Comparative Study of Grazing Efficiency between *Moina macrocopa* and *Branchinella thailandensis* to Control Phytoplankton Growth

Ratcha Chaichana[1]* and Nattawut Promwang[2]
[1]*Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, P.O. Box 1072 Kasetsart, Bangkok 10900, Thailand*
[2]*Center for Advanced Studies in Tropical Natural Resources, NRU-KU, Kasetsart University, Chatuchak, Bangkok, 10900,Thailand*

Abstract

Excessive growth of phytoplankton due to the addition of plant nutrients causes several negative impacts and lowers ecological values of water bodies. The concept of top-down control by zooplankton may help restore nutrient-enriched water bodies, already dominated by phytoplankton since zooplankton species have direct and substantial effects on phytoplankton communities through grazing. Therefore, in this study, an experiment to control phytoplankton by native zooplankton species was carried out. The species that were comparatively studied included *Moina macrocopa* (small body) and *Branchinella thailandensis* (large body). The results showed that in water with an abundance of *Chlorella* obtained from pure culture, *B. thailandensis* at the density of 1,000 individuals lowered *Chlorella* by 73% in comparison with *M. macrocopa*, which reduced biomass of *Chlorella* up to 70% after 48 hours of experiment. Statistical analysis indicated that the grazing efficiency of both species was not significantly different ($p>0.05$). Further investigation was carried out by using natural water collected from three nutrient-enriched ponds (namely A, B and C) at Kasetsart University. The experiment was set up for five days in the laboratory and it was discovered that after only two days, both species of zooplankton had reached maximum grazing efficiency and decreased phytoplankton populations. *M. macrocopa* reduced chlorophyll a in water collected from A, B and C up to 90.5%, 95.6%, and 91.4%, respectively. Similarly, *B. thailandensis* had reduced chlorophyll a to the following concentrations of 93.7%, 86.4% and 82%, respectively.

Key words: Zooplankton/ Grazing/ Eutrophication/ Water bodies/ Phytoplankton

1. Introduction

Phytoplankton blooms also known as eutrophication occur in natural and man-made water bodies around Thailand. The application of chemical fertilizers containing plant nutrients is a common practice by farmers to increase crop yields, but at the same time it contaminates water in the field (particular in paddy fields) which subsequently flows into rivers and

*Corresponding Author:
E-mail: fscircc@ku.ac.th

so on (Bennett et al., 2001; Pathak et al., 2004; Kaufman and Watanasak, 2011). In addition, expansion of industrial sectors throughout the country has increasingly contaminated plant nutrients in lakes and rivers (Braga et al., 2000), thus causing rapid deterioration of aquatic ecosystems as a result of excessive algal growth.

Based on the concept of restoration of nutrient-enriched water bodies, reduction of considerable growth of phytoplankton can be achieved by top-down control. As a primary consumer, zooplankton can consume a substantial portion of the phytoplankton (Cyr and Pace, 1992; Hanson and Butler, 1994; Kasprzak et al., 2002) and thus keep a lake phytoplankton-free. Several studies have demonstrated that the clear-water state is most likely at high grazing pressure of zooplankton on phytoplankton (Carpenter et al., 1985; Elser and Goldman, 1991; Jeppesen et al., 1997). There are several species that have proved to be effective in the control of phytoplankton including *Daphnia magna* and *D. pulex* (Moss et al., 1997). A pilot study on the control of phytoplankton by zooplankton in China showed that *D. magna* could graze single-cell phytoplankton and some *Microcystis flos-aquae* and the system had good removal efficiencies of phytoplankton up to 86.85% (Ma et al., 2009). In northern Poland, large-bodied zooplankton such as *D. magna* could control the density of all the phytoplankton size classes in comparison with small-bodied zooplankton (mainly *D. galeata, D. cucullata* and *Bosmina* spp.) that was able to control the density of small algae (< 50μm) (Dawidowicz, 1990). Generally large zooplankton species such as large *Daphnia* affect phytoplankton population more than small species such as rotifers (Vanni, 1987 and Moss et al., 1997).

