Utilizing the grazing effect of fresh water clams (*Unio douglasiae*) for the remediation of algal bloom during summer

Ki-Woong Nam, Jeong-Ryul Lee and Kyung-Il Park

Faculty of Applied Marine Biosciences, Kunsan National University, Gunsan 573-701, Republic of Korea

ABSTRACT

The occurrence of 'algal bloom', caused by the mass proliferation of phytoplankton, causes serious problems in streams and lakes in Korea. Therefore, in this study, the phytoplankton filter-feeding trait of *Unio douglasiae*, a type of freshwater clam, was used to reduce the algal bloom in outdoor water tanks during the summer. This involved the construction of a *U. douglasiae* cultivation apparatus, wherein 1,000 clams were divided into 8 rectangular baskets arranged in the shape of an empty square. The control tank was manufactured in exactly the same shape within the water tank, but without the addition of clams. The algal bloom-reducing effect of *U. douglasiae* was confirmed by the measurement of (and comparing between) the water quality at the center and periphery of the test and control cultivation apparatus. Water quality measurements included the measurement of water temperature, pH, turbidity, dissolved oxygen (DO) content, and chlorophyll-α concentrations; the water quality was measured twice a month between June and November 2014. The results of these analyses did not show a significant difference in water quality (temperature, pH, turbidity, DO) between the center and periphery of the test and control tanks. However, the chlorophyll-α concentration was observed to be much lower at the center of the test tank compared to that at the center and periphery of the control tank, as well as at the periphery of the test tank. This was believed to be a result of the *U. douglasiae* surrounding the center of the test tank, which prevented the influx of plankton from the periphery. Accordingly, the results of these analyses suggest the possibility that *U. douglasiae* cultivation could reduce the proliferation of algal blooms in lakes and streams during the summer. In particular, these results indicate possible improvements in *U. douglasiae* activity (reduction in algal blooms) by their effective arrangement in the water bodies.

Key words: algal bloom, *Unio douglasiae*, grazing effect, chlorophyll-α, clam cage

Introduction

Eutrophication refers to a state wherein a water system contains large quantities of carbon, nitrogen, and phosphorus; these conditions lead to the abnormal, large-scale proliferation of algae in water (Kim *et al.*, 1995; Park *et al.*, 1998; Kim *et al.*, 1999). The specific meanings of terms used to describe this phenomenon, such as green tide, water-bloom, or harmful algal-bloom (HAB) are different; however, they are sometimes assumed to refer to the same thing. The algal bloom or green tide phenomenon is visually unpleasant, causes public sanitation issues because of the toxic substances released by cyanobacteria, and is responsible for large-scale damage, such as ecosystem destruction caused by the depletion of dissolved oxygen and financial loss from the decline in the agricultural and industrial value of the water as well as its use for drinking purposes (Persson, 1982; Codd and Poon, 1988; Watanabe *et al.*, 1994).

Some methods suggested to reduce the damage caused by mass algal proliferation include the physical removal of algae, the use of chemical removers, and

biological methods (Wang *et al.*, 2011). Among the biological methods, aquatic plant cultivation (Shim and Han, 1998; Kong and Cheon, 1999) and the installation of artificial plant islands (Kwon, 1999) have been effectively used to reduce the amount of nutrient salts, the primary cause of algal blooms, in water bodies. On the other hand, algae that have already bloomed can be killed using ultrasound (Hedge, 2013), or can be reduced by the introduction of water fleas (Yan *et al.*, 2012) or filter-feeding shellfish (Reeder, 1989; Reeder and Vaate, 1992; Smit *et al.*, 1993), which eat algae.

The control of algal outbreak using shellfish involves the use of filter-feeders that filter phytoplankton, bacteria, protozoa, and abiotic particles of various sizes. This filtering mechanism has a large influence on the control of particles in water, and is known to cause changes in the colonies of aquatic plankton, increase transparency within the water system, and affect the cycling of substances within the aquatic ecosystem (Dame *et al.*, 1985; Reeders *et al.*, 1989; Arnott and Vanni, 1996; Vaughn and Hakenkamp, 2001; Hwang *et al.*, 2001; Kim, 2004).

