RESEARCH ARTICLE

Detection of suitable zooplankton from three taxonomic groups for application in biomanipulation

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Abstract: Many lentic water bodies of the world are under the threat of cultural eutrophication and experience problems associated with algal blooms. Biomanipulation is a useful technique applied to control algal blooms by deliberate alteration of an ecosystem through addition and removal of organisms. The feeding behavior of zooplankton has been applied in biomanipulation in controlling algal densities and to accelerate ecosystem recovery. For this, it is important to select the most efficient filter feeders. The present experiment was carried out to assess the grazing ability of five different zooplankton species belonging to three taxonomic groups, Cladocera, Copepoda and Ostracoda. Filtering efficiency of each algal species by selected zooplankton species were assessed by counting algal cells in each zooplankton introduced systems. Specific growth rate was calculated using the equation $(\ln N_1 - \ln N_0) / (t_1 - t_0)$ where, N_0 (cells/µl) and N_1 (cells/µl) are cell counts at times t_0 (day) and t_1 (day) respectively. The results indicated that zooplankton used in the experiment possess varied capacities of grazing on different species of algae. *Chlorella* sp. was well controlled by *Ceriodaphnia cornuta* whereas *Crucigenia* sp. was controlled better with the *Stenocypris* sp. Although species of *Scenedesmus* are favored by many zoopklankton as a food, it was better controlled by the members of the group Ostracoda and also by *C. cornuta*. Even though *Monoraphidium* sp. was controlled better by all the study species of zooplankton, *C. cornuta* showed the highest performances followed by ostracods.

Keywords: Eutrophic lentic systems, algal blooms, feeding behaviour.

INTRODUCTION

Surface waters, both lotic and lentic systems, experience algal blooms during trophic upsurge. High algal biomass in lentic freshwater systems including lakes and reservoirs commonly results from eutrophication mainly due to the loading of nutrients (Mustapha, 2009). Even though algae are necessary and beneficial to aquatic ecosystems, providing food and energy for nearly all other aquatic organisms, elevated levels of algal growth may negatively affect the health of aquatic systems. Further, nuisance algal blooms decrease aesthetic value of aquatic systems by reducing water clarity, creating unpleasant taste and odor. Extremely high levels of algae can make enough shade to prevent sunlight from reaching submerged aquatic macrophytes, limiting their growth or even causing their disappearance.

Nutrient enrichment in aquatic systems is a major cause for high algal biomass and now there is a considerable concern on this issue as it affects not only aquatic organisms but terrestrial organisms, including humans (Carpenter *et al.*, 1985; Smith, 1982). A classic traditional limnological approach in controlling algae is to understand lake ecosystem structure which orient at the food-chain concept and go through each trophic level: 1) nutrients, 2) phytoplankton, 3) zooplankton and 4) fish. Nevertheless, fish as top carnivores were not thoroughly considered as a component which may efficiently affect water-quality (Rigler, 1982). However, the use of biomanipulation as a tool for water management started in 1970's in small lakes and was later applied to large lakes due to the success of application (Lammens, 2001). Biomanipulation or biological restoration is seen as an option for restoring turbid lakes and reservoirs characterized by high phytoplankton biomass often with no aquatic plants to clear water through absorption of nutrients (Mustapha, 2009). Therefore, biomanipulation can be considered as a deliberate exploitation of interactions between the components of an aquatic ecosystem in order to reduce algal biomass (Wright and Shapiro, 1984). This method was introduced as an alternative to nutrient management and may provide a long

term effectiveness that nutrient management has failed to do in eutrophied lakes. Hence, biomanipulation is typically used in lakes that are small, shallow, and closed systems and it is likely to be the most successful in controlling algae in shallow eutrophic lakes (Mustapha, 2009). Further, it appears to produce desirable results in many applications. Unfortunately, the success in some cases can be limited and short term, thus further research is necessary to enhance the stability of biomanipulation (Shapiro, 1995). Therefore, a laboratory experiment was conducted to select the most suitable zooplankton from three selected groups for potential applications in biomanipulation.

MATERIALS AND METHODS

Collection of samples and culturing of species

For the collection of both phytoplanktons and zooplanktons, 20 reservoirs were selected to represent the three major climatic regions of Sri Lanka (Figure 1). Phytoplanktons were collected using plankton nets with 34µm and 70µm pore sizes, and 100µm pore size was used to collect zooplankton. Each sample was preserved in 4°C and was brought to the environmental research laboratory of the Department of Zoology, University of Peradeniya Sri Lanka for culturing (Zooplankton sampling protocol, 2011).

