APPLICABILITY EVALUATION IN THE PHYSIOLOGICAL ENERGETICS METHOD OF MUSSELS AS A BIOMANIPULATION TOOL

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APPLICABILITY EVALUATION IN THE PHYSIOLOGICAL ENERGETICS METHOD OF MUSSELS AS A BIOMANIPULATION TOOL

By

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A Thesis Submitted
In Partial Fulfillment of the Requirements
For the Degree of
Doctor of Engineering

Examination Committee:

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To the
DEPARTMENT OF URBAN AND ENVIRONMENTAL ENGINEERING
GRADUATE SCHOOL OF ENGINEERING
KYUSHU UNIVERSITY
Fukuoka, Japan
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CERTIFICATE

The undersigned hereby certify that they have read and recommended to the Graduate School of Engineering for the acceptance of this thesis entitled, ‘‘***Applicability Evaluation in the Physiological Energetics Method of Mussels as a Biomanipulation Tool***’’ by **Yuxian LIU** in partial fulfillment of the requirements for the degree of **Doctor of Engineering**.

Dated: October, 2014

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ABSTRACT

The research goals of the dissertation are directed toward the applicability evaluation in the physiological energetics method of the mussel *Anodonta woodiana* as a biomanipulation tool in Chinese Lake Taihu.

In **Chapter 1**, through literature review and the field investigation, the information of Lake Taihu was obtained. Then the alleviating eutrophication methods applied in practice and deficiency were summarized, furthermore, the research of biomanipulation methods and the application prospect of freshwater mussels were mentioned. Accordingly, the research objectives in this dissertation were proposed as: to clarify the feeding behavior of the mussel *A. woodiana* in freshwater ecosystems, especially on toxic *Microcystis* spp., which are the dominant algal species in Lake Taihu when algae blooming occurs; meanwhile, to evaluate the effect of toxic *Microcystis* spp. on *A. woodiana* in the physiological energetics method - scope for growth (SFG); furthermore, to clarify the interactions of *A. woodiana* and submerged macrophytes. And all these results can contribute to our understanding of the integrated ecological role of the mussel *A. woodiana* in the eutrophic lake, in addition the applicability of the mussel *A. woodiana* as a biomanipulation tool in Lake Taihu restoration.

In **Chapter 2**, the physiological evaluation method used in this research, SFG was explained, in which the significance, calculation methods, affecting factors were summarized.

As an important part of SFG, feeding behavior and the affecting factors of mussels were examined and further discussed in **Chapter 3**. The results in this chapter indicated that the filtration rates increased as the increase of algae concentrations till the critical cell density was reached, at which the pseudofaeces were expelled; above the critical cell density, the filtration rates decreased sharply as the algae concentration increased. The mussel *A. woodiana* reached the critical cell density at 4.3×10^7 cells mL^{-1} and 3.4×10^6 cells mL^{-1}, when the temperature was 20℃ and 25℃, respectively in this experiment.

In **Chapter 4**, a comparative study was carried out on the acute physiological responses to variable microalgae diets including toxic microcystins (MCs)-producing cyanobacteria *Microcystis aeruginosa* and non-toxic green algae *Scenedesmus obliquus*. The results showed that compared with the green algae *S. obliquus*, the mussel *A. woodiana* has a higher grazing ability on the toxic *M. aeruginosa*; furthermore, the effects of different algae diets on SFG of *A. woodiana* demonstrated that the toxic *M. aeruginosa* may supply 4.5 times more energy for *A. woodiana*'s potential growth than that *S. obliquus* could do.

In **Chapter 5**, the interactions between *A. woodiana* and algae blooming water were

examined. The 6-day feeding responses experiment was carried out with naturally blooming pond water and the mussels in the laboratory. The results indicated that toxic *Microcystis* spp. of colony and unicell in natural eutrophic water can be removed greatly by *A. woodiana*; moreover, the toxic *Microcystis* spp. were found to supply about 1.5 times more energy for *A. woodiana*'s potential growth after 6-day exposure, thus the mussels have the strong adaptation ability when they were exposed to toxic natural eutrophic water.

In **Chapter 6**, the mussels demonstrated a strong survival ability during exposure to natural eutrophic water containing high concentrations of MCs for 12 days. In order to clarify the survival mechanisms (feeding selective and detoxification mechanisms), all the conducted experiments from **Chapter 4** to **6** were summarized. A correlation analysis between the diet characters and mussels' physiological rates was performed and the results suggested that (1) filtration selectivity factors - MCs did not restrain the feeding behavior of *A. woodiana*; instead the exorbitant initial diet concentration could inhibit the filtration rates; (2) absorption selectivity factors - probably it is the different digestive enzyme activity in *A. woodiana*'s digestive tract that induced the prefer ingestion of *M. aeruginosa* to green algae cells, indicated by about 3 times higher absorption efficiency of *M. aeruginosa* than that of the green algae cells; (3) the possible detoxification process - in the liver of mussels, through the MCLR-Cys (cysteine conjugates of MCs) formation, MCs could be detoxified and then they were transferred to kidney and were excreted in the form of ammonia. In addition, more MCs were metabolized efficiently rather than accumulated in the liver, thus the MCs toxicity effect in *A. woodiana* did not reveal.

In **Chapter 7**, the experiments were performed with the mussel *A. woodiana* combined with the submerged macrophyte *Vallisneria asiatica* to figure out the interactions between them. The results showed that (1) the beneficial effect associated with mussels culture on the aquatic ecosystem depends on having aerobic sediments and thus mussels stocking density should be in a range named "ecological carrying capacity"; (2) the presence of submerged macrophytes can supply the required aerobic condition for mussels to play the effective role as a biomanipulation tool in the eutrophic freshwaters; (3) for the restoration of submerged macrophytes in the littoral zone, the sediment characteristics, such as composition and grain sizes, as vital factors need to be taken into account; (4) turbidity caused by the wind-induced sediment disturbance can be improved significantly with the existence of mussels, which can increase the possibility of successful restoration of submerged macrophytes.

Finally, in **Chapter 8**, the summary of the thesis and general conclusions were shown. In addition, the integrated ecological restoration method was proposed to be applied in Lake Taihu.

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I wish to thank Dr. SAKAI from Tokyo University, who has helped me measure the microcystins concentration in water samples, without which the scientific journal published on JSCE (Japan Society of Civil Engineering) could not be accepted.

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CHAPTER 1

General Introduction

Abstract

In recent years, the rapid economic development in the Taihu basin, mainly driven by increasing resource input and workforce, has resulted in overconsumption of natural resources. The natural environment of Lake Taihu has already deteriorated sharply, water pollution and eutrophication have become serious, which has become a big obstacle for the lake services, such as the drinking water resources. In order to improve water pollution and restore healthy aquatic ecology in Lake Taihu, in this chapter, firstly, the field investigation was conducted and the results indicated that water quality of East Taihu Lake is relatively healthy and there are abundant aquatic macrophytes communities, which can prevent overgrowth of algae, contribute to the increase of ecological system self-purification ability, and can keep the aquatic ecosystem in a clear water state at relatively high nutrients; however, in Meiliang Bay and the west coast, water pollution has become a serious problem, resulting in the disappearance of aquatic macrophytes communities. Algal blooms in these water areas have become increasingly evident due to nutrients inflow from the surrounding farmlands. In China, lake restoration from eutrophication always follows a process of source control, ecological restoration and catchment management. Thus, all the methods applied in Lake Taihu were summarized and the problems which are still existing were highlighted. Thereafter, the mussel was suggested as a potential tool to control eutrophication. Therefore, research goals in this study are directed toward the applicability evaluation of the mussel *Anodonta woodiana* in a physiological energetics method as a biomanipulation tool in Chinese Lake Taihu.

1.1 AN INTRODUCTION TO LAKE TAIHU

1.1.1 Hydrology and Drainage Basin

Lake Taihu, as the third largest freshwater lake in China, is in the middle to lower valley of the Changjiang River. At 30°55′40″-31°32′58″ N and 119°52′32″-120°36′10″ E, in the board Taihu plain, Lake Taihu is on the south side of the Changjiang delta. With the mean water depth as 1.9 m, Lake Taihu has the total area of 2,427.8 km^2, in which about 89.7 km^2 is occupied by 51 islands and islets, and the actual water area is 2,338.1 km^2. (**Sun and Rui, 2008**)

Lake Taihu is a typical shallow lake and the morphological characteristics of Lake Taihu are summarized in **Table 1-1** (**Sun and Rui, 2008**). From natural and anthropogenic causes, Lake Taihu has five bays: East Taihu Lake, Xukou Bay, Gonghu Bay, Meiliang Bay, and Zhushan Bay from east to west (**Sun and Rui, 2008**).

Table 1-1. Morphological characteristics of Lake Taihu[1,2] (**Sun and Rui, 2008**).

Catchment area (km^2)	Water area (km^2)	Lengh (km)	Width (km)	Mean depth (m)	Maximum depth (m)	Volume ($\times 10^8$ m^3)	Annual transfer coefficient
36,895	2,338.1	68.5	34	1.9	2.6	44.3	1.2

[1] The data were obtained by direct measurement on the topographic map produced in the 1980s.

[2] Excluding Wuli Bay, which is 5.7 km^2.

1.1.2 Development of the Water Quality in Lake Taihu

According to the Chinese Surface Water Quality Standard GB 3838-2002 (**CNEPA, 2002**), water quality is classified into five categories. The ranking is organized in order of increasing pollution levels (based on the criteria listed in **Table 1-2**): from Class Ⅰ to Ⅴ, indicating good, fairly good, slightly polluted, poor and hazardous. Class Ⅱ and Ⅲ water are suitable for drinking, swimming, and household use; Class Ⅳ water can be used for industry; Class Ⅴ water can only be used for agriculture irrigation. In the 1960s Lake Taihu was a Class Ⅰ-Ⅱ water body and then water quality severely deteriorated. As data from State Environmental Protection Bureau indicated, every 10 years, the water quality in Lake Taihu dropped by one grade (**State Environmental Protection Bureau, 2000)**. And by the year 2007, eutrophic and inferior Class Ⅴ water accounted for 83.5% of the total surface area of the lake (**Liu and Qiu, 2007**).

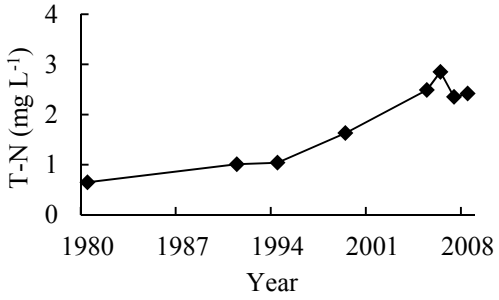


Fig. 1-1. T-N concentration annual changes. (**Hao et al., 2014**)

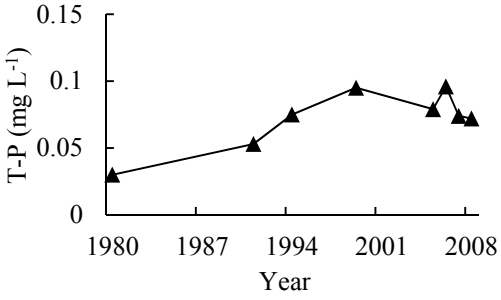


Fig. 1-2. T-P concentration annual changes. (**Hao et al., 2014**)

Table 1-2. The criteria of surface water quality for lakes or reservoirs (**CNEPA, 2002**).

Items	Surface water quality classification				
	Class Ⅰ	Class Ⅱ	Class Ⅲ	Class Ⅳ	Class Ⅴ
Water tempearature (℃)	Maximum week increase≤1		Maximum week increase≤2		
pH	6～9				
DO (mg L^{-1})	Satuation≥90%	≥6	≥5	≥3	≥2
COD_{Mn} (mg L^{-1})	≤2	≤4	≤6	≤10	≤15
COD_{Cr} (mg L^{-1})	≤15	≤15	≤20	≤30	≤40
BOD_5 (mg L^{-1})	≤3	≤3	≤4	≤6	≤10
T-N (mg L^{-1})	≤0.2	≤0.5	≤1.0	≤1.5	≤2.0
NH_3-N (mg L^{-1})	≤0.15	≤0.5	≤1.0	≤1.5	≤2.0
NO_2-N (mg L^{-1})	≤0.06	≤0.1	≤0.15	≤1.0	≤1.0
T-P (mg L^{-1})	≤0.01	≤0.025	≤0.05	≤0.1	≤0.2
Chlorophyll a (mg L^{-1})	≤0.001	≤0.004	≤0.01	≤0.03	≤0.065
Transparency (m)	≥15	≥4	≥2.5	≥1.5	≥0.5
Escherichia coli (L^{-1})	≤200	≤2000	≤10000	≤20000	≤40000

DO: dissolved-oxygen; COD_{Mn}: chemical oxygen demand by K_2MnO_4 oxidation method; COD_{Cr}: chemical oxygen demand by chromium oxidation method; BOD_5: biological oxygen demand; T-N: total nitrogen; T-P: total phosphorus.

As shown in **Fig. 1-1**, from 1980 to 2008, the annual average concentration of total nitrogen (T-N) in Lake Taihu increased approximately four times from 0.65 to 2.42 mg L^{-1}, and the annual average concentration of T-P (**Fig. 1-2**) increased from 0.03 to 0.07 mg L^{-1} (**Qin et al., 2007**).

1.1.3 Water Environment Status Survey on Lake Taihu (Hao et al., 2014)

1.1.3-1 Survey areas and sampling sites

East Taihu Lake is in the southeast of Lake Taihu. Its length is 27.5 km, the maximum width is 9.0 km, the entire water area is 131 km^2, and the average depth is 1.2 m. In addition, there are abundant aquatic macrophytes as shown in **Fig. 1-3** (left). Meiliang Bay is in the north part of Lake Taihu. Its water surface area is 124 km^2 and the average depth is 1.5 m. It is also the bay with serious algal blooms. In Lake Taihu, southeastern winds are prevailing, inducing the surface water current towards Meiliang Bay, especially in summer, which triggers the serious problem of concentrated algal blooms in this water area (**Fig. 1-3**, center). Although the population is less concentrated on the west coast of the lake, there are many farmlands in this area, water contamination of inflow rivers due to over fertilization has become serious, and the ecological system is starting to be destroyed.

The survey was conducted in East Taihu Lake, Meiliang Bay and the west coast of Lake Taihu and the sampling sites were indicated in **Fig. 1-4**. During July 26 ~ 30, 2011, the survey was conducted at 17 sites from E_1-E_{17} in East Taihu Lake; 20 sites from M_1-M_{20} in Meiliang Bay and 5 sites from T_1-T_5 at the mouth of some major inflow rivers in the west coast.

Fig. 1-3. Survey site at Lake Taihu (Left: East Taihu Lake; middle: Meiliang Bay; right: west coast).

1.1.3-2 Survey and analysis methods

Survey on water quality was performed with a multi-parameter water quality meter (Hydrolab Logger DS5X). At each site shown in **Fig. 1-4**, the water temperature, pH, DO, Chlorophyll a (Chl.a), EC, and turbidity were measured at 10 cm intervals towards the direction of water depth. Meanwhile, surface water (0.1m) was collected at each site using Heyroth water sampler (250 mL) for nutrients analysis. The collected water samples were analyzed for T-N using ultraviolet spectrophotometer and T-P using spectrophotometry (**State Environmental Protection Administration, 2002**).

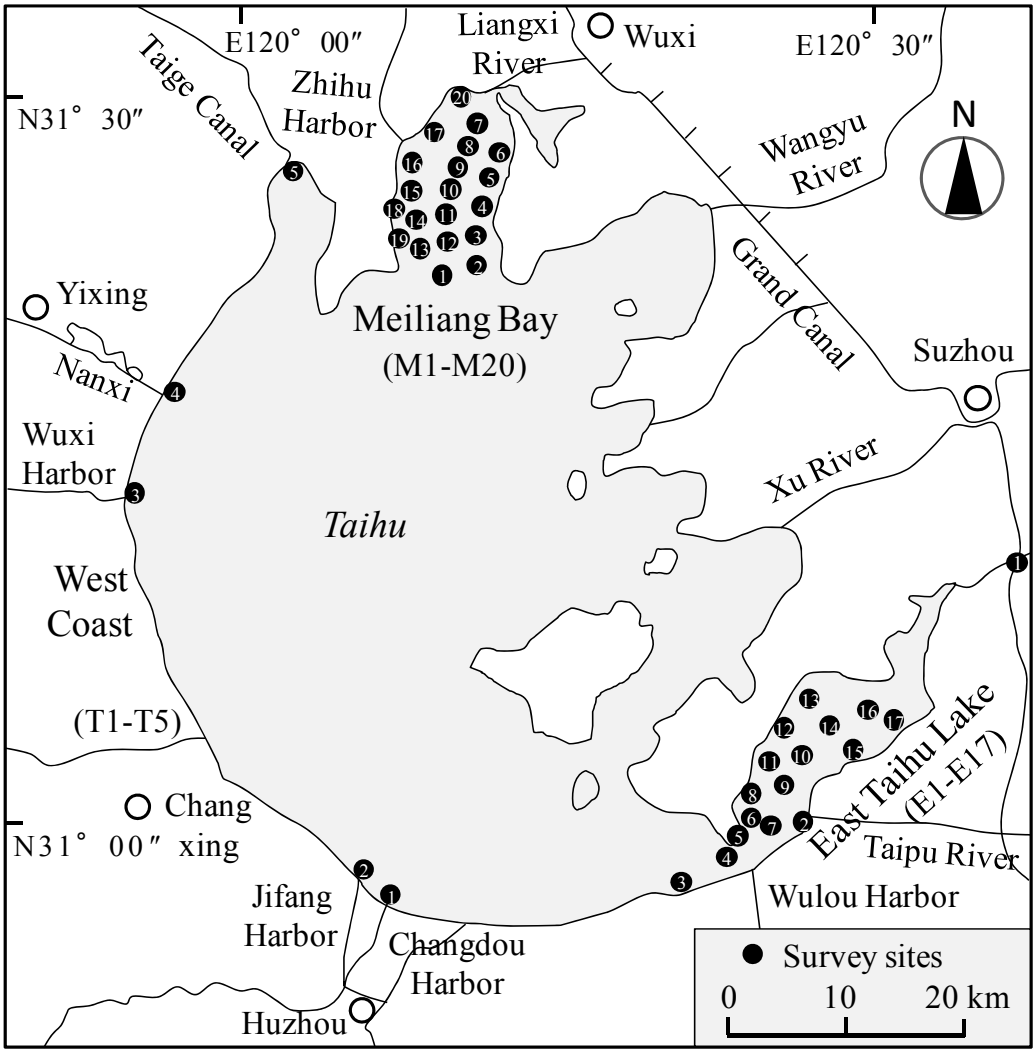


Fig. 1-4. Map of Lake Taihu and the location of sampling sites.

1.1.3-3 Water quality results and discussion

Fig. 1-5 (a)-(f) and **Fig. 1-6 (a)-(f)** show the planar distribution of water quality for surface water and bottom water of East Taihu Lake and Meiliang Bay survey sites. Since the survey was conducted in July, the water temperature **(a)** at every survey site was above 30°C. At one site near the coast of Meiliang Bay, the temperature reached to around 34°C, and algal blooms were evident in sites where water temperature was high. Because extra high water temperature is unsuited for the growth of most phytoplankton, which may be a reason for the dominance of high-temperature-tolerant *Microcystis* spp. in the waters.

pH **(b)** values were in the range of 8.0~9.8 and the water was generally alkaline.

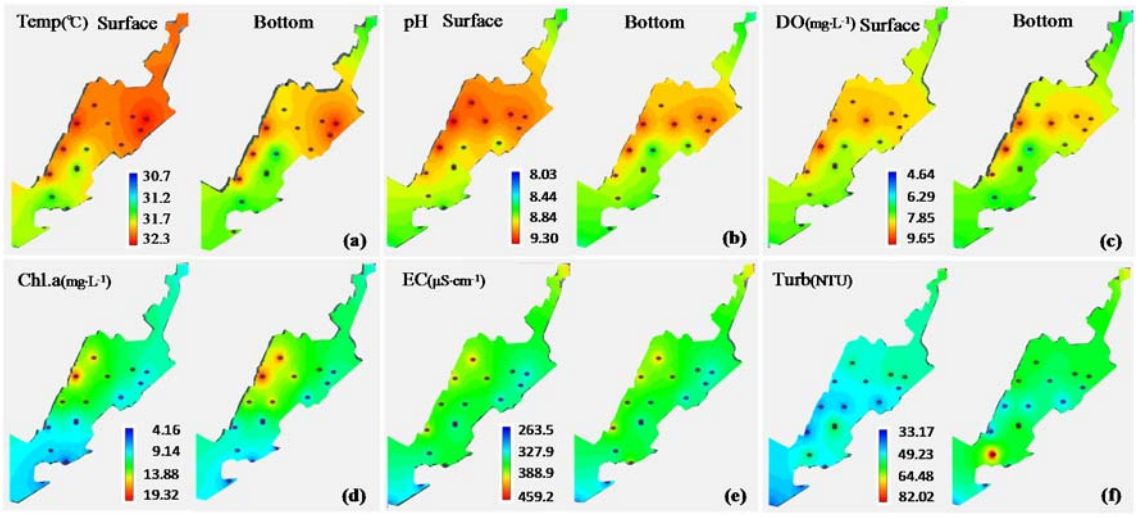


Fig. 1-5 (a)-(f). Planar distribution of water quality for surface water and bottom water at East Taihu Lake survey sites.

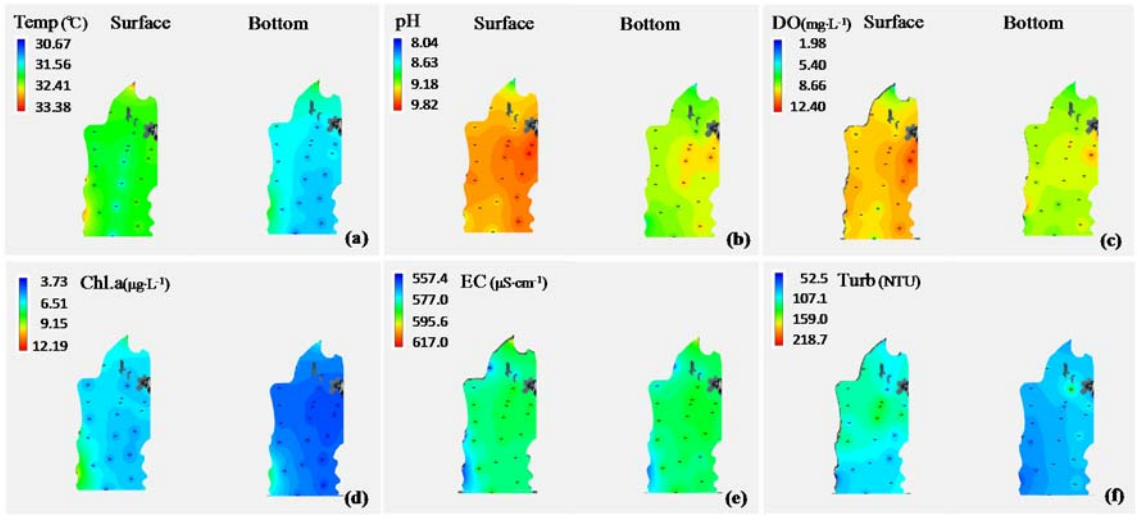


Fig. 1-6 (a)-(f). Planar distribution of water quality for surface water and bottom water at Meiliang Bay survey sites.

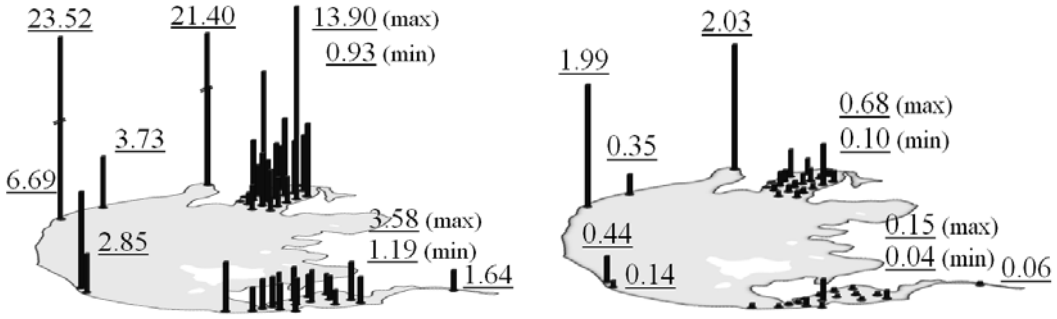
Soluble carbon dioxide (gas) in the water runs out while the pH value is rising, thus, photosynthesis of many aquatic macrophytes and phytoplankton becomes limited (**Watanabe et al., 2002**). However, *Microcystis* spp. can utilize bicarbonate ion in addition to soluble carbon dioxide (gas) (**Watanabe et al., 2002**), which probably aids *Microcystis* spp. in dominating over aquatic macrophytes and phytoplankton in the alkaline water environment.

Values of DO **(c)** for East Taihu Lake were 5.8～10.8 mg·L^{-1}, and 0.1～12.5 mg L^{-1} for Meiliang Bay, respectively. In Meiliang Bay, DO was 0.1 mg L^{-1}, and some spots

were in serious dysoxic condition, making the water environment become a place that is difficult for aquatic organisms to survive.

Values of Chl.a **(d)** in East Taihu Lake were from 2.3 to 20.5 μg L^{-1}, and there were some spots that showed high values. In Meiliang Bay, the values of Chl.a were from 4.4 to 9.6 μg L^{-1}, and they were generally the same between different spots. Chl.a is an important parameter which can reflect the eutrophication situation of Lake Taihu (**Qian and He, 2009**). The concentration of Chl.a was relatively higher of 9.8 μg L^{-1} for East Taihu Lake than that of 5.8 μg L^{-1} for Meiliang Bay, although as shown in **Fig. 1-3**, there were heavier blooming algae in Meiliang Bay. This is possibly because Hydrolab cannot measure Chl.a of water blooms exactly.

EC **(e)** values were high at all survey sites, which is likely caused by geographic or human activities. Turbidity **(f)** in East Taihu Lake was from 28 to 60 NTU, whereas in Meiliang Bay, the values tended to be higher, from 68 to 169 NTU. At some sites, the values were as high as several tens of times the levels at East Taihu Lake. During the site observation in Meiliang Bay, the phenomena were confirmed that the inflow of turbid water was from the watershed and the sediment of the lake was disturbed by wind waves.



(g) T-N (mg·L^{-1}) **(h)** T-P (mg·L^{-1})

Fig. 1-7. Distribution of T-N and T-P at survey sites.

Fig. 1-7 (g)-(h) shows the results of T-N and T-P of surface water taken at each survey site. Of these values, East Taihu Lake and Meiliang Bay had the minimum and maximum values observed within the watershed, respectively. These values were considerably higher on average compared to the baselines of 0.2 mg L^{-1} for T-N concentration and 0.02 mg L^{-1} for T-P concentration, which are the general levels that can induce eutrophication, (**Cui et al., 2009**). In East Taihu Lake, T-N values were from 1.19 to 3.58 mg L^{-1}, which is 6~18 times of the baseline (0.2 mg L^{-1}) and T-P values were from

0.04 to 0.15 mg L^{-1}, which is 2 to 7.5 times of the baseline (0.02 mg L^{-1}). While, in Meiliang Bay and the west coast, concentrations of T-N and T-P were considerably higher, 70 times higher than the baseline for T-N and 34 times higher than the baseline for T-P in Meiliang Bay; 117 times higher than the baseline for T-N and 101 times higher than the baseline for T-P in the west coast.

Since water quality of East Taihu Lake is relatively healthy, it is assigned as the water area that can maintain the stable clear state of the aquatic ecosystem in future. Due to the diversified ecosystem, it is difficult for any particular living organism to overgrow, and because of this, it is likely that such ecological effects have helped improve the water quality and prevent the overgrowth of algae. However, with the accumulation of the artificial feedstuff for crab culture poured into the lake and fecal matter excreted by the crabs, the nutrients loading here will possibly be increased, which has raised the increasingly more concern with algal blooms.

Meiliang Bay has two large inflow entrances: Liangxi River and Zhihu Harbor. The water quality here has been declined due to rapid economic development and urbanization of the surrounding area, which has brought fast increase of the sludge load that flows into the bay. Since high levels of organic matter and nutrients salt pose adverse effects on aquatic macrophytes, thus one possible reason for the vanishment of aquiherbosa is that such adverse effects posed on the macrophytes overwhelmed the macrophytes' self-purification capacity. Therefore, the loss of ecological self-purification capacity in the lake and the accumulation of blooming algae caused by the wind-driven current of the lake has increasingly aggravate the eutrophication in Lake Taihu, which probably can induce DO depletion in future.

