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Effects of cyanobacterium *Microcystis aeruginosa* on the filtration rate and mortality of the freshwater bivalve *Corbicula leana*

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Abstract: *We compared filtering rates (FR) and mortalities between freshwater filter-feeding bivalve Corbicula leana acclimatized (AM) and non-acclimatized (NAM) to an cyanobacterial bloom (mainly Microcystis aeruginosa) over seven days. Both mussel populations were simultaneously stocked into mesocosms constructed in littoral zones of a eutrophic lake at a density of 740 ind. m^{-2} for 16 days. NAMs decreased the concentration of chlorophyll-a 50% less than AMs. For the first seven days, the FRs (0.46~0.61) and mortality rate (slope = -30.2, r = -0.95) of NAMs were higher than those of AMs, possibly due to a sudden increase in unselective filtering. From the eighth day, NAM mortality decreased rapidly and then stabilized, becoming similar to that of AMs through the end of the experiment. Stocking both AMs and NAMs increased the NH_4-N concentration in the water, and the mortality rates of both mussel populations were correlated with NH_4-N. In contrast, other nutrients and microcystin concentrations were not significantly associated with mussel mortality. These results indicate that although a sudden introduction of non-acclimatized C. leana may partially control phytoplankton biomass for a short period, previous short-term acclimatization is needed to minimize mussel mortality.*

Key words: *Freshwater bivalve, Corbicula leana, Cyanobacteria, Filtration rate, Mortality*
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Introduction

Lots of data from laboratory and *in situ* studies support the powerful filtering abilities of bivalves (Hakenkamp *et al.*, 2001; Hwang *et al.*, 2004; Dionisio Pires *et al.*, 2005) and suggest potential roles for bivalves in water quality management strategies (Reeders and Vaate, 1990). Soto and Mena (1999) also demonstrated that the large freshwater mussel, *Diplodon chilensis*, could change salmon culture waters from hypertrophic to oligotrophic.

In Korea, the Asiatic clam *Corbicula leana* Prime is strictly confined to brackish water (*e.g.*, the Seomjin and Nakdong rivers), and no massive outbreaks of benthic bivalves have been recorded yet. Although this mussel has been consumed by people for at least a century, mass culture of the mussel has not been successful or sustainable in the field or laboratory, despite the high filtration activity of this organism (Hwang *et al.*, 2004). *C. leana* is of great ecological interest because it may be able to ingest the natural toxic cyanobacterium *Microcystis*; this study provides valuable new information in this regard.

Cyanobacterial blooms in eutrophic water comprise various biosestons, including phytoplankton that may occasionally be toxic, as well as nutrients and suspended particulate matter, making it difficult to predict mussel behavior in these waters. Within a confined environment, such as a laboratory or a mesocosm, repeated and short-term acclimatization of mussels to the cyanobacterial bloom water may allow us to understand differences or advantages in water quality improvement exhibited by mussels acclimatized (AM) or non-acclimatized (NAM). We proposed that 1) repeated or short-term acclimatization to bloom water would gradually increase the survival rate of AMs; and, 2) abrupt introduction of filter-feeder bivalves would increase the mortality of NAMs. The aim of this study was to compare filtering rates and mortalities between AM and NAM mussels in the field water containing a high biomass of the cyanobacterium *Microcystis aeruginosa*.

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Materials and Methods

Study organisms: The *Corbicula leana* used in this study were collected from the upstream (Gapyong) of the North Han River (Korea) by towing a hand-operated dredge behind a boat. The width of the stream ranged from 80-100 m, the depth was about 3.5 m, and the bottom comprised mainly mud and clay (< 20%) and pebbles (~80%). The captured mussels were carefully transferred to an artificial management system prepared with PVC material in the laboratory. This system uses dechlorinated tap water with washed sand as a bottom substrate and features a controlled temperature (18 ± 1°C), a controlled flow rate (8 l min^{-1}), and measured amounts of food (natural lake water). Light was controlled in amount (20 μmol photons $s^{-1}m^{-2}$) and duration (14 hr light and 10 hr dark). For acclimatization, some captured bivalves were immediately deposited in vinyl containers (10 m × 10 m) in the study

lake for seven days. Other mussels were placed in the artificial system.