The plate culturing of algae was done to obtain pure cultures. Bold's Basal Medium (BBM) was used for the plate culturing. An amount of 200 ml was taken from the culture media and 2.00 g of agar was added to the medium separately. The culturing media and other glassware were autoclaved under the pressure of 15 psi and temperature of 121°C for about 15-20 minutes. Then the autoclaved culture medium was transferred into petri dishes under sterile conditions and plates were covered until the agar medium solidified. Then each water sample from 20 reservoirs was diluted to the 10-3 dilution factor. An amount of 100 µl was taken from each water sample and was transferred into each solidified culture medium. Then the samples were spread with sterilized glass spreader. The culture plates were sealed with parafilm and allowed to incubate for 15-20 days under ambient temperature and light intensities. Then the pure cultures were isolated and used in the experiment. Five species of

phytoplankton which are highly abundant in the reservoirs showing relatively high growth rates were selected for the experiment, C*hlorella* sp., *Crucigenia* sp. *Scenedesmus dimorphus*, *Scenedesmus quadricauda* and *Monoraphidium* sp. Then the pure cultures were established following a standard dilution procedure by Anderson and Kawachi (2005). These five species proved to be relatively common in natural waters and easy to rear under laboratory conditions (Elliott *et al.,* 2006; Millie *et al.,*1992; Lurling and Roessink, 2006; Moisander *et al.*, 2003).

Cultivation of microalgae to understand the filtering capacity of each zooplankton was carried out using cow dung as the main nutrient source following the method outlined by Vaikosen *et al.* (2007). In this method, water samples from each reservoir were autoclaved and used as the media. Six grams of cow dung was autoclaved and 500 ml of boiling water was added into 3L glass containers. Then 2,000 ml of distilled water was added to the containers before covering them with mosquito nets. The containers were allowed to settle for one week.

A total of thirty containers were prepared for the experiment. After a week, 10 ml of the pure cultures (parent cultures) of each phytoplankton species were added to each container. For each phytoplankton species, six containers were prepared, leaving one as a control. These containers were kept for about 15 days under ambient temperature and light intensities to obtain mature and well established cultures of algae from each selected algal species. Before the introduction of zooplankton, algal counts were taken to estimate their initial cell densities.

