BIO-CONTROL OF *MICROCYSTIS AERUGINOSA* BLOOM USING VARIOUS AQUATIC ORGANISMS BY DUAL STABLE ISOTOPE (^{13}C AND ^{15}N) TRACERS

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(Received 7th Aug 2017; accepted 15th Jan 2018)

Abstract. The application of ^{13}C and ^{15}N labeled phytoplankton makes it possible to directly follow the pathway and transfer of food source (cyanobacteria) into consumers (aquatic organisms), in contrast to past studies where only changes in compositions of chlorophyll-*a*, clearity, and nutrients were taken as the evidence for these processes. To evaluate the effect of biocontrol by aquatic organisms (aquatic plants; *Iris pseudoacorus*, filter feeder bivalve; *Sinanodonta arcaeformis*, and *Unio douglasiae*, macroinvertebrate; *Caridina denticulate*, carnivore fish; *Pseudobagrus fulvidraco*, *Odontobutis platycephala*, planktivore fish; *Pseudorasbora parv*, and omnivore fish; *Misgurmus anguillicaudatus*) on large toxigenic cyanobacteria bloom (*Microcystis aeruginosa)* in the freshwater ecosystem, we conducted a biomanipulation test on *in situ* ponds using dual stable isotope tracers (^{13}C and ^{15}N). As a filter feeding bivalve, *S. arcaeformis* could incorporate more toxic cyanobacteria cells than *U. douglasiae*, demonstrating its larger detoxification capacity. Also, macroinvertebrate (*C. denticulate*) continuously assimilated to cyanobacteria species in combination with zooplankton and detritus, probably due to detoxification capacity. Indeed, the aquatic plants (*I. pseudoacorus*) seem to be nutrient uptakes in water column and inhibit to light attenuation, comparing to cyanobacteria species. As a primary consumer of phytoplankton, zooplankton (Copepoda) consumed to small and edible particles which is changed from inedible toxic filamentous cyanobacteria species through the grazing efficiency by aquatic organisms. However, various kinds of fishes hardly feed on toxic cyanobacteria directly. Our result suggests that the native species, like *Sinanodonta sp*. and *C. denticulate,* are very useful bio-control organisms on toxic cyanobacteria bloom rather than carnivore, omnivore and planktivore fish. Furthermore, if an aquatic plant that can not only remove nutrients but also provide habitats to aquatic organisms (zooplankton, bivalves and shrimps) is developed, it can help control toxic cyanobacteria blooms. Therefore, it is considered that the development and establishment of habitat of useful organisms is very necessary for water quality improvement. Our biomanipulation technique may provide a key tool for efficient management and restoration of eutrophied reservoirs.

Keywords: *bio-control, toxic cyanobacteria, Microcystis aeruginosa, stable isotope tracer, water management*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_931953

Introduction

Eutrophication in freshwater environment is a worldwide problem resulting in extensive blooms of blue-green algae including freshwater cyanobacteria (Beklioglu, 1999). It is well known that some freshwater cyanobacteria (i.e. *Microcystis*, *Anabaena* and *Oscillatoria*) may produce a wide range of toxins (cyanotoxins) as well as secondary metabolites under certain conditions of growth and environment factors (Figueiredo et al., 2004). Its potential toxicity including microsystins (Watanabe et al., 1992), cause a harmful effect to aquatic ecosystem, and decrease the biodiversity by eliminating other phytoplankton species (Bouvy et al., 2000), and can be harmful to many kinds of aquatic organisms including cladocerans (Boon et al., 1994; Matveev et al., 1994), copepods (Koski et al., 1999), shrimps (Engstrom et al., 2001; Kim et al., 2017a), bivalves (Bontes et al., 2007; Kim et al., 2017b) and fishes (Mohamed et al., 2003). To prevent toxic effect of cyanobacteria bloom in freshwater ecosystem, many countries have investigated for a long time (Perrow et al, 1997; Mehner et al, 2004). There are two accepted strategies for the control of water quality in nutrient-rich freshwater ecosystems (Wium-Anderson et al., 1982), which are to reduce the external nutrient load and to manipulate phytoplankton biomass through biological treatment in large water body, such as lakes and reservoirs (Carpenter et al., 1995). To control the external nutrient load is often expensive, it is difficult to obtain the positive results (Braband et al., 1990), while biomanipulation techniques which is increase biomass of zooplankton through reduction of planktivorous fishes by 'top-down' cascade showed significant effect (Shapiro et al., 1975). However, many researchers dispute to control of algal biomass with grazing by zooplankton, especially under toxic cyanobacteria blooms (Bernardi and Giussani, 1990; Degans and Meester, 2002). In such circumstances, and progressive biomanipulation approach, based on the direct control of phytoplankton by filter feeding plaktivorous fish appears to be more effective (Smith, 1985; Miura, 1990; Drenner et al., 1987). It has proved that filter-feeding fish, such as silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Artocarpus nobilis*) and tilapia (*Oreochromis niloticus*), are effective candidates for bio-control of plankton communities to eliminate cyanobactria, through suppress to phytoplankton for prey as well as zooplankton (Starling, 1993; Xie and Yang, 2000; Lu et al., 2002; Lu et al., 2006). However, these filter-feeding fishes are not common fishes in many countries, such as Korea and Japan, even though most intensively cultured fish species in Asia and comprise much of the production of Chinese and America aquaculture (Liang et al., 1981). On the contrary, An et al. (2009) and Kim et al. (2017a) argued that filter-feeding marcroinvertebrate (*Caridina denticulate*) as native species can easily reduce to blue-green algae than planktivore fishes, as a results, cyanobacteria accumulations might constitute up to 69% of biomass carbon in freshwater atyid shrimp (*Peristemia australiensis*) (Piloa et al., 2008). In addition, the use of bivalve species (Unionids) which is contribute to bio-control of eutrophication through bio-filter effectiveness by reducing algal biomass (Reeders et al., 1990; Soto et al., 1999; Kim et al., 2017b) are probably more suitable for shallow lake which mainly consist of soft substrate (mixture of mud and sand) instead of zebra mussel which is require hard substrate for settlement, with their high filtration rates on phytoplankton have been promoted as a tool in biomanipulation of lakes (Reeders et al., 1990). Also, aquatic plants are themselves part of the food web, and could be control massive algae bloom through competing for nutrients and other resources with phytoplankton and periphyton (Ozimek et al., 1990; van Donk et al., 1993).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_931953

Here, we compared assimilation efficiency of newly candidates aquatic organism as a bio-control, such as macroinvertebrate filter feeder (*C. denticulate*), bio-filter bivalve (*Unio douglasiae, Sinanodonta arcaeformis*) and aquatic palnt (*Iris pseudoacorus*), instead of famously bio-control planktivore fish (*H. molitrix, A. nobilis* and *O. niloticus*) under massive toxic cyanobacteria (mostly *Microcystis aerugionsa* and *Anabena spirodides*) bloom using dual stable isotope tracers (^{13}C and ^{15}N). Also, we investigated other planktivore fish (*Pseudorasbora parva*) as a bio-control, omnivore fish (*Misgurmus anguillicaudatus*) as a benthic scavenger, carnivore fish (*Pseudobagrus fulvidraco, Odontobutis platycephala*) as a regulating of other fish and macroinvertebrate biomass. The labeling isotope technique could be a possible method for tracking their fates in the natural biota, with involving energy sources and pathways (Peterson and Fry, 1987; Parker et al., 1989). The applications of ^{13}C- and ^{15}N-labeled phytoplankton make it possible to directly follow the assimilation pathways of carbon and nitrogen in aquatic organisms, in contrast to previous studies which only monitored changes in environmental conditions such as Chl. *a*, nutrients, turbidity and MC concentration (Benndorf et al., 1990; Jeppesen et al., 1997; An et al., 2010). This is the first study, to evaluate the bio-control efficiency through various candidate organisms under massive toxic cyanobacteria blooms in artificial pond using dual stable isotope tracers (^{13}C and ^{15}N).

Materials and methods

Experimental design for artificial pond

An artificial small pond is located in Sukmoon wetland (36°57'4"N, 126°37'5"E) at mid western region of South Korea (*Fig. 1*). The enclosure small ponds including muddy sediment consisted of two series of individual capacity 100 ton (size 10 × 5 × 2 m), having a reference and manipulated ponds (*Fig. 1*):

1. Reference pond (RP, in situ water containing massive biomass of *M. aerugionsa* and *A. spirodides*)
2. Manipulated pond (MP, in situ water containing massive biomass of *M. aerugionsa* and *A. spirodides* and aquatic organisms (bivalve ; *U. douglasiae, S. arcaeformis*, macroinvertebrate filter feeder; *C. denticulate*, carnivore fish ; *P. fulvidraco, O. platycephala*, planktivore fish; *P. parva*, omnivore fish ; *M. anguillicaudatus*, floating aquatic plant; *I. pseudoacorus*)

The candidate organisms were captured from agricultural reservoirs using hand nets (mesh size < 2 × 2 mm), kept for 2 weeks in water tanks and maintained at temperature of 25 ± 1°C, Dissolved Oxygen (DO) levels of 6±1 mg L^{-1}, pH levels of 7~8, and a photoperiod of around 16L:8D. Individuals were starved for 48 h prior to the beginning of experiment. The bivalves were collected by trawling in agricultural reservoir. They were kept in aquaria filled with reservoir water in a layer of sand, and kept at 17~20°C temperature under a 16L:8D regime. Individuals were fasted for 48 h prior to the experiment.