Various bivalves are living in the streams and freshwater lakes of South Korea. Of these, *Unio douglasiae*, a representative freshwater clam from Korea, presents several advantages, including a lower amount of excretion into the water, and higher chlorophyll-a-decreasing rate, compared to other mollusks (Kim, 2004). In particular, *U. douglasiae* displays an excellent capacity to feed on *Microcystis aeruginosa*, which is a major cause of summer algal blooms. Therefore, it is considered a useful organism for the control of algal blooms caused by eutrophication (Lee *et al.*, 2008).

So far, several studies analyzing the control of phytoplankton using *U. douglasiae* have been conducted in laboratories, or, when conducted outside, have focused on testing for short-term effects within 10 days; therefore, the reducing effect of *U. douglasiae* on algal blooms in actual streams and lakes remains to be confirmed. Therefore, the major objective of our study was to confirm the phytoplankton-reducing effect of *U. douglasiae* in a (constructed/man-made)

large-scale outdoor environment. In addition, the spatial arrangement of *U. douglasiae* was taken into account when performing the experiment with the aim of developing a high-efficiency algal bloom-removal apparatus; this included placing the clams in a square with an empty middle region, and comparing the chlorophyll-a concentrations in the center and periphery of the container.

Experimental Methods

1. Mollusk collection

Unio douglasiae used in this experiment were collected from the Boryeong region in Chungcheongnam-do, South Korea. Approximately 1,000 adult clams were selected and transported to the experimental setup. Subsequently, the length, height, breadth, and total mass of 100 arbitrary specimens were measured.

2. *U. douglasiae* cultivation facility

The *U. douglasiae* cultivation facility (test tank) consisted of 8 plastic boxes ($33 \times 25 \times 10$ cm^3) arranged in a square shape, with an empty space in the middle; holes (1 cm in diameter) were punched into each box at a distance of 1 cm to facilitate water permeability. The tanks were stacked up to a height of 3 boxes, and 62-63 clams were introduced into each of the boxes in the 1st and 2nd layers (from the bottom), for a total of 1000 clams. Buoys were placed in the uppermost layer, allowing the boxes to float (Fig. 1). The control tank was installed within an identical water tank at a distance of (approximately) 4 m from the test tank, utilizing the cultivation apparatus of the
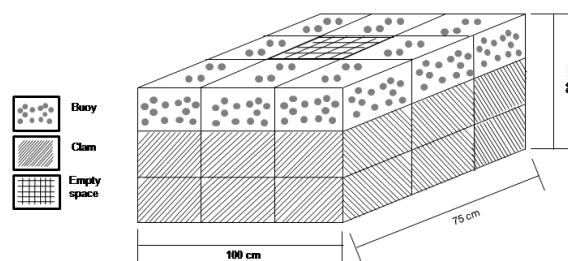

Fig. 1. Schematic diagram depicting a clam culture cage. No clam was added to the control cage.
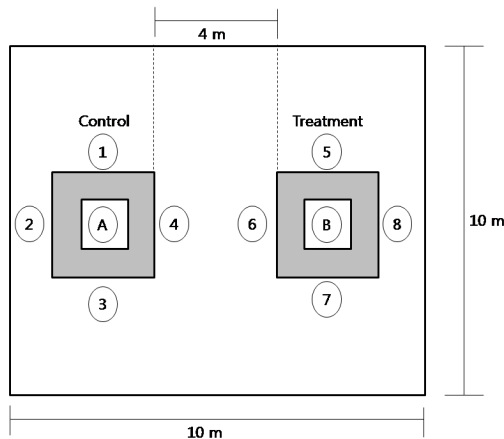
Fig. 2. Locations of water sampling in the treatment and control cages. **A**, center of the control cage; 1-4, sampling points around the control clam cage; **B**, center of the treatment clam cage; 5-8, sampling points around the treatment clam cage.

same material and size. The outdoor tanks were made of concrete ($10 \times 10 \times 1$ m³, 100 ton); the tanks were not provided with any additional water during the course of the experiment, aeration was conducted in a place without experimental apparatus.