Selection of zooplankton for the experiment was conducted by considering their multiplication rate, microhabitat in the natural environment as well as in the microcosm. Accordingly, five species of zooplankton which lead mainly planktonic life were selected including two Cladocera (*Ceriodaphnia cornuta*, *Diaphanosoma* sp.), two ostracoda (*Stenocypris* sp., *Notodromus* sp.) and one Copepoda (*Cyclpos* sp.).
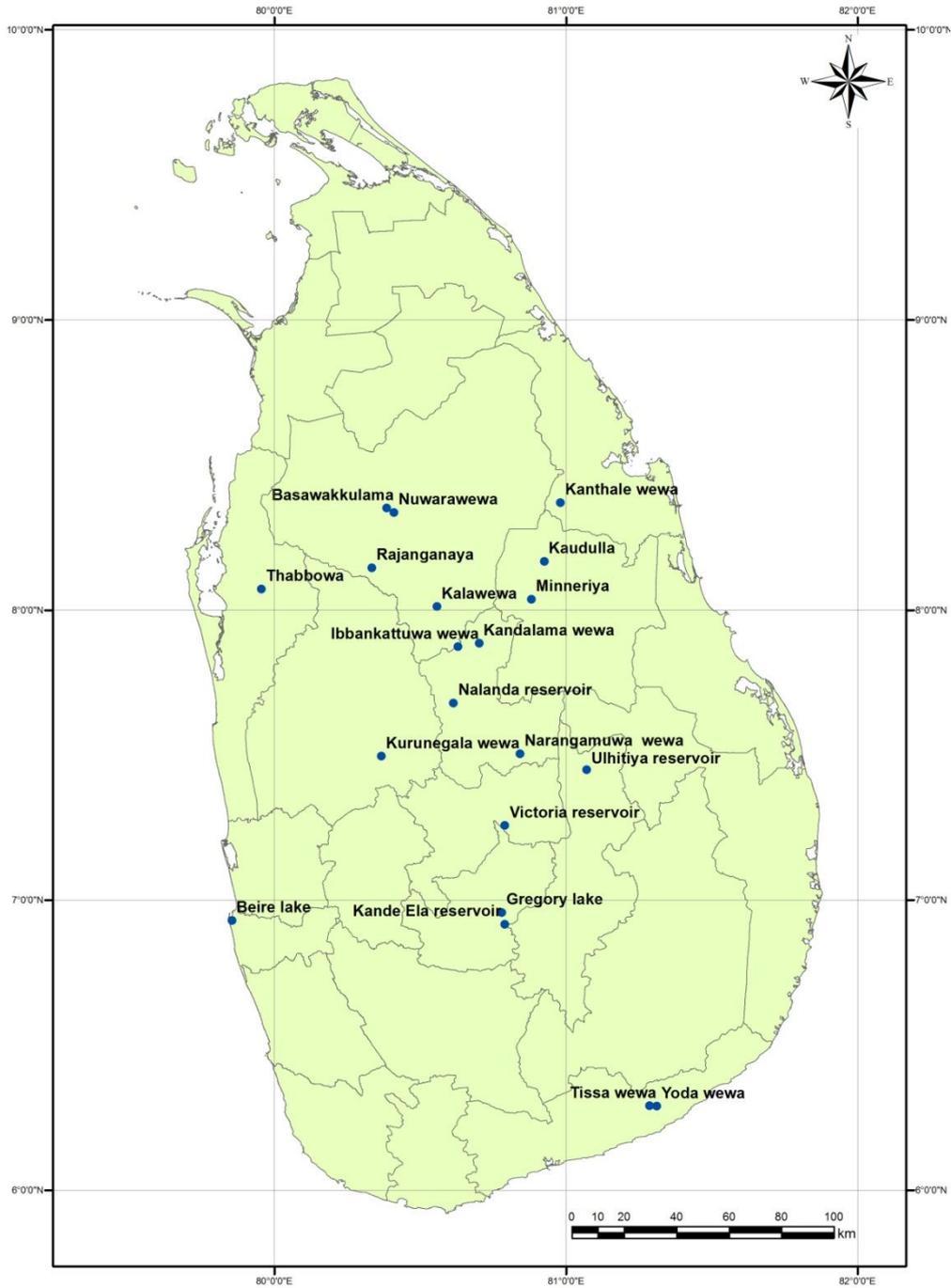
Figure 1: Locations of the 20 reservoirs in Sri Lanka that used to collect samples.

Introduction of zooplankton to the experimental setup

To each series of algae, five species of zooplanktons were introduced. Each container included one species of algae and one species of zooplankton. The containers maintained as the control included a single species of algae without introduction of any zooplankton species. Steps were taken to assure no contaminations. Thirty individuals from each zooplankton species were isolated and introduced to each container with algae.

Monitoring of the performances of each zooplankton species

After the introduction of zooplankton, the changes of cell density in each algal series were monitored to understand the filtering capacity of different zooplankton. The algal cell counts were taken every week for a period of three months using a Sedgewick rafter cell (PYSER – SGI Limited, United Kingdom). A research microscope (Carl Zeiss,GmbH, Konigsallee, 9-21, Germany) was used for the identification and counting of algal cells. Data were analyzed using Microsoft Excel (v. 2010). The specific growth rate of each algal species was calculated using the method explained by Lim (2006).

Data Analysis

The performances of each zooplankton species on controlling of algal densities were analyzed by correlation analysis using Microsoft Excel (v.2010) and by calculating specific growth rates. Specific growth rate = $(lnN_1-lnN_0) / (t_1-t_0)$ where, N_0 (cells/µl) and N_1 (cells/µl) are cell counts at times t_0 (day) and t_1(day). Specific growth rate of each algae with different zooplankton were compared to understand the filtering capacity of each zooplankton used in the experiment (Figure 3).

RESULTS

Growth pattern of the five algae species in control systems

The result of the experiment shows that the algae used in the study exhibits similar growth trends where the density of all five species of algae increased with the time. However, the initial cell density was different in each algal species, having the highest number in *Monoraphidium* sp.

and the lowest number in *Crucigenia* sp. (Figure 4).