For the in situ pond experiment, the individual numbers of candidate organisms were determined through the preliminary experiments; bivalve *U. douglasiae* and *S. arcaeformis* (5 ind. m^{-2}), macroinvertebrate filter feeder *C. denticulate* (3 ind. m^{-2}) andcarnivore fish *P. fulvidraco* (0.24 ind. m^{-2}). The carnivore fish (*O. platycephala*), planktivore fish (*P. parva*) and omnivore fish (*M. anguillicaudatus*) were lived originally in artificial pond.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_931953

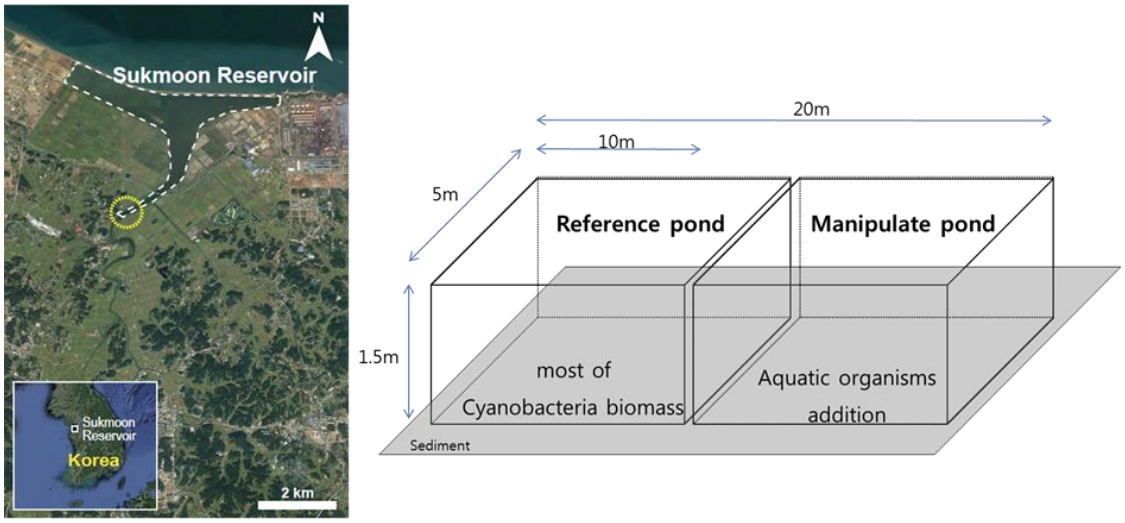

Figure 1. *The map shows to small pond experimental station in Sukmoon wetland. The filled circle represents to study site. The experiments were designed as reference pond (most of cyanobacteria biomass) and manipulated pond (aquatic organisms addition ; bivalve (U. douglasiae, and S. arcaeformi), macroinvertebrate filter feeder (C. denticulate), carnivore fish (P. fulvidraco and O. platycephala), planktivore fish (P. parva) and omnivore fish (M. anguillicaudatus)*

In situ artificial pond experiment

The artificial pond experiment was conducted while the massive toxic cyanobacteria bloom occurred, contributing to around 89% of cyanobacteria biomass during experimental period. The enclosures were filled by pumping the lakes water which mostly contained toxic cyanobacteria species. Each enclosure was maintained for two days to stabilize the water condition. As isotope tracers, $NaHCO_3$ (Isotech; $^{13}C > 99\%$) and $(NH_4)_2SO_4$ (Isotech; $^{15}N > 99\%$) were added to each pond, and maintain for a day. ^{13}C content of the disolved inorganic carbon (DIC) pool was increased up to about 5% and ^{15}N content of the ammonium pool was also enriched up to about 10%. The aquatic organisms (*I. pseudoacorus, C. denticulate, P. fulvidraco, U. douglasiae* and *S. arcaeformis*) were added into manipulate pond. The samples of water and aquatic organisms were collected at the 1st, 2nd, 3rd, 4th, 5th, 6th, 8th, 10th, 13th, 16th and 21nd day after addition.

Analysis of water quality parameters

Two replicate of water saples were collected to determine the concentrations of dissolved inorganic nitrogen (DIN : NH_4^+, NO_3^-+NO_2^-) and phosphate (PO_4^{3-}) in each pond. The concentrations of DIN and phosphate were measured by standard colorimetric techniques following the methods of Strickland and Parsons (1964) using UV-spectrophotometer (Carry 50). Chl-*a* concentration was determined by using a fluorescence spectrophotometer (Turner Design, 10R) after extraction with 90% acetone for 24 h. Water temperatures were measured using a multi-parameter water quality sensor (YSI Environmental Monitoring System 660, USA). All environmental parameters including nutrients, chlorophyll-*a* (Chl-*a)* and water temperature were monitored during the experimental period.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Enumeration and biomass determination of phytoplankton and zooplankton

Water samples for enumeration of phytoplankton cells were sedimented for 24 h, and a known volume of the concentrated sample was placed in a Sedgewich-Rafter counting chamber, where at least 300 cells were counted under 200-400× magnification. It is difficult to count some taxa (e.g., *Microcystis sp.*) as individual cell bases; thus, they were enumerated only as larger units. Phytoplankton cell density was determined on the basis of geometric solids that closely approximated each cell or colony shape (Wetzel and Likens, 1991). In addition, we selected to specific zooplankton taxa (copepoda) under microscope using micro-pipette, and placed on combusted 25 mm GF/F filter, and kept at -20°C refrigerator.

Analysis of stable isotope ratio

For analyzing C and N stable isotope ratio of particulate organic matter (mostly phytoplankton), water samples were passed into 20 μm size mesh to remove zooplankton, and remaining water samples filtered through pre-combusted (450°C, 24 h) glass fiber filters (GF/F Whatman) using gentle vacuum filtration. Zooplankton were collected with micropipette under a microscope, and filtered on pre-combusted (450°C, 5 h) glass fiber filters (GF/F Whatman) before freezing. All filters were fumed for 24 h with saturated hydrochloric acid (HCl) to remove inorganic carbon and dried completely using freeze drier. The biota sampling was carried out using hand nets (mesh size 2 × 2 mm) in manipulate pond. The bivalve samples were dissected in order to separate the digestive gland, stomach, muscle (abductor), gill and mantle. The macroinvertebrate and fish samples were divided into muscle and digestive gland. The plant samples were separated into leaf and root. All tissue samples were freeze-dried and then ground to a fine powder using grinder (FRITSCH-planetary mono mill, Pulverisette 6, Germany). The freezing and storage processes do not affect $\delta^{13}C$ and $\delta^{15}N$ values of biota tissue (Kim et al., 2013). Homogenized powder samples were decalcified with 1N HCl for at least 24 h to remove possible carbonates. But subsamples for $\delta^{15}N$ analysis did not be treated as it has been reported that HCl treatment affect $\delta^{15}N$ values (Kim et al., 2016). After the samples were re-dried using freeze drier, and ground to fine powder which was thoroughly mixed before analysis. Measurements of carbon and nitrogen stable isotopic ratios were performed with a continuous flow isotope ratio mass spectrometer (Isoprime 100; GV Instrument, U.K.) which coupled to an elemental analyzer (Vario Microcube, U.K.). Isotopic ratios were presented as δ values (‰) expressed relative to the Vienna PeeDee Belemnite (VPDB) standard and to atmospheric N_2 for carbon and nitrogen, respectively. Reference materials were IAEA-CH6 ($\delta^{13}C = -10.45 \pm 0.04$‰) and IAEA-N1 ($\delta^{15}N = 0.4 \pm 0.2$‰). The analytical precision was within 0.2‰ and 0.5‰ for carbon and nitrogen respectively. Isotope ratios were reported in per mil (‰) using standard delta notation (*Eq. 1*):

$$\delta X = \left\{\left(R_{sample} - R_{std}\right) / R_{std}\right\} \times 1000(‰) \quad \text{(Eq. 1)}$$

where X = ^{13}C or ^{15}N, R = $^{13}C/^{12}C$ or $^{15}N / ^{14}N$, and std (standard) = Vienna Peedee Belemnite (VPDB) for carbon or air N_2 for nitrogen, respectively.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_931953

For this study, the δ-values were converted to atom %, which is more appropriate for labelled samples. Conversion was done according to *Equation 2:*

$$A\ (\text{atom}\ \%)\ =\ 100/\left[1/\left\{(\delta\ \text{sample}\ /\ 1000+1)\times a_{ns}\right\}+1\right] \qquad \text{(Eq. 2)}$$

a_{ns} for carbon is 0.011180, for nitrogen is 0.0036765.