At the west coast of Lake Taihu, the rear mountains are the lake's main recharge areas. Although population in the west coast is small, there are many forests and farmlands, which have raised concerns on the load of inflow nutrients from over-fertilized farmlands through the rivers (**Cui et al., 2009**). Results of field survey indicated that the places marked with the highest concentrations of T-N and T-P, are also the water areas where algae often accumulate, due to the abundant supply of nutrients that enhances the growth of algae flowed in.

1.1.3-4 Phytoplankton survey results

As shown in **Table 1-3**, the total phytoplankton biomass in Meiliang Bay is much higher than that in East Taihu Lake. In both places, cyanobacteria were the dominant phytoplankton, in which *Microcystis aeruginosa* took up a large percentage. In Meiliang Bay, the total phytoplankton biomass was 6.7×10^5 cells mL^{-1}, 98% of which was *M.*

Table 1-3. The phytoplankton survey results in East Taihu Lake and Meiliang Bay.

Phylum	Species	E_{14}	M_{10}
		Biomass (cells mL^{-1})	
Cyanobacteria	*Anabaena* sp.1		740
	Anabaena sp.2	5,700	
	Aphanizomenon sp.	8,000	
	Aphanocapsa sp.	1,000	
	Oscillatoria sp.	2,480	
	Phormidium sp.	80	
	Microcystis aeruginosa	28,000	656,000
	Microcystis wesenbergii	1,860	6,000
	Microcystis sp.		2,000
Bacillariophyta	*Acanthoceras zachariasii*	8	
	Acnanthes sp.	8	
	Aulacoseira granulata	400	
	Aulacoseira itarica	25	
	Aulacoseira distans	12	
	Cocconeis placentura	4	
	Cocconeis sp.	4	1
	Crucigenia fenestrata	4	
	Cyclotella sp.	4	
	Cymbella sp.	2	
	Eunotia sp.	2	
	Frustulia vulgaris	4	
	Gomphonema sp.		1
	Melosira varians	8	
	Navicula spp.	12	4
	Nitzsthia spp.	40	1
	Stephanodiscus sp.		440
	Surirella sp.		2
	Synedra acus	44	1
	Synedra ulna	12	1
	Synedra sp.	8	1
Chlorophyta	*Actinastrum Hantzsschii*	16	
	Ankistrodesmus falcatus	8	
	Ankistrodesmus sp.	12	
	Cosmarium sp.	1	
	Chlorella sp.	90	4
	Dictyosphaerium pulchellum	16	
	Golenkinia paucispina	30	30
	Micructinum pusillum	16	
	Mougeotia sp.		9
	Oocystis sp.	16	4
	Oocystaceae		4
	Pediastrum duplex		8
	Pediastrum sp.	1	
	Planktosphaeria gelatinosa	32	
	Quadrigulla sp.	24	
	Scenedesmus acuminatus	8	
	Scenedesmus spp.	96	280
	Staurastrum sp.	1	
	Tetraedron trigonum	1	
	Tetraedron regulare	1	
Shannon-Wiener diversity index		1.97	0.13

aeruginosa; moreover, Shannon-Wiener diversity index in Meiliang Bay was 0.13, which was much smaller than that in East Taihu Lake (1.97), and this was probably caused by the excessive occurrence of *M. aeruginosa*.

1.1.3-5 Microcystin results

Occurrence and distribution of microcystins were investigated in Lake Taihu in summer 2011 by Sakai et al. The presence of microcystins in water systems can threaten human health. The World Health Organization microcystins guideline values are 1 μg L^{-1} in drinking water and 20 μg L^{-1} in recreational water. In their research, 50 μg L^{-1} of total cellular microcystins concentration was observed in the southern part of Lake Taihu; in the northern coastal areas, 20 to 44 μg L^{-1} of total cellular microcystins concentration was observed; at northern off shores, total cellular microcystins concentration was up to 4.8 μg L^{-1} (**Sakai et al., 2013**).

1.1.3-6 Macrophytes survey results

Table 1-4. Macrophytes at investigation places.

Aquatic vegetation	Vegetation species	Investigation places		
		East Taihu Lake	Meiliang Bay	West coast
Emerged macrophytes	*Phragmites communis*	++	+	++
	Nymphaea sp.	++		
Submerged macrophytes	*Potamogeton malaianus*	++		
	Vallisneria spiralis	++		
	Elodea nuttali	++		
	Myriophyllum spicatum	++		
	Ceratophyllum demersum	++		
	Hydrilla verticillata	+		
Floating-leaved macrophytes	*Nymphoi des peltata*	+		
	Trapa maximowiczii	+	+	
	Limnanthemum nymphoide.	+		
Free-floating macrophytes	*Spirodela polyrhiza*	+		

* 「+」 Existing; 「++」 Dominant species.

As shown in the **Table 1-4**, 12 species of aquatic macrophytes have been found in East Taihu Lake, of which submerged macrophytes were dominant. In addition, East Taihu Lake has the most abundant macrophytes species among the investigation places, which indicated the best water quality is in East Taihu Lake. However, seldom macrophtes have been observed neither in Meiliang Bay nor West coast, indicating the worse water

quality there.

1.1.3-7 Conclusions of water environment status survey on Lake Taihu

In Lake Taihu, the three water areas: East Taihu Lake, Meiliang Bay and the west coast have different water quality features and biological environment. Furthermore, aquatic macrophytes species composition and distribution depend on the aquatic environment. The deteriorated water quality induced by the evident algal blooms has made the environment inappropriate for the survival of other species of phytoplankton and aquatic macrophytes.

The water quality of East Taihu Lake is relatively healthy and there are abundant aquatic macrophytes communities, which can prevent overgrowth of algae and contribute to the increase of ecological system self-purification ability. However, in Meiliang Bay and the west coast, water pollution has become a serious problem, resulting in the disappearance of aquatic macrophytes communities. Algal blooms in these water areas have become increasingly evident due to nutrients inflow from the surrounding farmlands.

In order to improve water pollution and restore healthy aquatic ecology in Lake Taihu, technology that can prevent water environment pollution and measures that can control sustainable water environment are urgently needed.

1.2 ALLEVIATING EUTROPHICATION METHODS APPLIED IN PRACTICE AND DEFICIENCY

Aiming at eutrophication problem in Lake Taihu, many efforts concerning the eutrophication control has been conducted. In China, a process of source control, ecological restoration and catchment management was always performed in lake restoration from eutrophication. The source control included both the external nutrients loadings in the catchment and the internal loadings released from the sediment. Based on the source control, the ecological restoration was implemented in which the aquatic macrophytes reestablishment was always the key step. (**Qin et al., 2006**)

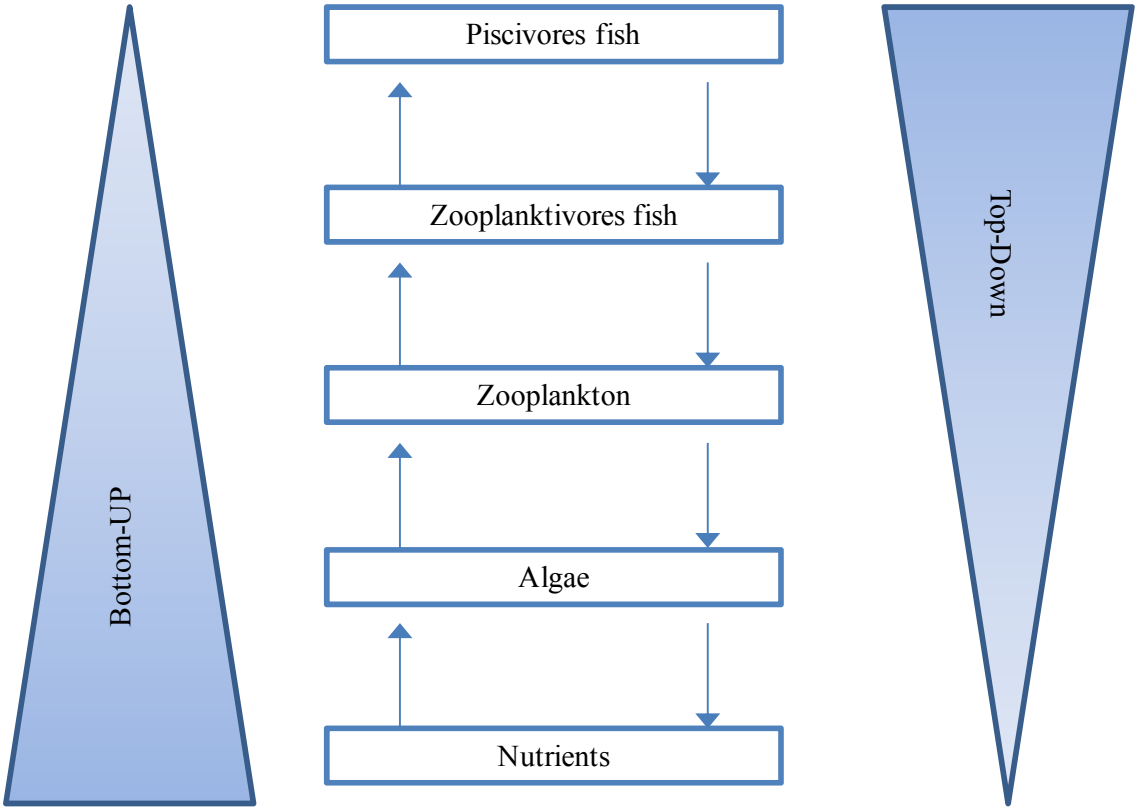


Fig. 1-8. The "Bottom-Up" and "Top-Down" methods.

Here the "Bottom-Up" and "Top-Down" methods will be explained. As shown in **Fig. 1-8** in the ecosystem, each group, such as the algae, zooplankton or piscivores, has a functional role. When nutrients are reduced to control algae, the method "Bottom-Up" is working, which most consistently works for controlling algae, on the other hand, if the numbers of grazers (organisms that eat other organisms) is increased or decreased to

control the numbers of lower level organisms is called "Top-Down" method or biomanipulation. Among all the techniques applied in the Lake Taihu in controlling eutrophication, the nutrients loading control and lake ecological restoration are the main two approaches, which can be classified into the "Bottom-Up" methods.

However, due to the insufficient understanding of eutrophication in shallow lakes, the results were often unsatisfied. Here all the methods applied in Lake Taihu are summarized and the problems which are still existing are highlighted.

1.2.1 Technologies in Control of Internal Nutrients Loadings in Eutrophic Lakes (Bottom-UP Method)

In restoration of shallow eutrophic lakes, the reduction of internal nutrients loadings is the key step for restoring the macrophytes dominant ecosystem.

Lake sediment accumulates a large quantity of nutrients. The phosphorous concentration in the lake is usually used to indicate the lake primary production and trophic level (**Vollenweider, 1968**). In natural lakes, most of the organic phosphorus was particulate-bound phosphorus (**Wetzel, 2001**). The particle-bound phosphorus will easily deposit into bottom after coming into the lake, which made the nutrients enriched in the sediment. These nutrients would be released to overlying water in certain conditions, and become the internal loadings (**Qin et al., 2006**).

Table 1-5. The ways of nutrients release from sediment.

Ways of nutrient release from sediment	Explanation
Molecular diffusion	Nutrient concentration gradient between the interstitial and overlying water
Dynamic disturbance	Wind and wave
Bio-disturbance	Benthic organisms
Bubble effluent	Etc.
Phytoplankton floating	Etc.
Transmission of aquatic plant root	Etc.

As shown in **Table 1-5**, there are several ways of nutrients release from sediment, (**Wetzel, 2001**). The nutrients release can be affected by many ambient factors, such as temperature, pH, redox condition (Eh), the content of iron and manganese and the wind-induced turbulence (**Moore and Reddy, 1994; Zhu et al., 2005**). For the pH value, when it is less than 2 or greater than 9, the nutrients release will increase obviously (**Moore and Reddy, 1994**).

Results of research conducted in Lake Taihu were consistent with the conclusion that the pH and Eh in the sediment can affect the nutrients release, transformation, adsorption and enrichment dramatically (**Qin et al., 2004**). In addition, investigation in Lake Taihu indicated that massive nutrients released into overlying water in short period accompanied with sediment resuspension caused by the wind-induced turbulence (**Zhu et al., 2005**). This phenomenon can often happen in shallow lakes, which makes the effect of nutrients reduction in water column of lakes unobvious.

Therefore, for restoration in the shallow eutrophic lakes, both internal loading control and external loading control should be strengthened. To select economic and effective measures for the internal loadings reduction, the factors such as water depth, pH, volume of lake, aeration ability, nutrients release rate and hydrological conditions should be considered (**Qin et al., 2006**).

1.2.1-1 The physic-chemical measures to control the internal loadings

Table 1-6. The physic-chemical measures to control the internal loadings.

Physic-chemical measures	Examples	Application conditions
Sediment oxidation	Aerating at bottom	(1) Small lakes or ponds; (2) hydrodynamics action is weak; (3) sediment surface is in anaerobic condition.
Chemical precipitation	Adding on aluminum, ferrous and calcium	
Physical measure	Sediment capping	

Generally speaking, the main factor inhibiting the phosphorus release (usually Fe-P release in reductive condition) is the redox condition when the pH of water is lower than 9 (**Qin et al., 2006**). With the physic-chemical measures shown in **Table 1-6** (**Kopacek et al., 2000**), the redox condition at the sediment-water interface will be changed from reductive condition to oxidizing condition, subsequently, the sensitive phosphate metallic compounds, i.e. the ferrous phosphate, transformed to oxidative condition, inhibiting the internal phosphorus release (**Deppe and Benndorf, 2002**). Thus, when pH in the water is lower than 9, these methods can effectively control the phosphorus release.

1.2.1-2 The physic-mechanical measures to control the internal loadings

(1) Water flushing is an effective method to dilute the nutrients in lakes.

From 2002 to 2003, the "Water diversion from Yangtze River to Lake Taihu" project was conducted in Lake Taihu. The success of this method depends on the availability of the clean source water and the strong enough flushing intensity. Generally, when about 10% -15% of the lake capacity can be flushed per day, the satisfied results can be achieved (**Qin et al., 2006**). This flushing method is usually effective, especially for the small size and shallow lakes; whereas, for large lakes, it could be limited in use because it is uneconomic. In addition, in this measure, nutrients-enriched water will be discharged from eutrophic lake to downstream, which will pollute the downstream areas. Thus in emergent situation it can be applied, and this method is only a temporary countermeasure and cannot control the eutrophication essentially.

(2) Sediment dredging is also an important measure in controlling the internal loadings in eutrophic lakes.

Although this method has been widely applied in many eutrophic lakes in China, few successful results were reported in larger lakes. The unsuccessful case of sediment dredging in Wuli Bay (**Fig. 8-2**) indicated that it had induced a series of problems in the local environment, including sediment resuspension, water transparency decrease, release of the internal nutrients and heavy metal ion (**Liu et al., 2006**). The investigation indicated that proper dredging is a temporary method, which can only improve the water quality in short term but cannot control internal loadings for a long term (**Fan et al., 2004**).

Furthermore, to determine whether a lake can be dredged, a comprehensive analysis is necessary. The sediment release rate, the nutrients content in sediments, sediment suspension or re-aeration intensity, pH and redox condition and the Fe and Mn content in the sediments should be included in this comprehensive analysis (**Qin et al., 2006**).

1.2.1-3 Biological measure to control the internal nutrients loadings

(1) Effective microorganisms, mainly including immobilized nitrobacteria-denitrifying bacteria, photosynthetic bacteria, actinobacteria, saccharomyces and lactobacillus, could be used to purify the eutrophic water in small lakes.

As reported by Li et al., immobilized nitrobacteria-denitrifying bacteria were used to treat the eutrophic lake water, and the results showed that immobilized nitrobacteria-denitrifying bacteria can remove total nitrogen, ammonium, and organics effectively. (**Li and Pu, 2000**).

In addition, as reported by Li et al., effective microorganisms, mainly composed of photosynthetic bacteria, actinobacteria, saccharomyces and lactobacillus, were used to control algal blooms in a small eutrophic lake in South China and they were reported to

be able to reduce Chl.a concentration and control algae blooms effectively, furthermore, they can improve water quality, as indicated by the decrease of concentration of chemical oxygen demand (COD), total nitrogen (T-N) and total phosphorus (T-P) after adding them. Accompanied with the decrease and sedimentation of algae in water column, the transparency was increased significantly indicating the improvement of water quality (**Li and Pu, 2000**).

Although the improvement of water quality was found in these small lakes, the mechanism was still unclear and the potential effects of effective microorganisms on the water ecosystem in the long-term is still unclear. Therefore, this method is still immature, thus cannot be promoted to be applied in large lakes.

(2) The bio-floating carpet technique with macrophytes cropped at eutrophic water surface, is another effective tool to purify the eutrophic water.

The principle of this technique is by assimilating, adsorbing and intercepting suspended particles throughout the roots of macrophytes, and by removing the ammonium, phosphorus and other pollutants to purify eutrophic water (**Qin et al., 2006; Liu et al., 2004**).

(3) The artificial aquatic macrophytes can be used to purify water, following the similar principle of purification by aquatic macrophytes, which can intercept the sestons, assimilate nutrients through attached microbes and periphytes.

This method was applied in the Lake Taihu to purify eutrophic water and preliminary result suggests that sestons can be removed, T-N, T-P concentrations can be reduced and water transparency can be increased (**Ji et al., 2004**).

1.2.2 Ecological Restoration to Control the Eutrophication

The lake eutrophication could occur naturally in the scale of centuries and millenniums, whereas human activities could speed up the eutrophication, making the anthropologic eutrophication happen in the time scale of decades (**Carpenter and Lodge, 1986**). Lake Taihu, as a shallow lake, the natural background of trophic level is high, which means it could be easily eutrophicated. It may be associated with the flooding events. During flooding period, a large amount of suspended particles and nutrients from upstream catchment would be transported to downstream and deposited in sediment of Lake Taihu. This is why the shallow lake has high baseline of trophic level than other lakes. Furthermore, massive nutrients released accompanied with the sediment resuspension induced by wind and wave, which would be the internal nutrients loadings.

This could further lead to less improvement of water quality in short term even if all the external loading is reduced (**Qin et al., 2006**). This could explain why it is difficult to control the eutrophication in Lake Taihu only through nutrients loading reduction or the Bottom-Up methods

1.2.2-1 Ecological restoration

The ambient environment changes induce the stress imposed on the lakes ecosystems. There is a critical threshold of stress-resisting ability for ecosystem, if this environmental stress is limited below the critical threshold, the ecosystem could resile to original state, and the system will be restored when the external stress is removed; if the external stress is greater than the critical threshold, ecosystem will drastically shift into the new ecosystem to fit the changed environmental conditions (**Scheffer et al., 2001**).

Accordingly, restoration is becoming an increasingly significant part of the improvement in water quality and the ecology of aquatic ecosystems. Restoration means the reestablishment of predisturbance aquatic functions and related physical, chemical, and biological characteristics (**Cairns, 1988; Lewis, 1989**). Thus, the objective of restoration is to help the ecosystem resile to the original state, both in function and structure. Often, restoration requires one or more of the following process: reconstruction of antecedent physical conditions; chemical adjustment of the soil and water; and biological manipulation, including the reintroduction of absent native flora and fauna or of those made nonviable by ecological disturbances.

1.2.2-2 The two stable states

Increasingly more evidences can prove that over a range of nutrients concentrations, shallow lakes can have two alternative states: a clear water state dominated by aquatic macrophytes, and a turbid state dominated by high biomass of algae (**Scheffer et al., 1993**). Macrophytes tend to keep the system in a clear water state at relatively high nutrients load through several mechanisms that (e.g. luxury uptake of nutrients, release of allelopathic substances, refuges for zooplankton, reduction of resuspension of sediment, stimulating denitrification) (**Carpenter and Lodge, 1986**). Therefore, return of aquatic macrophytes is vital for a successful restoration of shallow lakes at relatively high nutrients load (**Moss, 1990**).

1.2.2-3 Macrophytes restoration

The ongoing eutrophication in Lake Taihu has induced a decline or disappearance of

macrophytes. Increasing nutrients load may result in the increase of phytoplankton biomass and then creating water turbidity, which may induce light limitation and disappearance of submerged macrophytes (**Scheffer et al., 1993**).

It is proved that the existence of submerged macrophytes in the lakes can significantly reduce the nutrients released from sediment and improve the water quality (**Drenner et al., 1997**), in addition, submerged macrophytes can inhibit algal blooms through competing nutrients with algae. Thus, submerged macrophytes play an important role in the maintenance of clear water state. Due to the significance of submerges macrophytes in aquatic ecosystem, in order to restore ecosystem services and aquatic biodiversity, the key is to make efforts to induce backward shifts from the turbid, algal-dominated state to a clear state dominated by macrophytes, furthermore, the extent of return of submerged macrophytes are always used as the targets and success of the restoration efforts (**Bakker et al., 2012**).

Macrophyte restoration experiments to improve water quality started in China as early as midst of the 1990s. But so far few successful trials have been achieved, such as ecological restoration experiments conducted in Lake Taihu (**Li et al., 1996**). These experiments, basically, had restored the aquatic macrophytes by introducing the enclosure to separate the experimental area from the algal blooms and pollutant source, reducing the suspended particulates and increasing the water transparency, etc. For the aquatic plant cropping, technique such as community mosaic of aquatic macrophytes was employed (**Pu et al., 2001**). In all these experiments, at the beginning the water quality was obviously improved as the aquatic macrophytes grew and developed; but the artificial established macrophytes-dominanted ecosystem collapsed when the enclosure curtain was dismantled when the project was finished (**Qin et al., 2006**). This suggests that the artificial established macrophytes-dorminated ecosystem cannot be maintained stable and healthy without the corresponding environment. Thus, in order to establish the stable macrophytes-dominated system, the improvement of environment should be paid more attention; on the other hand, this indicates that our knowledge about the aquatic ecosystem is limited and insufficient to instruct the restoration of eutrophic lakes. Consequently, it could be unwise and blindness to over emphasize the macrophytes at present (**Qin et al., 2006)**.

1.2.2-4 Non-classical biomanipulation method-Top-Down

In order to control the severe algal blooms in eutrophic lakes in China, the non-classical biomanipulation was proposed by Chinese scientists to remove the blooming-algae by growing planktivores fish such as silver Carp and bighead Carp (**Xie and Liu,**

2001). They were validated to remove the blue-green algae effectively in situ enclosure experiments in Lake Donghu, Wuhan, China (**Xie and Liu, 2001**). From 9 May to 7 July in 2005, silver and bighead Carp were cultured in a large pen in Meiliang Bay of Lake Taihu for the control of *Microcystis* blooms; and the results showed that before June, the stocking of silver plus bighead Carp failed to achieve an adequate control of phytoplankton; after 7 June, silver and bighead Carp suppressed the *Microsystis* blooms (**Ke et al., 2009**).

However, this method remains controversial, because some authors reported that after stocking Carps, there is a stimulation of small sized plankton and an increase in the biomass of nanophytoplankton. Due to the size selectivity of the stocking Carps, for silver Carp only food particles with size larger than 10 μm can be filtered (**Smith, 1989**), and for bighead Carp, only food particles with size 17-3000 μm can be filtered. Moreover, some studies have reported that some colonial and filamentous cyanobacteria (such as colonial *Microcystis* spp.) remain viable after the intestinal tract of fishes and even increase their specific photosynthetic activity. Passage through silver Carps did no damage to cyanobacterial cells with mucous cover and only the nutrients from attached heterotrophic epibacteria (main components of mucous cover) were definitely assimilated (**Kamjunke and Mehner, 2001**). Thus, colonial *Microcystis* spp. were decomposed to unicellular *Microcystis* spp. and excreted into the water.

Therefore, in the review by Zhang et al., it was pointed that the initial phytoplankton community composition greatly impacted the success of this method. When there is a lack of macrozooplankton in eutrophic systems, the introduction of silver and bighead Carp could be effective management technique. Because nuisance blooms of large algal species cannot be controlled effectively by large herbivorous zooplankton, only if the eutrophic systems were dominated by these large sizes of blooming-algae, the non-classical biomanipulation can work efficiently, alternatively, in less eutrophic systems where nanophytoplankton dominated, this type of biomanipulation did not work efficiently (**Zhang et al., 2008**).

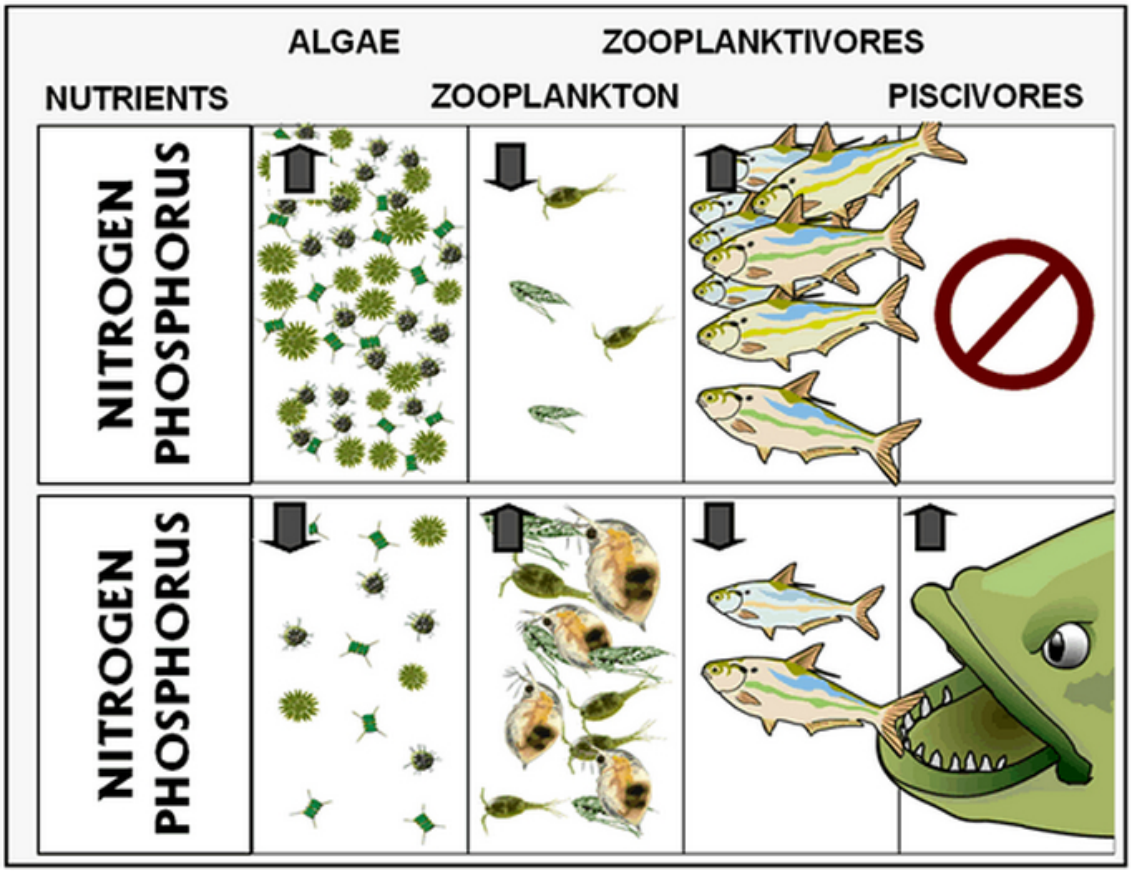
Moreover, it is blind to apply this method without evaluation the ecological effects such as the alternation of food-web and microbial food-web structure in Lake Taihu. It is still unclear whether the stocking of silver and bighead Carp can benefit the stable state of Lake Taihu ecosystem in the long term or not.

Therefore only viewing restoration at the whole-ecosystem level and incorporating a food-web perspective can contribute in a real way to ecological restoration efforts (**Donlan et al. 2003**).

1.3 BIOMANIPULATION METHODS AND FRESHWATER MUSSELS

1.3.1 Classical Biomanipulation Methods Process (Top-Down Method)

Biomanipulation is defined as "the manipulation of the food-web of aquatic ecosystems to increase the number of grazers of algae". The key point of classical biomanipulation is to increase the abundance and size of zooplankton (mainly large *Daphnia* species), which can contribute to enhanced grazing pressure on phytoplankton and, ultimately, clear water of lake (**Mehner et al., 2004**). Therefore, the fish community is always manipulated, by the increase of piscivorous fish biomass or the reduction of planktivorous fish (**Perrow et al., 1997**).



(Source from http://www.lmvp.org/Waterline/fall2005/topdown.htm)

Fig. 1-9. Trophic cascades.

Each box in the graphic (**Fig. 1-9**) to the right presents a "trophic" level, or food

group from nutrients to piscivores. Altering the higher trophic levels (e. g. piscivores) can lead to a "cascade" effect on the lower trophic levels. For example, with the absence of piscivores usually leads to an abundance of zooplantivores and algae (top row), while an increase in the number of piscivores eventually results in a decrease in the number of zooplanktivores and, theoretically, algae (bottom row). By adding piscivores, the grazing pressure is removed from the zooplankton and their numbers should increase. As a result, the algae population is more thoroughly grazed. The nutrients are generally not affected by this "biomanipulation".