Experimental design: The experiments were carried out during a cyanobacterial bloom (August 2003) in lake Ilgam, Republic of Korea (36°00' N, 140°02' E). The lake is small (55,661 m^2), shallow (mean and maximum depths = 1.5 m and 2.5 m, respectively), and hypertrophic (0.067 mg l^{-1} in total phosphorus (TP) and 1.150 mg l^{-1} in total nitrogen (TN). The water residence time is 288 days, and vertical stratification is not obvious over the year (Hwang *et al.*, 2004). The lake was constructed in 1957 and the Han River then flowed into the lake. However, since 1975, no river water has entered the lake due to urbanization between the river and the lake, and the lake has been gradually polluted with domestic wastewater. Nowadays, the lake is sustained with rain, snow, and some runoff. Since 1982, a cyanobacterial bloom (mainly *M. aeruginosa*) has been noted every year. Interestingly, the lake has no bloom period between March and May. During that period, the predominant plant is the submerged macrophyte *Potamogeton crispus*, and the lake becomes clear. The cyanobacterial blooms often begin with the senescence of the plant populations.

Six mesocosms were constructed at the littoral zone of the lake. They were 2 × 2 m in surface cross-section and 0.9-1.1 m deep, giving a volume of about 3.6-4.4 m^3. Each mesocosm was constructed of four stainless steel pipes that were pegged into the sediment to form a square with 5-m side length. The mesocosm walls were made of nylon-reinforced translucent polyester and were buried into the sediment to a depth of 0.5 m to ensure complete separation of the water columns inside the mesocosms from the surrounding lake water. Translucent sheets extended 20 cm above the mesocosm walls to prevent lake water from entering the mesocosms when waves were high. All mesocosms were open to the atmosphere and the sediment, and the light intensity at the surface of the mesocosms was, on average, 95% of that of the air above.

The study mesocosms were attended daily for 16 consecutive days from August 2 to August 19, 2003, during which time a cyanobacterial bloom occurred in the lake. The mussels were divided into two groups: AMs and NAMs. The NAMs were collected fresh from the same stream as the AMs three days before the start of the study and were sustained in the laboratory in dechlorinated tap water. The AMs were placed in cyanobacterial bloom water dominated by *M. aeruginosa* for seven days. Two mesocosms contained no mussels throughout the entire experimental period to serve as controls for the effects of mussel stocking (Control). Two mesocosms contained AMs and another two mesocosms contained NAMs. We introduced 2,960 individual *C. leana* (density = 740 ind. m^{-2}) into each of these mesocosms simultaneously. After mussel stocking, physicochemical including cyanotoxin (microcystin) and biological parameters in the AM and NAM mesocosms were monitored at the same time daily (11:00 AM).

Water quality: One-liter samples were siphoned from a depth of 0.3 m from each Control, AM and NAM mesocosm for water quality analysis. The samples were placed in polyethylene bottles pre-washed with weak acid and transferred to the laboratory within two hours. The water temperature was measured on-site, and the concentration of dissolved oxygen was measured in the laboratory using the azide modification method (APHA, 2005). Water samples were filtered through pre-combusted GF/F filters (Whatman) prior to analysis of chlorophyll-*a* concentration, suspended solids (SS; APHA, 2005), and dissolved inorganic nutrients. Dissolved inorganic phosphorus (DIP) was analyzed using the ascorbic acid method, and dissolved TP levels (DTP) were analyzed by persulfate digestion of the GF/F filtrate and lake water, followed by application of the ascorbic acid method (APHA, 2005). The particulate organic phosphorus (POP) was considered to be what remained when the DTP level was subtracted from the TP concentration. Dissolved inorganic nitrogen (DIN) and TN levels were measured from persulfate-treated samples, using the indophenol and cadmium reduction methods, respectively (APHA, 2005). Levels of microcystin-LR, a toxic protein, were determined using the enzyme-linked immunosorbent assay (ELISA) method of Ueno *et al.*, (1996).