Growth patterns of five species of algae in the presence of different zooplankton species

When consider the growth rate of each algal species, *Chlorella* sp. showed a gradual increase of cells with time compared to other species used in the experiment (Figure 4a). However, in the presence of zooplankton it is clear that the normal growth pattern of each algal species was disturbed, and showed a sharp decline of algal cells especially after the second week (Figure 4b). This situation was common to all the algal species used in the experiment. Interestingly, after the 3rd week, the density of algae appears to increase and after 4th week algal density started to decline again except in the presence of *Stenocypris* sp. This may be due to the ability of zooplankton to starve until they get used to the new type of food available in the system. After the 10th week, the density of *Chlorella* sp. was steady and completely controlled by the zooplanktons used in the experiment (Figure 4). When consider the overall performance of zooplankton in controlling *Chlorella* sp. the effectiveness of filtering in both the members of Cladocera (*Ceriodaphnia cornuta* and *Diaphanosoma* sp.) and *Notodromus* sp. is remarkable (Figure 2).

The growth of *Crucigenia* sp. appears to be different when compared to the growth pattern of other species of algae used in the experiment. Density of algae appears to be stable in the presence of *Diaphanosoma* sp. and after the 2nd week the algal cell number increased at a higher rate. In the presence of zooplankton, the algal cell density started to reduce after the 6th week and at the end of 12 week period the algae appear to be controlled by all the species of zooplankton used. However, the decline of density appears to be species specific and depends on both consumer and the producer. In the presence of *Stenocypris* sp. it shows a sharp reduction of algal cells after the 2nd week and continues to reduce the density until it reached a threshold level (Figure 4). Among the five species of zooplankton, the highest performance of controlling *Crucigenia* sp. growth was exhibited by *Stenocypris* sp. (Figure 3).
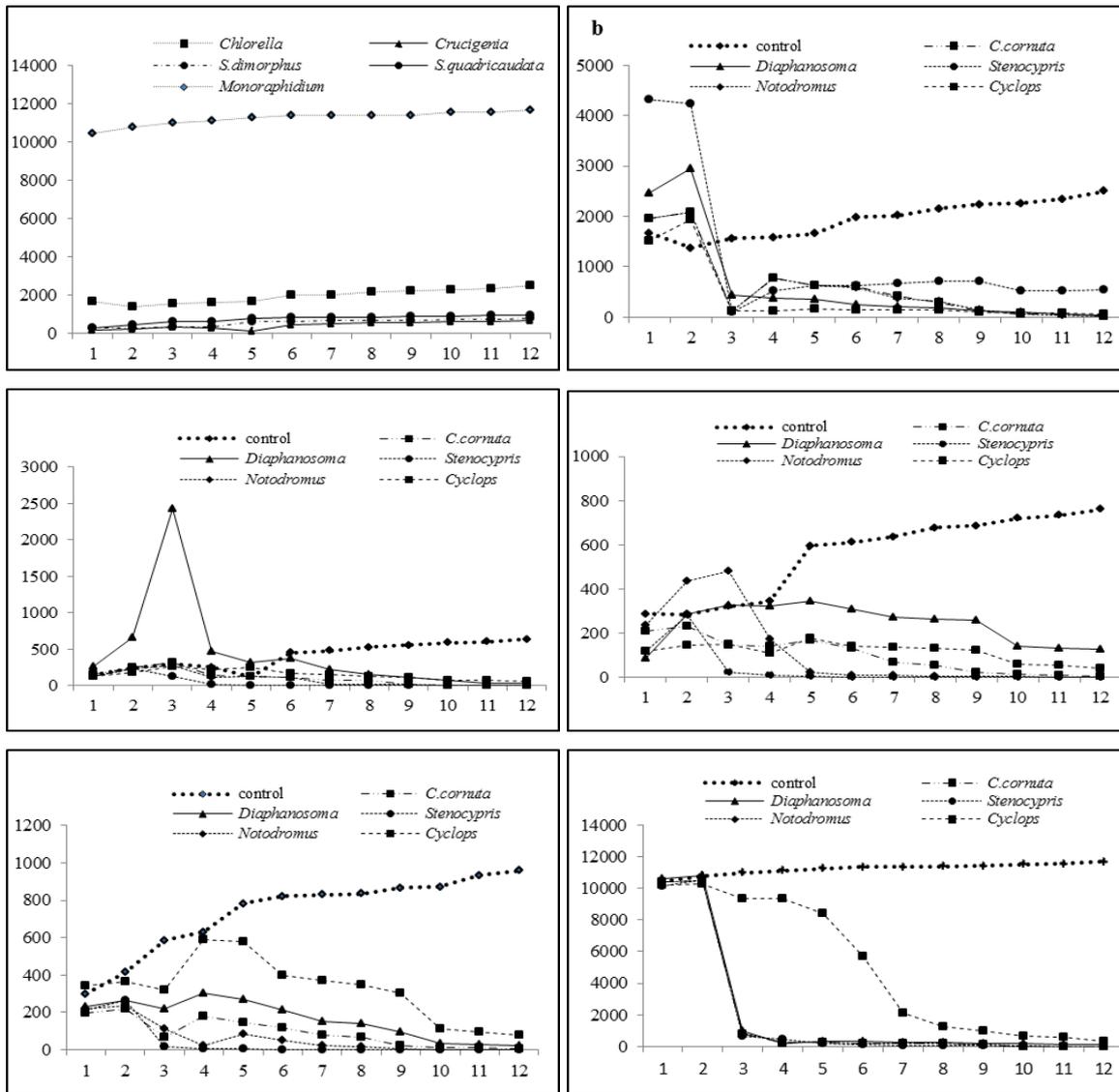
Figure 4: Line graphs showing the differences in growth patterns of algae in control systems (a) and with the presence of five species of zooplankton, *Chlorella* sp. (b), *Crucigenia* sp. (c), *S.dimorphus* (d), *S. quadricauda* (e), *Monoraphidium* sp. (f)
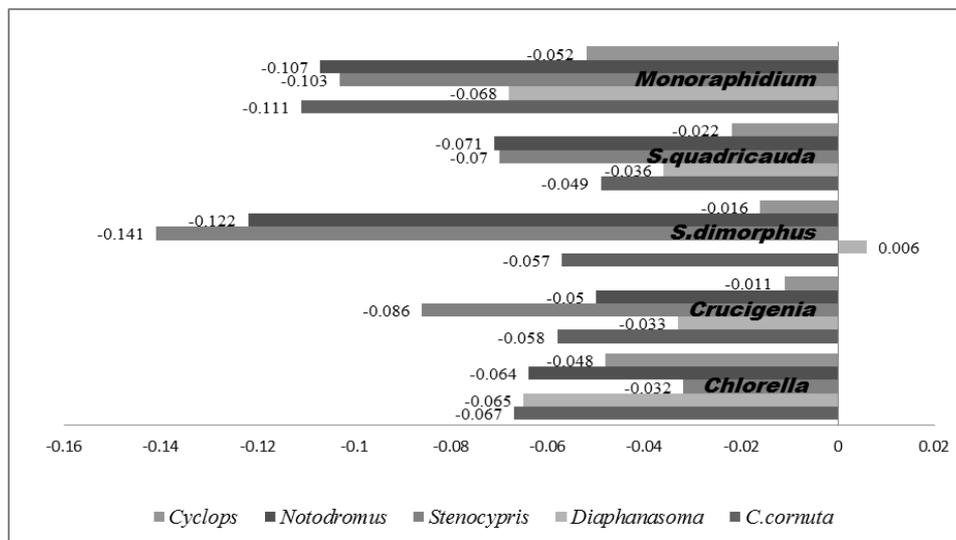