Determination of MCs concentration

The purification and analysis of MCs were carried out using the methods developed by Harada et al. (1988). For analyzing MCs concentration of the particulate organic matter (mostly phytoplankton, POM), water samples were passed into 20 μm size mesh to remove zooplankton, and remaining water samples filtered through pre-combusted (450°C, 24 h) glass fiber filters (GF/F Whatman) using gentle vacuum filtration. From a sample of freeze-dried *Microcystis* cell material, the MCs were extracted twice with 20 mL of 5% (v/v) acetic acid for 1 h while shaking at 140 rpm. The extract was centrifuged at 12,000 × *g*, and then the supernatant was applied to a C18 cartridge (Sep-Pak; Waters Association, Milford, MA, USA). The cartridge was rinsed with water and 20% methanol in water. The eluate from the cartridge with 90% methanol in water was evaporated to dryness, and the residue was dissolved in 100% methanol. The sample solution was analyzed on an HPLC (Agilent Technologies 1200 series, Santa Clara, CA, USA). The separation was performed on an ODS column (Cosmosil 5C18-AR, 4.6 mm × 150 mm) reverse-phase column and the mobile phase was a 0.1% formic acid:water solution with constant flow at 1 mL min^{-1}. The MCs were identified by their UV spectra and retention times, and by spiking the sample with purified standards of MC-LR (Sigma, Sigma-Aldrach, Yong In, Korea), MC-YR (Sigma, Sigma-Aldrach, Yong In, Korea) and MC-RR (Sigma, Sigma-Aldrach, Yong In, Korea). MCs concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR, MC-YR and MC-RR, Sigma, Sigma-Aldrach, Yong In, Korea).

Results

Environmental conditions in artificial pond

Total nitrogen (TN) concentration in reference pond showed higher value than manipulated pond on 3rd day after beginning the experiment, ranging from 1.5 to 1.8 mg/L, because floating aquatic plant continuously take up DIN in water column (*Table 1*). Total phosphorus (TP) concentration in reference pond showed a higher value until 5 days after beginning experiment, but it showed fluctuation since 5 days to end of the experimental period, probably due to resuspension of the phosphorus from sediment through windy turbulence and organisms movement (*Table 1*). Chl-*a* concentration in manipulated pond was dramatically decreased than that in the reference pond after beginning 2nd day, ranging from 257.6 to 15.6 μg/L (*Table 1*). It is indicated that toxic cyanobacteria bloom inhibited by aquatic organisms as their prey. Water temperature showed same pattern both in reference and manipulated pond, with slightly decrease after 23 October (*Table 1*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_931953

Table 1. *Analysis of environmental factors and MC concentration (-LR, -RR, -YR) between manipulate pond and reference pond (n = 5, Mean ± S.D.)*

Date	Chl.a (μg/L)		Water column DIN (mgl/L)		Water column DIP (mg/L)		MC concentration (ug/L)	
	Manipulate pond	Reference pond	Manipulate pond	Reference pond	Manipulate pond	Reference pond	Manipulate pond	Reference pond
Sep. 13	257.6±5.2	217.9±8.1	1.01±0.13	0.83±0.06	0.09±0.004	0.08±0.003	11.29± 2.15	11.21±0.19
Sep. 14	217.5±3.0	218.9±5.0	0.61±0.08	0.91±0.08	0.01±0.000	0.01±0.002	8.87±3.81	13.00± 0.87
Sep. 15	107.8±6.4	223.4±5.2	0.79±0.06	1.59±0.08	0.07±0.002	0.18±0.002	1.41±0.18	24.65±2.97
Sep. 16	115.7±5.0	227.2±5.2	0.76±0.23	1.25±0.26	0.09±0.007	0.12±0.004	3.41±0.33	14.07±1.41
Sep. 17	113.6±5.1	248.8±5.5	0.38±0.07	1.12±0.17	0.01±0.002	0.09±0.000	7.27±0.18	20.30±2.30
Sep. 18	87.2±6.2	166.0±6.4	0.62±0.00	1.08±0.06	0.07±0.007	0.05±0.000	4.92±0.23	13.56±2.53
Sep. 19	46.5 ±7.1	213.2±7.8	0.64±0.13	1.27±0.09	0.08±0.001	0.11±0.001	5.67±0.11	17.54±0.93
Sep. 21	47.8±8.8	213.9±8.2	0.80±0.08	1.45±0.08	0.10±0.001	0.08±0.006	4.65±0.62	13.77±0.58
Sep. 23	23.6±5.6	215.5±5.7	0.84±0.20	1.46±0.06	0.11±0.004	0.07±0.003	5.83±0.05	11.83±1.14
Sep. 26	22.8±5.0	155.7±5.0	0.71±0.17	1.76±0.17	0.09±0.000	0.09±0.002	18.77±1.89	21.27±3.15
Sep. 29	19.0±3.4	189.5±3.8	0.73±0.06	1.67±0.05	0.10±0.000	0.07±0.000	10.48±1.21	24.15±1.38
Oct. 3	15.6±2.1	156.1±2.9	0.93±0.09	1.87±0.21	0.09±0.001	0.10±0.000	5.88±0.53	9.14±0.46

MC concentration of POM

The MC concentrations of the POM in the reference pond and manipulated pond showed remarkable variation (*Table 1*). Within two days of beginning the experiment, the MC concentration showed a remarkable decrease of the POM in the manipulated pond while the reference pond maintained a relatively constant MC concentration through the end of the experiment. However, those values did increase slightly, possibly due to release from filter feeders as fecal pellets or expelled cyanobacteria cells in water column at last 7 days.

Enumeration and biomass determination of phytoplankton and zooplankton

The relative proportions of phytoplankton species at the beginning of manipulation experiment showed that the most dominant species was cyanophyceae (88.7%), next was bacillariophycae (7.5%), chlorophyceae (2.6%), crytophycee (0.6%), and dinophyceae (0.4%) were followed (*Table 2*). The cyanophyceae algae (*M. aerugionsa, A. spirodides, Synechocystis pevalekii, Aphanocapsa elachista* and *Chroococcus* sp.) were usually pre-dominant (89% of total phytoplankton biomass) in both pond at the beginning of experiment (*Table 2*). The cell density of phytoplankton in reference pond maintained similar biomass with small fluctuation during the entire experiment, but those in manipulated pond dramatically decreased (*Fig. 2*). When the experiment was ended, relative proportion cyanophyceae species in reference pond slightly changed from 88% to 82%, but those in manipulate pond was remarkably decreased from 88% to 21% during the study period (*Fig. 2*). It might be caused by biomanipulation effects that

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_931953

the aquatic organisms (zooplankton, bivalve, macroinvertebrate and fishes) reduce to cyanophyceae biomass as a prey, and prevent to decrease the biodiversity by eliminating cyanophyceae species. These results supported that aquatic organisms feeding on the ^{13}C and ^{15}N labeled phytoplankton, resulted in apparently enriched ^{13}C and ^{15}N atom % which was defined as dietary assimilation in their body during study period (Figs. *3, 4* and *5*). The rotifer was usually pre-dominant (93% of total zooplankton biomass) in all ponds, next was copepoda (4.6%) and cladocera (2.4%) were followed (*Table 3*). The cell density of zooplankton dramatically decreased from 12,594 to 795 cells mL^{-1} in manipulated pond than reference pond, ranging from 12,667 to 2,769 cells mL^{-1}, due to aquatic organisms fed on those cells (*Fig. 2*).