However, the ability of zooplankton to control phytoplankton biomass in lakes has been indicated to be more variable (**Brooks and Dodson, 1965; McQueen et al., 1986; Kerfoot et al., 1988.**) The success of biomanipulation can be impacted by many conditions: (a) planktivorous fish must be removed repeatedly or else they can become abundant again; (b) sustained reduction of the phytoplankton biomass by biomanipulation can be achieved only if the phosphorus loading is below the "biomanipulation efficiency threshold of the P-loading" (**Benndorf, 1995**); (c) even after the reduction of the external loading of phosphorus, and improvement of water quality can be often delayed, especially in summer, which was probably induced by the phosphorus release from the sediment pool (**Søndergaard, 2002**); (d) submerged macrophytes have been playing an important role in stabilizing clear water states (**Van Donk and Van de Bund, 2002**).

Moreover, cyanobacteria are inadequate food source for zooplankton. The feeding process of zooplankton can be inhibited severely by mechanical interference, which reduces the growth, reproduction and survival of zooplankton. In addition, some cyanobacteria can be toxic to zooplankton, inducing the cease of feeding. (**Lampert, 1987**).

1.3.2 Non-Classical Biomanipulation Method with Filter-Feeding Planktivorous Fish

In addition, if there are no large-bodied zooplankton in eutrophic lakes where nuisance algal blooms often occur in summer, and nutrients cannot be reduced to a certain low level, filter-feeding planktivorous fish have been attempted as an alternative biomanipulation method, based on the directly feeding on the undesirable bloom-forming blue-green algae (**Radke and Kahl, 2002; Leventer, 1979**). Since the 1970s, silver and bighead Carp have been applied to control algal blooms as menthioned in 1.2.2-4.

1.3.3 Biomanipulation Method with Mussels

In addition to filter-feeding planktivorous fish, benthic mussels can also consume significant quantities of phytoplankton. For example, the non-native zebra mussel *Dreissena polymorpha* was indicated to prevent the formation of the summer algal blooms in the Hudson River, after its invasion of the river in 1990 (**Smith et al., 1998**), in addition, it was found that the zebra mussels were an effective biomanipulation tool to reduce algal concentrations and thus to increase water clarity and light penetration (**Caraco et al**. **1997**).

Mussels were first suggested as a potential tool to control eutrophication by Officer et al. (**Officer et al.**, **1982**). In their study, using mathematical models, it is shown that a marine benthic mussel population might be expected to control algal growth if nutrients are abundant, water depth is shallow, the water residence time is long, and high densities of mussels are present. In freshwater mussels have also been suggested to be a potential biomanipulation tool to facilitate lake restoration. After calculating the filtration rate of populations of *D. polymorpha* in shallow lakes in the Netherlands, Reeders et al. found that populations of zebra mussels *D. polymorpha* can filter enough water to reduce phytoplankton concentrations obviously in the Netherlands (**Reeders and Vaate, 1990**).

1.3.3-1 How biomanipulation works

The concept of biomanipulation is based on the theory of two alternative stable states in shallow-lake ecosystems. As mentioned in the forgoing section, over a range of nutrients concentrations, there can be two alternative stable states in shallow lakes: a clear state dominated by submerged macrophytes; and a turbid state characterized by high algal biomass (**Scheffer et al., 1993**). Solely by reducing nutrients, water clarity cannot be improved effectively, but food web manipulation can shift the lake from turbid state to stable clear state (**Scheffer et al., 1993**).

There are probably many ecological mechanisms involved in the stabilization of both the turbid and clear state, among which turbidity and submerged macrophytes are always the main factors that can decide which stable state can be existed in the lake (**Scheffer et al., 1993**). For instance, as shown in **Fig. 1-10 (a)**, in the turbid state dominated by abundant algae, phytoplankton growth was promoted by the excessive nutrients loading and absence of algal grazers (mainly zooplankton). Sediment can be stirred up by waves or fish in shallow lakes with the absence of macrophytes. In this situation, high turbidity can inhibit the growth of submerged macrophytes, because the light availability becomes too low for submerged macrophytes to grow.

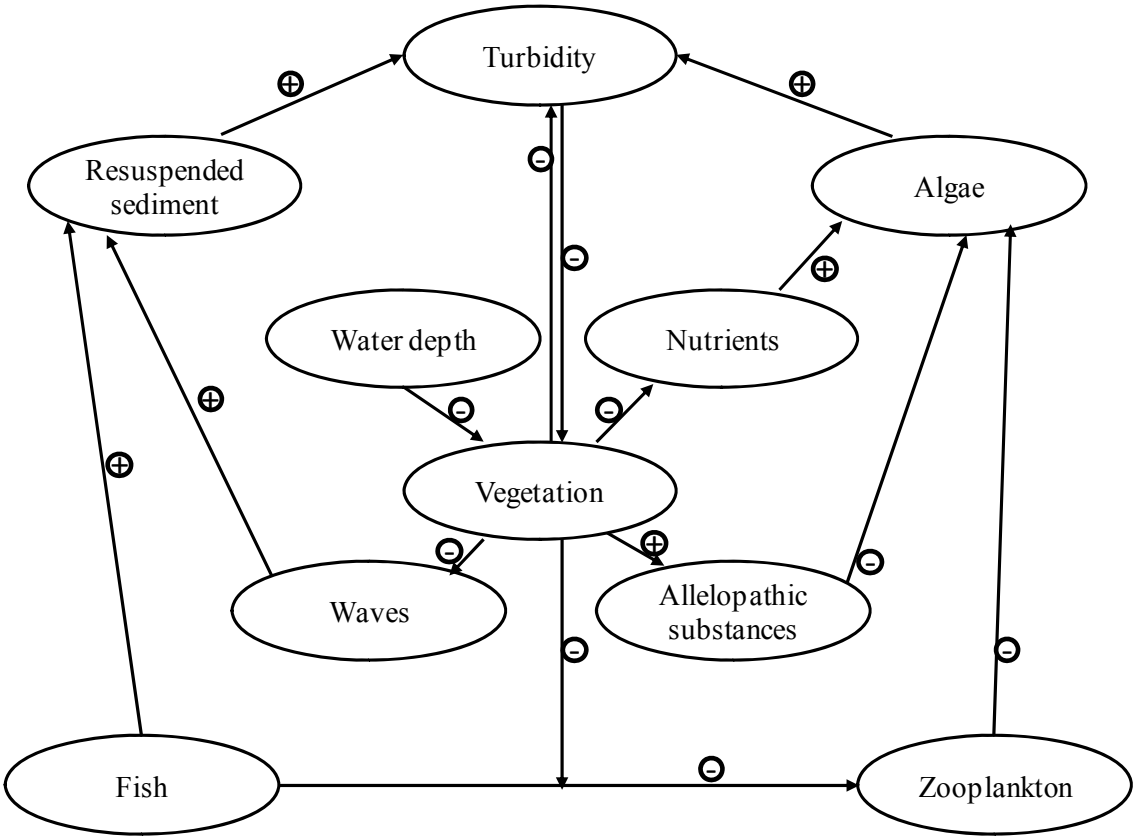
Biomanipulation, through increasing the numbers of algal grazers, can induce a “flip” from the turbid state to the clear state. In the case of “classical biomanipulation method”,

piscivorous fish biomass was increased or biomass of planktivorous fish was decreased, both of which can increase the biomass of zooplankton, the main algal grazers in freshwater lakes. Consequently, the excessive growth of algae can be inhibited by the increasing grazers, resulting in the decline of turbidity; meanwhile, with the decrease of planktivorous fish, less sediment was stirred up. Therefore, clarity can be improved remarkably, which stimulate the growth of submerged macrophytes, and in turn through competing with algae, the algal biomass was further reduced.

1.3.3-2 Freshwater mussels as biomanipulation tools

The introduction of freshwater mussels to a system can also result in a reduction in phytoplankton and turbidity (**Fig. 1-10 (b)**). Mussels can feed directly on phytoplankton, reduce turbidity through their filtering of all small particles (both algae and suspended sediment); in addition, their faeces and pseudofaeces can help bind the sediment together (**Prokopovich, 1969**), reducing resuspension of sediments.

(a)



(b)

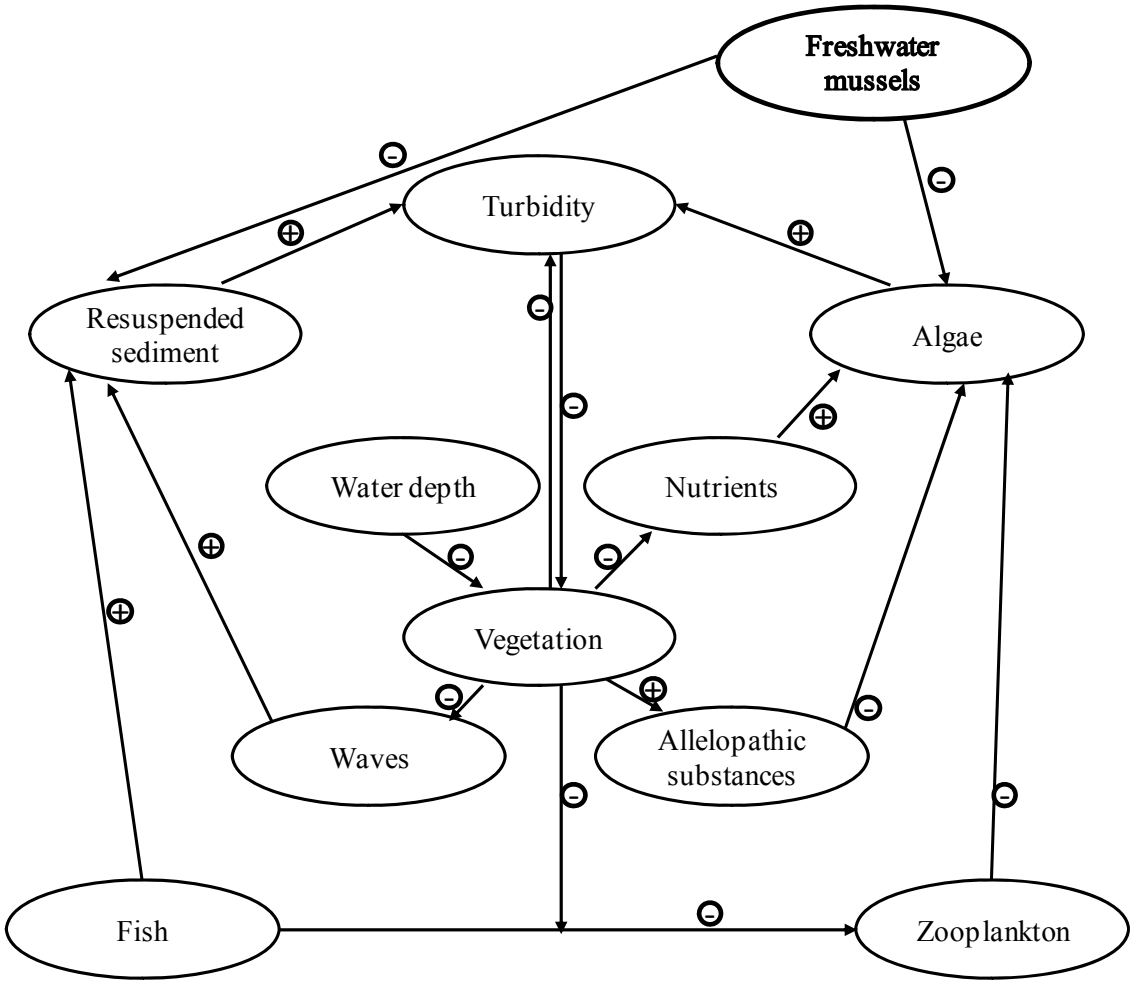


Fig. 1-10 (a). The main feed-back loops believed to be responsible for the existence of alternative stable states in shallow lake ecosystems (**Scheffer et al., 1993**); **(b).** the predicted effects of mussels on different components of these ecosystems (**Mclvor, 2004**).

Moreover, mussels can reproduce within the water body and maintain their populations over a much longer time-scale. The presence of mussels at the beginning of spring could reduce spring phytoplankton blooms which can induce the turbid state. Later in the season, mussels will form part of the network of negative feedbacks, and promote the abundance of submerged macrophytes, which stabilizes the clear-water state.

1.4 RESEARCH OBJECTIVES AND OUTLINE OF DISSERTATION

My primary research goals are directed toward the applicability evaluation in a physiological energetics method for the mussel *Anodonta woodiana* as a biomanipulation tool in Chinese Lake Taihu. Accordingly, the research objectives in this dissertation were proposed as: to clarify the feeding behavior of the mussel *A. woodiana* in freshwater ecosystems, especially on toxic *Microcystis* spp., which are the dominant algal species in Lake Taihu when algal blooms occur; meanwhile, to evaluate the effect of toxic *Microcystis* spp. on *A. woodiana* with the physiological energetics method-SFG (scope for growth); furthermore, to clarify the interactions of *A. woodiana* and submerged macrophytes, and all these results can contribute to our understanding of the integrated ecological role of the mussel *A. woodiana* in the eutrophic lake, in addition the applicability of the mussel *A. woodiana* as a biomanipulation tool in Lake Taihu restoration, pushing the lake from algae-dominated turbid state to macrophytes-dominated clear state.

Firstly, as shown in **Fig. 1-11,** in **Chapter 1**, through literature review and the field investigation, the information of the eutrophication situation in Lake Taihu was obtained. Then the alleviating eutrophication methods applied in practice and deficiency were summarized, furthermore, the research of biomanipulation methods and the application prospect of freshwater mussels were mentioned. Accordingly, the research objectives and the research flow chart of the PhD thesis were posed here.

Thereafter, in **Chapter 2**, the physiological evaluation method used in this research, SFG was explained, in which the significance, calculation methods, affecting factors were summarized.

As an important part of SFG, feeding behavior and the affecting factors of mussels were examined and further discussed in **Chapter 3**.

Based on the first three chapters, the application feasibility of the mussel *A. woodiana* were clarified through the following experiments described in **Chapter 4-7**.

In **Chapter 4**, we studied the acute physiological responses of the mussels on different phytoplankton cultured in laboratory, including the unicellular *Microsystis aeuginosa*.

Thereafter, in **Chapter 5**, the interactions between *A. woodiana* and algae-blooming water were examined, in which the grazing of mussels on phytoplankton in naturally blooming pond water dominated by toxic colonial *Microcystis* spp. was studied. If toxic cyanobacteria are ingested by *A. woodiana*, it is vital to know whether the cyanobacterial

toxins will pose a threat to their survival, or whether there are some possible survival mechanisms of *A. woodiana* in exposure to microcystins.

Subsequently, in **Chapter 6** *A. woodiana* was offered highly concentrated naturally blooming water, mainly containing colonies of toxic *Microcystis* spp. for 12 days, during which the feeding rates, metabolic rates were measured and finally, the potential SFG was calculated. Furthermore, the possible metabolization process of microcystins was inferred.

From these laboratory experiments, all the results indicate that the mussel *A. woodiana* can graze the toxic *Microcystis* spp. both of unicell and colony in blooming water, thereafter can still obtain the energy for its potential growth.

Accordingly, in **Chapter 7**, the interactions between *A. woodiana* and submerged macrophytes were clarified. Firstly, the relationship between mussels, nutrients and submerged macrophytes were studied, through which it was found that the existence of submerged macrophytes can be in favor of the removal of the excessive ammonia-nitrogen excreted by mussels; and then, the relation between submerged macrophytes, turbidity and mussels was researched, through which it was suggested that the presence of mussels can assist in the improvement of water turbidity induced by wind-induced sediment disturbance, which can increase chance of the submerged macrophytes restoration, especially in the littoral zone of Lake Taihu.

Consequently, in **Chapter 8**, the integrated ecological effects of *A. woodiana* were summarized. Therefore, the integrated ecological restoration method is proposed to be applied in Meiliang Bay, especially in the littoral areas. This integrated method includes the Ecological Dam (ED) and the Adjustable-Submerging Bed (ASB), through which the algae-dominated turbid state can be pushed into submerged macrophytes-dominated clear state in Mayliang Bay. Furthermore, this could further help create and maintain a clear water state in Chinese Lake Taihu.

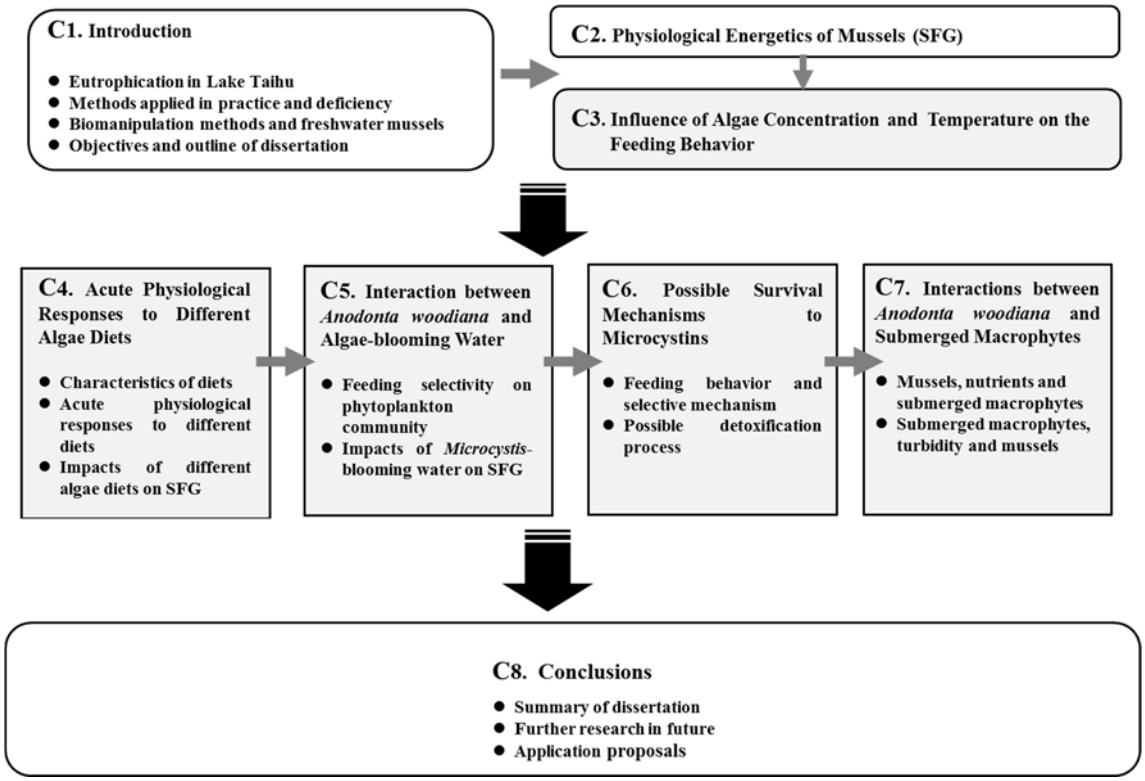


Fig. 1-11. The flow chart of PhD thesis.

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CHAPTER 2
Physiological Energetics of Mussels

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Abstract

In this chapter, firstly, a biological tool scope for growth (SFG) was introduced, which itself is the result of various vital functions (filtration, ingestion, absorption and respiration). It is a technique involving the calculation of the energy available for growth under standardized laboratory conditions. Since growth is the result of a combination of different physiological process involved in energy acquisition and consumption, the determination of growth in organisms is one of the most sensitive methods available for detecting, quantifying and identifying changes over time and space to the water quality of aquatic ecosystems. It consists of evaluating the energy acquired by an organism after absorbing the food it has ingested, and that lost in the respiratory and excretory processes being the net energy the organism obtains available for production (growth and reproduction). The presence of contaminants in the aquatic environment can break this energy balance, making SFG a marker for toxic stress. And then, the calculation method and affecting factors for SFG were described. Afterwards, the measurement methods and influencing factors for each component were demonstrated.

2.1 AN INTRODUCTION TO SCOPE FOR GROWTH

The increase in some dimension of the shell valves is usually used to describe growth in mussels. For example, length-the maximum distance between the anterior and posterior margins of the shell is the dimension of choice in mussels, however, in scallops shell height-the maximum distance between the dorsal (hinge) and ventral shell margins is used (**Fig. 2-1)** (**Gosling, 2003**).

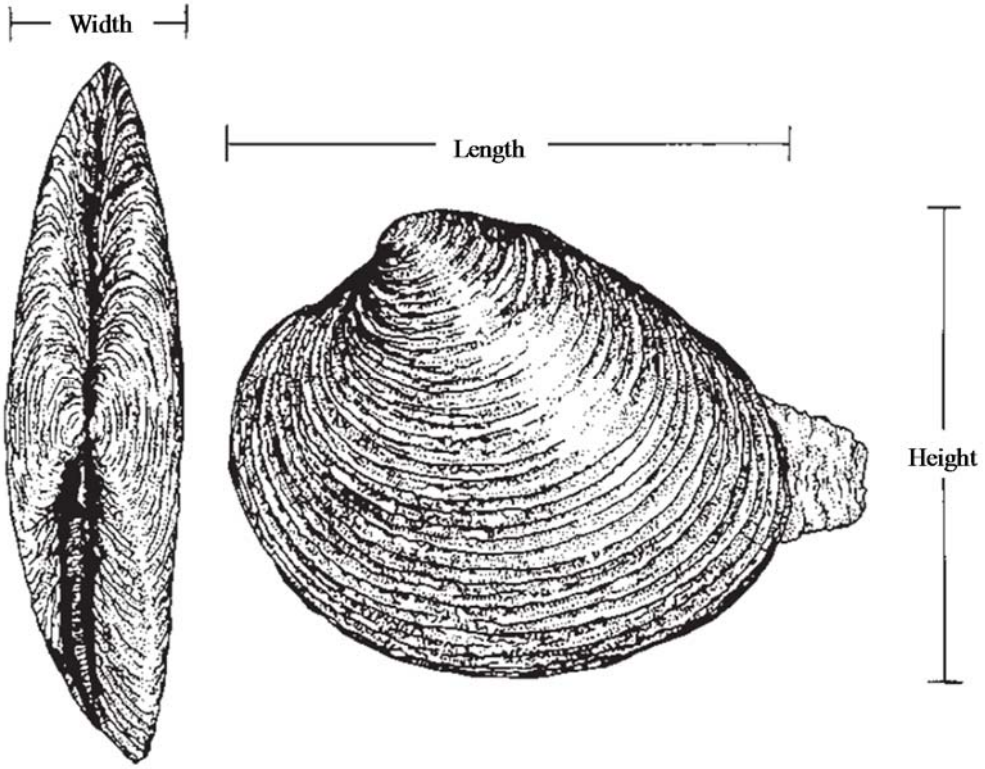

Fig. 2-1. The convention used for the main shell measurements in mussels from (**Gosling, 2003**).

In addition to the direct measurement of growth, there is an indirect method, which is based on the physiological energetics of the test individuals. Physiological energetics involves the study of energy balance within individuals, including not only the acquisition and cost of energy, but also the efficiency with which it is converted from one form to another (**Bayne and Newell, 1983**).

On one hand, physiological energetics measurements can provide information on the vital processes of energy acquisition, energy cost and thus energy available for growth

and reproduction, on the other hand, they can reflect some of the major mechanisms of toxicity. There are some important characteristics for this toxicological approach, such as minimal metabolic transformation of most bioaccumulated organic pollutants, high sensitivity to environmental levels of pollutants, quantitative (tissue) concentration-response relationships, integrated effects of contaminant mixtures, integrated biological results of complex mechanisms of toxicity and feasibility to both laboratory and field studies (**Widdows and Donkin, 1991**).

Consequently, in contrast to higher invertebrates and vertebrates, in which many contaminants are rapidly biotransformed and excreted (**Varanasi, 1989**), complex residues in tissues of mussels can largely reflect both qualitative and quantitative changes in pollutants in ambient environment (**Burns and Smith, 1981**), therefore, mussels are popularly applied in programs for pollution monitoring (e.g. "Mussel Watch", **Farrington et al., 1983**). For example, ascribed to its sedentary inherent character, widespread geographical distribution, large capacity for pollutants accumulations and its ease of sampling, the mussel *Mytilus galloprovincialis* is one of the most popular organisms in marine pollution studies (**Widdows and Donkin, 1991; Besada et al., 2011 a, 2011 b**).

In order to counteract the adverse effect of contaminants on their functioning, organisms exposed to environmental pollutants made various responses for adaptation. If such exposure is sustained over time and failed to be neutralized by the organism's defense mechanisms, it can affect the functioning of organism's biological systems, and furthermore, induce toxic effects that may inhibit organism's growth, reproduction and survival (**Albentosa et al., 2012**).

Thus the measurement of an organism's functional activity can be converted into a tool for environmental assessment. Scope for growth (SFG), due to being the result of various critical functions (filtration, ingestion, absorption and respiration), is one of these biological tools. It is a method mainly related to the calculation of the energy available for growth under standardized laboratory conditions. Since growth is the combined consequence of different physiological processes including both acquired and consumed energy, the measurement of growth in organisms is one of the most sensitive methods to detect, quantify and identify either temporal or spatial changes to the water quality of aquatic ecosystems. As the net energy the organism has available for production (growth and reproduction), growth of an organism includes measuring the energy acquisition absorbed from the ingested food, and energy consumption of the respiratory and excretory processes.

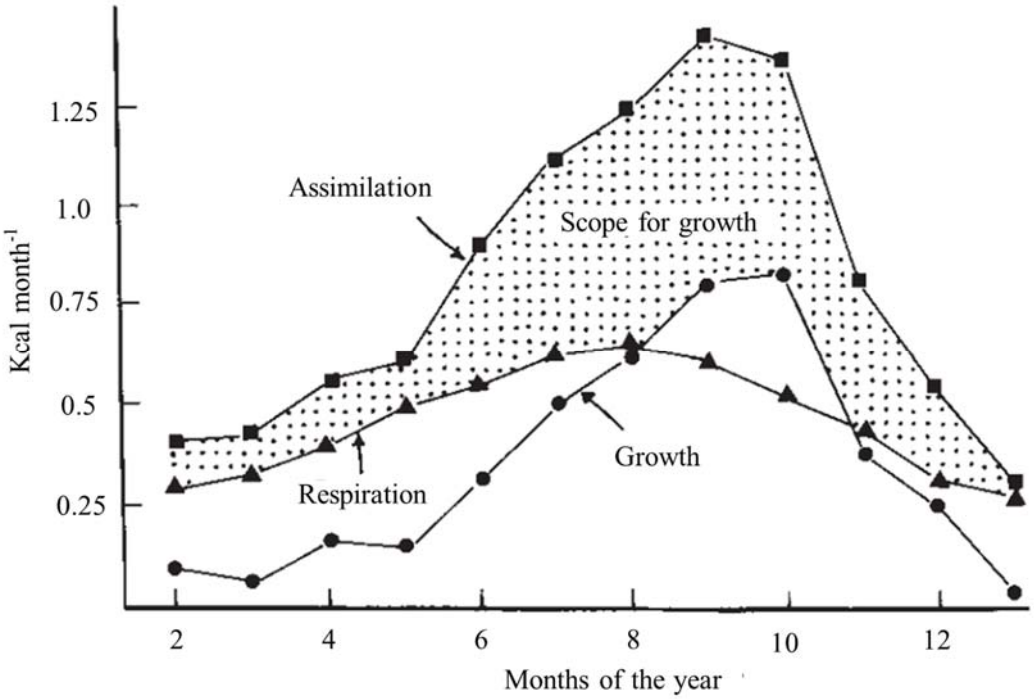

Fig. 2-2. Seasonal variations in energies of growth, respiration and assimilation in a 40 g inter-tidal oyster *Crassostrea virginica* over a 12 month period. The stippled area represents scope for growth. (**Dame, 1972; Bane and Newell, 1983)**.

The presence of pollutants in the aquatic environment changes this energy balance, making SFG an indicator for toxic stress (**Widdows and Johnson, 1988**).

In addition, SFG, as a biomarker at the individual level, has been widely applied in monitoring chronic pollution, acute pollution associated with a spill (**Fernandez et al., 2010**), and in laboratory studies on contaminant exposure (**Wang and Chow, 2002**). In all these cases, SFG values were calculated to determine the toxicity of most aquatic environmental contaminants and their effect on an organism's energy balance. Furthermore, it has been demonstrated that SFG values in mussels can be correlated with biodiversity metrics of the benthic communities where they inhabit (**Crowe et al., 2004**), indicating that SFG can be applied as a good biomarker of pollution both for individual organisms and for the population as a whole. (**Albentosa et al., 2012**).

Moreover, there is good consistence between growth rates estimated from energy budgets and growth rates measured by direct methods (**Fig. 2-2**) (**Bayne, 1998**), which further indicates that SFG can be used as an effective biomarker for organism's growth.