Moina macrocapa and *Branchinella thailandensis* are native to Thailand and abundant in most standing water bodies. However, their roles in suppressing the growth of phytoplankton are not known compared with studies of *D. magna* that are well documented. Therefore, in this study investigation and comparison of grazing efficiency between *M. macrocapa* (small-bodied zooplankton) and *B. thailandensis* (large-bodied zooplankton) were carried out. There are two sets of experiments including grazing efficiency of zooplankton on *Chlorella* that was obtained from pure culture in the laboratory (experiment I) and grazing efficiency of zooplankton on phytoplankton collected from natural water bodies (experiment II). The results will reveal whether these two species of zooplankton have a high enough potential to control and restore nutrient-enriched water bodies with excessive phytoplankton population.

2. Methodology

Experiment I (2.1) investigated grazing efficiency of zooplankton (small-bodied vs. large-bodied zooplankton) on *Chlorella* populations obtained from culture. Experiment II (2.2) determined grazing efficiency of zooplankton (small – bodied vs. large - bodied zooplankton) on phytoplankton in natural water collected from three ponds.

2.1 Comparison of grazing efficiency between zooplankton species in water with cultured Chlorella

Eggs of *B. thailandensis* were obtained from the Applied Taxonomy Research Centre, Faculty of Science, Khonkean University. Before culturing, the water was de-chlorinated for several days prior to the start of the experiment. The water was oxygenated for 24 hours and then 5,000 eggs of *B. thailandensis* were put in a white 5-litre container and placed in sunlight for 1 day until the eggs had developed into larvae. The larvae were then transferred into a black container with 20 L of water and oxygenated continuously. The larvae were fed a diet that composed of 50% of rice bran and 50% of dried *Spirulina* sp. (about 1.3 g) once a day. After 3 days, *B. thailandensis* larvae were approximately 0.3 cm (body length) and were ready to be used in the experiments. *M. macrocopa* with a body length of 1.3 mm was obtained from an aquatic animal diet shop.

An amount of 0.5 L *Chlorella* obtained from the Applied Taxonomy Research Centre was placed in an aquarium (15x40x20 cm) and then 10 L of water was poured into it. A mixture of 30 g of urea (46-0-0), 50 g of rice bran and 15 g of fertilizer (16-20-0) was ground and mixed. Subsequently 4 g of mixed medium was placed in each aquarium, which was positioned in a sunny area. After 5 days, *Chlorella* grew abundantly in the water.

The experiment contained 2 sets including a control set, without zooplankton and an experimental set, containing *M. macrocopa* and *B. thailandensis* at different densities (10, 100 and 1,000 individuals put in separated 250-milliliter flasks with *Chlorella*). Chlorophyll a was measured by extraction using acetone after 24 and 48 hours of releasing zooplankton. The efficiency value of both species of zooplankton was calculated by comparing decreases of chlorophyll a concentrations between the experimental and control sets. The efficiency of both species of zooplankton was analyzed by comparing the means of two independent sample groups. GraphPad was used to calculate an independent t-test.

2.2 Comparison of grazing efficiency between zooplankton species in water collected from 3 ponds

The water quality of 3 nutrient-enriched shallow ponds located in the Bangkhen campus of Kasetsart University, Bangkok was examined physically, chemically and biologically. Pond A had a water surface area of 300 m^2 and a depth of 1.7 m, whereas pond B was smaller, with a water surface area of 200 m^2 and a depth of 1.3 m. Pond C was the smallest water body, with a water surface area of 150 m^2 and a depth of 1 m. The 3 ponds contained different amounts of phytoplankton (18, 8 and 7 μgL^{-1} of chlorophyll a concentrations, respectively). 5 samples were collected in September 2009 from each pond. Temperature (^{o}C), transparency (m.), conductivity (μScm^{-1}) (YSI EC300), dissolved oxygen (mgL^{-1}) (YSI 550A) and total dissolved solids (mgL^{-1}) in each pond were measured in situ. Then, 2-liter water samples were collected for laboratory analysis of concentrations of soluble reactive phosphorus (SRP: μgL^{-1}), total nitrogen (TN: %), total phosphorus (TP: μgL^{-1}), chlorophyll a (μgL^{-1}) and suspended solids at the Environmental Science Department, Faculty of Science, Kasetsart University. Chemical analysis of water samples was based on standard methods for the examination of water and waste water (Greenberg et al., 1998). In addition, composition of phytoplankton and zooplankton was studied. 10 L of water were passed through a phytoplankton net (mesh size 20 µm) and a zooplankton net (mesh size 60 µm). Then, plankton samples were transferred into plastic bottles and preserved using 70% ethanol before they were examined under a microscope up to species level.

