time period. But *D. lumholtzi* is an effective grazer for *Chlorella* sp. that helps to control the algae within a shorter period of time. *Monoraphidium* sp., which is usually found in the surface water is effectively grazed by limnetic Cladocera (*Moina* sp. and *D. lumholtzi*) controlling the bloom. A similar situation was clearly demonstrated by *Navicula* sp. which is a both surface and bottom dwelling species. Large limnetic Cladocera, *Moina* sp. was a better grazer that helps reduce the cell numbers of *Cosmarium* sp. Therefore when selecting Cladocera species for the purpose of biomanipulation, types of algae and their microhabitats should be considered. Based on these information better candidates of Cladocera can be selected for applications in biomanipulation.

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