Filtration rate and mortality: To obtain the filtering rate (FR) of the mussels, the physical characteristics of 165 animals – length, width, wet weight, and ash free dry weight (AFDW) – were quantified. The AFDWs of mussels were measured according to Hwang *et al.* (2004). AFDWs of mussels were obtained from a linear regression between shell length and AFDW of the 165 mussels collected from the same habitat as the mussels used in the study; this correlation was used to determine AFDW because among the physical characteristics of mussels examined, shell lengths had the highest correlation with AFDW ($r^2 = 0.671$, n = 165, $p < 0.0001$). The FRs of AM or NAM mussels were calculated using the differences in chlorophyll-*a* concentrations between the control and treatment mesocosms at each sampling time, based on the Coughlan's (1969) formula and Alimov's (1969) clearance methods, FR (L $g^{-1}h^{-1}$) = V × ln (C/T) / (AFDW × t), where V is the volume of lake water in the mesocosm, C and T are the chlorophyll-*a* concentrations in the control (C) and treatment (T) mesocosms after time *t* (day), and AFDW is the ash-free dry weight (g). To measure the mortality of AMs and NAMs, dead mussels were collected and counted daily by means of three random samplings with the sediment grab. During the study, dead mussels were identified by the complete opening of shells, immobility, or the absence of a green mass or other aggregate near the exhalant siphon of the mussel. Then, these dead mussels were excluded when determining the AFDW values used to calculate

FRs. The significant growth of mussels (increase or decrease of length and weight) during the experimental 16 days, was not found.

Data analysis: Pearson's correlation analysis and principle component analysis was used to compare water quality parameters between the control and treatment groups and to understand relationship among water quality parameters, mussel mortality and filtration rates. Statistical significance was set at $p < 0.05$. Data were log-transformed when they did not fit a normal distribution. Statistical analyses were performed using software MVSP plus ver. 3.1 (Kovach Computing Services, 2002).

Results and Discussion

Over the 16-d experimental period, the levels of suspended solids (SS) and chlorophyll-*a* (chl-*a*) showed a similar pattern to the end of study, and slightly decreased and increased, respectively (Fig. 1). Our results indicate that even without acclimatization to the cyanobacteria, *C. leana* fed effectively on *M. aeruginosa* blooms. Particularly, NAMs were more effective than AMs in this regard in the period shortly after stocking (the first seven days), perhaps due to unselective filtering of the new food source. The chl-*a* in both the AM and NAM mesocosms decreased remarkably as compared to the control. Chl-*a* levels in the NAM mesocosms (the lowest level was 4.1 $\mu g\ l^{-1}$ on day 6) were 50% less than the levels in the AM mesocosms (the lowest level was 8.1 $\mu g\ l^{-1}$ on day 8) during the first eight days after mussel stocking. Thereafter, the NAM chl-*a* levels increased rapidly by as much as 30% per day to equal levels in the AM mesocosms (the highest level was 20 $\mu g\ l^{-1}$ on day 15). The FRs of the freshwater clam, *C. leana*, in the AMs remained constant and low. Instead, the FRs of the NAMs showed a marked peak on day 5, after which FRs diminished to a constant value similar to that of the AMs. The high FRs of the NAMs during the first week of the experiment can be attributed to the sudden exposure of these mussels to a new food source on introduction into the mesocosm.

These results clearly show that the Asiatic freshwater clam, *C. leana*, strongly decreased the levels of chl-*a* (by 50-90%) and SS (by 40-65%) in cyanobacterial bloom water; the magnitude of this effect was dependent on whether the mussels had been acclimatized to the bloom water prior to the experiment. The algae removal efficiency (%) of *C. leana* is similar to or higher than that of the zebra mussel; 59% to lake Huron (Fahnenstiel *et al.*, 1995) and 43% to lake Erie (Leach, 1993). During the initial week after stocking, we found that NAMs exhibited strong filtering activity (70–90%). As with chlorophyll-*a* removal levels, the FRs were also relatively high during the first week after NAM stocking (0.46-0.61 $lg^{-1}hr^{-1}$). The high initial FR and algal removal activity of NAMs decreased gradually, settling at 80-90% of the AM level within 16 days of stocking. Thus, the effects of prior acclimatization to cyanobacterial water on FR were limited to the first week after stocking.