3. Water quality analysis

The water quality of the cultivation area was measured and compared between the test (with clams) and control (without clams) groups every 2 weeks; the water was collected separately from the central and peripheral areas for quality analysis. This study was conducted for a total of 14 weeks (Fig. 2). The water quality was measured using a single value for the central area and an average value from 4 locations for the periphery. The water quality was measured using a water quality meter (WQC-22A Multi-parameter Water Quality Meter-22A; DKK-TOA, Tokyo, Japan), which facilitated the on-site evaluation of the water temperature, pH, conductivity, turbidity, and dissolved oxygen (DO) content.

4. Determination of the chlorophyll-α content

The change in chlorophyll-α concentration was measured as follows: water samples (1 L) were filtered using a GF/C filter (Whatman Inc.; GE Healthcare,

Buckinghamshire, UK); chlorophyll-α was extracted using 90% acetone for 24 h in a cool, dark environment. The extract was separated by centrifugation for 20 min, and the absorbance of the supernatant was measured to determine the chlorophyll-α content.

5. Size and weight of *U. douglasiae*

The individual length, height, breadth, and total weight of 100 randomly selected clams was measured immediately prior to and after the experiment.

6. Statistical analysis

Each group was subjected to a T-test, using the SPSS v.12.0 software platform (SPSS Inc., Chicago, IL). The significance between mean values was determined at a confidence level of 95%.

Experimental Results

1. Water quality analysis

Measurements of the water temperature in the cultivation facilities during the clam cultivation period showed a consistent temperature of 26°C from June to July; however, the temperature increased to 28°C at the end of July, subsequently decreasing to approximately 18°C by the end of the experimental period (mid-November). These results were the same in the control and test tanks (Table 1). The differences in pH, turbidity, and DO content between the test and control tanks was also not statistically significant, except for the values obtained in early July. The water quality at the central portion of the clam cultivation facilities was generally similar to that at the periphery; this was maintained throughout the test period (Table 2).

2. Size and weight of *U. douglasiae*

The *U. douglasiae* measured on the 10th of June, 2014 (at the start of the experiment) had an average individual length of 70.78 ± 10.97 mm and a total weight of 40.99 ± 16.84 g. The clams measured at the end of the experiment (16th November 2014) displayed an average individual length of 73.06 ± 06.84 mm, and a total weight of 44.77 ± 11.22 g (Table 3).

Table 1. Water qualities at the peripheral points of the treatment and control clam cages

Date	WT (℃)		pH		Turbidity (NTU)		DO (mg/L)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
Jun 10	26 ± 0	26 ± 0	8.32 ± 0.16	8.50 ± 0.19	154.0 ± 8.94	148.0 ± 8.37	9.08 ± 0.03	8.99 ± 0.05
Jun 24	26 ± 0	26 ± 0	8.36 ± 0.15[a]	8.42 ± 0.08[b]	152.0 ± 13.04	148.0 ± 8.37	9.06 ± 0.02	8.97 ± 0.02
Jul 08	26 ± 0	26 ± 0	8.97 ± 0.03[a]	8.82 ± 0.06[b]	163.6 ± 5.59[b]	175.2 ± 5.36[a]	9.11 ± 0.06[a]	8.94 ± 0.06[b]
Jul 22	28 ± 0	28 ± 0	8.20 ± 0.10[a]	7.94 ± 0.21[b]	146.0 ± 11.40	158.0 ± 4.47	9.11 ± 0.06	9.05 ± 0.05
Aug 05	25 ± 0	25 ± 0	7.86 ± 0.15	7.86 ± 0.15	134.0 ± 15.17	144.0 ± 15.17	9.01 ± 0.02	9.07 ± 0.06
Aug 19	24 ± 0	24 ± 0	7.72 ± 0.25	7.68 ± 0.13	134.0 ± 15.17	152.0 ± 19.24	9.11 ± 0.06	9.05 ± 0.05
Sep 09	22 ± 0	22 ± 0	7.76 ± 0.11	7.70 ± 0.10	144.0 ± 11.40	152.0 ± 8.37	9.11 ± 0.04[a]	9.04 ± 0.04[b]
Nov 16	18 ± 0	18 ± 0	6.80 ± 0.07	6.80 ± 0.07	144.0 ± 5.48	148.0 ± 8.37	8.06 ± 0.04	8.03 ± 0.07