Figure 3: Effectiveness of five selected zooplankton species in controlling five species of algae.

The growth pattern of *S. dimorphus* was different from other algal species. Although irregularities occur at different intervals, the alga was well controlled by the zooplankton used in the study, especially in the presence of *Stenocypris* sp., where a complete removal of the alga was observed. In addition, the alga was well controlled by *Notodromus* sp. after the 6th week of the experiment (Figure 4).

When considering the growth pattern of *S. quadricauda* with other zooplanktons, a rapid reduction was seen after the 6th week. Compared to other species, there was a total reduction of algal cells in the presence of *Stenocypris* sp. and *Notodromus* sp. Further, *Diaphanosoma* sp. and *C. cornuta* also showed a remarkable filtering ability of *S.quadricauda*. However, when compared to other species the filtering capacity of *Cyclops* sp. was low (Figure 4). In the experiment, both species of *Scenedesmus* were effectively controlled by two species of ostracods than the other zooplankton species used in the experiment (Figure 3).

Unlike the other species of algae, the initial cell density of *Monoraphidium* sp. was high. In the presence of zooplankton with high filtering capacity, there was a sharp decline of algal cells after the 2nd week. The control of *Monoraphidium* sp. was almost similar with all the zooplankton species used in the experiment except by *Cyclops* sp. With the presence of *Cyclops* sp. the algal cell reduction was gradual (Figure 4). Finally, the best performances of algal control were carried out by *C.cornuta* and both members of ostracods.

DISCUSSION

With the increase biomass of algae, several negative effects can be observed especially in natural water bodies. Balanced aquatic systems are characterized by having certain qualities and quantities of biological, chemical and physical components. When the quality and quantities are disturbed, the system becomes imbalance. With the increasing levels of algal biomass, changes could be occurred within the system. It will directly affect the other biological components of the systems by disturbing the food webs and force other species to leave the habitat. Indirectly, it will cause changes in the chemical and then the physical components as well. Mostly this will affect reduction of water quality by reducing the dissolved oxygen level.

Therefore, lake restoration is important where systems have high algal biomasses. This concept can be applied through bottom-up and top-down force. This process can be explained based on which part of an organism's life cycle is being affected. In bottom-up control, it regulates the organism's number due to limited supplies of resources that affects its production of new biomass. In this situation, the nutrients are the responsible component which affects the production of new algal biomass. In top-down control, population regulation can be done through altering the death rates stimulated by predators or herbivores that attack another species or biomass. In this situation, through controlling the zooplankton densities, the phytoplankton biomass can be regulated.

Algal cultivation can be done under variety of environmental conditions but the ideal conditions for higher production of algae need to consider the factors that determine their growth rates and productivity of different algal species or strains (Garofalo, 2009).Therefore, in this experiment, steps were taken to provide essential environmental conditions that allow the algae to grow. According to the results obtained from the experiment, all species of zooplankton selected have the ability to reduce the algal density in different capacities. When simulated systems are considered, in the zooplankton absent systems, all the five species of algae showed an increasing trend of the density indicating that the algae growth is continuous during the period of 12 weeks (Figure 4).

At the beginning of the experiment, *Monoraphidium* sp. had the highest cell density and *Crucigenia* sp. had the lowest cell density (Figure 4). Therefore, the present results also agree with Bogen *et al*. (2013) where a *Monoraphidium* sp. was identified as an oleaginous species with ability to grow rapidly. Even under nitrogen limited conditions, it composed of predominantly C18:1 and C16:0 fatty acids and this could be the reason why *Monoraphidium* sp. was well filtered by all the zooplankton used in the experiment. In addition, it has been revealed that the *Monoraphidium* sp. is tolerant to relatively wide pH ranges and salt concentrations of up to 1.0 % NaCl, in which the cells exhibit larger structures. The surface waters of Sri Lanka contain higher amounts of Na and Cl unlike the common scenario of lentic systems of the world (Yatigammana and Cumming,

2016). Such a change of ionic composition may have helped increas the density and even the size of cells of the species. According to Sterner (1993), biochemical contents of primary producers varied with their growth rate and therefore, herbivores feeding on food with rapid growth rate have different diets than herbivores feeding on low growth rate. Our results indicate that except *Cyclops* sp. all the other zooplankton used in the experiment showed very rapid filtering capacities and help to reduce *Monoraphidium* sp. during the first two weeks (Figure 4). Therefore, it is clear that the growth of *Monoraphidium* sp. can be controlled by all the species used in the experiment. Hence, in the applications of biomanipulation, integrated use of zooplankton may bring better results than the use of single species.

Chlorella sp. is a single-celled green microalgae found in freshwater known as a super food that contains higher nucleic acids (RNA/DNA) than any other food, with an energy producing potential (Yecong *et al.*, 2011). In our experiment, *Chlorella* sp. showed gradual growth over time during the period of 3 months. However, even with robust growth trend, *Chlorella* sp. was well controlled by all the zooplankton species used in the experiment (Figure 4). *Chlorella* sp. has high tolerance to extreme environments, with the ability to grow even in industrial wastes (Kalana *et al.*, 2015). Further, it is used to remove nutrients from the waste water and the biomass is used for biofuel production as it contains high percentage of lipids (Yecong *et al.,* 2011). Unlike other species of algae used in the experiment, *Chlorella* sp. was well controlled after the third week by all the species of zooplanktons (Figure 4). According to Pipes and Koutsoyannis (1962), the sunlight is a strong environmental factor that limits the growth of *Chlorella* sp. than any other environmental conditions. In our study, all the steps were taken to provide equal environmental conditions including sunlight for all the cultures of *Chlorella* sp. Therefore, it is apparent that the growth was controlled by the zooplankton grazers. Among the zooplankton used in the experiment, *C. cornuta* was identified as the most effective zooplankton grazer that control the growth of *Chlorella* sp. (Figure 3).