Unlike both chl-*a* and SS, three species of phosphorus increased simultaneously after mussel stocking, with greater increases occurring in the NAM than in the AM (Fig. 2). TP concentrations in AM (average = 52.1 $\mu g\ l^{-1}$, range = 50.0-57.0) and NAM (average = 60.4 $\mu g\ l^{-1}$, range = 56.1–68.0) were significantly higher (ANOVA, $p < 0.001$ for all) than TP in the control mesocosms (average = 36.4 $\mu g\ l^{1}$, range = 32.2-38.0); other form of phosphorus showed similar patterns. TN in the AM and NAM also increased over time and was sustained near the mean level (2.6 mg l^{-1}), whereas the control level gradually decreased toward the end of the study (Fig. 3). In the case of nitrite plus nitrate, the levels in AM mesocosms increased quickly during the initial eight days of the experiment (the highest value was 0.007 mg l^{-1}) and then decreased suddenly, whereas nitrite plus nitrate levels in both the control and NAM mesocosms were similar, fluctuating between 0.0001 and 0.002 mg l^{-1} over the study period. The levels of ammonium ion (NH_4-N) in the AM and NAM mesocosms showed a similar pattern. During the first seven days of the experiment, the NH_4-N levels in the NAM mesocosms (mean = 0.966 $\mu g\ l^{-1}$) were significantly higher than those in the AM mesocosms (mean = 0.801 mgl^{-1}; ANOVA, $p < 0.001$). Thereafter, the pattern reversed, indicating a drastic decrease in the ammonia level in NAM mesocosms.

There was a distinct difference in daily mortality between AMs and NAMs (Fig. 4); the NAMs suffered much more severe mortality on the eighth day after stocking (slope = -30.152, r = -0.95, p = 0.000347) as compared to the AMs (slope = -3.953, r = -0.94, p = 0.000528). However, 12 days after mussel stocking there were no significant differences in mortality between the AM and NAM mesocosms (ANOVA, $p > 0.5$). These findings indicate that both the FR and mortality of *C. leana* were strongly influenced by acclimatization to cyanobacterial bloom water. The differences between AMs and NAMs began to diminish after the first week of the mesocosm experiment. The death rates of *C. leana* in the mussel-containing mesocosms showed significant positive correlations with the levels of NH_4-N ($r = 0.8377$, $p < 0.001$); the correlation was more significant in AM mesocosms (r = 0.95) than in NAM mesocosms (r = 0.56). Results of PCA (Fig. 5) on physicochemical and biological parameters in AM and NAM mesocosm also indicate that ammonia concentration increased after mussel stocking without acclimatization can be determined the mussel mortality. The levels of microcystin-LR ranged between 0.0997 and 0.0782 $\mu g\ l^{-1}$ in the AM and between 0.0875 and 0.0575 $\mu g\ l^{-1}$ in the NAM ; there were no significant differences before and after mussel stocking (ANOVA, p>0.5) (data not shown), suggesting a cyanotoxin (microcystin) was beyond the mussel mortality.