Table 2. Water qualities at the center of the treatment and control clam cages

Date	WT (℃)		pH		Turbidity (NTU)		DO (mg/L)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
Jun 10	26	26	8.3	8.3	160	140	9.08	8.99
Jun 24	26	26	8.3	8.4	160	140	9.08	8.99
Jul 08	26	26	8.9	8.7	166	172	9.02	8.93
Jul 22	28	28	8.3	7.9	150	160	9.12	8.99
Aug 05	25	25	8.1	7.9	130	130	9.05	9.04
Aug 19	24	24	7.3	7.8	130	130	9.12	8.99
Sep 09	22	22	7.6	7.7	140	140	9.08	8.99
Nov 16	18	18	6.8	6.8	150	140	8.05	7.94

Table 3. Sizes and total weights of *Unio douglasiae* reared in a cage during the experimental period

Date	Shell length (mm)	Shell height (mm)	Shell width (mm)	Total weight (g)	Note
Jun 10	70.78 ± 10.97[b]	38.41 ± 7.70[b]	26.27 ± 5.50[b]	40.99 ± 16.84[b]	Beginning of experiment
Nov 16	73.06 ± 06.84[a]	41.09 ± 3.48[a]	29.72 ± 4.27[a]	44.77 ± 11.22[a]	End of experiment

3. Chlorophyll-α concentration in the cultivation tanks

The chlorophyll-α concentration in the control and test cultivation tanks rose drastically (to its highest value) at the start of the study in early July; however, the concentration decreased rapidly subsequently in both tanks. The average chlorophyll-α concentration at the periphery (based on the measurement at 4 peripheral points) of the test tank was determined to be 69.2 μg/L, which was lower than that at the periphery of the control tank (81.4 μg/L); the chlorophyll-α concentration at the periphery of the test tank remained below that of the control tank even at the end of July, when the concentration began to decline (Fig. 3). However, the chlorophyll-α
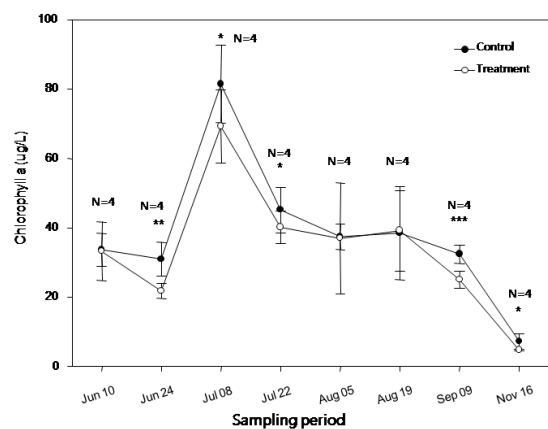
Fig. 3. Seasonal changes in mean chlorophyll-α concentrations measured from the periphery of the clam culture cages.
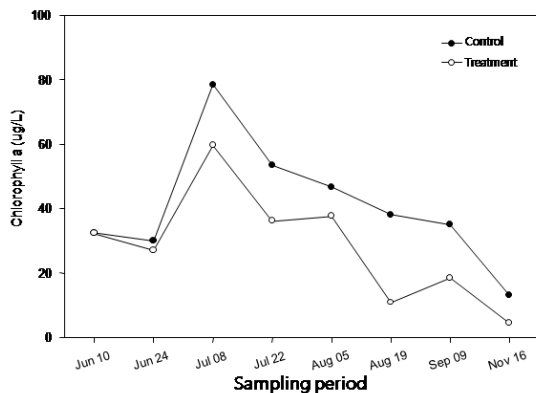
Fig. 4. Seasonal changes in chlorophyll-α concentrations measured from the center of the clam culture cages.

concentrations in the periphery of the control and test tanks were similar (40 μg/L) from August.