2.2 CALCULATION METHODS

A variety of components of the balanced energy equation are measured to assess energy balance in an individual (**Winberg, 1960**):

$$C = P + R + F + U \quad (2\text{-}1)$$

Where food consumption (C) is the primary source of energy input; (P) represents energy expenditures allocated in production of shell, soft tissue and gametes; (R) presents energy lost by respiratory heat; (F) is faecal energy losses; (U) is energy lost as excretory products. The absorbed ration (A) is the actual amount of material digested and is represented by C - F. The efficiency with which the ingested ration (C) is absorbed, A/C is called the absorption efficiency (AE).

By rearranging the energy balance equation we get the production expressed as

$$P = A - (R + U) \quad (2\text{-}2)$$

Production (P) is, therefore, the difference between energy gains from the absorbed ration (A) and metabolic losses through respiration and excretion (R + U). Only if the energy gains from A exceeds total metabolic losses, an organism can allocate energy to growth or reproduction This energy, surplus to metabolic demands, is referred to as "scope for growth" or SFG (**Warren and Davis, 1967**), although a better term might be "net energy balance" (**Bayne, 1998**), as shown in **Fig. 2-2**.

As seen above, SFG equals to total production (P), and is the sum of the reproductive output (P_g) and growth of somatic tissue and shell (P_s). Either P_s or P_g is usually measured under controlled laboratory conditions or alternatively, P can be measured indirectly as "scope for growth". SFG measured in laboratory under appropriate conditions, has been demonstrated to realistically forecast production in the field (**Griffiths and Griffiths, 1987**).

Reproductive output (P_g) can be measured directly by inducing animals to spawn and collecting the gametes, or from estimates of total gonad volume and counts of mature oocytes in serially sectioned gonads. Alternatively, P_g can be deduced indirectly from comparisons of gonads weight before and after spawning. **Table 2-1** demonstrates data on P_g as a proportion of total production (P) in a selection of mussel populations. Mean P_g/P is 44%, but there is huge inter-specific variation, with values ranging between 16

Table 2-1. Reproductive output (P_g) in relation to total production (P) in kJ m^{-2} yr^{-1} in mussels.

Species	C	P_s	P_g	P	$P_g/P \times 100$
Aulacomya ater	27499	3.4	5.8	9.2	63
Mytilus edulis	-	8.9	4.8	13.7	35
Choromytilus meridionalis	831890	1.6	4.8	6.4	75
Crassostrea gigas (mature)	10140	0.4	20.7	21.1	98
Crassostrea virginica	8811	17.7	3.4	21.1	16
Ostrea edulis	134-468	6.4	5.5	11.9	47
Mercenaria mercenaria	5400	5.6	4.7	10.3	46
Chlamys islandica	55711	1	0.4	1.4	28
Patinopecten yessoensis	-	22.7	4.3	27	16
Mean		7.5	6	13.5	44

Energy budget parameters for a variety of mussel populations: P_s (somatic growth), P_g (reproductive output) and production (P) are each expressed as a percentage of consumption (C; kJ m^{-2} yr^{-1}). Dashes indicate that no measurement was made. The enormous variation in C mainly reflects differences in population density (10-10000 individuals m^{-2}). Adapted from **Griffiths and Griffiths (1987)**.

and 98% (**Gosling, 2003**).

Choromytilus meridionalis populations have been reported to have similar variations (**Griffiths and Griffiths, 1987**). The variations are due to factors such as age (size) and variable environmental changes. For example, with the increase in body size, an investment in gamete production is generally increasing (**Gosling, 2003**).

SFG is usually measured under laboratory conditions in a short time. *AE* is measured by comparing the proportion of the organic matter in the food and faeces of animals kept in individual experimental chambers (**Conover, 1966**). Oxygen consumption (R) is measured using an oxygen electrode to record the decrease in oxygen partial pressure for individual animals maintained in closed glass respirometers held in a temperature controlled water bath (**Widdows and Johnson, 1988**). Ammonia excretion (U) is measured by taking water samples at regular intervals from individual animals maintained in air-saturated filtered water (**Widdows, 1985**). In addition, the large quantities of mucus in pseudofaeces produced by bivalves should also be included under excretion. However, loss of this potential energy in mucus has not been reported (**Hawkins and Bayne, 1992**). The physiological responses A, R and U are transformed into energy equivalents (J g^{-1} h^{-1}), and thus SFG is expressed in these terms. Calculation of C, A, R and U (all J g^{-1} h^{-1}) is as follows (**Widdows and Johnson, 1988**)

$$C = Filtration\ rate \times POM \times 23\text{J mg}^{-1}\text{ash-free dry weight} \quad (2\text{-}3)$$

$$A = C \times Absorption\ efficiency \quad (2\text{-}4)$$

$$R = VO_2\ (\text{mL } O_2\ \text{g}^{-1}\ \text{h}^{-1}) \times 20.33\ \text{J mL}^{-1}\ O_2 \quad (2\text{-}5)$$

$$U = Excretion\ rate\ (\text{mg } NH_4^+\ \text{g}^{-1}\ \text{h}^{-1}) \times 19.4\ \text{J mg}^{-1}\ NH_4^+ \quad (2\text{-}6)$$

Where *POM* (mg L^{-1}) is the amount of particle organic material per liter in input water samples, VO_2 (mL O_2 g^{-1} h^{-1}) is the rate of oxygen consumed by individual mussels.

The methods that are applied in production estimation are not only suitable for individual animals, but also for whole populations, or even to communities. Based on *C*, *A*, *R* and *E* of different-sized individuals estimated under laboratory conditions, integrated with field measurements on population density, age structure, production of populations can be estimated. The results are generally expressed in terms of energy flux per unit area of habitat kJ m^{-2} yr^{-1}. Production (*P*), expressed as a percentage of consumption, along with P_g and P_s values for various mussel species are shown in **Table 2-1**. Production values vary from as low as 1-2% in the scallop *Chlamys islandica*, to over 20% for the two oyster species *Crassotrea gigas* and *C. virginica*, and the scallop *Patinopecte yessoensis*. Such variation can be caused by inter-specific growth differences (somatic and reproductive), and differences in recruitment, immigration, mortality and emigration. The variation in P_g and P_s estimated between species shown in **Table 2-1** can largely reveal age structure differences. (**Gosling, 2003**)

2.3 FACTORS AFFECTING GROWTH

Many factors can influence growth in mussels, of which food supply is recognized to be the most significant. Since without food, it is impossible for mussels to grow steadily (**Seed and Suchanek, 1992**). Nevertheless, food supply to mussels is affected by factors such as temperature, aerial exposure, water depth and population density. Meanwhile, these factors can often synergistically interact, thus it is usually difficult to quantify the precise effect of a single environmental factor on growth in natural populations of mussels. Besides, endogenous factors that are inherent to the organism, e.g. genotype and physiological status, interact in a complex way with environmental factors. (**Gosling, 2003**)

2.3.1 Environmental Factors

2.3.1-1 Food

Food supply, as the most important factor can determine mussel growth in both hatchery and wild populations (**Gosling, 2003**). In the wild, it has been indicated that growth is correlated with phytoplankton abundance (**Utting, 1988**). Nevertheless, increasingly more evidence shows that large filtration capacity of mussels can induce growth to be limited by phytoplankton. Therefore, in order to satisfy their energy demand, mussel populations must exploit other non-phytoplanktonic carbon resources from re-suspended sediment, a complex mixture of benthic microflora, microalgae, fine organic detritus and quantities of inorganic material. It has been shown that in appropriate concentrations sediment suspensions can stimulate growth of mussels, both under laboratory conditions and field experimental conditions (**Gosling, 2003**).

Whether re-suspended material can be used as a food source to mussels depends on several factors: (1) compared with benthic-dwelling culture method, suspended culture method makes this food source more readily available to mussels; (2) due to excessive turbidity over muddy bottoms, sandy bottoms are more suitable for the potential growth of mussels. The seston diluted with inorganic particles has poorer seston quality and also progressive clogging the mussel filtering organ; (3) other factors such as the hydrography features of an area and weather patterns also influence re-suspension. (**Gosling, 2003**)

2.3.1-2 Tidal exposure

Submersion is a dispensable condition for mussels to feed, thus the longer a species is in aerial exposure the less time it has to feed. Therefore, the animals in the high intertidal zone revealed obviously lower growth rates compared to animals that are permanently submerged in the tide. (**Gosling, 2003**)

Species have different tolerance ability to aerial exposure. But for most mussel species a value of around 50% exposure indicates the point of zero growth, which means the energy required for metabolism during aerial exposure exceeds that available during the feeding period, and thus SFG declines to zero (**Seed and Suchanek, 1992**).

2.3.1-3 Temperature

Water temperature changes with latitude and there is a wide recognition in the literature that mussels from low latitudes have more rapid growth rate at ambient temperature compared with conspecifics from higher latitude. Nevertheless, growth rate is not a linear function of temperature. (**Orensanz et al., 1991**)

In addition, growth is modulated by the reproductive cycle, which is temperature-dependent; food supply, while mainly light/nutrients limited, is also temperature-dependent. (**Gosling, 2003**)

2.3.1-4 Salinity

The responses of mussels to changes in external salinity includes the closure of shell valves and the regulation of the intracellular concentrations of ions, amino acids and other small molecules to maintain a relatively constant cell volume. Firstly rates of feeding and respiration are decreased but when osmotic equilibration is reached, they can gradually recover. The period mussels require to completely adapt depends on the extent of the initial salinity change (**Almada-Villela, 1984**).

Little has been reported on the effect of salinity on SFG, but the excretory losses induced by low and fluctuating salinity can be regarded as a potential important energy loss (**Bayne and Newell, 1983**).

2.3.1-5 Stock density

In order to determine optimal stocking densities for scallop in various stages of the life cycle, many researchers have verified the effect of a range of stocking densities on scallop growth. Most of them have reported that there is a negative correlation between stocking density and growth (**Gosling, 2003**).

One possible reason is that food availability per individual was reduced with a greater stocking density. Another possible reason is that higher stocking density induced reduction in space, which leads to increased physical contact between individuals, with more frequent irritation and retraction of the mantle, or valve closure, and finally results in less feeding (**Cote et al., 1993**).

2.3.1-6 Water depth and flow (Gosling, 2003)

Compared with deeper waters, the shallow waters are more productive due to relatively higher temperatures.

However, in view of the fouling (other organisms colonized on scallop culture nets) changes with depth, results will be changed. Near the surface larger quantities of barnacles and hydroids are colonized on scallop nets and this can reduce the water flow and suspended food particles available for scallops. Furthermore, the fouling organisms, many of which are suspension feeders, can probably compete with the scallops for food resources.

In general, it is observed that there are declined growth rates for suspension feeding mussels in areas with lower current speed and higher population densities. Possible reasons are the decrease in seston supply and furthermore food limitation in these areas. Therefore, compared with mussels held on the shore, those held in suspended culture can grow much more rapidly under comparable conditions; nevertheless, at excessively high current speeds, feeding activity are inhibited and thus growth rates are declined reduced.

2.3.1-7 Pollutants

Mussels, have been widely used as biomarkers in the assessment of the effects of specific toxicants on physiological responses such as mortality, shell valve gape, scope for growth, shell and tissue growth (**Widdows and Donkin, 1991**).

Furthermore, mussel embryos and larvae have been applied as various bioassays to evaluate the toxic effect of marine pollutants on the growth of mussels both in the laboratory and field conditions (**Geffard et al., 2001**). Generally, compared with shell growth, tissue growth is more sensitive to toxicants. In addition, it is tended that adults are about 4-10 times as sensitive as larvae to many pollutants including Cu, hydrocarbons, TBT and sewage sludge. This can be explained by the larvae rely on energy reserves rather than direct feeding, in addition, they do not have a developed nervous system, which is an important site of toxic action (**Widdows and Donkin, 1991**).

In addition, other factors such as light, storms can modulate growth. Furthermore,

low dissolved oxygen concentrations in water (**Baker and Mann, 1992**), toxic algal blooms (**Chauvaud et al., 1998**) and inter-specific competition have also been indicated to reduce mussel growth.

2.3.2 Endogenous Growth Factors

Definitely, the variation for growth in mussel has a genetic basis.

In addition, for all animals general metabolism is limited by the surface area available for oxygen diffusion. Metabolic rate is proportional to a constant power of the body weight as described above by the allometric equation:

$$Y = aX^b \tag{2-7}$$

In this case, Y is the metabolic rate (such as oxygen consumption), X is body size, a represents metabolic rate of an animal of unit weight, b is the exponent. While the value of a varies according to factors such as temperature, b approximates 0.75 (**Hawkins and Bayne, 1992**). Owing to various exogenous and endogenous factors, the proportion of energy allocated to somatic growth or reproduction varies widely between species, and even between populations of the same species

Moreover, neurosecretory cells of the central nervous system can regulate the molluscan growth through hormone and innate rhythms (**Toullec et al., 1992**).

2.4 MEASUREMENT METHODS AND FACTORS

2.4.1 Filtration Rates Measurement and Factors

Most of mussels use gills for feeding. This method of feeding is called suspension or filter feeding because suspended particles from the water pumped through the mantle cavity are removed by the gill with their different ciliary tracts. The mantle cavity is divided into inhalant and exhalant chambers by the gills. Firstly, water enters through the inhalant siphon, and then cilia on the gills and mantle surface drive the water from the inhalant to exhalant chambers, thereafter the water exits by the exhalant siphon. Both siphons have a muscular velum, the inner fold of the mantle, and water flow can be regulated through the mantle cavity. (**Gosling, 2003**)

2.4.1-1 Experimental techniques used to measure filtration rates of mussels

Filtration or pumping rates are defined as the volume of water through the gill in a unit of time. The clearance rate is that volume of water completely cleared of particles per unit of time. Only if all particles presented to the gill are removed from suspension, the clearance rate equals to the filtration rate. (**Gosling, 2003**)

The two processes, clearance and filtration, are independent processes in mussels. Due to this reason, the gill can have dual function both in feeding and in respiration. In order to accommodate various particle concentrations in the incoming water, the size of the gill can be regulated themselves, and the beat frequency and pattern of the latero-frontal cirri can be changed by mussels (**Gosling, 2003**). However, variations in particle concentration cannot affect the ventilation current created by the lateral cilia, and thus gas exchange is unimpaired (**Bayne et al., 1976 a**). It is believed that the general mantle surface plays a significant role in gas exchange (**Gosling, 2003**).

Clearance rates in mussels can be determined directly or indirectly. For those species where the inhalant and exhalant currents can be easily separated, the direct method is most successful (**Gosling, 2003**). In this method, water exits by the exhalant siphon is physically separated from the surrounding water, using tubes inserted into the exhalant siphon or a rubber apron places over the mussel (**De Bruin and Davids, 1970**). In this method flow from the exhalant siphon can be measured directly, but clearance rates may be underestimated attributed to disturbance to the mussel and pressure gradients across the gills (**Riisgård, 2001**).

In addition, clearance rates can be determined in indirect methods. The mussel is submerged in water containing suspended particles for a set period of time, and the volume of the water is known. Usually algae cells, organic and inorganic powders, or even bacteria are used as the suspended particles (**Gosling, 2003**). There is a decrease in particle concentrations over time due to the filtration of mussels, and the filtration rate is determined from the exponential decrease (e.g. verified as a straight line in a semi-log plot) in algal concentrations as a function of time using the equation:

$$CR = (V/[wt]) \ln[C_0/C_t] \quad (2\text{-}8)$$

In which CR is the clearance rate (L g^{-1} h^{-1}), V is the volume of water cleared (L), w is the fresh weight of the mussels in each vessel (g), t is the duration of the experiment (in hour), C_0 is the particle concentration (mg L^{-1}) at 0 or one time step before t and C_t is the particle concentration at time t. In the vessels with mussels the particle concentration was corrected for changes observed in the control vessels without mussels.

Techniques applied in this method include the flow-through chamber method, through measuring the reduction in particle concentrations in a water current flowing past the mussels (**Stanczykowska et al., 1976**); the suction method, where tubes are placed above the inhalant and exhalant siphons of the mussels, and the difference in particle concentration of samples taken at different flow rates from these tubes is measured (**Kryger and Riisgård, 1988**); and the clearance method, through measuring the reduction in concentration of a particle through time in a closed chamber (**Pusch et al., 2001**).

Nevertheless, these methods measure the "clearance rate" of particles, i.e. the volume of water cleared of particles per unit time, rather than the filtration rate. In view of the condition in which the clearance rate is equivalent to the filtration rate is that 100% of particles are removed by the mussel, particles of a size which are regarded to be 100% efficiently retained by the mussel species under study (for unionids, particles should be greater than 4 μm in diameter; **Jørgensen et al., 1984**) are selected in these methods.

2.4.1-2 Disadvantage of this method

There are several deficiencies in methods involving closed systems, such as accumulated excretory products, decreased oxygen concentrations, declining particle concentrations, all of which can, in different degrees, change normal filtration behavior.

However, flow-through systems where the animals are kept in a chamber through which the particle suspension flows at a constant rate, have been able to overcome these

problems. In order to prevent recirculation, water is pumped at high speed through the chamber, and particle concentration in the inflow water is maintained constant.

In field experiments, clearance rates can be measured in bio-deposits method, in which bio-deposits are directly collected. They can be calculated according to the ratio: *clearance rate* (L h^{-1}) = (mg inorganic matter egested as faeces and pseudofaeces per hour)/ (mg total inorganic matter in seawater per liter). Due to the ease to set up, this method has been applied successfully for several field studies (**Pouvreau et al., 2000**).

Riisgård has reviewed various methodologies, in which the reliability of these techniques has been questioned; in addition, it was pointed out that there have been experimental flaws in many of the methodologies applied in previous studies, which has been regard as the sufficient reason to explain the various filtration rates reported in the literature (**Riisgård, 2001**). Furthermore, it was indicated that of the techniques mentioned above, reliable filtration rates can only be estimated through the suction method and the clearance method, when they are used under optimal laboratory conditions (**Mclvor, 2004**).

2.4.1-3 External factors affecting filtration rates

(1) Particle concentration

The concentration of particulate material in the medium can control pumping rates largely. A very dilute suspensions cannot be filtered by mussels, and in this way mussels can conserve energy during periods when particulate food is insufficient, such as in winters (**Gosling, 2003**). Therefore, filtration can only be started in mussels when particle concentrations reach a critical threshold level (**Gosling, 2003**). For example, in Riisgård's study, it was reported that when the particle concentration of *Rhodomonas baltica* is 3-10 cells μL^{-1}, filtration rate has been maximized in mussels *Mytilus edulis*; however, excessively high concentrations can restrain the filtration behavior, thus when the concentration is increased above 15 cells μL^{-1}, valve gape has been reduced in the mussels, ultimately inducing a decrease in filtration rates (**Riisgård, 1991**). According to his inference, the relatively low growth rates acquired in laboratory studies may be owing to the use of unnaturally so high concentrations that can induce valve closure, metabolism reduction and biosynthesis/growth reduction (**Gosling, 2003**).

Accompanied with increasing particle concentrations, the rate of pseudofaeces (material cleared from suspension but rejected before ingestion) production increases. The net ingestion rate can be obtained by subtracting the rate of pseudofaeces production from filtration rate (**Gosling, 2003**). In Foster-Smith's study, it was reported that ingestion

rate of *M. edulis* gradually elevated up to a concentration of 300 cells μL^{-1} and then kept constant up to 800 cells μL^{-1}. Thus, for this species, a constant filtration rate can be maintained over a wide range of cell concentrations, but pseudofaecal production rapidly increased and finally the ingestion rate can be maintained consistent (**Foster-Smith, 1975**).

(2) Temperature

There is a remarkable influence of temperature on filtration rates in filter-feeding mussels. Since with elevating temperature, metabolic expenditure (measured by O_2 consumption) elevates, mussels can adapt these high temperatures instead of rapid weight loss by concomitantly increasing energy acquired from feeding food (**Gosling, 2003)**.

Furthermore, in order to clarify the influence of algae concentrations and temperatures on the feeding behavior of mussels, a series of microcosm experiments were carried out, and more information can be found in **Chapter 3**.

In addition, in natural environment, flow rates of water current in the mussels' habitat, chemicals and sanity of water column in mussels' habitat can also affect the filtration rates (**Gosling, 2003)**.

2.4.2 Absorption Efficiencies Measurement and Factors

The efficiency by which ingested ration is absorbed is named the absorption efficiency (*AE*). Food assimilated is defined as that amount of the food ingested which is taken in through membranes of the digestive organs of an animal (**Conover, 1966**).

2.4.2-1 Experimental techniques used to measure absorption efficiencies of mussels

AE cannot be obtained accurately through measuring the quantity of a radioisotope accumulated in the body of an animal fed labeled food unless it is corrected for losses caused by recycling (**Conover, 1966**). One the other hand, to measure assimilation directly, the quantitative recovery of zooplankton faeces is required, and this process can be difficult even in laboratory experiments because faeces pellets are easily torn open or reingested by actively feeding animals. Moreover, "pseudofaeces" formed from uningested organic matter, are difficult to separate from true faeces (**Conover, 1966**).

However, there is a method that only the percentage assimilated is required, in which neither the quantitative recovery of faeces nor knowledge of the amount of food eaten is needed; thus, this method can also be applied in the field almost as readily as in the laboratory (**Conover, 1966**).

This method is dependent on the assumption that only the organic component of the food is significantly affected by the digestive process (**Conover, 1966**). It is required only to obtain the ratio or fraction of organic matter for a food sample and a faeces sample to calculate percentage of assimilation (**Conover, 1966**).

$$AE(\%) = (F' - E')/[(1 - E') \times F'] \times 100 \quad (2\text{-}9)$$

Where *F*' is the organic ratio of the food and *E*' is the organic ratio of faeces.

The organic ratio can be obtained by the ratio of ash-free dry weight to dry weight (AFDW/DW). Food samples for dry weight determination were filtered onto tared, glass-fiber filters. Samples and blanks (filters without food) were dried at 60-70℃ for at least 12 hours. Filters and samples were weighed on a microgram balance. Weight of sample was calculated by difference from the filter tare, after correcting for any weight change in the blank.

Ash-free dry weight was determined by difference from a second weighing after igniting filters in a muffle furnace at 450℃.

There are several collection ways of fecal pellets, depending on the type of experiment and food source: (1) if feeding was heavy, sufficient faecal material could be picked up with a fine-bore squeeze bulb pipette in 5 or 10 minutes; (2) when faeces were scarce, they can be separated from the food culture by filtration onto a suitable bolting cloth (usually No. 25 Nytex); (3) sometimes faecal pellets were collected in the field with a suspended trap made from an 8-inch (20 cm) plastic funnel fitted with a shell vial in which the sample accumulated. Thus, collected faeces were compared with a surface sample of particulate matter taken at the time the traps were set out; (4) alternatively, zooplankton were fed with natural particulate matter and the excreted faeces were collected by pipette or filtration as described above (**Conover, 1966**). Field samples were frozen and returned to the laboratory for analysis (**Conover, 1966**).

2.4.2-2 Factors affecting mussel absorption efficiencies

AE is functionally interrelated with gut capacity, the residence time for food in the gut and the filtration rate. Altering the rate of filtration and gut residence time is a powerful method for a mussel in order to adapt to various food source (**Bayne and Newell, 1983**). In his study, the mussel *Mytilus edulis*, was fed diets of different organic content, and the results indicated that for mussels feeding on a diet of lower organic content, the filtration rate was elevated, a higher percentage of filtered material as pseudofaeces was rejected and the selection efficiency for higher organic content in filtered matter was

increased. Due to these adaptation behaviors, mussels can maintain organic gut content regardless of varieties both in food quality and quantity (**Bayne and Newell, 1983**). In addition, it is believed that adjustment of *AE* can occur over a period of days induced by physiological changes, whereas altering in filtration rate and productuion of pseudofaeces occur in much shorter time scales (**Bayne et al., 1993**).

Moreover, *AE* can be enhanced by the increased synthesis of digestive enzymes, which can accelerate the rates of enzymatic breakdown of ingested material and create more space in the gut for ingested material (**Wong and Cheung, 2001**).

2.4.3 Respiration Rates Measurement and Factors

2.4.3-1 Experimental techniques used to measure respiration rates of mussels

Oxygen consumption rate ($\dot{V}O_2$) can be measured by placing an individual animal in a closed chamber sealed with an oxygen probe that is connected to an oxygen meter. Oxygen concentration (PO_2) is recorded at regular intervals and is not allowed to drop below 80% saturation, unless the specific aim of the experiment is to examine the effects of declining PO_2 on $\dot{V}O_2$ (**Shumway and Koehn, 1982**). A control chamber without an animal is used to correct for bacterial respiration, electrode drift, etc. (**Labarta et al. 1997**). To gain a full understanding of $\dot{V}O_2$ in a given species, measurements should be made at different times of the year, include animals of different sizes, and at different reproductive stages. Oxygen consumption can also be used as an indirect measure of metabolic rate, *MR* (**Gosling, 2003**).

2.4.3-2 Factors affecting respiration rates

(1) Size

The relationship between body size (usually expressed as weight) and the rate of oxygen consumption ($\dot{V}O_2$) is generally described by the allometric equation

$$Y = aX^b \tag{2-7}$$

Where Y is the rate of $\dot{V}O_2$, X is the body weight and a, the intercept, and b, the slope, are fitted parameters (**Gosling, 2003**). The intercept a is a measure of $\dot{V}O_2$ of an individual of unit weight (or length), and its value changes depending on environmental conditions and on the species being tested. The slope b is a measure of the rate of increase in $\dot{V}O_2$ with size. It was reported that the value of b in eleven different mussel species ranged

between 0.44 and 1.09, with a mean value of 0.728±0.130. (**Gosling, 2003**)

(2) Food

Without particulate food, VO_2 reduces to a steady state condition, named the "standard rate", which is characterized with an animal with shell valves open but demonstrating minimal feeding activity. When the mussel is returned to feeding, VO_2 is marked increased in to the "active rate". There can be as much as a five-fold difference between these two rates. "Routine rates" are between active and standard rates, which depend on variations in filtration or ventilation rates that in turn are dependent on factors such as animal size, season, and gametogenesis (**Bayne et al., 1976 a**). There is complex relationship between VO_2 and food supply, involving both consumed energy associated with the mechanical process of filtration and the physiological costs of digestion and excretion (**Gosling, 2003**).

(3) Temperature

Temperature is one of the major factors regulating VO_2 and *MR* in marine mussels. Variations in temperature cause two types of responses. If the animal is suffered a sudden change in temperature it responds by a change in VO_2 which may initially result in overshoot, following by a period of stabilization that occurs over a period of minutes or hours (**Gosling, 2003**).

If the temperature change lasts over days or weeks, VO_2 can be adjusted gradually to a level comparable with that before the temperature change (**Bayne and Newell, 1983**). However, these responses are variable among mussels.

2.4.4 Ammonia Excretion Rates Measurement and Factors

2.4.4-1 Experimental techniques used to measure excretion rates of mussels

Another component of metabolic loss is expressed by the products of excretion. The kidneys and pericardial glands are the major excretory organs, although excretory products are possibly also lost across the general body surface and especially across the gills (**Bayne et al., 1976 a**).The excretion is urine, containing a high concentration of ammonia, and small amounts of amino-nitrogen, urea and uric acid-nitrogen (**Table 2-2**) (**Gosling, 2003**).

Of the N absorbed by mussels from the ingested food, the majority is used for tissue growth and some is excreted as urine (**Bayne et al. 1976 a**).

The excretion rate (*ER*; mg NH_4^+-N h^{-1}) measurement was performed

simultaneously with the filtration rate measurement. After the collection of Chl.a with GF/C filter paper (Whatman), the filtered water samples were measured for ammonium ion concentration. Ammonium ion concentration was plotted against time of measurement and excretion rate was calculated from the slope of the decrease of ammonium ion concentration over time (ammonium ion concentration was measured every 30 minutes). The amount of energy used in excretion (*U*) was calculated for each individual by multiplying *ER* by assuming an energy content ammonium ion of 19.4 J mg^{-1} NH_4^+ **(Widdows, 1985**).

Table 2-2. Nitrogenous compounds in the urine of some mussels (**Gosling, 2003**).

Species	Excreted components as % of total measured nitrogen (N)				Reference
	NH_4-N	Urea-N	Amino-N	Uric acid-N	
Crassostrea virginica	68	8	21	3	Hammen (1968)
Modiolus demissus	62-75	0	25-38	-	Lum & Hammen (1964)
Mercenaria mercenaria	66	0	30	4	Hammen (1968)
Mya arenaria	94	6	-	-	Allen &Garrett (1971)
Mytilus californianus	100	-	0	-	Bayne & Schullard (1977)
Summer	71	-	29	-	Bayne & Schullard (1977)
Winter	97-100	0	0-3	-	Bayne & Schullard (1977)

Nitrogen excretion rates are extremely variable in mussels; average values for a range of species varied from 9.6 to 94.7 g NH_4^+ g^{-1}FW d^{-1} (**Bayne et al., 1976 a**).