Table 2. *Major species and relative proportions (%) of phytoplankton taxa in the initial artificial pond in Sukmoon wetland at the beginning of mesocosm*

Class	**Species**	**Cell density (cells mL^{-1})**	**Relative proportion (%)**
Cyanophyceae			88.72
	Microcystis aerugionsa	53,200	
	Anabena spirodides	9,160	
	Synechocysits pevalekii	2,640	
	Aphanocapsa elachista	1,740	
	Chroococcus sp	1,410	
Chlorophyceae			2.68
	Secenedemus sp	1,380	
	Staurastrum sp	310	
	Monoraphidium contortum	370	
Crytophycee			0.62
	Cryptomonas erosa	480	
Dinophyceae			0.44
	Ceratium hirundinella	340	
Bacillariophycae			7.53
	Synedra acuse	480	
	Synedra sp	260	
	Navicula sp	300	
	Awlacoseira granulata	2,560	
	Awlacoseira sp	220	
	Nitzscchia holsatica	420	
	Cyclotella comta	1,540	

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_931953

Table 3. *Major species and relative proportions (%) of zooplankton taxa in the initial artificial pond in Sukmoon wetland at the beginning of mesocosm*

Class	Species	Cell density (cells mL^{-1})	Relative proportion (%)
Cladocera			2.43
	Bosmina longispina	156	
	Diaphanosoma brachyurum	148	
Copepoda			4.66
	Cyclops strenuous	140	
	Daphnia retrocurva	142	
	Nauplius	301	
Rotifera			92.91
	Ascomorpha ecaudis	2,621	
	Asplanchna herriciki	284	
	Brachionus calyciflorus	128	
	Brachionus diversicornis	346	
	Filinia longiseta	801	
	Filinia terminalis	124	
	Keratella cochlearis	5,014	
	Keratella valga	1,521	
	Polyathra dolichoptera	112	
	Trichocerca birostris	542	
	Trichocerca branchyura	0	
	Trichocerca capucina	38	

^{13}C and ^{15}N atom (%) of POM, Copepoda and aquatic organisms

The ^{13}C and ^{15}N atom % of particulate organic carbon (POC) and particulate organic nitrogen (PON) in POM (mostly phytoplankton) in reference and manipulated ponds showed similar variation trends (*Fig. 3*). Within one day after addition of the tracers, ^{13}C and ^{15}N atom % were remarkably enriched in the POM through active phytoplankton assimilation, but manipulated pond showed, mostly a little bit, lower atom% value rather than reference pond after 3rd day. However, those values were saturated and declined slightly at the end of time. The incorporated ^{13}C and ^{15}N atom % of copepod after 2nd day in both ponds showed a clear enrichment, demonstrating that copepoda fed the ^{13}C and ^{15}N labeled POM as a diet (*Fig. 3*). ^{13}C and ^{15}N atom % value in copepod were higher in manipulated than reference pond after 3rd day in beginning experiment, indicating copepoda grazing was larger in manipulated pond.

The incorporation rates of carbon and nitrogen into muscle and digestive gland of the aquatic organisms were calculated based on the increased ^{13}C and ^{15}N atom % through dietary assimilation. Most aquatic organisms showed continuously apparent enrichment of ^{13}C and ^{15}N tracers in their tissues during the experiment period (*Figs. 4, 5* and *6*). According to different bivalve species, *U. douglasiae* showed 1.08~1.11 ^{13}C and 0.36~0.61 ^{15}N atom % respectively, *S. arcaeformis* showed a 1.08~1.20 ^{13}C and 0.36~1.28 ^{15}N atom % respectively during experimental period in manipulated pond (*Figs. 4, 5* and *6*). The different atom % might be on account of different

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assimilation capacity between two freshwater bivalve species. As a macroinvertebrate, *C. denticulate* showed apparently enrichment of ^{13}C and ^{15}N tracers rather than other species, 1.08~1.24 for ^{13}C atom % and 0.36~1.27 for ^{15}N atom %, demonstrating that those organisms has higher assimilation efficiency to toxic cyanobacteria cells through feeding activity (*Figs. 4*, *5* and *6*). Among the fish species, incorporated ^{13}C and ^{15}N atom % in *M. anguillicaudatus* species showed 1.08~1.12% and 0.36~0.59% respectively, *P. parva* showed 1.08~1.12% and 0.36~0.72% respectively, *P. fulvidraco* showed 1.08~1.12% and 0.36~0.58% respectively, and *O. platycephala* showed 1.08~1.13% and 0.36~0.60% respectively, during study period in manipulated pond (*Figs. 4*, *5* and *6*). The incorporated ^{13}C and ^{15}N atom % among the fishes were various probably due to differential feeding type such as carnivore, planktivore and omnivore. As a floating aquatic plant, *I. pseudoacorus* showed 1.08~1.08 ^{13}C and 0.37~2.13 ^{15}N atom % respectively, during experimental period in manipulated pond (*Figs. 4*, *5* and *6*). *I. pseudoacorus* showed significantly enriched ^{15}N atom %, due to assimilating inorganic nitrogen in water column through its root, however it was hardly enriched ^{13}C atom % because it uptakes carbon dioxide from the atmosphere through its leaf rather than root.

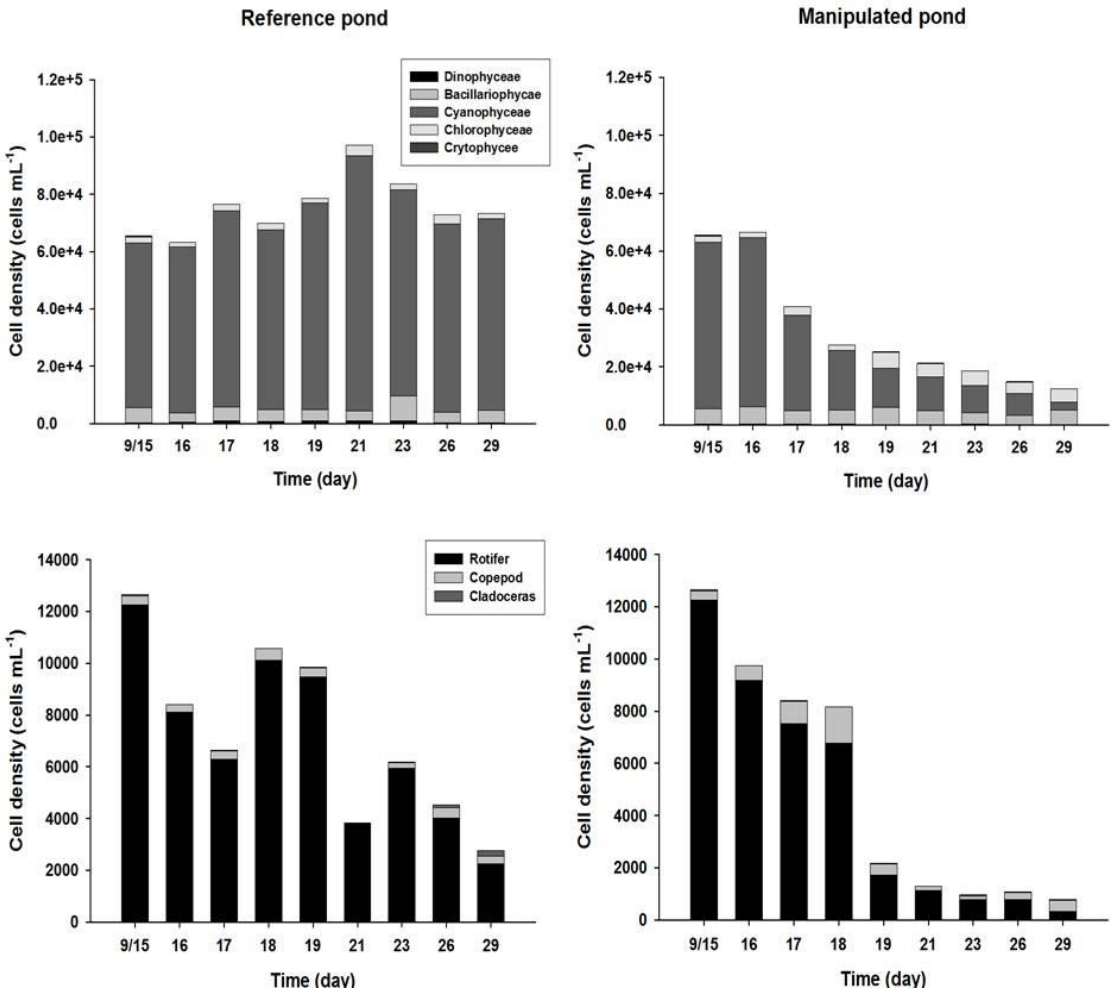

Figure 2. *Temporal variation of phytoplankton and zooplankton groups to the each total biomass in the two kinds of ponds (reference and manipulated ponds) during study period*