Water samples of 5 L were collected from each of the 3 nutrient-enriched ponds for the experiment. *M. macrocopa* and *B. thailandensis* at the densities of 100 and 1,000 individuals were placed separately in 250 mL flasks filled with water collected from each pond. Efficiency of 2 zooplankton species to reduce phytoplankton was obtained by decrease of chlorophyll a that was measured every 24 hours for 5 days.

3. Results

3.1 Comparison of grazing efficiency between zooplankton species in water with cultured Chlorella

In the first 24 hours, *M. macrocopa* at the densities of 10 and 100 individuals reduced chlorophyll a concentrations slightly (Figure 1). In contrast, *M. macrocopa* in flasks containing the 1,000 individuals decreased chlorophyll a up to 43% compared with the initial chlorophyll a level. After 48 hours, the reduction in chlorophyll a by the 10, 100, and 1,000 individuals of *M. macrocopa* was 7, 30 and 70%, respectively.

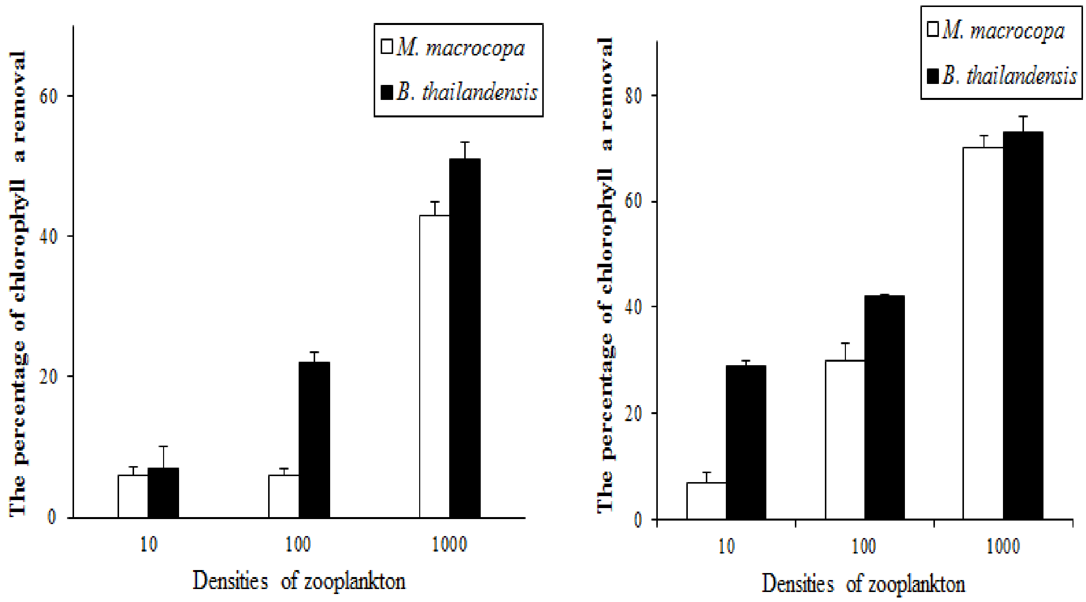

Figure 1: Comparison of grazing efficiency between *M. macrocopa* and *B. thailandensis* at different densities of 10, 100 and 1,000 individuals to reduce chlorophyll a after 24 h (left) and 48 h (right)

Grazing efficiency of *B. thailandensis* to reduce *Chlorella* was also similar to *M. macrocopa.* The result showed that in the first 24 hours, *B. thailandensis* at the densities of 10, 100 and 1,000 individuals reduced chlorophyll a by 7, 22 and 51%, respectively. After 48 hours, *B. thailandensis* in flasks containing 1,000 individuals decreased chlorophyll a level by 73%. In addition, *B. thailandensis* at the densities of 10 and 100 individuals reduced chlorophyll a by 29% and 42%, respectively after 48 hours.

Statistical analysis indicated that both species of zooplankton at the density of 1,000 individuals performed at maximum grazing efficiency in reducting of *Chlorella* and grazing efficiency between the two species was not significantly different after 24 hours ($P > 0.05$) and 48 hours (P > 0.05) at the 95% confidence level.