Scenedesmus is one of the most common genera of microalgae with diverse applications including biofuel production, wastewater treatment, bio fertilizer, a foliar spray, a seed primer to evaluate seed germination, plant growth, and crop yield for some tomato plants (Gonzalez and Jesus, 2014). However, when they exist as blooms especially in natural systems they could cause both economic and environmental damages. So it has become important to understand the ecology and growth patterns of this important microalga. Consequently, our experiment helps to understand the growth patterns and the control of the species using an environmentally friendly technique. All the zooplankton species used in the experiment were able to control the growth of both *Scenedesmus* species after the 5[th]week (Figure 4). However, a variation of filtering by different zooplankton was noticed during the 12 weeks of experimental period. For example, the growth of *Scenedesmus dimorphus* was controlled better by *Stenocypris* sp. while in *Notodromus* sp. system, the cell density of *Scenedesmus dimorphus* increased initially and after the 3rd week the cell density started to reduce (Figure 4). According to Lürling and Roessink (2006), *Scenedesmus* species initially inhabit in benthic environment and then disperse to the other areas with the increase of cell density. So the initial increase of cell density of *Scenedesmus dimorphus* in *Notodromus* sp. (Ostracoda) system could be related to pattern of colonization of the alga in aquatic systems. *Notodromus* sp. is an ostracod species that inhabit in the water column. At the initial stage of algal colonization, *Notodromus* sp. may not be able to control the growth of alga as the habitat of grazer does not overlap with the algal habitat. On the other hand, unlike a large ostracod, *Stenocypris* sp. and *Notodromus* sp. are small and therefore the efficiency of filtering may be lower until they establish in the system. Hence, it is apparent why the both ostrcods showed a similar pattern of grazing and filtering of the algae. In addition in *Scenedesmuss quadricauda* system the grazing pattern of *Cyclops* sp. was different. After the 3rd week the cell density increased and again it started to reduce (Figure 4). Unlike *Scenedesmus dimorphus*, *Scenedesmus quadricauda* is armed with floatation spine. Therefore, the *Cyclops* sp. may not have been adjusted to feed on the floating individuals initially. However, when compared to the grazing of three types of zooplanktons, cladocera, ostracoda and copepoda, both species of *Scenedesmus* were well controlled by

ostracoda species than other two groups (Figure 3).

     Crucigenia sp. used in the experiment showed a diverse relationship with the tested zooplankton. However, a maximum negative growth rate (-0.086) was demonstrated with *Stenocypris* sp. and the lowest with *Cyclops* sp. (-0.011). Even though *Crucigenia* sp. shows irregularities in growth pattern, after the 6th week all the zooplankton used in the experiment show filtering of *Crucigenia* sp. to low cell numbers (Figure 4).Unlike other species of algae used in the experiment, *Curicigenia* sp. appeared to form compacted colonies with several cells. According to the specific growth rates in the systems with ostracod as a grazer showed the highest preference by *Stenocypris* sp. (Figure 3). In *Stenocypris* sp., *Crucigenia* sp. showed a sharp decline of cells in the 4th week (Figure 4). Even *Notodromus* sp. and *C.cornuta* reduced the cell number during the same week but the reduction was not as high as with *Stenocypris* sp. (Figure 4).This situation suggests how the size variations of zooplanktons affect the reduction of cell numbers. Further, specific growth rates suggest that all the species of zooplankton have the ability to control *Crucigenia* sp. However, as they exhibit some variations depending on their size, it would be encouraging to use larger size zooplanktons to get better results.

CONCLUSION

According to our study, grazing behaviors of five selected zooplankton species were differed, which could be related to characteristics of algae such as their ecology, morphology and growth characteristics. Benthic and limnetic *Scenedesmus* sp. was better controlled by the members of the group Ostracoda. *C. cornuta,* also a good filter feeder, has the potential to control of all algal species especially *Chlorella* sp. and *Monoraphidium* sp. The present study shows that the application of zooplanktons can be done with prior selection of their efficiencies in controlling specific algal species.

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