2.4.4-2 Factors affecting excretion rates

Factors such as body size, food, temperature and salinity can affect excretion rates (**Gosling, 2003**).

It has been reported that variability in excretion rates can be owing to feeding history and the gametogenic stage of the animals being analyzed. For example, it has been pointed in the study of Bayne and Scullard that in *Mytilus edulis* nitrogen excretion peaks prior to spawning, which is followed by a progressive decrease to minimum rates in autumn and early winter (**Fig. 2-2**) (**Bayne and Scullard**, **1977**). Just before spawning there are minimum glycogen reserves for mussels and they must catabolize protein to adapt to nutritional stress. The consumption of protein reserves under stress conditions has been further proved by the results of Bayne and Thompson for starved *M. edulis* in the laboratory; and it has temperature (6℃) (**Bayne and Thompson**, **1970**). In natural

environment, there is a trend that oxygen consumption decreases during starvation andconsequently the O:N ratio is reduced obviously. These changes are most obvious in smaller individuals, probably due to more rapid consumption been found that protein was consumed more rapidly at higher (16℃) than at lower rates of glycogen reserves and higher weight-specific metabolic rates

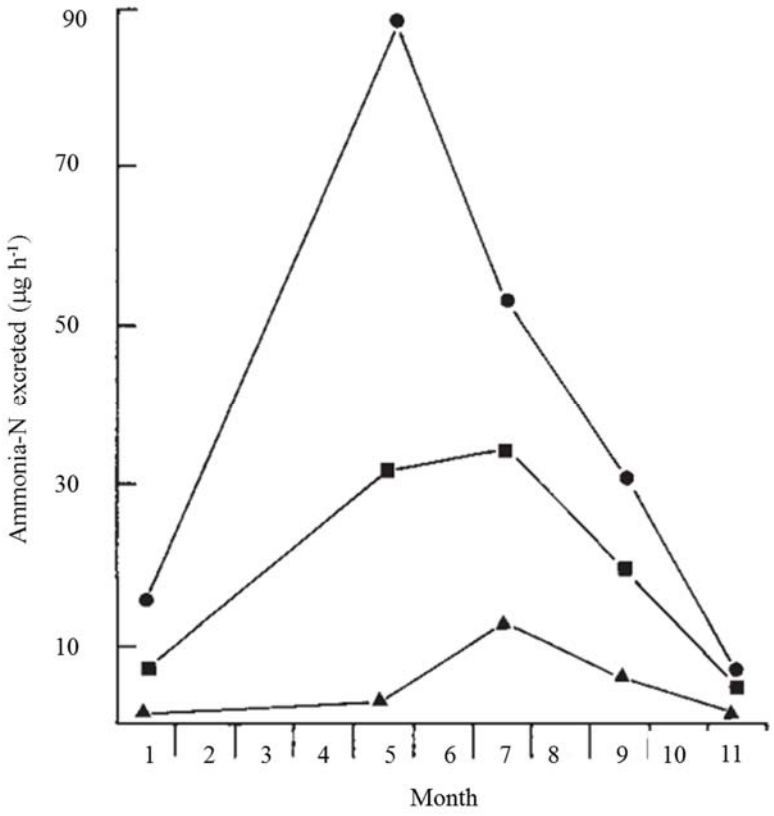

Fig. 2-2. Rates of excretion of ammonia (NH_4^+-N) by the mussel *Mytilus edulis* of 0.2 g (▲), 1.0 g (■) and 2.0 g (●) dry flesh weight during different months of the year. (**Bayne and Scullard, 1977)**.

than larger individuals. In the autumn, when glycogen reserves are high, but food is scarce, ammonia excretion rates are low, which indicates that glycogen is preferentially being used to meet metabolic requirements (**Gosling, 2003**).

Salinity also influences excretion rates. Increased amounts of ammonia and amino acids are excreted when animals are initially exposed to reduced salinities. However, excretion rates return to normal after a period of time and the duration depends on the extent of the salinity decline (**Allen and Garrett, 1971**). Generally, a mussel's first response to variations in salinity is shell closure. Consequently the tissues are independent of osmotic changes in the external medium. In short-term however, salinity change

induces the animal compelled to regulate cell volume by its demand for oxygen and food (**Bayne, 1976 b**). Or else, it may open, begin pumping, and then die because the sanity change is over the ability of mussels to regulate cell volume (**Gosling, 2003**).

2.4.5 O/N

The atomic ratio of oxygen consumed to ammonia excreted can provide the index of the balance in an animal's tissues between the catabolism rates of protein, lipid and carbohydrate substrates. A low value of ~ 10 represents considerable protein is catabolized, while higher values indicates that greater percentages of lipid or carbohydrate are being metabolized (**Bayne and Newell, 1983**).

Mayzaud and Conover has reviewed the process responsible for natural and experimentally-induced changes in the ratio and in the discussion of their relative importance in relation to a variable natural environment, and it has been reported that during starvation the O:N *ratio* is dependent on the availability of energy reserves and the use of body protein (**Mayzaud and Conover, 1988**). Under natural feeding conditions, the value of the ratio depends on the use by the animal of each biochemical fraction assimilated. Using theoretical computations, it can be shown that pure protein catabolism will result in O:N *ratio* in the range 3 to 16, while equal amounts of lipid and protein catabolism will yield values between 50 and 60 (**Mayzaud and Conover, 1988**). In addition, other factors, such as seasonal events in the life cycle, biochemical composition of the body and food quality are important reasons for the variations (**Mayzaud and Conover, 1988**).

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CHAPTER 3
Influence of Algae Concentrations on the Feeding Behavior of *Anodonta Woodiana*

CHAPTER 3

Influence of Algae Concentrations on the Feeding Behavior of *Anodonta Woodiana*

Abstract

The filtration and ingestion rates of *Anodonta woodiana* were determined in relation to food concentrations at two different temperatures 20℃ and 25℃, respectively, using pure suspensions of the green algae *Chlorella* sp. in concentrations ranging from 4.0×10^5 to 3.4×10^8 cells mL^{-1}. The mussels reached the critical cell density at 4.3×10^7 cells mL^{-1} and 3.4×10^6 cells mL^{-1}, when the temperature was 20℃ and 25℃, respectively. For both temperature conditions, when the algae solution concentration was lower than the critical cell density, the filtration rates increased as the increase of algae concentrations till the critical cell density was reached, at which the pseudofaeces were expelled; above the critical cell density, the filtration rates decreased sharply as the algae concentration increased. With increasing algae concentrations, the mass-specific ingestions increased steadily, although there was a slight fluctuation when the algae concentration was around the critical cell density.

3.1 INTRODUCTION

The mussel *Anodonta woodiana* is widely distributed throughout Chinese freshwaters and is an important economic peal mollusk; as a benthic suspension filter feeder, it is also capable of filtering a variety of sestonic particles, including phytoplankton, detritus, small zooplankton and bacteria, as well as dissolved organic matter (**Liu et al., 1979**).

In China, *A. woodiana* is considered an useful animal, because: (1) it is the natural main food source for the healthy culture of *Eriocheir sinensis* (**Guo et al., 2005**) and (2) intensive *A. woodiana* has been promoted as a tool in biomanipulation of lakes in China and strong suppression of phytoplankton, apparent changes of phytoplankton community structure and the improvement of water transparency were observed (**Yang et al., 2008**), although large-scale applications have not been successful so far.

Table 3-1. Basic information about the mussel *Anodonta woodiana.*

Family	Unionidae
Higher Group	Bivalvia
Specific name	*Anodonta woodiana* (Chinese pond mussel)
Biology	Slowly running rivers or eutrophic ponds, commonly found in muddy sediment
Size	(120-200) × (100-170) × (30-45) mm

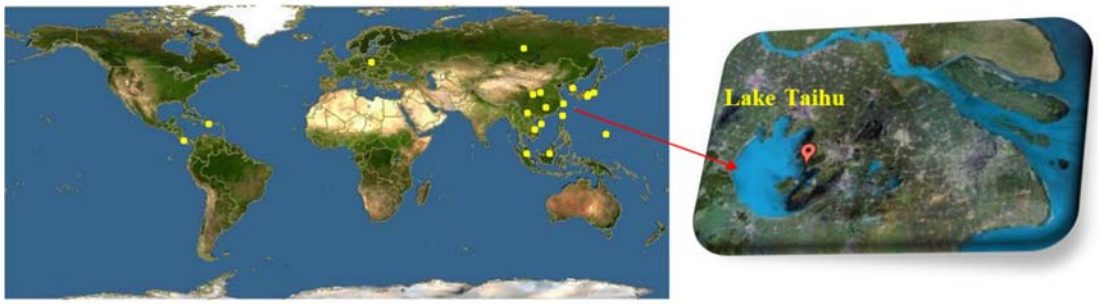

Fig. 3-1. *Anodonta woodiana* (I. Lea, 1834) distribution in the world (places with yellow dots indicate the places with *Anodonta woodiana*).

In mussels particles are filtered from the water column at first. In the mantle cavity (where gills and palps are situated), ingestible particles may enter directly into the mouth,

whereas unwanted particles are sorted on the gills and palps, embedded in mucus and then expelled as pseudofaeces (**Dionisio Pires, 2005**) in the surrounding water, where they can easily disperse. Pseudofaeces may contain live algae cells (**Roditi et al., 1997**) which can be return alive into the water.

Some *in situ* experiments were performed with *A. woodiana* to study the filtering capacity, the filtration rate and selective feeding behavior. The results showed that there was a relationship between filtration rates and total suspended substances; the filtration rate was strongly related to the body weight and the selective feeding behavior was not significant (**Wu et al., 2005**). However, the knowledge of feeding habits and, hence, the determination of filtration and ingestion rates according to algae concentrations and temperature, which is important for understanding the nutritional biology of filter-feeing mussel, is still unavailable.

The study covered by this chapter was aimed at analyzing the influence of algae concentrations on the feeding behavior of *A. woodiana* at two different temperatures under laboratory conditions. Therefore, the feeding behavior of *A. woodiana* on the green algae *Chlorella* sp., at five different concentrations and two different temperatures were investigated in experiments. The results obtained may lead to a better understanding of the feeding mechanism of *A. woodiana*, which are relevant for studies of high efficiency culture, as well as for the possible application of this mussel in biomanipulation of turbid shallow lakes (**Wu et al., 2005**).

3.2 MATERIALS

3.2.1 Collection and Maintenance of *Anodonta Woodiana*

A. woodina mussels were supplied by Kanoya City (Kagoshima, Japan). The mussels were collected with a trawl and kept at 4℃ before transporting them to the laboratory. Immediately upon arrival, the mussels were transferred to 35 L aquaria with 30 L aerated dechlorinated tap water at room temperature (13℃-15℃), under a light: dark regime of 12:12 h for at least one week, before being used for experiments. They were daily fed with green algae *Chlorella* sp. at stable levels. The water was completely refreshed three times per week.

Fig. 3-2. The image of the mussels *Anodonta woodiana* used in this experiment.

Table 3-2. The biological indexes of the mussels used in each group.

Group	Individual weight (g)	Average weight (g)	Shell length (mm)
1	125.8	138.6	112.8
	151.3		135.1
2	170.3	167.4	134.9
	164.6		130.3
3	150.4	147.8	137.9
	145.2		138.9
4	166.2	162.3	133.1
	158.5		131.1
5	216.6	195.2	119.7
	173.8		129.9

3.2.2 Grazing Experiments

One week before the start of grazing experiments, 10 active mussels were selected and placed in a tank filled with 20 L aerated dechlorinated tap water. The mussels were gradually acclimated from the temperature in the aquaria (13℃) to that in the thermostatic chamber (20℃).

They were fed daily with *Chlorella* sp. One day before the experiments, mussels were acclimated to the experimental algae at the concentration to be used in the experiments. Before placing in the grazing vessels mussels were gently cleaned with a brush under running de-ionized water to remove phytoplankton adhered to the shell and not fed for 4 h in clean aerated dechlorinated tap water to clear the guts (**Lei et al., 1996**).

Table 3-3 (a). The initial algae solution conditions of each group in the grazing experiment.

Group	DO ($mg\ L^{-1}$)	Initial algae concentration ($\times 10^4$ cells mL^{-1})	Temperature (℃)
1	9.3	50	20
2		190	
3		4298	
4		14063	
5		33803	

Table 3-3 (b). The initial algae solution conditions of each group in the grazing experiment.

Group	DO ($mg\ L^{-1}$)	Initial algae concentration ($\times 10^4$ cells mL^{-1})	Temperature (℃)
1	8.5	40	25
2		335	
3		2624	
4		9390	
5		12529	

Grazing experiments were performed in the thermostatic chamber, with *A. woodiana* feeding on the green algae *Chlorella* sp. as the single food resources. The mussels were exposed to 2 L algae solution with a range of five food concentrations. **Table 3-3 (a)** and

(b) show the concentrations of *Chlorella* sp. used at the start of the experiment (the algae cell numbers were counted directly with the bacteria counting chamber, Erma Tokyo, 0710). Per grazing vessel, two mussels were placed. Experimental vessels with only phytoplankton and no mussels were used as controls to check for changes in phytoplankton concentrations. The algae solution was stirring gently each time before suspended food was sampled at certain intervals of time to keep food in suspension and homogeneous.

3.3 METHODS

3.3.1 Measurement Methods

3.3.1-1 The indirect method of filtration rates measurement

Of the two principle methods available for the measurement of the rate of passage of water through the mantle cavities of mussels, the indirect method was chosen. The advantages and disadvantages of both methods and the modifications are summarized by Ali (**Ali, 1970**).

The indirect method can provide information on the filtration rate, i.e., the volume which is filtered clear within a certain period of time ("swept-clear volume"). The indirect method requires a measurement of the concentration of the suspended particles at certain intervals of time, in order to calculate the filtering rate.

Mussels were fed green algae, *Chlorella* sp., with a mean diameter of 8-10 μm. Freshwater unionids are known to retain particles greater than 4 μm in diameters with 100% efficiency (**Jørgensen et al., 1984**). Therefore the clearance rates measured in these experiments are assumed to be equivalent to the filtration rate of mussels.

3.3.1-2 Sampling analysis method

Grazing experiment for Group 3, Group 4 and Group 5, samples were measured by means of ultraviolet spectrophotometer (UV-1600PC, Shimadzu, Japan) to determine Chl.a concentrations. A sample of 10 mL was taken on certain intervals from the grazing vessels and measured with the spectrophotometer at 665 nm and 750 nm. Considering the lower initial algae concentration for Group 1 and 2, 10 mL samples were collected from the center of the food solutions and fixed with Lugol-solution for further determination of the algae concentration using algae counting method, with the bacteria counting chamber (Erma Tokyo, 0710) under an inverted microscope (Olympus BHS-BHT) at ×100 magnification.

There is a linear relationship between the Chl.a concentration over the range of concentrations used in the grazing experiments for Group 3, Group 4 and Group 5, as measured with the spectrophotometer, and cell density, which is counted under the inverted microscope. The relationship is described by the following equation:

$$Y = 4E - 05X + 94.195 \quad (3\text{-}1)$$

In which Y is the concentration of Chl.a (μg L^{-1}), X is the cell density (10^6 cells mL^{-1}, R^2 is found to be 0.99.

3.3.2 Calculation Method

Grazing was measured by calculating clearance rates of the mussels on the different food concentrations. The filtration rate (*FR*, mL g FW^{-1} h^{-1}) is calculated as the volume of water (mL) from which the mussel has removed all of the food particles per unit time (**Bunt et al., 1993**). The ingestion rates (*IR*, cells g FW^{-1} h^{-1}) were determined by the following formula, which has been used in modified form by many other authors (**Coughlan, 1969**; **Beiras et al., 1993**):

$$FR = (V/[wt])\, ln[C_0/C_t] \quad (3\text{-}2)$$

$$IR = (V/[wt])(C_{0-}\, C_t) \quad (3\text{-}3)$$

In which V is the volume of the food suspension (2000 mL), w is the fresh weight of the mussels in each vessel (g), t is the duration of the experiment (in hour), C_0 is the algae concentration (cells mL^{-1}) at 0 or one time step before t and C_t is the algae concentration at time t. In the vessels with mussels the algae concentration was corrected for changes observed in the control vessels. In the experiments, a proportion of the filtered cells may return to the water in the form of pseudofaeces and hence their Chl.a may be reanalyzed. The filtration rates and ingestion rates reported here for the mussels experiments are therefore net filtration rates and net ingestion rates.

Filtration rates were calculated for the total experimental period and for different time intervals between 0 and 3 hour.

3.4 RESULTS

3.4.1 Filtration Rates as Functions of Algae Concentrations

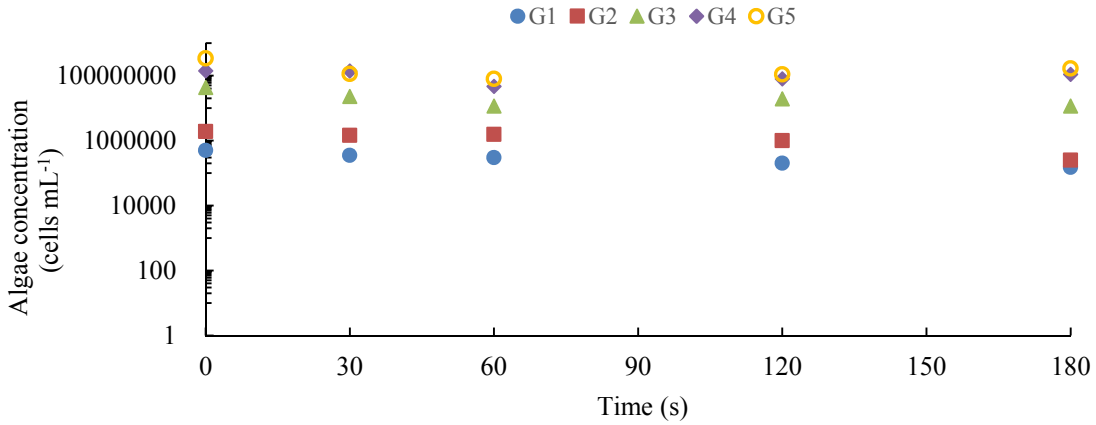

Fig. 3-3 (a). The algae concentration (cells mL^{-1}) changes during the feeding experiment for each group (G1-Group 1, G2-Group 2, G3-Group 3, G4-Group 4, G5-Group 5) at 20℃.

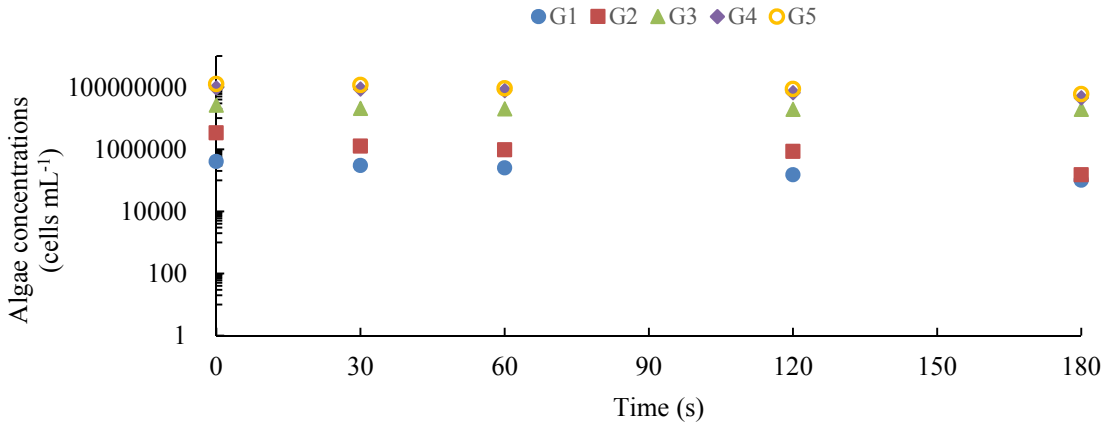

Fig. 3-3 (b). The algae concentration (cells mL^{-1}) changes during the feeding experiment for each group (G1-Group 1, G2-Group 2, G3-Group 3, G4-Group 4, G5-Group 5) at 25℃.

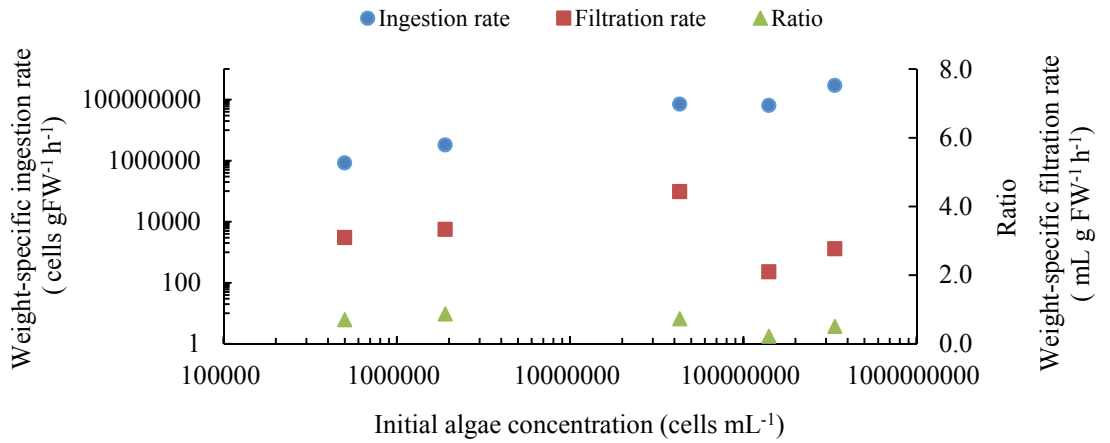

Fig. 3-4 (a). Weight-specific ingestion rate (cells g FW^{-1} h^{-1}), weight-specific filtration rate (mL g FW^{-1} h^{-1}), and ratio of cells filtered in algae solutions offered related to various food concentrations of *Chlorella sp*. at 20℃.

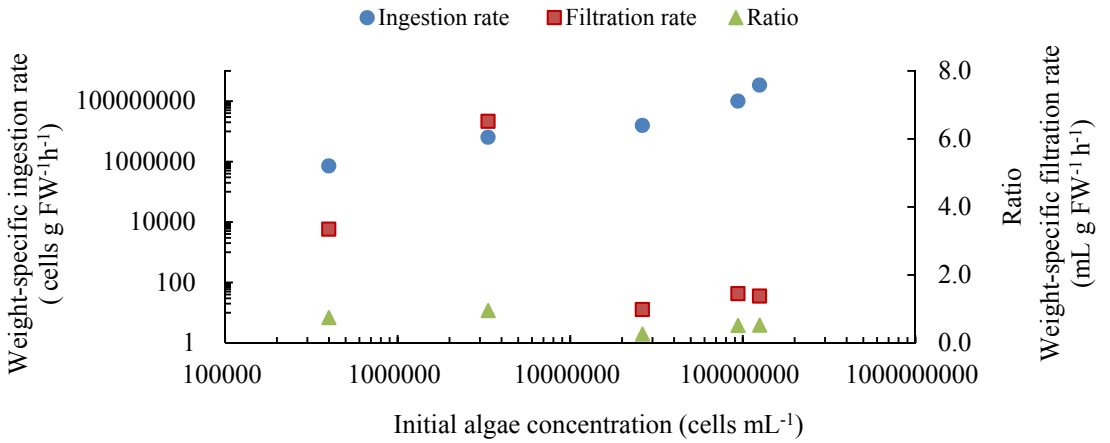

Fig. 3-4 (b). Weight-specific ingestion rate (cells g FW^{-1} h^{-1}), weight-specific filtration rate (mL g FW^{-1} h^{-1}), and ratio of cells filtered in algae solutions offered related to various food concentrations of *Chlorella sp*. at 25℃.

The algae concentration (cells mL^{-1}) changes during the feeding experiment for each group (G1-Group 1, G2-Group 2, G3-Group 3, G4-Group 4, G5-Group 5) at 20℃ and 25℃ are shown in **Fig. 3-3 (a)** and **(b)**.

It is obvious from the results of both series of experiments that an significant correlation exists between algae concentrations offered and filtered volume per hour

(expressed as the filtration rate) shown in **Fig. 3-4 (a)** and **(b)**: the filtration rates increased with increasing algae concentrations at lower algae concentrations (from 5.0×10^5 to 4.3×10^7 cells mL^{-1}, 20℃; from 4.0×10^5 to 3.4×10^6 cells mL^{-1}, 25℃), whereas at higher algae concentrations (from 4.3×10^7 to 3.4×10^8 cells mL^{-1}, 20℃; from 3.4×10^6 to 1.3×10^8 cells mL^{-1}, 25 ℃), the decrease trend of filtration rates with increasing algae concentrations was observed. Furthermore, at the algae concentration of 4.3×10^7cells mL^{-1}, the filtration rate of the mussels at the 20℃ reached the peak value; whereas the filtration rate of the mussels at 25 ℃ reached the maximum value at the algae concentration of 3.4×10^6 cells mL^{-1}.

Similarly, the percentages of algae removed from solution (expressed as ratio of algae removed shown in **Fig. 3-4 (a)** and **(b)**) have demonstrated the same trend to that of filtration rates with algae concentrations: the ratio values increased with increasing algae concentrations at lower algae concentrations (from 5.0×10^5 to 1.9×10^6 cells mL^{-1}, 20℃; from 4.0×10^5 to 3.4×10^6 cells mL^{-1}, 25℃), whereas at higher algae concentrations (from 1.9×10^6 to 3.4×10^8 cells mL^{-1}, 20℃; from 3.4×10^6 to 1.3×10^8 cells mL^{-1}, 25℃), ratio values decreased with increasing algae concentrations. Furthermore, at the algae concentration of 1.9×10^6 cells mL^{-1}, the ratio value of the mussels at the 20℃ reached the peak value; whereas the filtration rate of the mussels at 25℃ reached the maximum value at the algae concentration of 3.4×10^6 cells mL^{-1}.

3.4.2 Ingestion Rates as Functions of Algae Concentrations

In **Fig. 3-4 (a)** and **(b)**, the ingestion rates are plotted against the algae concentrations. At the two different temperatures, with increasing algae concentrations, the absolute cell numbers which were swept off the solution per mussel fresh weight (g) per hour (expressed as weight-specific ingestion rates) increased steadily, although the water passing through the gills (shown as the filtration rate) decreased steadily. When temperature was 20℃, within the range of 5.0×10^5 cells mL^{-1} to 1.9×10^6 cells mL^{-1}, the ingestion rate increased in accordance with the algae concentrations offered; while when the algae concentrations were in the range from 1.9×10^6 cells mL^{-1} to 4.3×10^7 cells mL^{-1}, the curve tended to fall off slightly, possibly due to the production of pseudofaeces within these algae concentrations.

3.5 DICUSSION

3.5.1 Influence of Algae Concentrations on Filtration Rates

The filtration rates here increased with increasing algae concentrations until 4.3×10^7 cells mL^{-1} at 20℃ and 3.4×10^6 at 25℃, respectively, after which, there was rapid decrease. Similarly, as mentioned on Page 48 in **Chapter 2**, in Riisgård's study, it was reported that when the particle concentration of *Rhodomonas baltica* is 3-10 cells μL^{-1}, the filtration rate has been maximized in mussels *Mytilus edulis*; however, excessively high concentrations can restrain the filtration behavior, thus when the concentration is increased above 15 cells μL^{-1}, valve gape has been reduced in the mussels, ultimately inducing a decrease in filtration rates (**Riisgård, 1991**).

The ratio calculated as the percentage of the number of algae removed shows the same trend to that of the filtration rate. It is suggested that algae concentrations (*Chlorella* sp.) higher than 4.3×10^7 cells mL^{-1}greatly disturbed the filtration behavior and filter mechanism of *A. woodina*, thus the filtration rate dropped sharply to very low value (**Fig. 3-3 (a)**). A further increase in algae concentration did not result in an obvious increase in the filtration rate. Similar phenomenon happened to the experiment at 25℃, and 3.4×10^6 cells $mL^{-1}$was the "critical cell density" (**Loo san off, 1942**), the algae concentration at which a tolerable disturbance of filtration activity of mussels occurs. At this particle density the mussel still filters normally, and produces no pseudofaeces. At food concentrations higher than the "critical cell density", a steady decrease in filtration rate occurs together with an increasing rate of pseudofaeces production. A reduction in the energy-expending activity seems to be advantageous for mussels under conditions of excessively high food concentrations, since energy might be wasted by over increasing filtration rates.

With increasing particle concentrations, the rate of pseudofaeces (material cleared from suspension but rejected before ingestion) production was observed to increase during the experiment period. As mentioned that, the filtration rates measured here are the net filtration rates, including the effect of the dispersed pseudofaeces.

3.5.2 Influence of Algae Concentrations on Ingestion Rates

At the two different temperatures, with increasing algae concentrations, ingestion

rates increased steadily. Similarly as reported by Khalil in the clam *Tapes decussatus*, the ingestion rates increased with elevated algae concentrations (**Khalil, 1996**).