3.2 Comparison of grazing efficiency between zooplankton species in water collected from 3 ponds

Water quality values measured in situ show general environmental information of the studied ponds (Table 1). The results indicated that pond A had the highest concentration of SRP (156.11 μgL^{-1}). Pond C and Pond B in comparison with pond A had lower SRP concentrations of 55.89 and 50.59 μgL^{-1}, respectively. The highest TP (1,300 μgL^{-1}) was detected in pond C. and in ponds A and B, TP values were 460 and 360 μgL^{-1}, respectively. As for total nitrogen, pond A had the highest total nitrogen by 0.58%. In ponds B and C, total nitrogen was 0.45% and 0.39%, respectively. Chlorophyll a concentrations were the highest in pond A (18.13 μgL^{-1}) and lower in ponds B (8.25 μgL^{-1}) and C (7.70 μgL^{-1}). The results of water quality especially plant nutrients reflected concentrations of chlorophyll a among the 3 ponds. This is because higher densities of phytoplankton in pond A compared with other ponds may be strongly linked to high concentrations of plant nutrients, especially soluble reactive phosphorus that can be readily up taken by phytoplankton.

Table 1: Water quality measured from 3 selected ponds in Kasetsart University (n=5)

Parameters	Pond A	Pond B	Pond C
Temperature (^{o}C)	30.7±1.4	31.1±1.2	29.4±1.6
Transparency (m)	0.35±0.1	0.44±0.2	0.45±0.2
Conductivity (μscm^{-1})	392±28.6	465±37.8	273±12.4
Dissolved Oxygen (mgL^{-1})	3.71±0.7	3.54±0.4	3.12±0.6
Total dissolved solid (mgL^{-1})	0.23±0.02	0.27±0.08	0.16±0.04
Total suspended solid (mgL^{-1})	7.5±2.2	5.5±1.2	6.0±1.5

A total of 15, 8 and 6 species of phytoplankton were found in ponds A, B and C, respectively (Table 2). The dominant species of phytoplankton found in pond A was *Phacus longicauda* whereas *Euglena oxyuris* and *Cylindrospermopsis raciborskii* were abundant in ponds B and C, respectively. The densities of phytoplankton in pond A, B and C were 4,915, 1,260 and 790 cells L^{-1}, respectively.

5 species of zooplankton were found in pond A at the density of 670

individuals L-1 (Table 3). There were 6 species of zooplankton in pond B at the density of 160 individuals L-1 and in pond C there was only 1 species of zooplankton at the density of 80 individuals L-1. The dominant species of zooplankton in both ponds A and B included Brachionus caudatus and the copepod nauplii were found abundantly in pond C.

Table 2: Species of phytoplankton recorded in ponds A, B and C (actual numbers of phytoplankton)

Species	Pond A	Pond B	Pond C
Aulacoseira granulata	417		
Crucigenia apiculata	83		
Cylindrospermopsis raciborskii			330
Dictyosphaerium pulchellum	250		
Euglena acus	750	120	
Euglena oxyuris var. charkowiensis	250	450	172
Navicula sp.	500		64
Oscillatoria lemnitica		50	
Pediastrum duplex	83		
Pediastrum duplex var. clathramtum	250		
Pediastrum obstusum	167	105	18
Pediastrum simplex		75	
Pediastrum simplex var. duodenarium		60	
Phacus longicauda	1,000		
Phacus ranula	80		
Phacus tortus	833	80	46
Scenedesmus amatus	95	320	790
Scenedesmus bernadii	92		
Tetraedron glacile	65		
Total (cells L^{-1})	**4,915**	**1,260**	**790**

Table 3: Species of zooplankton in ponds A, B and C

Species	Pond A	Pond B	Pond C
Anuraeopsis coelata	167	40	
Branchionus angulanis	3		
Branchionus caudatus	250	60	
Copepod nauplii	167	20	80
Clalanoid copepod		20	
Trichocerca capucina	83	10	
Unidentified bryozoan		10	
Total (individuals L^{-1})	**670**	**160**	**80**

Grazing efficiency of *M. macrocopa* and *B. thailandensis* on phytoplankton in water collected from 3 ponds was similar to a previous study using *Chlorella*. It was found that *M. macrocopa* at the density of 1,000 individuals clearly decreased the chlorophyll a level in all three ponds by the second day of the experiment. And the chlorophyll a level of water from ponds A, B and C had decreased by 90.48, 95.67 and 91.35%, respectively. After the second day, the chlorophyll a level was almost constant or decreased slightly (Figure 2).