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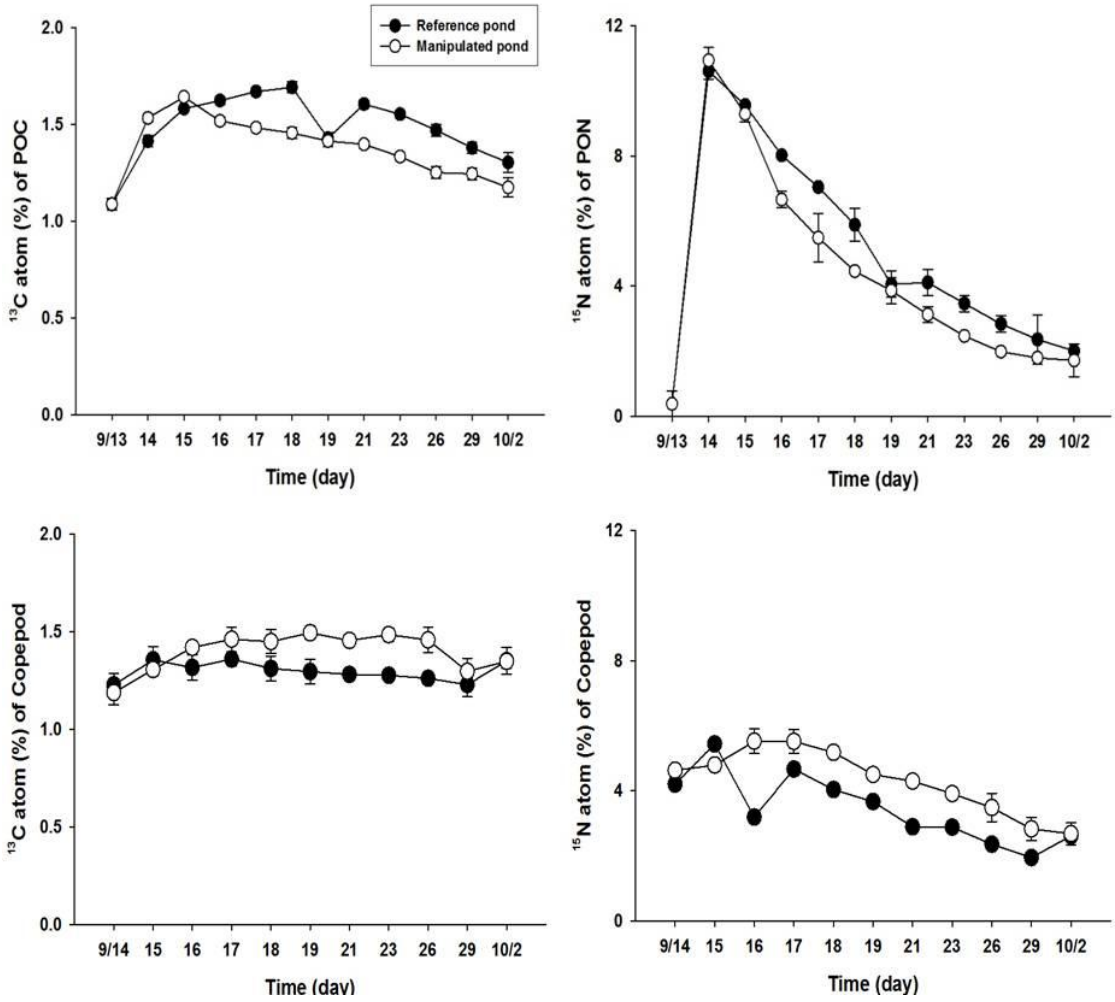

Figure 3. *The ^{13}C and ^{15}N atom (%) of POM and Copepod in reference and manipulated ponds during study period*

Discussion

Comparing grazing by differential Sinanodonta bivalve species

In this study, the incorporated ^{13}C and ^{15}N atom % of aquatic organisms means that newly photosynthesized phytoplankton cells were assimilated into those cells. Even though similar *Sinanodonta* freshwater bivalve species were used, the incorporated ^{13}C and ^{15}N atom % of *U. douglasiae* bivalve showed lower value rather than other bivalves during entire experiment period (*Fig. 4*).

This result reflects that *U. douglasiae* has smaller capacity to assimilate toxic cyanobacteria derived diet, and affected by the occurrence of toxic-producing cyanobacteria bloom. In this study, we did not determine cyanotoxic compound like microcystin (MC) in aquatic organisms. However, it is well known that cyanobacteria, especially members of the genera *Mycrocystis*, *Anabaena*, *Aphanizomenon* and *Oscillatoria*, produce MC as a common toxic and potentially harmful compound in the freshwater environments (Figueiredo et al., 2004). Relative proportion percent of phytoplankton taxa in this study, *Mycrocystis* and *Anabaena* species consist of 88%. Indeed, hepatoxins like MC are produced by *M. aeruginosa* (Rinehart et al., 1994).

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Therefore, two kinds of bivalves as well as aquatic organisms should be influenced by toxic stress through harmful MC compounds in our study. For that reason, we assumed that *U. douglasiae* species could not assimilate any more and excreted their prey in the form of feces and pseudofeces into the water column, demonstrating its limited ability to incorporate toxic-producing cyanobacteria cells, while we found that *S. arcaeformis* bivalves continuously assimilated toxic-producing cyanobacteriacells into their body as a prey, demonstrating its ingestion of those cells (*Fig. 4*). To explain differential incorporated atom % between each bivalves, toxic cyanobacteira bioaccumulation and tolerance need to be considered (*Fig. 4*), because toxic cyanobacteria species, likes a *Mycrocystis* and *Anabaena*, impact on prey ingestion rate of bivalve (Bontes et al., 2007). Bioaccumulation pattern of cyanobacterial toxins was different according to bivalve species. Yokoyama and Park (2002) reported that MC concentration in *U. douglasiae* was higher than *S. woodiana* and *C. plicata* bivalve, indicating that some kinds of bivalves are quite tolerant to cyanobacterial toxins and are able to depurate these cyanotoxins efficiently. It is known that MC can conjugate with glutathione (GSH) and ultimately degrades to MC-Cys and increase water solubility, consequently reducing the toxicity and enhancing excretion of MC (Kondo et al., 1992). In this study, *S. arcaefomis* bivalves showed higher assimilation rate, comparing to *U. douglasiae* species, it means that they tend to depurate these cyanotixins efficiently, or excrete to out-interior as a form of feces and pseudofeces rather than *U. douglasiae* species. However, we did not consider excretion without digestion and assimilation because incorporated C and N atom % was continuously enriched in *S. arcaefomis* which means to assimilate toxic cyanobacteria cells as a their prey (*Fig. 4*). Therefore, it seems that *U. douglasiae* slowly expel the toxic cyanobacteria cells that are not ingested and digested in stomach and digestive gland. Therefore, our results demonstrate that *S. arcaefomis* bivalves would ingest newly photosynthesized toxic cyanobacteria cells into its body, but that it may have an immunological system to depurate toxic microcystin, in contrast to *U. douglasiae*.

In this study, ^{13}C and ^{15}N atom % of five different organs (muscle, digestive gland, mantle, gill and stomach) among the three bivalves were determined during the experimental period. The incorporation rate which means synthesized newly tissue through the isotopically enriched new diet reflects the metabolic breakdown of old tissue synthesized during feeding on a previous diet and its subsequent replacement by tissue synthesized from the new diet. It is widely accepted that enrichment processes are tissue-specific. Raikow and Hamilton (2001) demonstrated that stable isotope enrichment of tissues (gland and muscels) in unionid bivalves reflected short-term and long-term assimilation of nutrients. Furthermore, differences in isotopic ratios for each tissues and turnover rates have been observed in mussel *Pecten masimus* (Lorrain et al., 2002) *and Mytilus galloprovincialis* (Deudero, 2009). In this study, bivalves showed a different isotopic enrichment among the tissues, showing more enriched in the digestive gland and stomach organ during entire experiment period because those organs have relatively faster turnover rate and reflects short-term assimilation records (Raikow and Hamilton, 2001) (*Figs. 5* and *6*).

Indeed, muscle organ of bivalves showed lower enriched atom % than digestive gland organ because of slower turnover and relatively long-term integration of energy source (Raikow and Hamilton, 2001). The mantle and gills tissues indicated relatively low incorporation rate because these organs have slow turnover rates integrating diet isotopic signatures over a longer time period than other tissues such as digestive gland

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which has faster turnover rates (Lorrain et al, 2002). Also, Hill et al. (2009) reported that mantle tissue replaced its carbon isotopes faster than gill parts, while gill tissue was faster than adductor muscle in the mussel *Perna perna* because mantle is the main energy source for sustaining gonadal development.