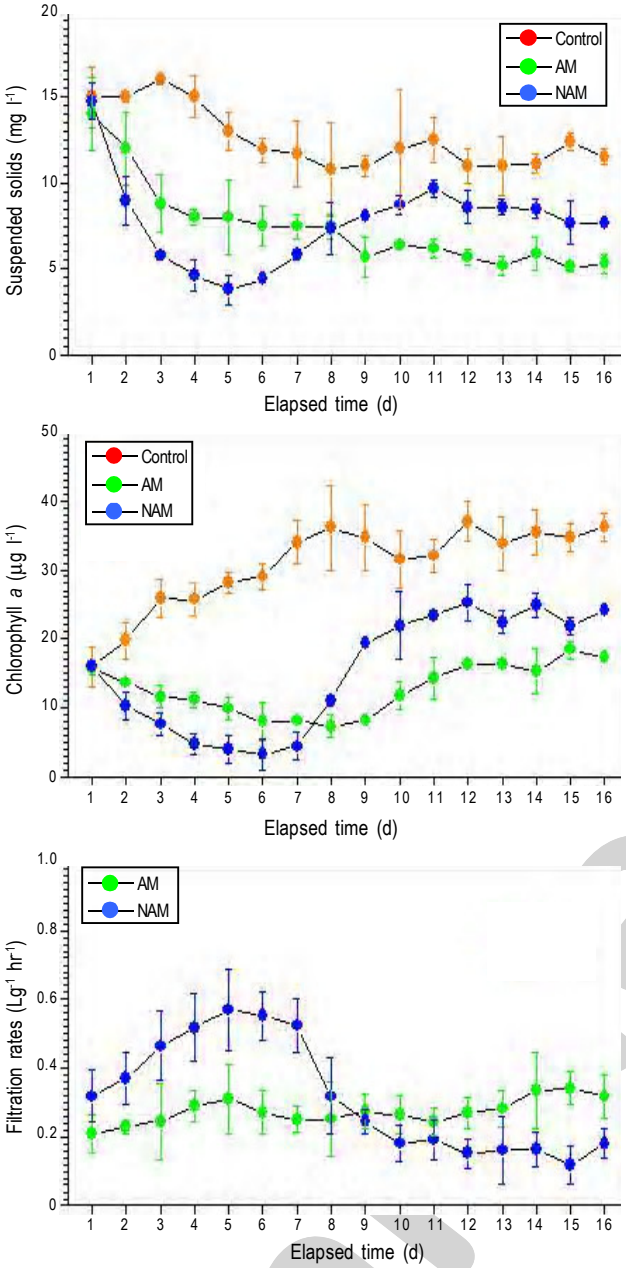

Fig. 1: Concentrations of suspended solids, chlorophyll-*a* and filtration rates in acclimatized (AM) and non-acclimatized (NAM) mussel mesocosms