Similarly, *B. thailandensis* at the densities of 100 and 1,000 individuals caused a decrease in chlorophyll a in the 3 ponds too. Chlorophyll a decreased clearly during the first 2 days of the experiment, especially at the density of 1,000 of *B. thailandensis* that reduced the chlorophyll a level from pond A by 93.74%. The chlorophyll a levels of water collected from ponds B and C were decreased by 86.40% and 82.04%, respectively. After the second day of the experiment, the level of chlorophyll a changed only slightly.

4. Discussion

Moina macrocopa and *Branchinella thailandensis* are both effective in reducing the biomass of *Chlorella.* In addition, a greater density of zooplankton caused a greater reduction in the chlorophyll a level. The results were consistent with other studies that grazing rates were positively related to zooplankton biomass and negatively related to food concentration (Pyr and Pace, 1992). In addition, *B.*

thailandensis at densities of 10 and 100 was more effective at reducing the level of chlorophyll a than *M. macrocopa*. This is likely because *B. thailandensis* has relatively larger body size than *M. macrocopa*. Quiblier-Llobera et al., (1996) revealed that the grazing impact of smaller zooplankton, such as copepods (<17% day $^{-1}$), was relatively low compared with that of cladocerans, with the greatest grazing impacts amounting to 36% day $^{-1}$. However, the study of Cyr and Pace (1992) showed different results in that communities dominated by large zooplankton did not tend to have higher grazing rates than communities dominated by small zooplankton, which did not agree with our study results. At a density of 1,000 of *M. macrocopa* and *B. thailandensis*, the efficiency of both species of zooplankton in reducing the number of phytoplankton was not statistically different. Although *M. macrocopa* was smaller than *B. thailandensis*, the high density of *M. macrocopa* could reduce efficiency levels equally as the larger zooplankton.

Grazing effectiveness between the two zooplankton species in reducing phytoplankton numbers in nutrient-enriched water from 3 ponds shows interesting results. The zooplankton experiment indicated that both species of zooplankton at densities of 100 and 1,000 efficiently reduced the number of phytoplankton with different species and sizes within the first two days of the experiment. Both *M. macrocopa* and *B. thailandensis* were able to reduce levels of chlorophyll a over 80%, which was more effective than in natural water bodies where daily grazing by crustaceans produced reductions of 2–21% of the chlorophyll (Pyr and Pace, 1992). In the experiment, the zooplankton was more efficient, but this was possibly because they were present in higher densities. Moreover, grazers in enclosure experiments may have been active all day, while those in the natural water bodies entered the open water zone (epilimnion) only at night, since during the day, the zooplankton were primarily in deep waters and, thus, grazing activity in the upper five m was low (Lampert and Taylor, 1984; 1985; Lampert et al., 1986).

After the second day of the experiment, the amount of chlorophyll was constant and this is likely because most of the phytoplankton had been grazed by *M. macrocopa* and *B. thailandensis.* Thus, a small amount of phytoplankton remained the same. As the zooplankton levels dropped due to gradual mortality, the efficiency of the zooplankton to graze phytoplankton in general began to decrease or was constant. Figure 2 shows that the level of chlorophyll a in the control sets also decreased. The possible explanation is that the water from the 3 ponds was not filtered to remove zooplankton and thus, some zooplankton species may have been present in the water of the control set during water collection or perhaps a decrease of chlorophyll was due to nature.