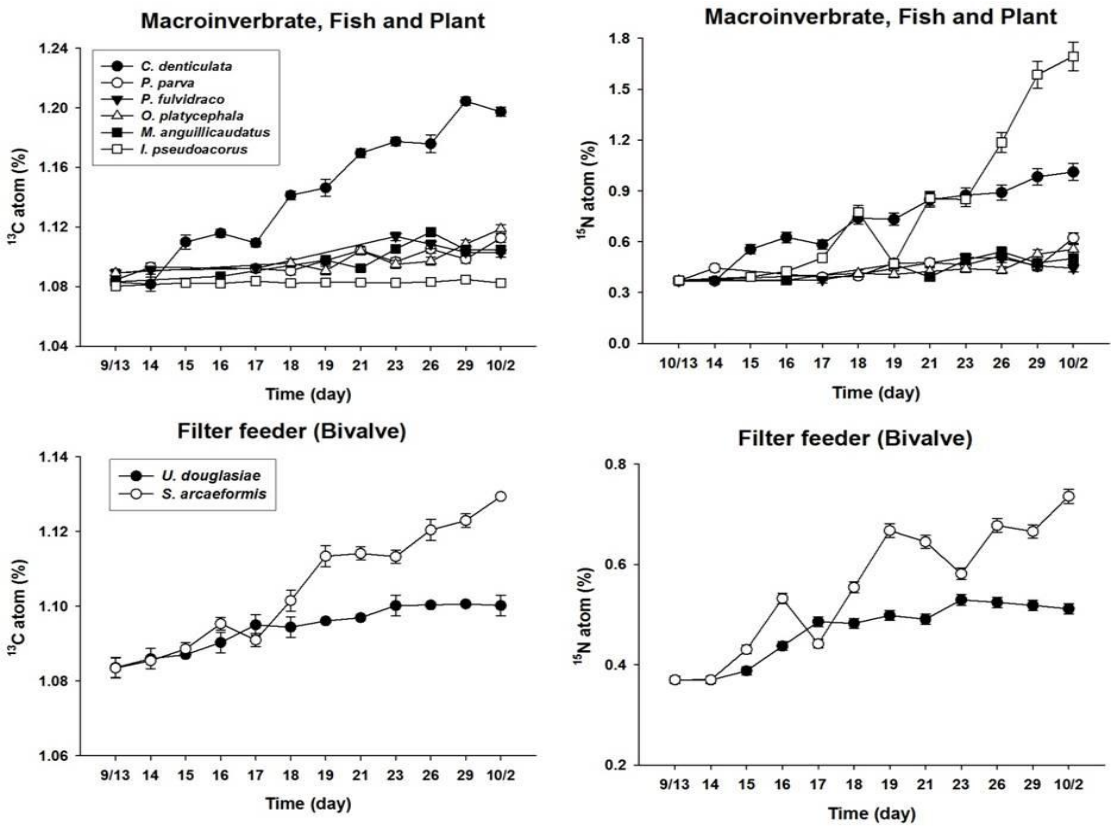

Figure 4. *Incorporation of ^{13}C and ^{15}N atom (%) into predator (fish and macroinverbrate) and plant in manipulated pond during study period*

From a management perspective, freshwater bivalves are efficiently filter feeders capable of depleting suspended particles in water column (Kim et al., 2011), so it may be a feasible method for water quality control (Reeders and Bij de Vaate, 1990; Soto and Mena, 1999). Among the freshwater bivalves, the zebra mussels (*D. polymorpha)* showed higher clearance rate in single cells of the *Microcystis* (Baker et al., 1998), a reverse gradient exists between those mussel densities and the cyanobacterial biomass (Ibelings et al., 2003), so those mussels could be used as a potential tool in the biomanipulation of shallow lakes, suffering from harmful cyanobacterial blooms (Reeders and Bij de Vaate, 1990). However non-native mussel species (*D. polymorpha*) threat to native mussel because invasive species often differ greatly from native species in resource use and trophic interactions, they have great potential to negatively affect ecosystems (Naddafi et al., 2007). Therefore, we sure that for shallow lakes or reservoirs with soft substrates, unionids are better adapted to these habitats than zebra mussels that hard substrate for settlement. For that reason, we recommend the native bivalve species, likes unionids, than famously invasion species (*D. polymorpha*) for biomanipulation on harmful cyanobacteria bloom in lakes or reservoirs. Unionid

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mussels can filter large amounts of the water column, as long as their biomass is large (Vaughn et al., 2004). However, the research of common native freshwater bivalve (unionid mussels, like *Sinanodonta* sp. and *Unio* sp.), especially toxic cyanobacteria bloom conditions, was very unique except below citation. Therefore, our result is very important information to evaluate bio-control effects. Yokoyama and Park (2002) revealed that *Sinanodonta* bivalves persisted to toxic cyanobacteria bloom and showed lower bioaccumulation patterns of MC, and *Sinanodonta*'s grazing capacity, per individual, is equal or higher than *D. polymorpha* (Bontes et al., 2007). For example, *Sinanodonta*bivalves showed ×12 higher efficiencies of clear unit per square meter per unit of water volume than *Dreissena* mussels (Pires et al., 2005; Bontes et al., 2007). *Sinanodonta* bivalves had a larger effect on the primary productivity of toxic cyanobacteria, demonstratinga more realistic achievement for biomanipulation. Also, *Sinanodonta* (*S. arcaefomis* and *S. woodiana*) should incorporate more toxic cyanobacteria cells than *U. douglasiae*, probably due to its larger detoxification capacity (Kim et al., 2017b). As a result, *S. arcaefomis* and *S. woodiana* could be useful organisms to reduce massive toxic cyanobacteria blooms in eutrophic agricultural reservoirs and lakes.

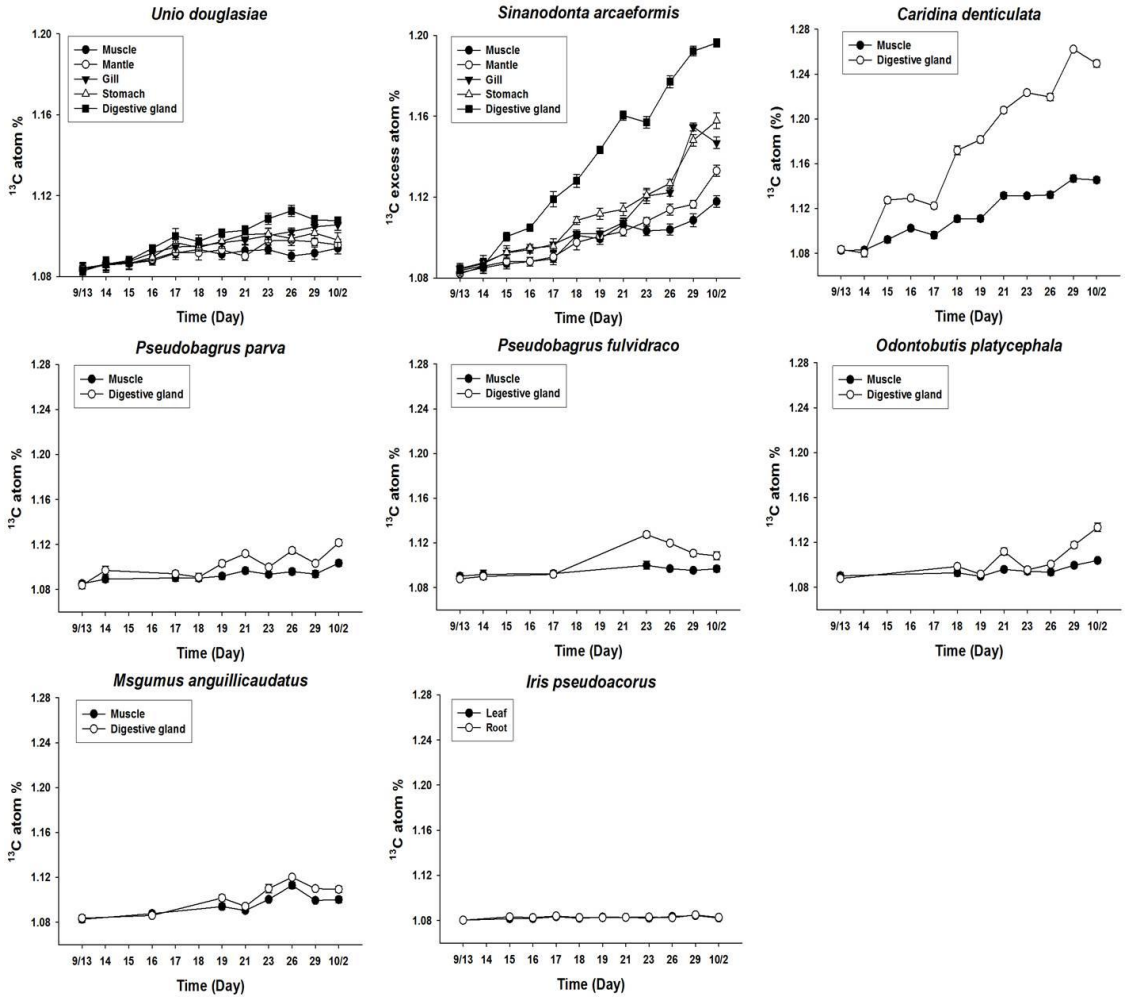

Figure 5. *Incorporation of ^{13}C atom (%) into part of aquatic organisms tissue in manipulated pond during study period*