Tammes and Dral (**Tammes and Dral, 1973**) reported in their experiments of *Mytilus edulis*, that the amount of retention (cited authors used the word "straining") may change although the quantity of water-pumped is more or less constant. Therefore, the filtering of particles is not directly correlated to the filtration rate, i.e., the filtered volume of water per unit time (**Schulte, 1975**). Thus, ingestion rates did not demonstrate consistent trend with filtration rates shown in **Fig. 3-3 (a)** and **(b)**.

Although in this experiment, limited by the algae concentrations offered were probably not high enough for ingestion rates to reach a peak value and then keep constant. Based on the decrease of filtration rates at excessively high concentrations of algae and the increase of pseudofaeces production, it can be inferred that there is an algae concentration, after which the increasing ingestion rates of *A. woodiana* can be constant. In this way, the gut content can become saturated with organics, without over burden from food.

In addition, the temperature can affect the value of critical cell density in some degree; the mussels reached the critical cell density at 4.3×10^7 cells $mL^{-1}$and 3.4×10^6 cells mL^{-1}, when the temperature was 20℃ and 25℃, respectively. Further research needs to be carried out in future on the effect of temperature on the feeding behavior of *A. woodiana*.

3.6 CONCLUSIONS

The results in this experiment indicated that:

(1) The "critical cell concentration", seems to be an optimum food concentration for mussels, at which filtration activity is reduced to low energy-consuming filtration rate (swept-clear volumes, ventilation) and all food particles are ingested, no pseudofaeces being produced or dispelled. The mussel *A. woodiana* reached the critical cell density at 4.3×10^7 cells mL^{-1} and 3.4×10^6 cells mL^{-1}, when the temperature was 20℃ and 25℃, respectively.

(2) For both temperature conditions, when the algae solution concentration was lower than the critical cell density, the filtration rates increased as the increase of algae concentrations till the critical cell density was reached, at which the pseudofaeces were expelled; above the critical cell density, the filtration rates decreased sharply as the algae concentration increased. With increasing algae concentrations, the mass-specific ingestions increased steadily, although there was a slight fluctuation when the algae concentration was around the critical cell density.

(3) Based on the decrease of filtration rates at excessively high concentrations of algae and the increase of pseudofaeces production observed in the experiment, it can be speculated that there is an algae concentration, after which the increasing ingestion rates of *A. woodiana* can become constant. In this way, the gut content can become saturated with organics, without over burden from food.

Therefore, algae concentrations can control the feeding rates of the mussel *A. woodiana,* on the other hand, through the regulation of filtration rates and pesudofaeces production rates, the food particles for ingestion can be modulated in order to adapt to a wide range of algae concentrations.

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CHAPTER 4

A Comparison of Acute Physiological Responses to Different Algal Diets

CHAPTER 4

A Comparison of Acute Physiological Responses to Different Algal Diets

Abstract

The mussel *Anodonta woodiana* is widely distributed throughout Chinese freshwaters and is an important economic pearl mollusk. Recently, *A. woodiana* as a biomanipulation tool in Chinese lakes due to its strong suppression of phytoplankton, has been attracted increasingly more attention. In order to examine whether *A. woodiana* can exert grazing pressure on *Microcystis* sp. and to evaluate the different effects of each algal diet on mussels' potential growth, a comparative study was carried out on the acute physiological responses to variable microalgal diets including toxic microcystin-producing cyanobacteria *Microcystis aeruginosa* and non-toxic green algae *Scenedesmus obliquus*. The values of filtration rates, absorption efficiencies, oxygen consumption rates and ammonia excretion rates of *A. woodiana* were measured and finally the scope for growth (SFG) value as a measure of metabolic energy balance for *A. woodiana* was calculated and compared. The results showed that the mussel *A. woodiana* has a higher grazing ability on the toxic *M. aeruginosa* compared with the green algae *S. obliquus*; furthermore, the effects of different algal diets on SFG of *A. woodiana* demonstrated that the toxic *M. aeruginosa* may supply more energy for *A. woodiana*'s potential growth. These results indicated that *A. woodiana* has strong adaptation ability when they were exposed to toxic *M. aeruginosa* solution in this study, which implied that there is high application feasibility of *A. woodiana* as a toxic *Microcystis*-blooming controller in practice.

4.1 INTRODUCTION

Lake Taihu is the third largest freshwater lake in China and situated in the Yangtze delta. Since 1980's, however, the development of industry and agriculture in the lake region, as well as a rapid increase in the population has resulted in pollutants being produced and discharged into rivers and the lake. With the deterioration of inflow water quality, eutrophication and cyanobacterial blooms have occurred. Recently, cyanobacterial blooms have extended its coverage and persisted throughout the summer, which affected the function of the lake as a drinking water supply (**Qin et al., 2007**).

To solve the cyanobacterial blooms problem and obtain a normal lake ecosystem, many approaches have been applied to control growth of phytoplankton. One of the extensively used approaches is biomanipulation, using filter feeders, including zooplankton (**Gerasimova et al., 2002**).

Farmed silver carp and bighead carp are the main focus of the non-traditional biomanipulation technique (**Xie and Liu, 2001**), and in recent years, more studies have been reported about the utilization of silver carp and bighead carp in managing algal community structure. However, experimental stocking of silver carp for managing phytoplankton biomass in lakes and ponds has often failed to reduce algae (**Laws and Weisburd, 1990**). As mentioned in **Chapter 1**, page 19, due to the size selectivity of the stocking Carps, for silver Carp only diets particles with size larger than 10 μm can be filtered (**Smith, 1989**), and for bighead Carp, only diets particles with size 17-3000 μm can be filtered. Moreover, some studies have reported that some colonial and filamentous cyanobacteria (such as colonial *Microcystis* spp.) remain viable after the intestinal tract of fishes and even increase their specific photosynthetic activity. Passage through silver Carps did no damage to cyanobacterial cells with mucous cover and only the nutrients from attached bacteria were definitely assimilated (**Kamjunke and Mehner, 2001**). Thus, colonial *Microcystis* spp. were decomposed to unicellular *Microcystis* spp. and excreted into the water.

Other than these filter-feeding fishes, mussels are also the important consumers on phytoplankton especially when they are abundant. However, only zebra mussel (*Dreissena polymorpha*) has been extensively studied before. In the previous studies, the zebra mussel was found to successfully coexist with cyanobacterial blooms and believed to be an important biofilter for its preferential grazing on *Microcystis aeruginosa,* irrespective of whether these cyanobacteria are toxic or not (**Dionisio Pires et al., 2005**).

To explore whether the mussels other than zebra mussels can exert potential grazing

pressure on bloom-forming algae, the mussel *Anodonta woodiana* was chosen, which is widely distributed throughout Chinese freshwaters and is an important native economic peal mollusk. In Lake Taihu, where *Microcystis* sp. blooms frequently especially in summer, abundant *A. woodiana* can be sampled in the lakes especially in the littoral zones.

Previous studies have generally focused on the effects of mussels on water body. The mussel *A. woodiana*, as a tool in biomanipulation of lakes in China has been attracted increasing more attention, and strong suppression of phytoplankton, apparent changes of phytoplankton community structure and the improvement of water transparency were observed (**Yang et al., 2008**).

However, the effects of different algal diets qualities on the physiological responses of the mussel *A. woodiana*, that integrates all the physiological processes, including feeding activities like filtration and absorption, metabolism like respiration and ammonia excretion and ultimately energy balance, known as scope for growth (SFG), have not been considered yet.

The SFG measurement, which measures the energy available for potential growth, has been widely used in many ecotoxicological studies (**Widdows et al., 1995**).

This measurement of physiological energetics, normally in terms of SFG, can provide instantaneous assessment of the growth process of mussels as affected by environmental stress and pollution. SFG is measured by integrating several physiological parameters, including filtration rate, absorption efficiency, respiration rate and ammonia excretion rate, all of which can directly affect the energy available for growth, maintenance and reproduction of mussels.

Although this physiological biomarker has been widely applied in assessing the physiological condition of mussels, there is no study that applied this biomarker to determine the effects of different algal diets qualities on freshwater mussels. Therefore, the objective of this study is to examine the most popular native mussel *A. woodiana* can exert grazing pressure on *M. aeruginosa* and to introduce SFG related to energetic balance of *A. woodiana* to evaluate the influence of different diets including toxic (microcystin-producing cyanobacteria *M. aeruginosa*) and non-toxic (green algae *Scenedesmus obliquus*) on mussels' potential growth. The results obtained may lead to a better understanding of the feeding selective mechanism of *A. woodiana*, which are relevant for the possible application of this mussel in biomanipulation as toxic cyanobacterial blooms controller in turbid shallow lakes.

4.2 MATERIALS

4.2.1 Production of Algal Species

Due to the fact that *M. aeruginosa* is the common dominant species in the blooms, the toxic strain of *M. aeruginosa* is used as the diets fed to the mussels. *S. obliquus* is also the diets fed in the experiments as a comparison.

Toxic *M. aeruginosa* strain NIES-90 and green algae *S. obliquus* strain NIES-2279 were obtained from National Institute for Environmental Studies (NIES), Ibaraki in Japan. Both algal species were cultured under controlled conditions (25 ℃; 12h light:dark cycle).

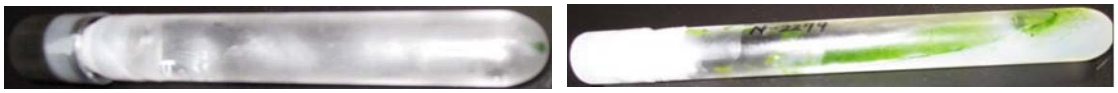


Fig. 4-1. *Microcystis aeruginosa* strain NIES-90 (left) and *Scenedesmus obliquus* strain NIES-2279 (right).

For toxic *M. aeruginosa* strain NIES-90, the culture medium MA (**Ichimura, 1979**) was used; for non-toxic green algae *S. obliquus* strain NIES-2279, the culture medium C (**Provasoli and Pintner, 1959**) was used.

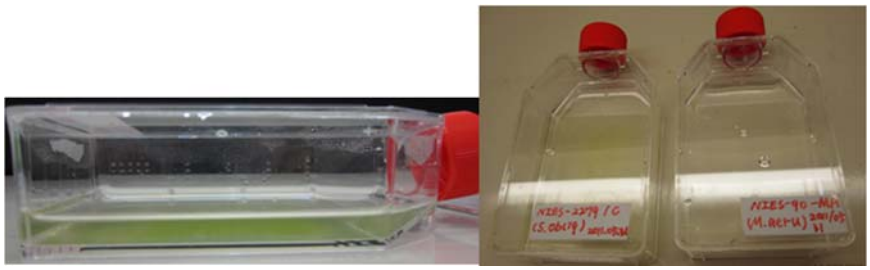


Fig. 4-2. *Scenedesmus obliquus* strain NIES-2279 (left) and *Microcystis aeruginosa* strain NIES-90 (right).

Each strain was used in its exponential stable phase of growth. Cultured algae were separated from culture medium by centrifugation and they were diluted in aerated dechlorinated tap water. In order to obtain comparable masses of suspended matter in each diet, the initial Chl.a concentration was corresponding to around 35 μg L^{-1} in the diets treatments. There were three diets treatments: (1) toxic *M. aeruginosa* (2) *S. obliquus*, and (3) a mixture of 50% toxic *M. aeruginosa* and 50% *S. obliquus*. The characteristics of algal diets were listed in **Table 4-1**.

4.2.2 Mussels

The mussels of *A. woodiana* were supplied by Gunma Prefecture in Japan. The mussels were kept at 4℃ before transporting them to the laboratory. Immediately upon arrival, the mussels were transferred to 35 L aquaria with 20 L aerated dechlorinated tap water at controlled temperature 25℃, under a light:dark regime of 12h:12h for at least one week, before being used for experiments. The water was completely refreshed three times per week. 24 individuals of *A. woodiana* (the mussel length of 9.1 ± 0.3 cm and fresh-weight of 79.6 ± 8.7 g) were chosen with similar sizes to remove the effects caused by physiological sizes. Before introduction to the beakers, mussels were gently cleaned with a brush under running de-ionized water to remove phytoplankton adhered to the mussel. They were kept in clean aerated dechlorinated tap water with artificial diets, 2 days before the experiment, they were not fed for 2 days to clear the guts.

4.3 METHODS

Grazing experiments of *A. woodiana* on three different diets were carried out simultaneously. For each diet condition, two mussels in a beaker containing 2,000 mL of each experimental diets solution with constant aeration were used with three replicates, simultaneously; in addition, beakers with diets, but without mussels, were used as a control to check for growth of phytoplankton cells during the experiments. The beakers were placed in an incubator at 25 ± 0.5℃. After fed with different algal diets for four hours, the SFG in mussels were quantified fed with each diets solution for treatment microcosms.

4.3.1 SFG Measurement and Calculation

4.3.1-1 Filtration rates measurement

The filtration rate (*FR*, mL g^{-1}FW h^{-1}) of each individual mussel, defined as the volume of water cleared per unit time was determined by the time course of the decrease of algal density due to mussel filtration. The experiment began when the mussel valve of the mussel opened. Chl.a concentration in the feeding beaker was measured every one hour, for a total of 4 hours.

The algae solution was stirring gently to keep diets in suspension and homogeneous. Each time suspended diets were sampled at certain intervals of time. The Chl.a concentration was measured by fluophotometer, and *FR* was determined by the following formula, which has been used in modified form by many other authors (**Coughlan, 1969**):

$$FR = (V/[wt]) \ln[C_0/C_t] \quad (4\text{-}1)$$

In which *V* is the volume of the diets suspension (2 L), *w* is the fresh weight of the mussels in each vessel (g), *t* is the duration of the experiment (in hour), C_0 is Chl.a concentration (μg L^{-1}) at 0 or one time step before *t* and C_t is the particle concentration at time *t*. In the vessels with mussels the particle concentration was corrected for changes observed in the control vessels.

The amount of ingested or consumed energy (*C*) was calculated for each individual by multiplying *FR* by the amount of particle organic material (*POM*) per liter in input water samples and assuming an energy content of algal material of 23 J mg^{-1} (**Widdows and Johnson, 1988**).

4.3.1-2 Respiration rates measurement

The respiration rate (μg O_2 g^{-1}FW h^{-1}) of mussels was measured by respirometers in enclosed chambers (1,200 mL) full of oxygen saturated tap water after filtration rate measurement experiments. The mussels were isolated from the treatment vessels after the filtration rate measurement and transferred into corresponding enclosed chambers. A chamber without a mussel was used as a control. Tap water in the chamber was mixed by a magnetic stirrer placed on the bottom. The oxygen concentration in the solution was detected by an oxygen electrode (LDO-HQ30d, HACH). Measurement of oxygen concentrations commenced when the animals opened their mussel valves and lasted for 1 hour in darkness. The initial and final oxygen concentrations were measured and the respiration rate was calculated from the decrease of oxygen concentrations in 1 hour. The amount of energy used in respiration (*R*) was calculated assuming an energy content of 0.456 J μmol$^{-1}O_2$ (**Gnaiger, 1983**).

4.3.1-3 Absorption efficiencies measurement

The absorption efficiency of the organic matter by mussels was determined by the ratio method (**Conover, 1966**). This method assumed that the absorption of the inorganic component of diets was insignificant during the digestive process. After the respiration rate measurement, the mussels were placed into three vessels with 2 L aerated dechlorinated tap water. Any faeces egested were collected with pipettes after 12 hours. The faeces were filtered onto a preweighted GF/F filter (Whatman). The filters were subsequently ashed in a muffle furnace at 450℃ for 1 h, and the loss of organic matter was calculated. The absorption efficiency (*AE*) was calculated by comparing the ratio of organic and inorganic materials between the diets and faeces, by the following equation (**Conover, 1966**):

$$AE(\%) = (F' - E')/[(1 - E') \times F'] \times 100 \quad (4\text{-}2)$$

Where *F'* is the ash-free dry weight:dry weight ratio (AFDW/DW) of the food and *E'* is the AFDW/DW ratio of faeces.

4.3.1-4 Excretion rates measurement

Another component of metabolic loss is presented by the products of excretion. The excretion rate (*ER*; mg NH_4^+-N h^{-1}) measurement was performed simultaneously with the

respiration rate measurement. After the measurement of the respiration rate, the filtered water samples were measured for ammonium ion concentrations with the Dionex Ion Chromatography ICS-2100_ICS-1100_AS (Thermo Fisher Scientific, U.S.A.). The ammonium ion concentration was measured at 0-hour and 1-hour and the excretion rate was calculated from the increase of ammonium ion concentrations in 1 hour. The amount of energy used in excretion (*U*) was calculated for each individual by multiplying *ER* by assuming an energy content ammonium ion of 19.4 J $mg^{-1}NH_4^+$ (**Widdows and Johnson, 1988**).

4.3.1-5 SFG Calculation

The SFG, which defined the energy available for growth and reproduction and was calculated by the following equation (**Widdows and Johnson, 1988**):

$$\mathrm{SFG} = C \times AE - R - U \tag{4-3}$$

Where *C* is the consumed energy, *AE* is the absorption efficiency, *R* is respired energy, and *U* is excreted energy.

4.3.2 Statistical Analysis

For all feeding data, physiological parameters were compared using one-way analysis of variance (ANOVA). All statistical analysis was carried out with Excel.

4.4 RESULTS

4.4.1 Characteristics of Diets

Chl.a concentrations were adjusted to approximately 35 μg L^{-1} for the three diets tested (**Table 4-1**; range: 30.4-38.7 μg L^{-1}), but there were not significant variations between diets (one-way ANOVA, $F<F_{0.05}$). POM concentrations did not differ statistically (range: 105-120 mg L^{-1}; one-way ANOVA, $F<F_{0.05}$). An organic content between 93.6-95.6% was obtained for the three diets (**Table 4-1**) and no significant differences were observed between them (ANOVA on arcsine transformed data, $F<F_{0.05}$). Size spectra obtained with the microscope showed that *S. obliquus* was obviously larger than *M. aeruginosa.* Microscope observations showed that the stain *M. aeruginosa* NIES-90 consisted of single cell suspensions, while *S. obliquus* NIES-2279 consisted of colonies.

Table 4-1. Charactistics of the experimental diets fed to *Anodonta woodiana*.

Strain code	Shape	ESD (mm)	Microcystin (μg L^{-1})	Chl.a (μg L^{-1})	POM (mg L^{-1})	Organic content (%)
Microcystis aeruginosa NIES-90	Single cell	3.0 - 4.5 (**Juhel et al., 2006**)	1.5	38.7	120.0	95.6
Scenedesmus obliquus NIES-2279	Colony	9.1-18.1 (**Dionisio Pires et al., 2004**)	No microcystin	30.4	105.0	93.6
Mixture	Colony	-	0.8	37.0	112.5	94.9

POM: particulate organic matter; ESD: equivalent spherical diameter. Number of measurement=3.

4.4.2 Acute Physiological Responses to Different Algal Diets

4.4.2-1 Grazing rates on different algal diets

The Chl.a concentration changes during four hours in beakers for filtration rates

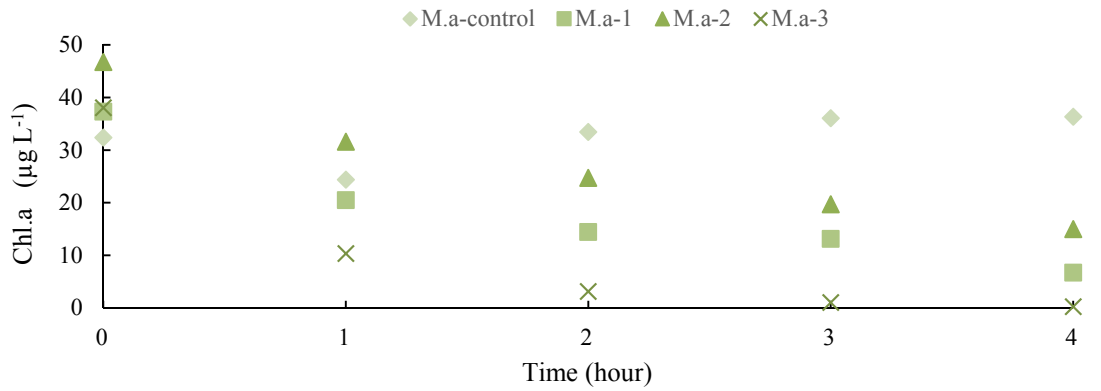


Fig. 4-3 (a). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of mussels fed on the diet *Microcystis aeruginosa*.

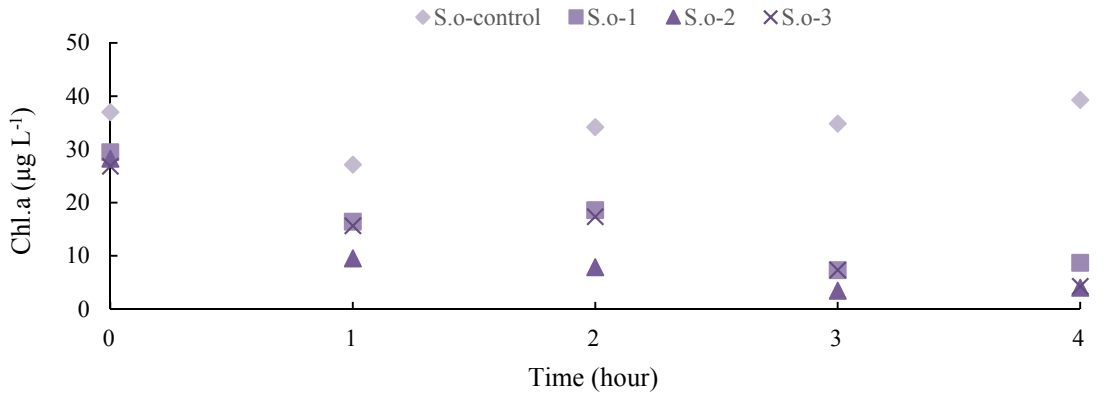


Fig. 4-3 (b). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of mussels fed on the diet *Scenedesmus obliquus*.

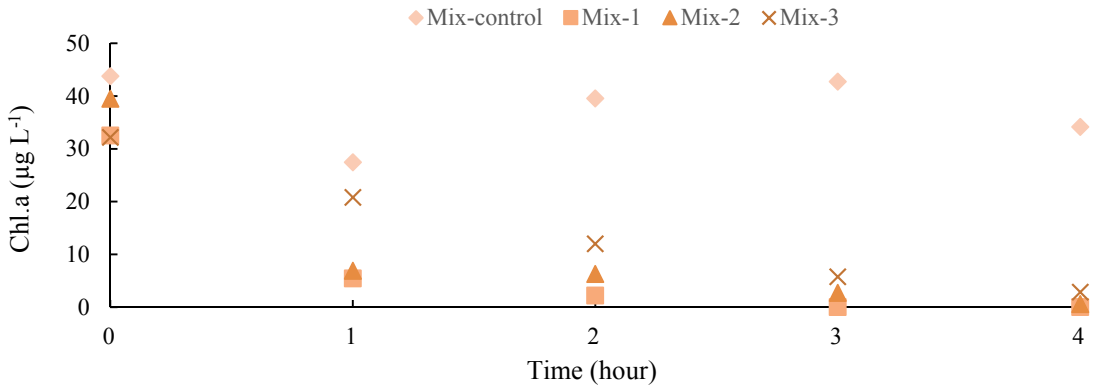


Fig. 4-3 (c). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of mussels fed on the mixed diet.

Table 4-2. The AFDW/DW ratio in faeces and food for *Anodonta woodiana* feeding on different diets.

Strain code	*F′*	*E′*
Microcystis aeruginosa NIES-90	0.96	0.75
		0.71
		0.64
Scenedesmus obliquus NIES-2279	0.94	0.90
Mixture	0.95	0.92

measurement of mussels fed on different diets are shown in **Fig. 4-3 (a)**, **(b)** and **(c)**. The weight-specific filtration rate and absorption efficiency of *A. woodiana* on each diet solution are shown in **Fig. 4-4**. A comparison of the mean filtration rate (*FR*) calculated with the three diets offered to mussels showed a significant statistical difference (**Fig. 4-4**, one-way ANOVA, $F>F_{0.05}$) ranging from 6.0 mL g^{-1}FW h^{-1} for *S. obliquus* NIES-2279 to 14.4 mL g^{-1}FW h^{-1} for the mixture diet. The mean absorption efficiency was significantly higher for mussels fed on the algae *M. aeruginosa* (89.0%) than those fed either on the mixture diet, or *S. obliquus* (40.9% and 38.5%, respectively) (one-way ANOVA, $F>F_{0.05}$, **Fig. 4-4**).

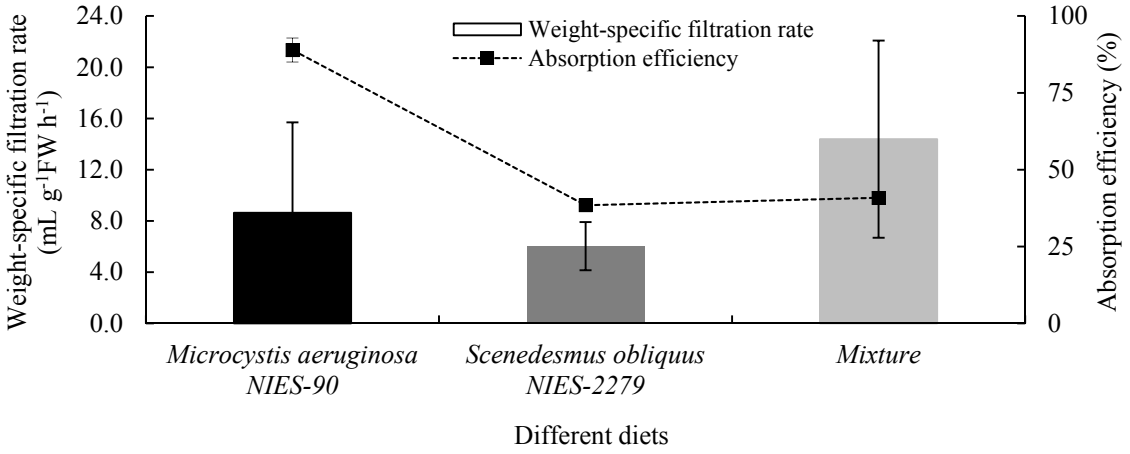


Fig. 4-4. The weight-specific filtration rate (mL g^{-1}FW h^{-1}) and absorption efficiency (%) of *Anodonta woodiana* feeding on different diets. Error bars are S.E. (n=3).

4.4.2-2 Metabolic rates on different algal diets

The metabolic rates of *A. woodiana* feeding on different diets were shown in **Fig. 4-5**. A comparison of the three metabolic rates indicator, respiration rates, amongst the three different diets were significantly statistically different (**Fig. 4-5**, one-way ANOVA, $F>F_{0.05}$). Mean respiration rates of the mussels fed with the mixture diet were observed significantly higher than those fed with each of the single diet. Meanwhile, the respiration rates of mussels fed with *M. aeruginosa* were obviously higher than those fed with *S. obliquus*. Mean values of respiration rates ranged from 2.7 μg O_2 g^{-1}FW h^{-1} for *S. obliquus* to 14.4 μg O_2 g^{-1}FW $h^{-1}$for the mixture diet.

Ammonia excretion rates among mussels fed with each diet were not obviously different (**Fig. 4-5**, one-way ANOVA, $F<F_{0.05}$). Furthermore, as shown in **Fig. 4-5**, mean values of ammonia excretion rates ranged from 0.7 μg NH_4^+ g^{-1}FW h^{-1} for the diet *M. aeruginosa* to 1.1 μg NH_4^+ g^{-1}FW h^{-1} for the mixture diet.

Table 4-3. The DO concentration changes during one hour in beakers for respiration rates measurement of mussels fed on the mixed diet.

Group	DO concentration (mg L^{-1})								
	M.a-1	*M.a*-2	*M.a*-3	*S.o*-1	*S.o*-2	*S.o*-3	Mix-1	Mix-2	Mix-3
0-hour	8.51	8.80	8.64	8.43	8.60	8.45	8.71	8.45	8.74
1-hour	7.13	8.09	7.75	8.07	7.53	8.26	7.07	7.11	7.37

Table 4-4. The ammonium ion concentration changes during one hour in beakers for respiration rates measurement of mussels fed on the mixed diet.