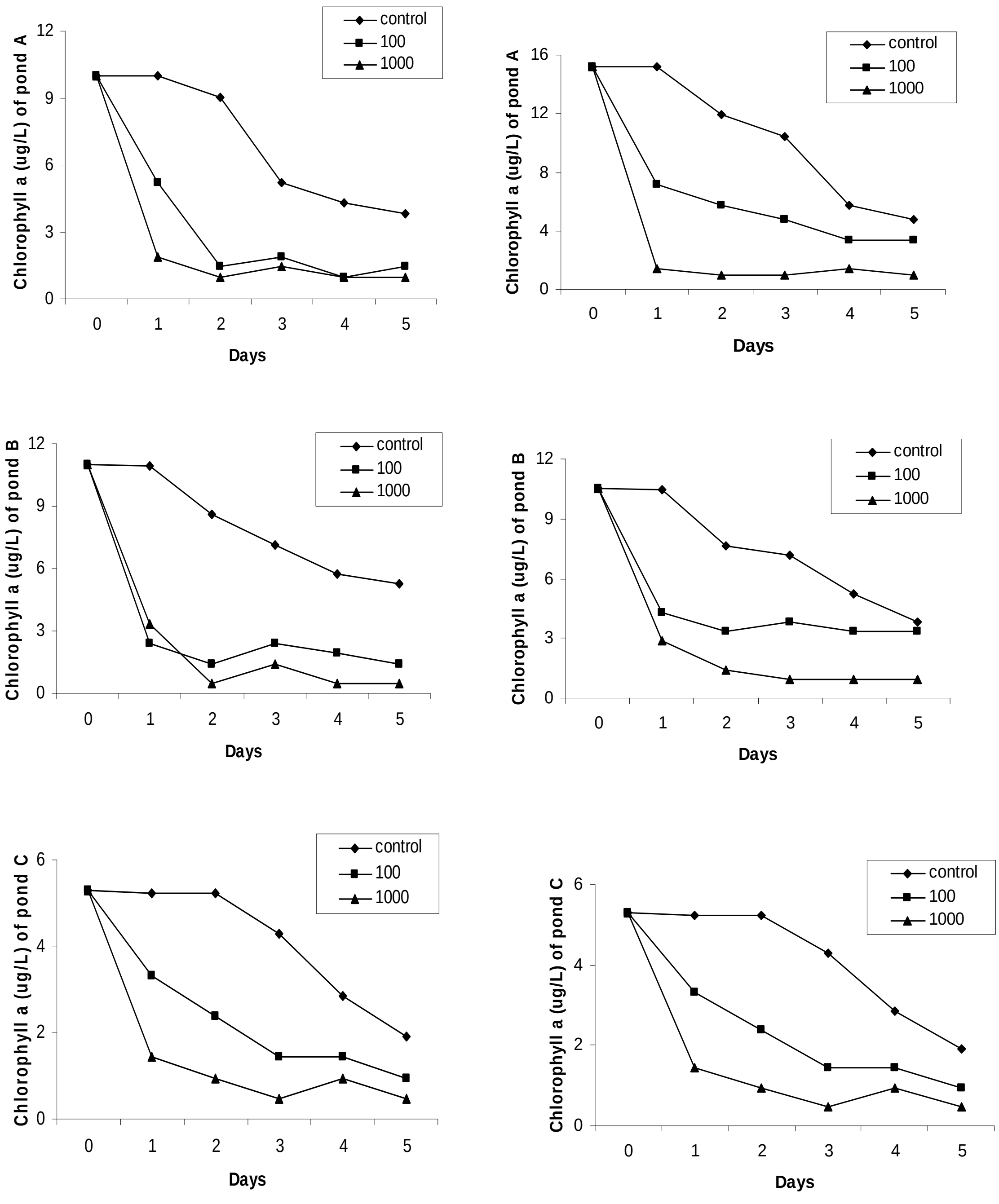

Figure 2: Comparison of grazing efficiency between *M. macrocopa* (left) and *B. thailandensis* (right) to reduce chlorophyll a in water brought from 3 ponds during 5 days of experiment

5. Conclusions

Both *Moina macrocopa* and *Branchinella thailandensis* have a high potential to control the growth of phytoplankton. In particular, the grazing efficiency of both zooplankton species (small and large bodies) on phytoplankton increased when the densities of *M. macrocopa* and *B. thailandensis* increased. Therefore, nutrient-enriched (eutrophic) water bodies dominated by phytoplankton could be restored by *M. macrocopa* and *B. thailandensis*. Clear water plant dominated water bodies resulted in a great variety of aquatic plants and animals as well as making use of natural resources of restored lakes by people.

6. Acknowledgement

Authors would like to express my sincere thanks to Department of Environmental Technology and Management, Faculty of Environment for lab facilities at Kasetsart University, Bangkok Thailand.

7. References

Arnott, E. and Vanni, M.J. 1993. Zooplankton assemblages in fishless bog lakes: influence of biotic and abiotic factors. **Ecology** 74(8): 2361-2380.

Bennett, E.M., Carpenter, S.R. and Caraco, N.F. 2001. Human impact on erodable phosphorus and eutrophication: a global perspective. **BioScience** 51(3): 227-234.

Boonmak, P., Saengphan, N. and Sanoamuang, L. 2007. Biology and fecundity of two fairy shrimps, *Streptocephalus sirindhornae* (Sanoamuang, Murugan, Iekers and Dumont) and *Branchinella thailandensis* (Sanoamuang, Saengphan and Murugan). **Khon KeanUniversity Research Journal** 12: 25-131.