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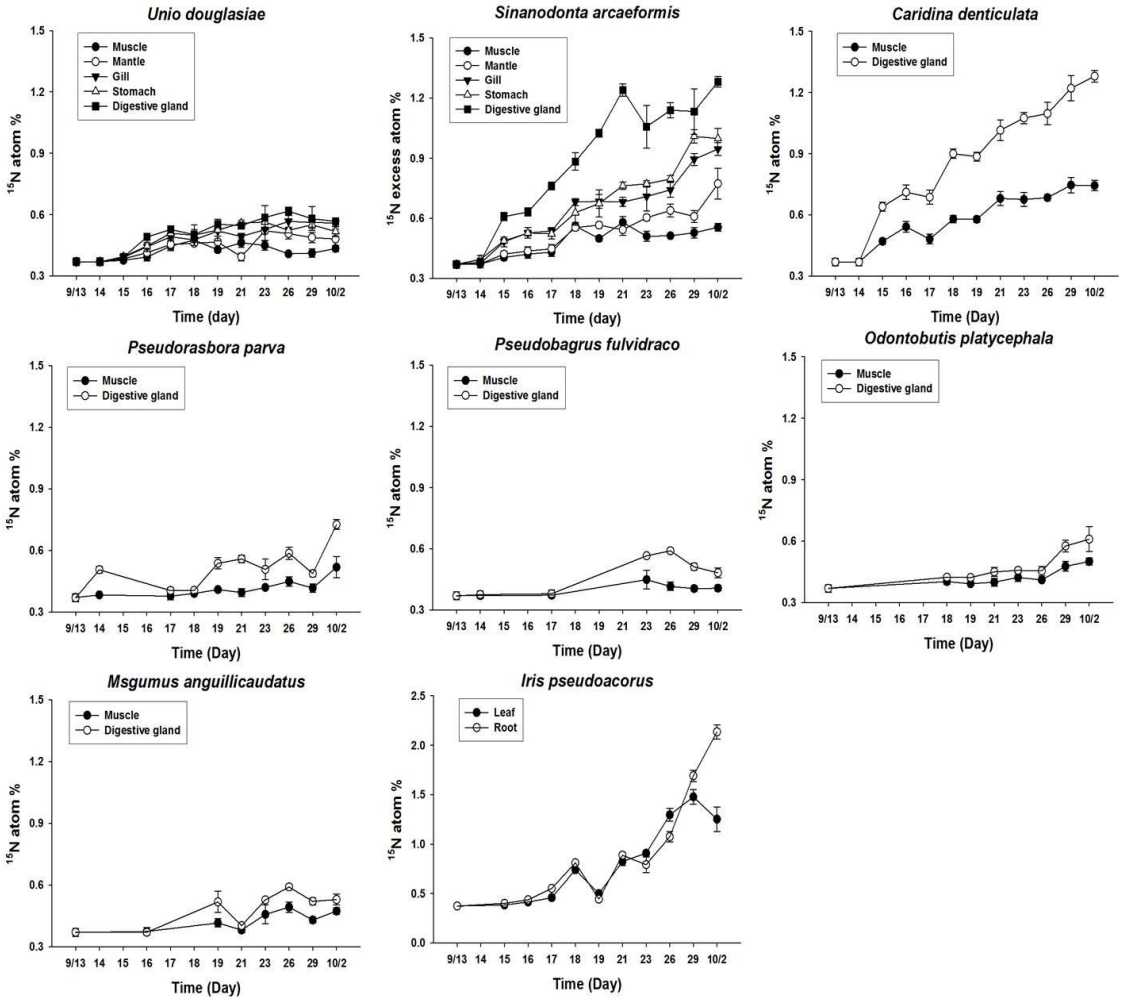

Figure 6. *Incorporation of ^{15}N atom (%) into individually part of aquatic organisms tissue in manipulated pond in Sukmoon wetland during study period*

Assimilation correlation between macroinvertebrate and various fish species

In the past study, *C. denticulate* did not easily uptake cyanobacteria as a prey because it contained poor nutrition and toxicity for aquatic invertebrates (Proter and Orcutt, 1980). However, our data appears that *C. denticulate* accumulated large amounts of isotopically enriched ^{13}C and ^{15}N prey cell, comparing to planktivore, omnivore, carnivore fishes and other bivalve species (*Fig. 4*). The enriched ^{13}C and ^{15}N of *C. denticulate* in our study, we could assume that *C. denticulate* also continuously assimilated enriched cyanobacteria species as a diet. However, we couldn't convince that *C. denticulate* uptake to *M. aeruginosa*, because *C. denticulate* may be to expel the toxic cyanobacteria cells that are not ingested and digested in stomach and digestive gland, in the form of feces and pseudofeces, which is released into the water column. If so be that the *C. denticulate* undigested foods and released into the water column, the ^{13}C and ^{15}N atom % probably highly enriched in digestive gland tissue and slightly enriched in their muscle tissue, due to hardly transport energy from digestive gland to muscle tissue. However, the ^{13}C and ^{15}N atom % of both digestive gland and muscle tissue in *C. denticulate* always clearly enriched until ending of experiment (*Fig. 4*), it is

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indicate that *C. denticulate* fed upon their enriched *M. aeruginosa* through digestion and assimilated energy in their body.

As a food source, cyanobacteria accumulations in *C. denticulate* may not only include the transfer of C and N, but also the transfer of cyanobacteria hepatotoxins or related compounds. Despite toxic cyanobacteria blooms influence aquatic organisms in manipulated pond during the study period, how *C. denticulate* could show the highest ^{13}C and ^{15}N values except aquatic plant for ^{15}N atom % value (*Fig. 4*). Some reports introduced detoxification that the organism enzymatically can form a conjugate of the hepatotoxin, which helps the organism to survive under cyanobacterial stress (Pflugmacher et al., 1998). This detoxification occurs in various aquatic organisms such as macrophytes, bivalves and fishes (Wiegand et al., 1999). However, toxic inhibition enzyme is still unclear in the macroinvertebrate. Our results may offers new possibility that *C. denticulate* have an immunological system to depurate toxic microcystin, due to actively assimilate toxic cyanobacteria as shown higher ^{13}C and ^{15}N atom % values. Therefore, *C. denticulate* is key-stone taxa that are capable of removing cyanobacteria algal populations. Many research supported our hypothesis that macroinvertebrate was possible for bio-control on massive toxic cyanobacterial bloom through laboratory and field study. An et al. (2010) supported that macroinvertebrates, *C. denticulate*as a same species of our study and *P. paucidens*, were effective candidates for phytoplankton control, compared to fishes in *M. aeruginosa* bloom condition through mesocosm experiment, and Kim et al. (2017a) reported that *C. denticulate* actively assimilated toxic *M. aeruginosa* through stable isotope tracer experiments. Moreover, mysid shrimps (*M. relicta*) showed high survival in the presence of toxic cyanobacteria through laboratory experiment (Engstrom et al., 2002), *M. mixta* didn't showed to increase mortality during experiment period where mysids were exposed to high concentrations of toxic *N. spumigena*, which suggests that they may be tolerant against cyanobacterial toxins (Engstrom et al., 2001). Therefore, we suggest that *C. denticulate* is veryeffective candidates for inhibiting of toxic cyanobacteria blooms, may be achieve favorable responses, such as a reduction in algal biomass and attainment of clear water.

As a management perspective, it is very important to control cyanobacterial blooms to reduce their harmful effects on aquatic ecosystems. In previously, it has proved that filter-feeding fish, such as silver carp (*H. molitrix*), bighead carp (*A. nobilis*) and tilapia (*O. niloticus*), are effective candidates for bio-control of plankton communities to eliminate cyanobacteria, but it is consider that amount of feces by various fishes were also excreted into water column which leads to excessive nutrient loading problem. Indeed, it has a problem about invasion species and it is difficult to sustain and stabilize fish and zooplankton community through feeding mechanism by biomanipulation, probably owing to the complexity of natural ecosystems. Therefore, we recommend that *C. denticulate*, which is native species, can apply to control toxic cyanobacteria blooms as a newly candidate bio-control species. The *C. denticulate* species could be a useful aquatic organism for bio-control through feeding activity, and are efficient filter feeders capable of depleting suspended particles, including phytoplankton, zooplankton, detritus and other abiotic particles in water column.