Group	Ammonium ion concentration (mg L^{-1})								
	M.a-1	*M.a*-2	*M.a*-3	*S.o*-1	*S.o*-2	*S.o*-3	Mix-1	Mix-2	Mix-3
0-hour	0	0	0	0	0	0	0	0	0
1-hour	0.113	0.059	0.087	0.075	0.065	0.064	0.100	0.073	0.167

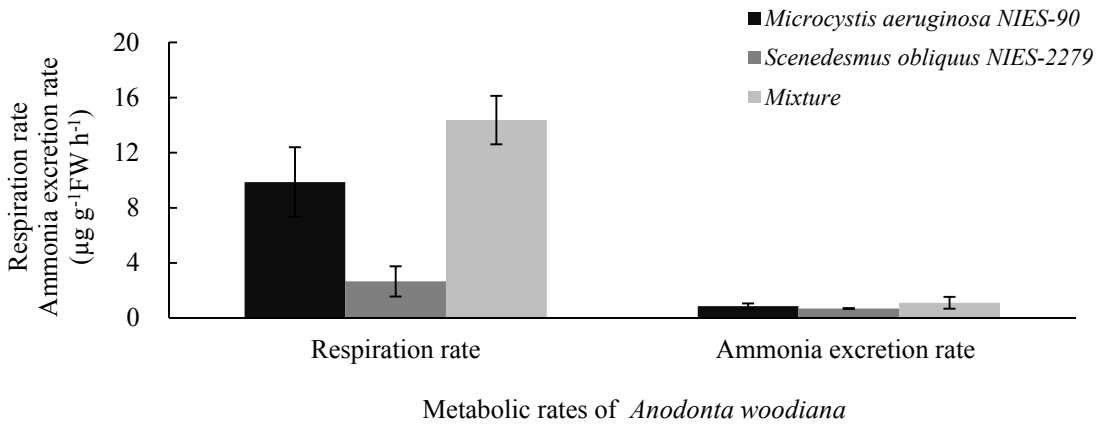


Fig. 4-5. The metabolic rates (μg g^{-1}FW h^{-1}) of *Anodonta woodiana* feeding on different diets. Error bars are S.E. (n=3).

4.4.3 SFG of *Anodonta Woodiana* on Different Algal Diets

The energy equivalents of mussels are presented in **Table 4-2**. The energy used for growth as represented in SFG, differed significantly among the three algal diets (one-way ANOVA, $F>F_{0.05}$), ranging from 5.9 J g^{-1}FW h^{-1} for *S. obliquus* to 27.2 J g^{-1}FW h^{-1} for *M.*
aeruginosa. SFG in mussels was significantly higher for the mussels fed with *M. aeruginosa* than those fed with the other two diets, mainly contributed by the highest filtration rates.

4.5 DISCUSSION

4.5.1 The Predominant Factors of Diets Characteristics That Influence Mussels' Feeding Behavior

As for many freshwater and marine suspension feeders, feeding-process of zebra mussels are influenced by environmental factors such as temperature (**Bayne et al., 1977**) but also by diets quality and diets quantity. In this study, *A. woodiana* was fed three algal diets with different characteristics.

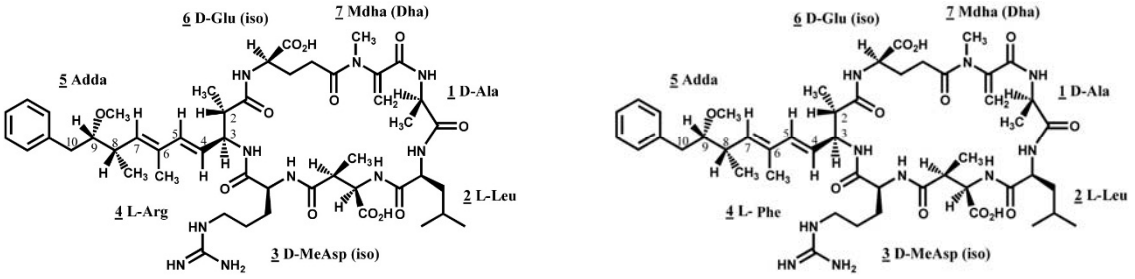
In the case of algal suspensions, diets quantity can be expressed as the Chl.a concentration and particulate organic matter (POM). Each diet was supplied to the mussels at comparable levels of POM in order to eliminate any effect of diets quantity on mussels' physiological parameters. Furthermore, each algae suspension was offered at bloom levels corresponding to approximately 30 μg L^{-1} of Chl.a, which is in the middle of the range of values found in Lake Taihu.

Diets quality can be expressed in different ways: organic:inorganic ratio of seston, toxin content and also polyunsaturated fatty acids (PUFAs) content. The shape and size of algal cells are also parameters that potentially affect the feeding behavior of zebra mussels.

For all the three diets tested, organic content was high (higher than 90%), corresponding to an organic:inorganic ratio of about 9:1 and not significantly different between diets. According to Schneider et al. (**Schneider et al., 1998**), with such a high organic content, zebra mussels reported with high feeding parameters, particularly high absorption efficiency and high SFG.

Microcystins (MCs) are a family of cyclic heptapeptides isolated from cyanobacteria, and their structures were determined in 1984, since when 67 related compounds have been isolated (**Botes DP et al., 1984; Budde WL et al., 2006**). MC-LR (shown in **Fig. 4-6 (a)**) is the most popular member among the microcystins family, containing leucine (L) and arginine (R) in position 2 and 4, respectively; MC-LF is the variant of MC-LR, containing the phenylalanine (F) in position 2 as shown in **Fig. 4-6 (b)** (**Mayumi et al., 2006**). Two of the three algal suspensions contained MCs in a concentration of 1.5 μg L^{-1} and 0.8 μg L^{-1}, respectively. Our toxin measurement using enzyme-linked immunosorbent assay (ELISA) method did not allow separate quantification of MC-LR or MC-LF but gives a total estimation of MCs present in cyanobacterial cells. Therefore,

in our experiments, the impacts of MCs on the mussels' feeding behavior may be the result of a combined effect of the toxins present in both variances.



Microcystins (abbreviation)	Molecular weight	1	2	3	4	5	6	7
Microcystin-LR (MC-LR)	994	D-Ala	L-Leu	D-MeAsp	L-Arg	Adaa	L-Glu	Mdha
Microcystin-LF (MC-LF)	985	D-Ala	L-Leu	D-MeAsp	L-Phe	Adaa	L-Glu	Mdha

Adda: [(2*S*, 3*S*, 8*S*, 9*S*)-3 amino-9-methoxy-10-phenyl-2, 6, 8-trimethyldeca-4 (*E*), 6 (*E*)-dienoic acid, Mdha: *N*-methyldehydroalanine, MeAsp: *β-erythro*-methyl-D-Asp.

Fig. 4-6 (a). molecular structure of microcystin-LR and **(b).** molecular structure of microcystin-LF and related compounds. (Modified from **Mayumi et al., 2006**)

The three diets have different shapes and sizes, *S. obliqure* presenting the largest cell type in the form of cell colonies, and the strain of *M. aeruginosa* corresponds to the smallest cell type. Analysis of each diet's characteristics therefore shows that the predominant factor that could influence the mussel's feeding behavior was diets quality, particularly toxicity, and cell shape and size. Moreover, no reports are available in the literature concerning the lipopolysaccharides content of these algal diets. Furthermore, temperature conditions and the dose of diets supplied met the requirement for a high feeding response.

4.5.2 Impacts of Different Algal Diets on Acute Physiological Responses

4.5.2-1 Filtration rates

As shown in **Fig. 4-4**, the weight-specific filtration rate of *A. woodiana* values obtained in this study were approximately fell in the range of values found by Wu et al. (**Wu et al., 2005**). In their study, the mussels *A. woodiana* with freshweight of 50-100 g were used to filter the eutrophic lake water with algal concentrations ranged from 4.6×10^6 to 5.9×10^7 cells L^{-1} from July to September, and the average value of filtration rates was 8.9 mL g^{-1}FW h^{-1}, which is lower than the maximum value of 14.4 mL g^{-1}FW h^{-1} for the

mixture diet found in this study. The possible reasons maybe that the diet and other experimental conditions, such as temperatures for the two experiments were different, which can induce the difference in the filtration rates.

In addition, in this study, the filtration rates of the mussel *A. woodiana* were significantly higher in the mixture diets treatment than those in single diets treatments. This is in accordance of the findings of Liu et al. (**Liu et al., 2009**) who observed higher filtration rates of the mussels *Unio douglasiae* and *Corbicula fluminea* on a mixture of *S. obliquus* and toxic *M. aeruginosa* than the single diets alone. Dionisio Pires et al. (**Dionisio Pires et al., 2004**) also observed a higher filtration rate of zebra mussel on a mixture of *S. obliquus* and *M. aeruginosa* than *Scenedesmus* alone.

Moreover, it was proposed by Dionisio Pires et al. (**Dionisio Pires et al., 2004**) that the sensory quality of the diets may affect the feeding activity of the mussels and hence their pumping rate. Thus, it can be suspected that the sensory impact of mixed *S. obliquus* and *M. aeruginosa* on the mussels might be greater than that of *S. obliquus* or *M. aeruginosa* alone, thus resulting in higher pumping and clearance rates in the mixture diets treatment.

Furthermore, it was noted that clearance rates obtained with the green algae *S. obliquus*, which are considered as highly desirable diets were much lower than them obtained with *M. aeruginosa*. Mussel's filtration gill selection is mainly affected by algae sizes. As the algal diets characteristics of size spectra obtained with the microscope showed that *S. obliquus* was obviously larger than *M. aeruginosa*. *M. aeruginosa* used here are single cells (cells range from 2.6 to 5.4 μm in diameter) instead of the colonies in natural water body; *S. obliquus* is colonial green alga consisting of cells aligned in a flat plate. It is probably that *M. aeruginosa* cells were in the range *A. woodiana*'s gill preferred.

In addition, this indicates that the toxic *M. aeruginosa* in this experiment did not exert acute effects on the grazing of *A. woodiana* in present research and on *Unio douglasiae* in previous research (**Liu et al., 2009**). When very toxic *M. aeruginosa* contained predominately MC-LF, which is known to be one of the most potent MC variants was supplied in the algae suspension at high levels, caused the mussels to reduce their filtration rate significantly (**Juhel et al., 2006 a**). Thus, it can be explained that in the present study, the MC-LF concentration in the algae suspension maybe not sufficient to induce the negative effect on mussels' filtration rates.

4.5.2-2 Absorption efficiencies

In this study the absorption efficiencies of *A. woodiana* on *M. aeruginosa* were

obviously higher that the values obtained by *A. woodiana* on *S. obliquus* and the mixture diets. This was similar to the results reported by Liu et al. In their study, it was also found that both the mussels *Unio douglasiae* and *Corbicula fluminea* excreted more *S. obliquus* cells than *M. aeruginosa* cells in the excreted produces thus both the mussels they used preferred to ingest *M. aeruginosa* cells to *S. obliquus* (**Liu et al., 2009**). Meanwhile, they gave the possible reason was that *S. obliquus* cells have thick cell walls which makes them indigestible in the digestive tracks (**Liu et al., 2009**). Previous studies report that zebra mussels showed a preference for the low-toxicity strain *M. aeruginosa* CCAP1450/06 contained 7.4 μg L^{-1} of the MC-LR variant to the green algae *Chlorellla vulgaris* (**Juhel et al., 2006 a**).

However, we inferred that it is the different digestive enzyme activity in *A. woodiana's* digestive tract that induced the prefer ingestion of *M. aeruginosa* to the green algae. As it was reported by Fei et al. that the ratio of amylase to protease enzyme activities (A/P) in the stomach of *A. woodiana* was quite high, which indicated that *A. woodiana* has stronger digestive ability on cyanobacteria that mainly contains of starch than green algae that mainly contains of protein (**Fei et al., 2006**). In a word, the toxic *M. aeruginosa* did not exert effects on the grazing of *A. woodiana* in present research, on the contrary *A. woodiana* preferred to graze on the toxic *M. aeruginosa* to the green algae *S. obliquus*.

4.5.3 Impacts of Different Algal Diets on SFG of *Anodonta Woodiana*

Table 4-5. Energy equivalent of *Anodonta woodiana* on different diets.

Strain code	*A* (J g^{-1} h^{-1})	*R* (J g^{-1} h^{-1})	*U* (J g^{-1} h^{-1})	SFG (J g^{-1} h^{-1})
Microcystis aeruginosa NIES-90	27.34	0.14	0.02	27.16
Scenedesmus obliquus NIES-2279	5.94	0.04	0.01	5.90
Mixture	8.25	0.20	0.02	8.03

A: energy absorbed from the food; *R*: energy consumed by respiration; *U*: energy consumed by ammonia excretion; SFG: energy available for growth.

As the endpoint of these experiments, the net energy balance (SFG) was calculated and indicated the *M. aeruginosa* as the diet supplied the mussel with the highest SFG value compared with either *S. obliquus* or the mixture diet. Furthermore, the least SFG

value supplied by the *S. obliquus* as the diet was mainly due to the significant production of 'pseudodiarrhoea', which is pseudofaecal material known to be rich in mucus (**Juhel et al., 2006 b**). Since molluscan mucus is known to be expensive to produce.

In addition, as it is reported by Harold (**Harold, 1968**), the ratio of SFG to *A* (named net growth efficiency K_2) may range from 20% to 90%; high K_2-values must also be looked upon as an adaptive response to environmental conditions; growth efficiencies under laboratory conditions are often higher than in the field. However, as shown in **Table 4-2**, the SFG value measured in this study were over 90% of the *A* value. One possible reason for the much lower consumed energy by respiration and ammonia excretion is that the respiration rate and ammonia excretion rate measured here were standard metabolic rates without diets which are lower than active metabolism rates.

Ultimately, the toxic *M. aeruginosa* did not restrain the grazing behavior of *A. woodiana* instead supplied the highest SFG value for the mussels' growth. In some degree, this indicated that the mussel *A. woodiana* accumulated and depurated MCs, although, we could not distinguish whether it was MC-LR or the MC-LF variant.

As an instantaneous measurement of the energy available for growth, increased SFG indicates that the processing of *M. aeruginosa* by the mussels *A. woodiana* is a profitable mechanism as a large amount of energy was recovered by *A. woodiana* feeding on the *M. aeruginosa.*

4.6 CONCLUSIONS

The results of acute physiological responses on different diets by *A. woodiana* and different SFG values obtained from different diets indicated that:

(1) The mussel *A. woodiana* preferred to graze on the toxic *M. aeruginosa* compared with the green algae *S. obliquus*.

(2) The toxic *M. aeruginosa* supplied 4.5 times more energy for *A. woodiana*'s potential growth than that *S. obliquus* could supply; although toxic algal blooms have been reported to reduce mussel growth as mentioned in **Chapter 2-3**.

(3) On the other hand, *A. woodiana* showed strong adaptation ability exposed to toxic *M. aeruginosa* solution.

Thus, the results in this experiment indicated that there is quite high feasibility to use *A. woodiana* as a toxic *Microcystis*-blooming controller in the practice.

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CHAPTER 5

Interactions Between *Anodonta Woodiana* and Algae-Blooming Water

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Interactions Between *Anodonta Woodiana* and Algae-Blooming Water

Abstract

Sinanodonta (*Anodonta*) *woodiana* is widely distributed throughout Chinese freshwaters and is an important economic pearl mollusk. In order to evaluate the application feasibility of *A. woodiana* as a *Microcystis*-blooming removal tool, the 6-day feeding responses experiment was carried out with naturally blooming pond water and the mussels in laboratory. In this experiment, phytoplankton abundances and community structure were analyzed on 0-day and 6-day for both control and treatment microcosms; also, filtration rates, absorption efficiencies, respiration rates and ammonia excretion rates of *A. woodiana* were measured on 0-day and 6-day and finally the scope for growth (SFG) value as a measure of metabolic energy balance for *A. woodiana* was calculated and compared. The results showed that *Microcystis* spp. of colony and unicell were reduced obviously on the 6-day; meanwhile, after 6-day's exposure to *Microcystis*-blooming pond water, the SFG value for *A. woodiana* increased. These results indicated that toxic *Microcystis* spp. in natural eutrophic water can be removed greatly by *A. woodiana*; moreover, the mussels have strong ability for adaptation when they were exposed to toxic natural eutrophic water. Therefore, it can be inferred that there is high application feasibility of *A. woodiana* as a *Microcystis*-blooming controller in practice.

5.1 INTRODUCTION

The mussel *Sinanodonta* (*Anodonta*) *woodiana* (**Zhao et al., 2011**) is widely distributed throughout Chinese freshwaters and is an important economic pearl mollusk; as a benthic suspension filter feeder, it is also capable of filtering a variety of sestonic particles, including phytoplankton, detritus, small zooplankton and bacteria, as well as dissolved organic matter (**Liu et al., 1979**). In Chinese Lake Taihu, where *Microcystis* spp. blooms frequently especially in summer, abundant *A. woodiana* can be sampled in the lakes especially in the littoral zones.

Previous studies have generally focused on the effects of mussels on water body. The mussel *A. woodiana* has been promoted as a biomanipulation tool of lakes in China and strong suppression of phytoplankton, apparent changes of phytoplankton community structure and the improvement of water transparency were observed (**Yang et al., 2008**). Yet relatively little is known about the grazing effect of *A. woodiana* on toxic cyanobacterial blooms and the effects of long-term exposure to natural eutrophic water on the growth and physiological energetics.

In **Chapter 4**, laboratory experiments were carried out to compare the mussel *A. woodiana*'s acute physiological responses to variable microalgae diets, including toxic microcystin-producing cyanobacteria *Microcystis aeruginosa* NIES-90 and non-toxic green algae *Scenedesmus obliquus*, and it was found that *A. woodiana*'s has higher grazing ability on the toxic microcystin-producing cyanobacteria *M. aeruginosa* than that of non-toxic green algae *S. obliquus* (**Liu et al., 2014**).

However, *M. aeruginosa* cultured in the laboratory was unicellular and could not form colonial type as in natural blooms. Moreover, the effects of long-term immersion to natural eutrophic water with both colonial and unicellular *Microcystis* spp. on the growth and physiological energetics, which is an integration of several physiological processes, of mussels have not been widely studied yet.

"Scope for growth" (SFG) measurement, which measures the energy available for growth, as mentioned in **Chapter 2** has been widely used in many ecotoxicological studies (**Widdows et al., 1995**). This measurement of physiological energetics, normally in terms of SFG, can provide instantaneous assessment of the growth process of mussels as affected by environmental stress and pollution. SFG is measured by integrating several physiological parameters, including filtration rates, absorption efficiencies, respiration rates and ammonia excretion rates, all of which can directly affect the energy available for growth, maintenance and reproduction of mussels. Many studies have indicated the

advantages of assessing the physiological status of mussels by measurement of SFG rather than individual physiological process (**Bayne et al., 1983**). Although this physiological biomarker has been widely applied in assessing the physiological condition of mussels, there is no study that applied this biomarker to determine the effects of natural *Microcystis*-blooming water on freshwater mussels. With a growing concern on the impact of eutrophic water, it is clear necessary to assess whether eutrophic water has a negative impact on the growth of shellfish in this region.

Therefore, the aim of this chapter is to examine whether the most popular native mussel *A. woodiana* can exert grazing pressure on naturally blooming *Microcystis* spp. through evaluating the effect of *A. woodiana*'s feeding behavior on phytoplankton abundances and community structure and then assess whether eutrophic water has a negative impact on the growth of *A. woodiana* through introducing SFG related to energetic balance of *A. woodiana*.

Thus, in this study microcosm experiments with naturally *Microcystis*-blooming water which contained large quantities of colonial *Microcystis* spp. were performed. The 6-day feeding responses experiment was carried out with naturally blooming pond water and the mussels in laboratory. In this experiment, a comparative analysis of the algae presented in the control tank and treatment tanks on 0-day and 6-day was performed; in addition, the SFG values of mussels on 0-day and 6-day were compared. These results may lead to a comprehensive assessment of the application possibility of *A. woodiana* as a *Microcystis*-blooming removal tool in eutrophic shallow lakes.

5.2 MATERIALS

A. woodiana was supplied by Gunma Prefecture in Japan. The mussels were kept at 4℃ before transporting them to the laboratory. Immediately upon arrival, the mussels were transferred to 35 L aquaria with 20 L aerated dechlorinated tap water at controlled temperature 25℃, under a light:dark regime of 12 h:12 h for at least one week, before being used for experiments. The water was completely refreshed three times per week. Source water applied in our test was from a pond in the central part of Chikushino city, Fukuoka, Japan. In the pond, the cyanobacterial bloom was a growing problem due to increasing eutrophication resulting from agricultural pollution and domestic sewage.

The pond was characterized by summer cyanobacterial bloom (*Microcystis* spp.) from June to October. Besides *Microcystis* spp., some other species such as *Nitzschia* spp. and *Chlorella* sp. were also observed by microscopy in the pond water. The characteristics of raw water: pH is 8.3, total nitrogen and phosphorous are 3.0 and 0.08 mg L^{-1}, respectively, Chlorophyll a is 177.7 μg L^{-1} and phytoplankton concentration is 5.8×10^5 cells mL^{-1}.

5.3 METHODS

The experiments were performed at controlled conditions (25±1℃, 12h light:dark cycle, 3400 lux) in four tanks (40 cm × 25.2 cm × 26.2 cm). One tank was set as control filled with 20 L *Microcystis*-blooming pond water with a layer (10 cm) of pond sediment (the same pond to resource water) without mussels; the other three replicated tanks were set as treatment at the same condition with mussels. The mussels of similar size (shell length of 7.5 ± 0.4 cm and fresh-weight of 42.2 ± 7.7 g) were chosen and kept in the three replicated treatment tanks (the mussels number n = 3 per tank). During the experiment, constant aeration was supplied. Sediments were supplied to simulate the natural condition and the mussels' habitat. Before introduction to the tanks, mussels were gently cleaned with a brush under running de-ionized water to remove phytoplankton adhered to the shell and not fed for 2 days in clean aerated dechlorinated tap water to clear the guts (**Smaal and Widdows, 1994**).

Mussels were fed with *Microcystis*-blooming pond water for 6 days. We quantified the SFG in mussels on 0-day and 6-day for treatment microcosms.

5.3.1 Phytoplankton Abundances and Community Structure Analysis and Identification

15 mL water samples for phytoplankton community structure analysis were collected on 0-day and 6-day in the control and treatment microcosms. They were preserved with 5% formalin. After complete mixing, samples were counted directly through a 0.1 mL counting chamber (MPC-200, Matsunami Company) using an inverted microscope (Olympus BHS-BHT) at 400 magnification. Specific identification of phytoplankton was made according to the Illustration of the Japanese Freshwater Algae (**Hirose and Yamagishi, 1977**).

In addition, with regard to the microcystins (MCs) effect on *A. woodiana's* physiological behavior, 20 mL *Microcystis*-blooming water was collected for MCs analysis. After filtered with GFC filter paper (Whatman), the filtered water sample was measured for the extracellular MCs concentration with the enzyme-linked immunosorbent assay (ELISA) test kit. (Tokiwa Chemical Industries Co. LTD.) and the toxin measurement in ELISA method did not allow separate quantification of MC-LR or MC-LF but gave a total estimation of MCs presented in cyanobacterial suspensions.

5.3.2 SFG Measurement and Calculation

5.3.2-1 Filtration rates measurement

The filtration rate (*FR*, mL g^{-1}FW h^{-1}) of each individual mussel, defined as the volume of water cleared per unit time was determined by the time course of the decrease of algal density due to mussel filtration. The experiment began when the shell valve of the mussel opened. The Chl.a concentration in the feeding beaker was measured every one hour, for a total of 4 hours on 0-day and 6-day.

The algae solution was stirring gently to keep food in suspension and homogeneous. Each time suspended food was sampled at certain intervals of time. The Chl.a concentration was measured by fluophotometer, and *FR* was determined by the following formula, which has been used in modified form by many other authors (**Coughlan, 1969**):

$$FR = (V/[wt]) \ln[C_0/C_t] \quad (5\text{-}1)$$

In which *V* is the volume of the food suspension (2 L), *w* is the fresh weight of the mussels in each beaker (g), *t* is the duration of the experiment (in hour), C_0 is the Chl.a concentration (μg L^{-1}) at 0 or one time step before *t* and C_t is the particle concentration at time *t*. In the beakers with mussels the particle concentration was corrected for changes observed in the control beakers.

The amount of ingested or consumed energy (*C*) was calculated for each individual by multiplying *FR* by the amount of particle organic material (*POM*) per liter in input water samples and assuming an energy content of algal material of water samples and assuming an energy content of algal material of 23 J mg^{-1} (**Widdows and Johnson, 1988**).

5.3.2-2 Respiration rates measurement

The respiration rate (μg O_2 g^{-1}FW h^{-1}) of mussels was measured by respirometers in enclosed chambers (1200 mL) full of *Microcystis*-blooming water used in filtration rate measurement experiments on 0-day and 6-day. The mussels were isolated from the treatment tanks after the filtration rate measurement and transferred into corresponding enclosed chambers. A chamber without a mussel was used as a control. Pond water in the chamber was mixed by a magnetic stirrer placed on the bottom. The oxygen concentration in the pond water was detected by an oxygen electrode (LDO-HQ30d, HACH).

Measurement of oxygen concentrations commenced when the animals opened their shell valves and lasted for 2 hours in darkness. The oxygen concentration was measured every 30 min and plotted against time of measurement, and then the respiration rate was calculated from the slope of the decrease of oxygen concentrations over time during two hours. The amount of energy used in respiration (*R*) was calculated assuming an energy content of 0.456 J $\mu mol^{-1}O_2$ **(Gnaiger, 1983)**.

5.3.2-3 Absorption efficiencies measurement

The absorption efficiency of the organic matter by mussels was determined by the ratio method (**Conover, 1966**). This method assumed that the absorption of the inorganic component of food was insignificant during the digestive process. After the respiration rate measurement, the mussels were placed into three beakers with 2 L aerated dechlorinated tap water. Any faeces egested were collected with pipettes after 12 hours. The faeces were filtered onto a preweighted GF/F filter (Whatman). The filters were subsequently ashed in a muffle furnace at 450℃ for 1 h, and the loss of organic matter was calculated. The absorption efficiency (*AE*) was calculated by comparing the ratio of organic and inorganic materials between the food and faeces, by the following equation (**Conover, 1966**):

$$AE(\%) = (F' - E')/[(1 - E') \times F'] \times 100 \quad (5\text{-}2)$$

Where *F'* is the ash-free dry weight:dry weight ratio (AFDW/DW) of the food and *E'* is the ash-free dry weight: dry weight ratio of faeces.

5.3.2-4 Excretion rates measurement

Another component of metabolic loss is presented by the products of excretion. The excretion rate (*ER*; mg NH_4^+-N h^{-1}) measurement was performed simultaneously with the filtration rate measurement. After the measurement of the respiration rate, the filtered water samples were measured for the ammonium ion concentration with Ion Chromatography ICS-2100_ICS-1100_AS. The ammonium ion concentration was measured at 0-hour and 2-hour and the excretion rate was calculated from the increase of ammonium ion concentrations in 2 hours. The amount of energy used in excretion (*U*) was calculated for each individual by multiplying *ER* by assuming an energy content ammonium ion of 19.4 J $mg^{-1}NH_4^+$ (**Widdows and Johnson, 1988**).

5.3.2-5 O/N *ratio*

Another biomarker used to indicate the physiological state of the organism in this case exposed to *Microcystis*-blooming pond water is the quantification of oxygen-nitrogen atomic ratio (O/N). This biomarker is the result of the quantification division of oxygen uptake and the quantification of ammonia excretion in atomic equivalent. It was calculated to determine the proportion of protein relative to carbohydrate and lipid catabolized for energy metabolism (**Bayne, 1976**).

5.3.2-6 SFG calculation

The SFG, which defined the energy available for growth and reproduction and was calculated by the following equation (**Widdows and Johnson, 1988**):

$$\mathrm{SFG} = C \times AE - R - U \tag{5-3}$$

Where C is the consumed energy, AE is the absorption efficiency, R is respired energy, and U is excreted energy.

5.3.3 Statistical Analysis

A paired *t*-test was used to determine the differences in all the mussel physiological parameters between 0-day and 6-day, respectively. In addition, for all the energy values, the differences between 0-day and 6-day were also tested with the paired *t*-test.

All statistical analyses were carried out with SPSS 16.0 for Windows.

5.4 RESULTS

5.4.1 Phytoplankton Abundances and Community Structure

Table 5-1. Phytoplankton abundances and community structure in the control and treatment tanks on 0-day and 6-day.

Algae		Control	Treatment	Control	Treatment
		0-day		6-day	
		(cell mL^{-1})			
Cyanophyceae	*Chroococcus* spp.	-		200	1,200
	Phormidium sp. (f)	92,000		300	1,400
	Oscillatoria sp. (f)	-		150	60
	Microcystis spp. (colony)	26,800		124,480	25,027
	Microcystis spp. (unicell)	460,000		340,000	144,000
Chlorophyceae	*Dictyosphaerium* sp.	-		-	320
	Gloeocystis sp.	-		10	250
	Schroederia sp.	-		-	200
	Chlorella sp.	10		-	-
Bacillariophyceae	*Cyclotella* sp.	-		-	40
	Nitzschia spp.	1,400		3,200	1,800
Total		580,210		468,340	174,297

* (f): filament; -: unobserved.

The initial phytoplankton was mainly dominated by *Microcystis* spp., including high densities of unicellular and colonial types.