Braga, E., Bonetti, C.V.D., Burone, L., and Filho, J.B. 2000. Eutrophication and bacterial pollution caused by industrial and domestic wastes at the Baixada Santista estuarine system, Brazil. **Marine Pollution Bulletin** 40(2): 165-173.

Carpenter, S.R., Kitchell, J.F., and Hodgson, J.R. 1985. Cascading trophic interactions and lake productivity. **BioScience** 35(10): 634-639.

Chaichana, R. 2008. **Birds and the eutrophication of a system of small**

lakes. PhD Dissertation, University of Liverpool. 337 pp.

Cyr, H. and Pace, M.L. 1992. Grazing by zooplankton and its relationship to community structure. **Canadian Journal of Fisheries and Aquatic Science** 49(7): 145-1465.

Dawidowicz, P. 1990. Effectiveness of phytoplankton control by large-bodied and small-bodied zooplankton. **Hydrobiologia** 200-201(1): 43-47.

Elser, J.J. and Goldman, C.R. 1991. Zooplankton effects on phytoplankton in lakes of contrasting trophic status. **Limnology and Oceanography** 36: 64-90.

Fermin, A.C. 2007. Freshwater cladoceran Moina macrocopa (Strauss) as an alternative live food for rearing sea bass Lates calcarifer (Bloch) fry. **Journal of Applied Ichthyology** 7(1): 8-14.

Hanson, M.A. and Butler, M.C. 1994. Responses of plankton, turbidity and macrophytes to biomanipulation in a shallow prairie lake. **Canadian Journal of Fisheries and Aquatic Science** 51: 1180-1188.

Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T., Pedersen, L.J. and Jensen, L. 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. **Hydrobiologia** 342/343: 151-164.

Kasprzak, P., Benndorf, J., Mehner, T., and Koschel, R. 2002. Biomanipulation of lake ecosystems: an introduction. **Freshwater Biology** 47: 2277-2281.

Kaufman, A. and S. Watanasak. 2011. Farmers and fertilizers: a socio-ecological exploration of the alternative agriculture movement in Northeastern Thailand. **Environment and Natural Resource Journal** 9(3): 1-11.

Lampert, W. and Taylor, B.E. 1984. In situ grazing rates and particle selection by zooplankton: effects of vertical migration.**Verhandlungen-Internationale Vereinigung fuer Theoretische und Angewandte Limnologie**. 22: 943-946.

Lampert, W. and Taylor, B.E. 1985. Zooplankton grazing in a eutrophic lake: implications of vertical migration. **Ecology** 66: 68-82.

Lampert, W., Fleckner, W., Rai, H. and Taylor, B.E. 1986. Phytoplankton control by grazing zooplankton: a study on the spring clear-water phase. **Limnology and Oceanography** 31(3): 478-490.

Livengood, E.J. and Chapman, F.A. 2007. **The ornamental fish trade: an introduction with perspectives for responsible aquarium fish ownership**. IFAS Extension, University of Florida. 8 pp.

Ma, H., Cui, F., Liu, Z., and Fan, Z. 2009. Pilot study on control of phytoplankton by zooplankton coupling with filter-feeding fish in surface water. **Water Science and Technology** 60(3): 737-743.

Moss, B., Madgwick, J,. and Phillips, G. 1997. **A guide to the restoration of nutrient-enriched shallow lakes**. W.W.Hawes, London.

Norman, K.E., Blakely, J.B. and Chew, K.K. 2009. The occurrence and utilization of the cladoceran Moina macrocopa (Straus) in a kraft pulp mill treatment lagoon. **Proceedings of the World Mariculture Society** 10(1-4): 116-1210.

Quiblier-Lloberas, C., Bourdier, G., Amblard C. and Pepin, D. 1996. Impact of grazing on phytoplankton in Lake Pavin (France): contribution of different zooplankton groups. **Journal of Plankton Research** 18(3): 305-322.

Pathak, B.K., Kazama, F., and Iida, T. 2004. Monitoring of nitrogen leaching from a tropical paddy field in Thailand. **Journal of Scientific Research and Development** 6: 1-11.

Vanni, M.J. 1987. Effects of nutrients and zooplankton size on a structure of a phytoplankton community. **Ecology** 68(3): 624-635.

Wu, L., Xie, P., Dai, M., and Wang, J. 1997. Effects of silver carp density on zooplankton and water quality: implications for eutrophic lakes in China. **Journal of Freshwater Ecology** 12(3): 437-444.