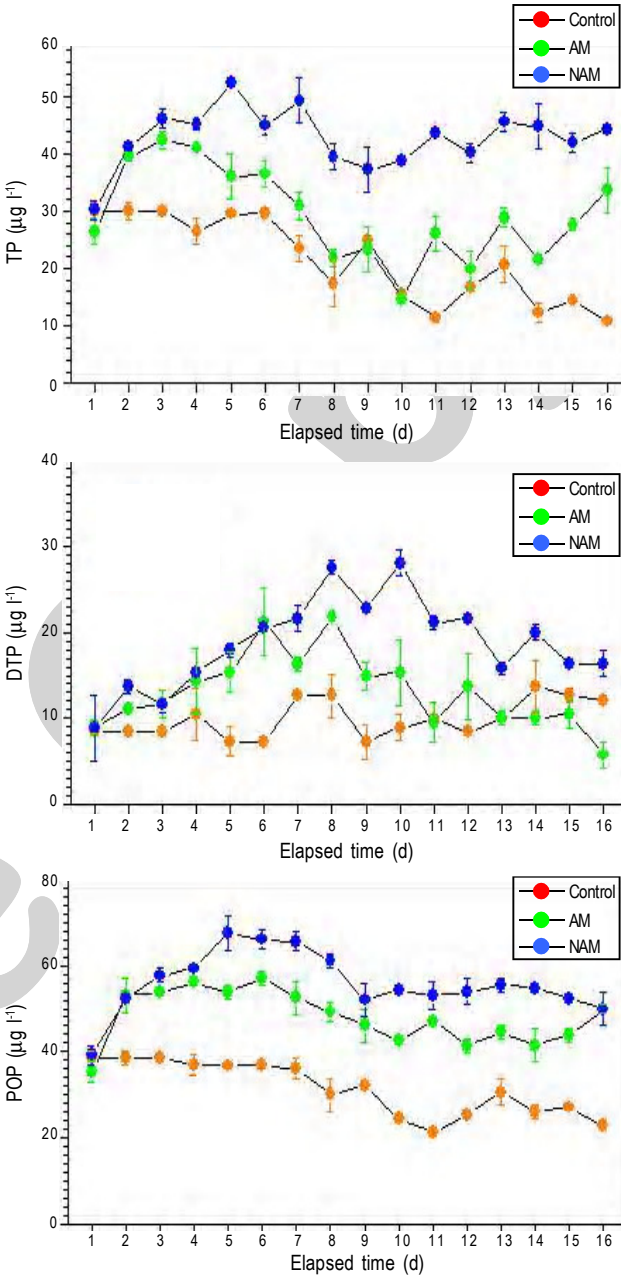

Fig. 2: Concentrations of total phosphorus (TP), dissolved total phosphorus (DTP), and particulate organic phosphorus (POP), in both acclimatized (AM) and non-acclimatized (NAM) mussel mesocosms

Our study revealed that during the period immediately following stocking, high FRs was accompanied by high mortality, particularly of NAMs. Owen and Cahoon (1991) observed a 50% death rate within 15 hours of water temperatures exceeding 30°C, with water conditions becoming anaerobic thereafter. This high temperature-induced mussel death occurs due to lack of oxygen and increased ammonia toxicity (Buddensiek *et al.*, 1993; Kleydman *et al.*, 2004; Yamamuro and Koike, 1993). In conditions similar to those in this study, Belanger (1991) observed 100% mortality within 13 days when mussels were continuously exposed to 0.74 mgl^{-1} NH_4-N at 24°C. Interestingly, our study clearly shows a positive correlation between the mussel death rate and NH_4-N concentration. The maximum level of NH_4-N observed was 163.0 µg l^{-1}, which is below that reported to be lethal to the mussel (Belanger, 1991). Similarly, microcystin-LR levels (< 0.1 µgl^{-1}) did not reach lethal concentrations. The reason for the lack of increase in dissolved microcystin-LR in AM and NAM mesocosms after mussel stocking is not known. It is possible that a considerable amount of time in necessary to dissociate or lyse colonial *Microcystis* cells, which are surrounded with mucilaginous materials when released from the mussel.