Freshwater fishes including *O. platycephla, P. fulvidraco* (carnivore fish), *P. parva* (planktivore fish) and *M. anguillicaudatus* (omnivore fish) had a lower atom % value rather than bivalves and marcoinvertebrate species (*Fig. 5*). *P. fulvidraco* and *O. platycephla* species is a carnivore fish in stream in Korea, feeding mainly on insect, invertebrate and small fishes (Han et al., 2001; Iwata et al., 1988). ^{13}C and ^{15}N atom %

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of *P. fulvidraco* slightly enriched owing to those consuming ^{13}C and ^{15}N incorporated invertebrate such as *C. denticulate* and zooplankton, but had a lower values than their prey during the entire experimental period. Furthermore, ^{13}C and ^{15}N atom % in *P. fulvidraco* were decreased distinctly after 26th October, while those in *O. platycephla* increased (*Fig. 6*). *M. anguillicaudatus* as a omnivorous fish is a bottom-dwelling scavenger, feeding mainly on organic material, tubifex worms and other small aquatic organisms (Kim et al., 2002). *P. parva* is a widely distributed planktivore in the littoral zones of freshwater ecosystem in China, Japan and Korea (Masuda et al., 1988). We expected that ^{13}C and ^{15}N atom % of *P. parva* and *M. anguillicaudatus* might be increased during the study period because their prey is mainly enriched phytoplankton or deposition organic matter, but ^{13}C and ^{15}N atom % in *P. parva* slightly increased while it was decreased after 29th October in *M. anguillicaudatus*. These results demonstrate that it slowly assimilate their prey, because toxic microcystins in cyanobacteria may to influence on feeding capacity in fishes. Bioaccumulation of microcystins in fish has been well documented (Malbrouck et al., 2006). Therefore, ^{13}C and ^{15}N atom % of all fishes showed lower assimilation rate, compared to those in bivalve, zooplankton and macroinvertebrate during the entire experimental period. Xie et al. (2005) found that the large amount of microcystins in *H. molitrix*as a phytoplanktivorous fish was detected in the intestines, also Specziár et al. (1997) reported that cyanobacteria *M. aeruginosa* was more abundant in phytoplanktivorous than omnivorous fish, since omnivorous fish normally consume zooplankton and detritus. Among the fishes in our study, planktivore and omnivore fishes consumed mainly phytoplankton or organic matter, so they may be affected directly by toxic cyanobactria. Therefore, we suggest that these fishes couldn't directly control massive algal bloom, even though some filter feeding silver carp and bighead carp (*Aristichthys nobilis*) fishes drastically reduced cyanobacteria (*M. aeriginosa*) bloom (Miura, 1990). In this study, we proposed that in applied biomanipulation, fish reductions may have positive effects on phytoplankton reduction and increased water clarity in dependent of their effects on zooplankton grazing, because they may influence on cyanobacteria bloom through P-supply, due to either by excretion or by sediment bioturbation.

Impact of floating macrophytes on cyanobacteria community

Here, as a floating aquatic plant *I. pseudoacorus* which is a part of the freshwater ecosystem, was added in manipulation pond to reduce cyanobacteria bloom. In this study, *I. pseudoacorus* species showed 1.07~1.08 ^{13}C and 0.37~2.13 ^{15}N atom % respectively, in manipulated pond (*Figs. 5* and *6*). It was hardly enriched ^{13}C atom % because plant used carbon dioxide from the atmosphere through their leaf. However, it showed higher ^{15}N atom % value (*Fig. 4*) and decreased TN concentration in manipulated pond because of absorbing the nutrients by *I. pseudoacorus* in water column, thus continuously inorganic nitrogen is accumulated in their leaf and root (*Table 1*). Macrophytes can assimilate preferential nutrients, like nitrate and phosphate, from the water column, because floating macrophytes maintain a competitive advantage over microalgae through increased nitrogen uptake (Barko and James, 1998). However, nutrient availability in macrophyte beds may depend on both the macrophyte species and density (Sondergaard and Moss, 1998). In this study, the floating *I. pseudoacorus* covered about 10% of the water surface area. So, we consider self-shading effects, especially floating macrophytes, that has been shown to significantly reduce phytoplankton abundance in vegetated areas because of lower light irradiation to the

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water column under the floating macrophytes (Ozimek et al., 1990). Also, the chemical substances excreted from macrophytes have long been suspected to suppress phytoplankton growth (Hutchinson, 1975). Some studies have reported that the production and excretion of alleochemicals by aquatic macrophytes may be an effective defense strategy against other photosynthetic organisms, like epiphyton and phytoplankton, in the competition for light and nutrients (Gopal and Goel, 1993). For the above reason, our results assumed that the growth of phytoplankton could be regulated by aquatic plants, which is likely to plan an important role in the suppression of phytoplankton biomass, so floating plant (*I. pseudoacorus*) should be possible manipulation to control massive cyanobacteria blooms.

Influence on zooplankton (copepod) feeding process through bio-control

Our data showed that cell density of zooplankton decreased from 12,667 to 2,769 cells mL^{-1} and from 13,001 to 795 cells mL^{-1} in reference and manipulated pond, respectively during study period (*Fig. 2*). The reason for decreasing cell density of zooplankton in reference pond might be toxic cyanobacteira effects which reduce grazing pressure by zooplankton (DeMott et al., 1991), while aquatic organisms such as fishes can influence zooplankton biomass as a prey in manipulated pond. Although the cell density of zooplankton dramatically decreased was in manipulated pond, the ^{13}C and ^{15}N atom (%) of copepoda showed higher value than reference pond (*Fig. 3*). Why dietary assimilation rate of copepoda in manipulated pond was increased than reference pond even though zooplankton had a pressure by aquatic organisms? The cyanobacteria are inedible prey size, mostly colonial or filamentous, for most species of zooplankton, and only small colonies or dispersed cells of cyanobacteria can be ingested (Jarvis et al., 1987). For this reason, it is generally assumed that aquatic organisms may induce shortened breakage cells of larger filaments of cyanobacteria species through the feeding process, allowing the later consumption of cyanobacteria by zooplankton species. It is well known that each zooplankton species differs in its selective feeding patterns depending mainly on prey size (Pagano et al., 1999). Eventually, there is the possibility of breakage of filaments by some aquatic organisms (e.g., bivalve, macroinvertebrate, various fishes) in this study, with suggests that inedible cyanobacterial filaments may be changed efficiently grazing small size and edible particles by zooplankton species (e.g., copepod). In this study, zooplankton species consumed breaking down of cyanobacteria aggregate even though cyanobacteria has a toxic effect to zooplankton, demonstrating enriched ^{13}C and ^{15}N atom (%) of zooplankton species in manipulated pond. Therefore, bio-control using aquatic organisms could be change shape and size of cyanobacteria aggregates through their feeding activity, thus could change grazing efficiency of zooplankton.

Ecosystemic effects

Methods for the purification of contaminated water using various kinds of organisms are within the category of ecological engineering water quality improvement techniques, including aquatic vascular plants, algae, zooplankton, and fish and shellfish. From the viewpoint of the living organism, the technology of improving the water quality by using organisms is to grow by absorbing the nutrients. However, from the viewpoint of the user, it is possible to expect a more economical, long-term and environmentally friendly effect as compared with the engineering technique. Especially,

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development of submerged plants can contribute to the improvement of water quality by not only eliminating nutrients but also by providing habitat useful for zooplankton and invertebrates through feeding on algae. In addition, the installation of the artificial plant island can partially inhibit the light, thereby contributing to the suppression of the occurrence of algae, and has various indirect effects such as an aesthetic point of view. Therefore, it is considered that the development and establishment of habitat of useful organisms is very necessary for water quality improvement. Also water quality improvement through the use of biological food webs is to inhibit algae growth by increasing algae feeding rates using organisms with higher rates of algae feeding (such as bivalves, shrimps, etc.). This will be more effective in connection with the development and installation of the useful biological habitat described above.

Conclusion

As a filter feeding bivalve groups, *S. arcaeformis* has a larger feeding capacity rather than *U. douglasiae* on cyanobacteria bloom condition. Also, macroinvertebrate (*C. denticulate*) continuously assimilated their diet through detoxification for toxic prey quality. Furthermore, aquatic plants (*I. pseudoacorus*) compete nutrients uptakes and light availability with cyanobacteria in the water column. This study assumed that zooplankton (Copepoda) consumed small particles of cyanobacteria which is induced by shortened breakage cells of longer filamentous cyanobacteria by aquatic organism through the grazing behavior. However, various kind of fishes (carnivore; *P. fulvidraco*, *O. platycephala*, planktivore; *P. parva*, omnivore; *M. anguillicaudatus*) hardly feed on toxic cyanobacteria directly. Especially, development of submerged plants can contribute to the improvement of water quality by not only eliminating nutrients but also by providing habitat useful for zooplankton and invertebrates through feeding on algae. Furthermore, if an aquatic plant that can not only remove nutrients but also provide habitats to aquatic organisms (zooplankton, bivalves and shrimps) is developed, it can help control toxic cyanobacteria blooms. Therefore, it is considered that the development and establishment of habitat of useful organisms is very necessary for water quality improvement. Our biomanipulation technique may provide a key tool for efficient management and restoration of eutrophied reservoirs.

Acknowledgements. This work was supported by the National Institute of Environment Research R&D of Korea government (NIER) and National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2016R1E1A1A01943004).

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