Meanwhile, the chlorophyll-α concentration at the center of the test tank was lower than that at the periphery of the same test tank (or otherwise) throughout the experiment; the chlorophyll-α concentration at the center of the test tank was much lower than that at the periphery or the center of the control tank (Fig. 4). The chlorophyll-α concentration at the center of the test tank was 59.68 μg/L during the experimental stage when the chlorophyll-α concentrations were highest (early July); this was much lower than the chlorophyll-α concentrations at the center (78.44 μg/L) and periphery of the control tank (81.40 μg/L). This concentration was also low compared to the concentration observed at the periphery of the test tank (69.20 μg/L). This trend was maintained up to the end of the experiment. In particular, during the stage of the experiment when the chlorophyll-α concentration decreased rapidly (mid-αugust), the chlorophyll-α concentration at the central area of the test tank (with clams) was only 10.83 μg/L, compared to that at the test tank periphery (39.17 μg/L), and the control tank center (38.08 μg/L) and periphery (38.42 μg/L).

Discussion

Water from Korean streams and lakes is widely used for agricultural, industrial, and drinking purposes. However, the water bodies are characterized by very large changes in volume depending on the season. Therefore, a large number of dams and weirs have been constructed to alleviate these seasonal changes. However, these dams and weirs weaken the flow of water, leading to an increased possibility of eutrophication resulting from the influx of pollutants from the outside; this would ultimately lead to mass proliferation of phytoplankton in freshwater ecosystems, typified by the 'green tide' phenomenon (Lee *et al.*, 2014; Rhew *et al.*, 2014).

Such mass proliferation of phytoplankton decreases the amount oxygen dissolved in the water; some phytoplankton species are also known to release toxic substances into the water. Therefore, various technologies are being developed to reduce phytoplankton proliferation in large water bodies. In particular, methods that utilize shellfish feeding on phytoplankton have the advantage of minimizing human artificial interference, as they utilize the food chain of the natural ecosystem. Smith *et al.* (1998) reported a 17-fold decrease in the phytoplankton content in the Hudson River (New York, USA) after the introduction of zebra mussels (*Dreissena polymorpha*) to the water. Although this addition resulted in a number of negative consequences, zebra mussel being an ecosystem-disrupting species, this study ultimately revealed the very strong possibility of aquatic plankton removal using freshwater bivalves. In particular, studies have shown that the use of freshwater shellfish for the reduction in phytoplankton content is ecologically friendly, unlike other chemical or physical removal methods (Rose *et al.*, 2014). However, the superiority of freshwater shellfish has usually been tested in laboratories or, when tested in outdoor tanks receiving the influence of real sunlight, for a very short period (10 days). Therefore, this method must be further researched to facilitate commercialization.

The Ministry of Environment of Korea declares an algal bloom (green tide) alert when the chlorophyll-α concentration in streams and lakes exceeds 25 μg/L (Water Information System, 2014). The chlorophyll-α

concentration measured in the outdoor water tanks was much higher than this threshold throughout the course of this experiment. This excluded the mid-November period; the decrease in temperature in the tank water led to the development of severe algal bloom. During this time, the outbreak of phytoplankton in the area protected by *U. douglasiae* was reduced by 10-70%, reinforcing the possibility of *U. douglasiae* use for algal bloom removal from outdoor environments, as well as the laboratory.

However, the cultivation of *U. douglasiae* for the reduction of aquatic plankton is not very feasible. Yang *et al.* (2004) reported that the concentration of clams required to reduce the aquatic plankton would amount to approximately 0.1% of the total volume of water. In addition, the pseudofeces excreted during mass cultivation results in an additional increase in the organic matter. Consequently, a plankton-reducing effect using *U. douglasiae* can be obtained by establishing appropriate management techniques for the mass cultivation of clams (Park *et al.*, 2008).

In order to solve the problem associated with mass clam cultivation and to have the plankton-reducing effect, we conducted growing the clams in 16 rectangular baskets, which were used to fashion a clam cultivation facility in the shape of a square frame with an empty middle region. The chlorophyll-a concentration was observed to be lower in the center of the test tank than in the center of the control tank, as well as the periphery of the test tank. This was attributed to the *U. douglasiae* surrounding the center of the test tank, which prevented the influx of plankton from the periphery. Therefore, we believe that this method of arranging clams could be useful for the efficient management of *U. douglasiae*, and could facilitate the reduction in plankton concentration in areas with limited intake.

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