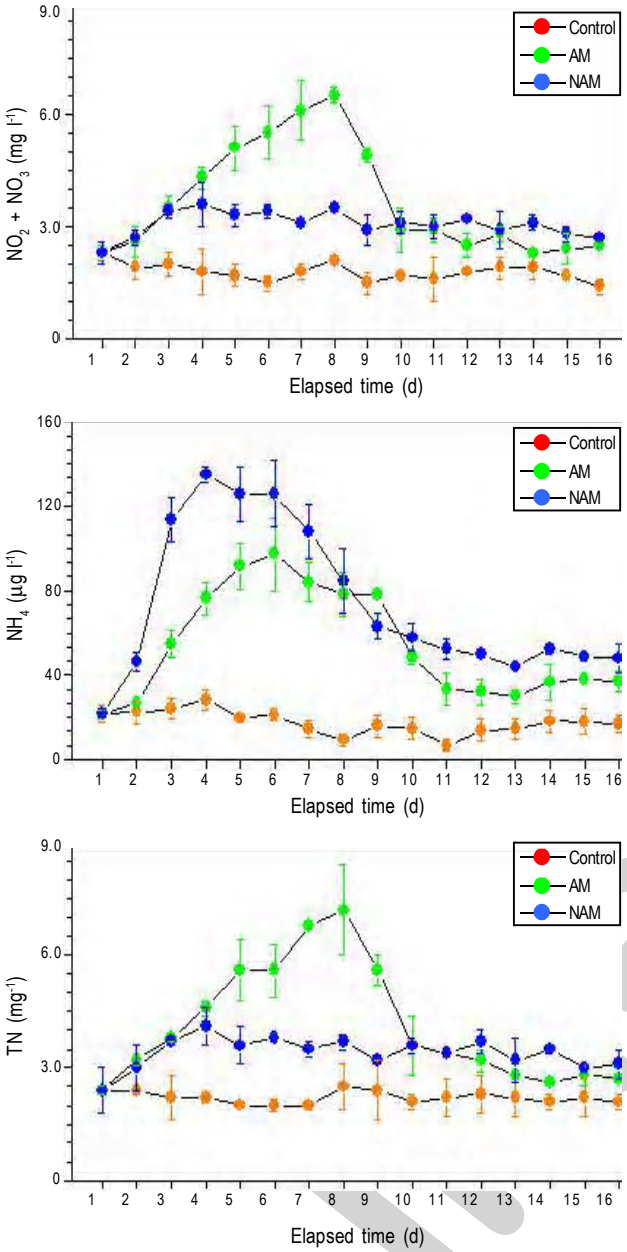

Fig. 3: Concentrations of total nitrogen (TN), ammonium ion (NH_4-N), and dissolved inorganic nitrogen (NO_2+NO_3) in both acclimatized (AM) and non-acclimatized (NAM) mussel mesocosms

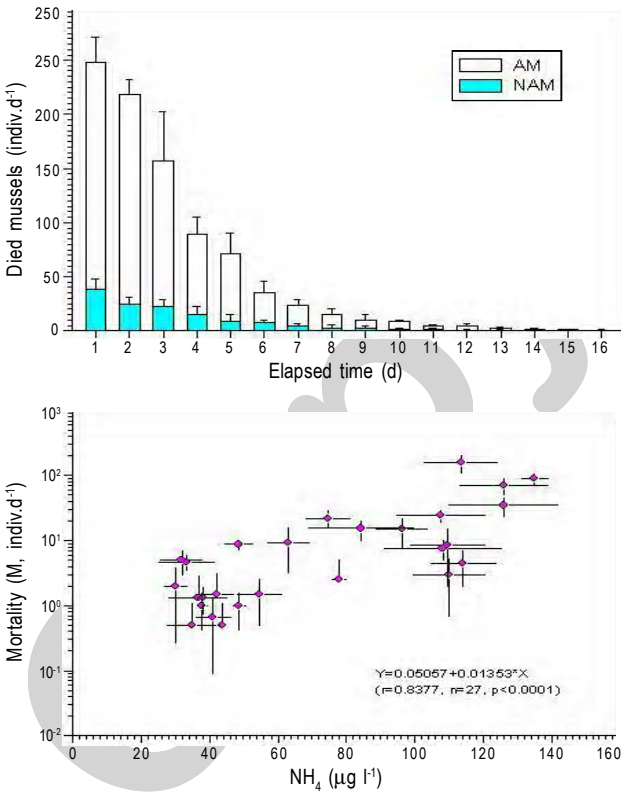

Fig. 4: Mortality of *Corbicula leana* and the relation with ammonium ion (NH_4-N) concentrations in both acclimatized (AM) and non-acclimatized (NAM) mussel mesocosms

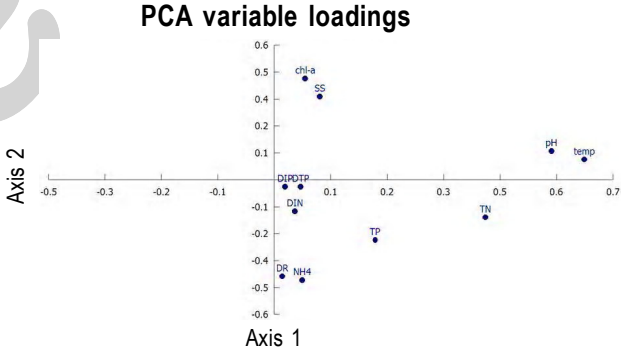

Fig. 5: Principal component analysis on the physicochemical and biological parameters in both acclimatized (AM) and non-acclimatized (NAM) mussel mesocosms.

In conclusion, the results of this study suggest that the filter-feeding bivalve *C. leana* can mitigate cyanobacterial bloom in eutrophic lakes. However, the continued study is needed in order to escape the high death rate of NAMs during the early stage of the field application likely due to physiological shock such as increase of nutrient and cyanobacterial toxin levels.

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