After 6 days, the total phytoplankton biomass in the control tank decreased slightly compared with that on 0-day. Correspondingly, there was a much more obvious decrease of total phytoplankton biomass, reduced to less than one third of the initial concentration on 6-day in treatment tanks with mussels, which indicated that the mussels have significant grazing ability on the eutrophic pond water.

Through 6 days, Cyanophyceae, Chlorophyceae and Bacillariophyceae showed different variation trends in the control tank and treatment tanks.

Colonial *Microcystis* spp. have grown to about five times of that on 0-day in the control tank without mussels; however, in the treatment tanks with mussels' grazing, there was a slight decrease of colonial *Microcystis* spp., which indicated the colonial *Microcystis* spp. can be effectively filtered by *A. woodiana*.

Meanwhile, unicellular *Microcystis* spp. decreased in the control tank and treatment tanks. It dropped slightly in the control tank, while it decreased more significantly to less than one third of the initial concentration in the treatment tanks, revealing the strong grazing effect of *A. woodiana* on unicellular *Microcystis* spp.

Similarly, *Phormidium* sp. dropped remarkably in each tank. Whereas, absolute concentrations of *Phormidium* sp. in the treatment tanks were higher than in the control tank, which indicated that the feeding effect of *A. woodiana* can stimulate the growth of *Phormidium* sp. in some degree.

For Chlorophyceae, the total biomass in the control tank kept stable, while both the species and total biomass of Chlorophyceae in the treatment tanks went up markedly, revealing that the feeding behavior of mussels in the favor of the Chlorophyceae growth.

Furthermore, the total biomass of Baclillariophyceae in each tank climbed. The appearance of *Cyclotella* sp., although of small quantities, indicated the mussels' feeding behavior benefited the growth of Baclillariophyceae; the biomass of *Nitzschia* spp. grew up in both cases, while the quantities in the treatment tanks was less than the control tank, reflecting the mussels' feeding ability on *Nitzschia* spp.

5.4.2 SFG of Mussels

The weight-specific filtration rate and absorption efficiency of *A. woodiana* on 0-day and 6-day are shown in **Fig. 5-1 (c)**. After 6-day's exposure to *Microcystis*-blooming water, the weight-specific filtration rate increased obviously (*t*-test, $P<0.05$), while the absorption efficiency was reduced significantly (*t*-test, $P<0.05$).

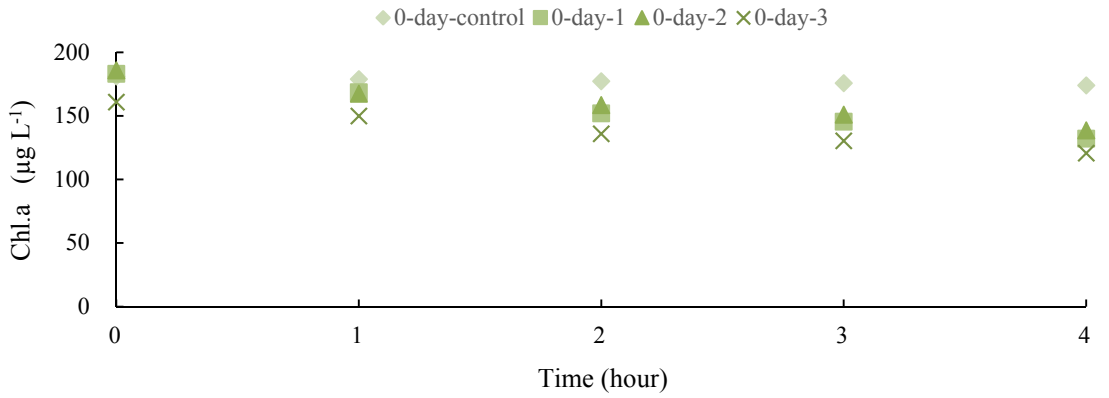

Fig. 5-1 (a). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of *Anodonta woodiana* on 0-day.

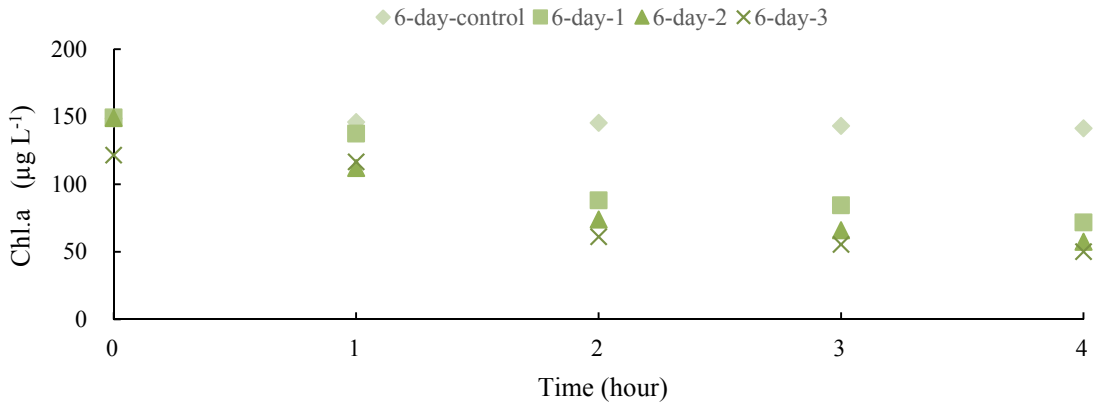

Fig. 5-1 (b). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of *Anodonta woodiana* on 6-day.

Table 5-2. The AFDW/DW ratio in faeces and food for *Anodonta woodiana* feeding on different diets.

	0-day-1	0-day-2	0-day-3	6-day-1	6-day-2	6-day-3
F'	0.88	0.88	0.89	0.70	0.53	0.61
E'	0.31	0.34	0.35	0.46	0.53	0.38

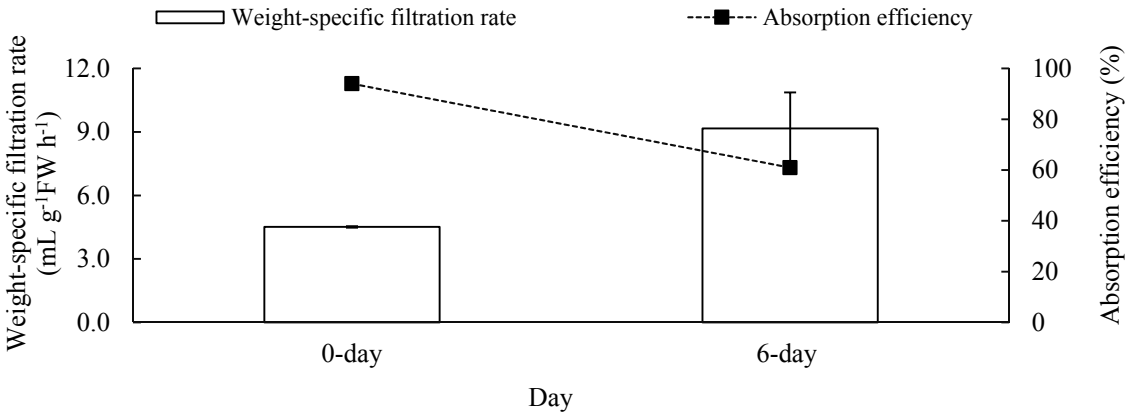

Fig. 5-1 (c). The weight-specific filtration rate (mL g^{-1}FW h^{-1}) and absorption efficiency (%) of *Anodonta woodiana* on 0-day and 6-day. Error bars are S.E. (N=9)

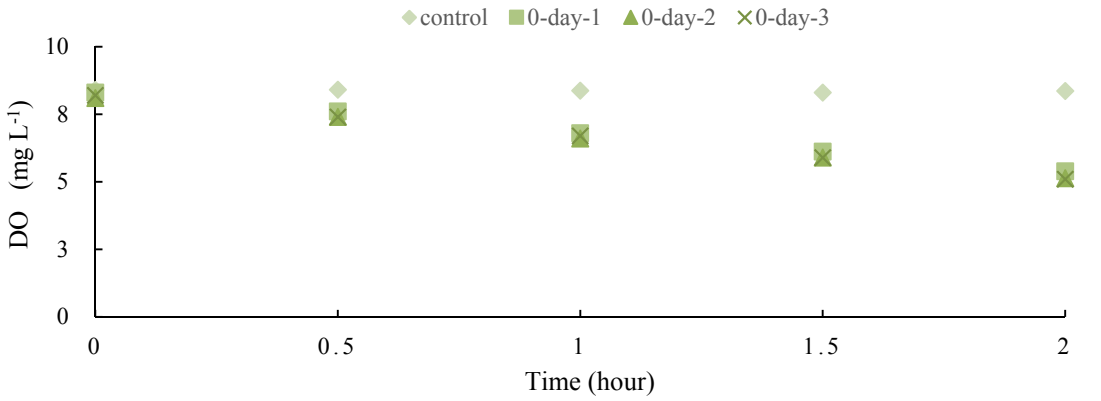

Fig. 5-2 (a). The DO concentration changes during two hours in beakers for respiration rates measurement of *Anodonta woodiana* on 0-day.

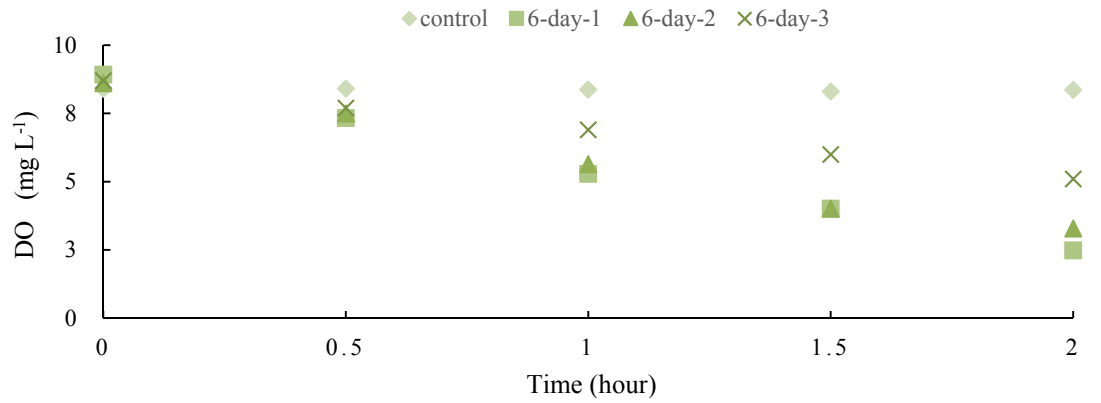

Fig. 5-2 (b). The DO concentration changes during two hours in beakers for respiration rates measurement of *Anodonta woodiana* on 6-day.

Table 5-3. Ammonium ion concentration changes for ammonia excretion rates measurement of *Anodonta woodiana* on 0-day and 6-day.

Time	Ammonium ion concentration (mg L^{-1})					
	0-day-1	0-day-2	0-day-3	6-day-1	6-day-2	6-day-3
0-hour	0.134	0.140	0.168	0.717	0.837	0.405
2-hour	0.555	0.592	0.543	0.997	1.440	0.906

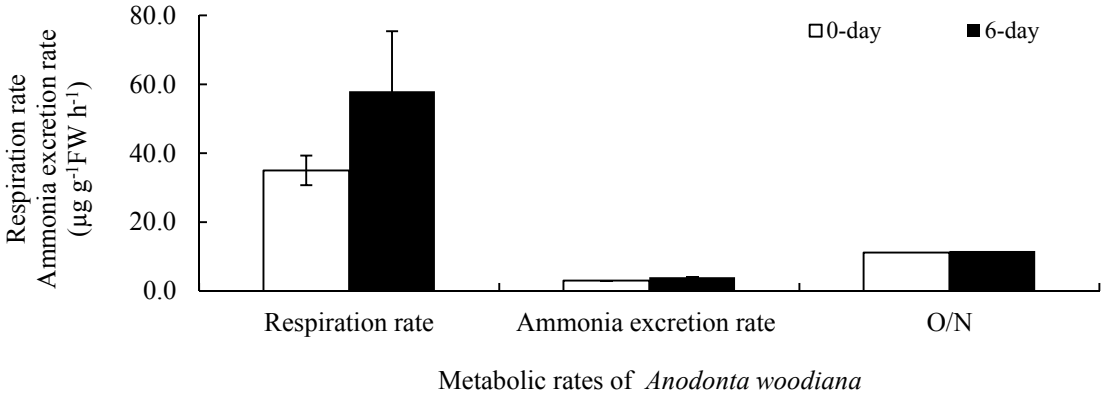

Fig. 5-2 (c). The metabolic rates (μg g^{-1}FW h^{-1}) of *Anodonta woodiana* on 0-day and 6-day exposed to *Microcystis*-blooming pond water. Error bars are S.E. (N=9).

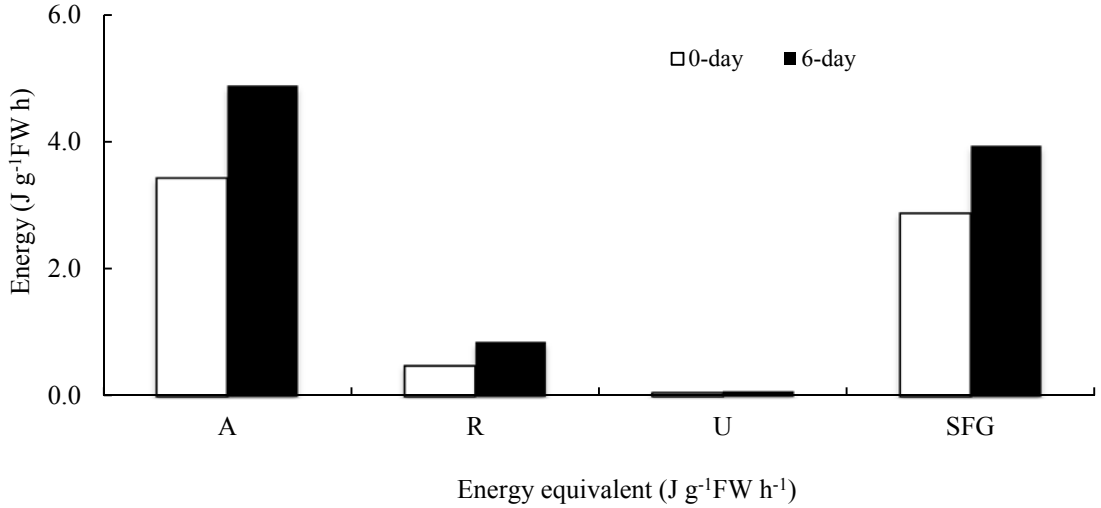

Fig. 5-3. Energy equivalent (J g^{-1}FW h^{-1}) of the physiological responses of *Anodonta woodiana* on 0-day and 6-day. *A*: energy absorbed from the food; *R*: energy consumed by respiration; *U*: energy consumed by ammonia excretion; SFG: energy available for growth.

As shown in **Fig. 5-2 (c)**, the respiration rate of 6-day was almost twice of that on 0-day. There was a slight but not obvious increase (*t*-test, $P>0.05$) for the ammonia excretion rate and O/N values through 6-day's exposure. O/N is the atomic ratio of oxygen consumed to ammonia excreted and it can provide the index of the balance in an animal's tissues between the catabolism rates of protein, lipid and carbohydrate substrates, which represents the degree to which protein is utilized in energy metabolism by invertebrates.

In this experiment, they were all in the range of 3-16 from 0-day to 6-day.

The energy equivalents of mussels are presented in **Fig. 5-3**. SFG in mussels was significantly increased (*t*-test, $P<0.05$), mainly contributed by the increasing filtration rate, whereas the energy respired and excreted was not greatly affected after 6-day's exposure.

5.5 DISCUSSION

5.5.1 Feeding Selectivity of *Anodonta Woodiana* on Phytoplankton

This experiment is performed to illustrate the feeding activity effect on phytoplankton community. Obviously, *A. woodiana* can effectively control the total biomass of phytoplankton by virtue of the feeding activity, especially colonial and unicellular *Microcystis* spp.

The interaction between mussels and phytoplankton community is complex, particularly because mussels can affect algal community composition both directly and indirectly (**Bastviken et al., 1998**). The direct effects of mussel activities on the phytoplankton community include: (i) selective removal (preferential clearance); (ii) selective ingestion (pre-ingestive selection in the mantle cavity); or (iii) differential digestion (post-ingestive selection) of phytoplankton. Indirectly the mussels could alter phytoplankton community by (1) altering nutrient or light regimes in a way that may favor certain phytoplankton groups; (2) by removing phytoplankton from the water column at such a high rate that faster growing species will become relatively more abundant (**Bastviken et al., 1998**).

Direct and indirect mechanisms may act simultaneously and a resultant change in phytoplankton species composition at the whole-system level could be caused by a combination of all of these effects (**Bastviken et al., 1998**).

Thus, the different responses of different species of algae to mussels' grazing behavior might be caused by the mussels' feeding selectivity. As for many freshwater and marine suspension feeders, feeding-process of mussels is influenced by food quality and food quantity (**Hawkins et al., 2001**).

In the case of algal suspensions, food quantity can be expressed as Chl.a concentrations and particulate organic matter (POM). Food quality can be expressed in different ways: organic: inorganic ratio of seston (**Schneider et al., 1998**), toxin content (**Engstrom et al., 2001**) and also polyunsaturated fatty acids content (**Demott and Muller-Navarra, 1997**). The shape and size of algal cells are also parameters that potentially affect the feeding behavior of mussels.

In the control tank, although cultured in the same conditions, different species of algae demonstrated different variation trends, probably due to the different competitive ability and growth speeds. The colonial *Microcystis* spp. have the higher photosynthesis capability, due to their more visible vacuoles and less lipid droplets, which make the

colonial *M. aeruginosa* have better buoyancy ability for migration than the unicellular *M. aeruginosa* (**Sherman and Webster, 1994**). Furthermore, vertical migration provides competitive advantages for colonial *M. aeruginosa*. Therefore, during only 6 days, the colonial *Microcystis* spp. can grow rapidly. For *Phormidium* sp. and unicellular *Microcystis* spp., the obvious decrease can be caused by the weak adaptation and competitive abilities in the laboratory microcosm. The similar phenomenon was reported by Yang et al (**Yang et al., 2008**). In their study, it was also found that in the control bucket of eutrophic lake water without mussels, *Microcystis* spp. (no information of unicellular species or colonial species was mentioned) declined obviously during one week.

Nevertheless, the laboratory microcosm method is still popularly used on the study of mussels' feeding selectivity and SFG measurement. It enables us to use naturally blooming water in the lab microcosm, isolated from other environmental variables, and evaluate the mussels' feeding effect on phytoplankton community. Thus, it can cover the shortage of aggregating ability deficiency for pure cultured *M. aeruginosa*, which can not form the colony of *M. aeruginosa* under laboratory conditions.

5.5.2 Impacts of *Microcystis*-Blooming Water on Mussels

The weight-specific filtration rates of *A. woodiana* values obtained in this study were similar to the values obtained by Wu et al. (**Wu et al., 2005**) who showed that the filtration rates of different mussels (with individual weight ranging from 50 g to 100 g) ranged from 2.5 mL g^{-1}FW h^{-1} to 9.0 mL g^{-1}FW h^{-1}. In their study, the mussels of *A. woodiana* with freshweight of 50-100 g were used to filter the eutrophic lake water with algal concentrations ranged from 4.6×10^6 to 5.9×10^7 cells L^{-1} from July to September.

Larger filtration rates on *Microcystis*-blooming pond water were accompanied by larger oxygen consumption rates which supplied energy for the active feeding behavior.

Of the N absorbed by mussels from the ingested diets, the majority is used for tissue growth and some is excreted as urine (70% of which is NH_4^+, 0% to 13% urea, and 5% to 21% amino-N) (**Bayne et al., 1976**). Feeding and metabolic behaviors are effected each other.

As mentioned in **Chapter 2**, on the page of 55, the atomic ratio of oxygen consumed to ammonia excreted can provide the index of the balance in an animal's tissues between the catabolism rates of protein, lipid and carbohydrate substrates. A low value of ~ 10 represents considerable protein is catabolized, while higher values indicates that greater percentages of lipid or carbohydrate are being metabolized (**Bayne and Newell, 1983**).

O/N represents the degree to which protein is utilized in energy metabolism by invertebrates. Under natural feeding conditions, the value of the ratio depends on the use by the animal of each biochemical fraction assimilated. Using theoretical computations, it can be shown that pure protein catabolism will result in the O/N *ratio* in the range 3 to 16, while equal amounts of lipid and protein catabolism will yield values between 50 and 60 (**Mayzaud and Conover, 1988**). In this experiment, they were all in the range of 3-16, which indicated protein was the main metabolic substrate from 0-day to 6-day. As mentioned before, large quantities of *Microcystis* spp. were removed by the mussels as the main food.

Furthermore, in the study of De la Fuente G. et al., the nutritional characteristics of *Microcystis* spp. that occurred naturally in a Guatemalan lake were determined and they were proved to have a high protein content 55.6% (**De la Fuente et al., 1977**). Thus, from 0-day to 6-day, the algae absorbed by the mussels mainly constituted of large quantities of *Microcystis* spp., had high protein contents, which probably induced the O/N *ratio* both on 0-day and 6-day to fall in the range of 3-16.

After 6-day's filtration, *Microcystis* spp. in the treatment tanks were decreased obviously, which means the high nutritional food with 55.6% protein content for *A. woodiana* was less than one third of that on the 0-day. Thus, the absorption efficiency for *A. woodiana* was decreased due to the low nutritional content of food in pond water. Meanwhile, in order to acquire enough energy to maintain metabolic activity, filtration rates were increased through the increased volume of filtered water that passed the gill of *A. woodiana.*

With regard to the microcystins (MCs) effect on *A. woodiana's* physiological behavior, the toxin value was 0.36 μg L^{-1} in this study. MCs measurement in ELISA method did not allow separate quantification of MC-LR or MC-LF but gave a total estimation of MCs presented in cyanobacterial suspensions. As reported by Dittmann and Borner that the extracellular MCs are usually <10% of total MCs (**Dittmann and Borner, 2005**), thus the total MCs of the food suspension used in this study may be estimated higher than 3.6 μg L^{-1}.

After 6-day's exposure to the toxic *Microcystis* blooming water (MCs>3.6 μg L^{-1}), significantly increased SFG for *A. woodiana* was observed, mainly due to the increasing filtration rate as shown in **Fig. 5-3**. It can be inferred that MCs in this study did not restrain the filtration rate and the potential growth of *A. woodiana*.

5.6 CONCLUSIONS

The results from the 6-day feeding responses experiment performed with naturally *Microcystis*-blooming pond water, indicated that for the mussel *A. woodiana*:

(1) Both unicellular and colonial *Microcystis* spp. can be effectively removed by *A. woodiana*. It also indicated that *A. woodiana* can potentially grow well after their feeding, demonstrated by the increased SFG value.

(2) The toxic *Microcystis* spp. were found to supply about 1.5 times more energy for *A. woodiana*'s potential growth after 6-day exposure; on the other hand, *A. woodiana* can survive and showed strong adaptation ability when it was exposed to toxic *Microcystis* solution.

Therefore, the mussel *A. woodiana,* which is widely distributed throughout Chinese and Japanese freshwaters, can be promoted as a tool in biomanipulation of eutrophic lakes in China and Japan associated to its powerful filtering capacity on suppression of *Microcystis s*pp. and adaptation ability when exposed to toxic *Microcystis*-blooming water.

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CHAPTER 6

Possible Survival Mechanisms of *Anodonta Woodiana* Exposed to Microcystins

CHAPTER 6

Possible Survival Mechanisms of *Anodonta Woodiana* Exposed to Microcystins

Abstract

In **Chapter 4** and **5**, a series of laboratory experiments was performed and the results suggested that *A. woodiana* could graze both the pure cultured *Microcystis aeruginosa* and the natural blooms of toxic *Microcystis* spp. in unicellular and colonial forms, greatly. Furthermore, in the subsequent long-term grazing experiment performed in this chapter, the mussels demonstrated strong survival ability during exposure to natural eutrophic water containing microcystins (MCs) for 12 days. In order to clarify the survival mechanisms (feeding selective and detoxification mechanisms), all the conducted experiments were summarized and a correlation analysis between the diet characters and mussels' physiological rates was carried out. The results showed that (1) MCs did not restrain the feeding behavior of *A. woodiana*; instead the exorbitant initial diet concentration could inhibit the filtration rates; (2) the absorption efficiency increased with the elevation of the toxic algae *Microcystis* spp. concentration; (3) there was an obvious positive correlation between ammonia excretion rates and microcystin concentrations. Finally, combined with the phenomena in the long-term grazing experiment, the possible detoxification mechanism in this mussel was inferred.

6.1 INTRODUCTION

In Chinese Lake Taihu, where *Microcystis* spp. frequently bloom especially in summer, *A. woodina* has been found in the lake especially in the littoral zones (**Yang et al., 2005**).

In addition, a similar phenomenon has been reported for *A. woodiana* in the Lake Suwa, Japan. In the Lake Suwa where the mussel samples were collected, dense water blooms of *Microcystis* spp. occurred every year, and in surface water, the concentrations of microcysins (MCs) were estimated to be in a range from 40 to 100 μg L^{-1} (**Watanabe et al., 1997**).

The coexistence of mussels with high concentrations of MCs indicated that there must be a strategy for the mussels to overcome toxicity in exposure to cyanobacteria, which enables them to survive and reproduce.

In **Chapter 4** and **5**, in order to assess the application feasibility of *A. woodiana* as a *Microcystis* blooms removal tool in eutrophic shallow lakes, a series of laboratory experiments was performed: (1) *A. woodiana*'s acute physiological responses to different microalgae diets (**Liu et al., 2014**) (2) 6-day feeding response experiment (**Liu et al., 2013**), and the results suggested that not only the pure cultured *Microcystis aeruginosa* but also the naturally blooming toxic *Microcystis* spp. of unicell and colony can be grazed greatly by *A. woodiana*; moreover, in the following long-term grazing experiment (**Liu et al., 2013**), the mussels themselves have shown strong adaptation ability when they were exposed to toxic natural eutrophic water for 12 days (**Liu et al., 2013**) and this will be explained in detail in **Chapter 6**. In accordance with the phenomena of *A (S). woodiana*'s survival both in the Lake Taihu and Suwa, these results indicated that there are probably survival mechanisms (feeding selective and detoxification mechanisms) that aid the mussels in surviving when they are exposed to toxic cyanobacterial blooms in the field.

However, most research related to MCs depuration has been focused on the detoxification process on mammals and fish (**Kondo et al., 1996; Wiegand et al., 1999**), there are few studies that describe the mechanism of MCs metabolized in freshwater mussels when they are exposed to the highly toxic *Microcystis* spp. blooming water.

Therefore, the objectives in this study are to figure out the feeding selective mechanism and to deduce the possible detoxification mechanism to the high concentrations of MCs, based on the phenomena found in the long-term grazing experiment.

6.2 MATERIALS

6.2.1 *A. Woodiana*'s Acute Physiological Responses to Different Microalgae Diets

A (S). woodiana (shell length of 9.1 ± 0.3 cm) were chosen with similar sizes to remove the effects caused by physiological sizes. Pure cultured toxic *Micosystis aeruginosa* strain NIES-90 and green algae *Scenedesmus obliquus* strain NIES-2279 were used in their exponential stable phase of growth. There were three diet treatments: (1) toxic *M. aeruginosa* (total MCs concentration was 1.55 μg L^{-1}) (2) *S. obliquus* (3) a mixture of 50% toxic *M. aeruginosa* and 50% *S. obliquus* at the same initial Chl.a concentration of 35 μg L^{-1} (**Liu et al., 2014**). Grazing experiments of *A. woodiana* on three different diets were carried out simultaneously. For each diet condition, two mussels in a beaker containing 2,000 mL of each experimental diets solution with constant aeration were used with three replicates, simultaneously; in addition, beakers with diets, but without mussels, were used as a control to check for growth of phytoplankton cells during the experiments. The beakers were placed in an incubator at 25 ± 0.5℃. After fed with different algal diets for four hours, the SFG in mussels were quantified fed with each diets solution for treatment microcosms. More details for this experiment were demonstrated in **Chapter 4**.

6.2.2 6-Day Feeding Responses Experiment

The experiment was performed at controlled conditions (25±1℃, 12h light:dark cycle, 3400 lux) in four tanks (40 cm × 25.2 cm × 26.2 cm, filled with 20 L *Microcystis*-blooming pond water). The mussels of similar size (shell length of 7.5 ± 0.4 cm) were chosen and kept in the three replicated treatment tanks (3 mussels per tank). Mussels were fed with *Microcystis*-blooming pond water (the initial Chl.a concentration was 120.0 μg L^{-1} and the initial MCs>3.6 μg L^{-1}) for 6 days.

In addition, with regard to the microcystins (MCs) effect on *A. woodiana's* physiological behavior, 20 mL *Microcystis*-blooming water was collected for MCs analysis. After filtered with GFC filter paper (Whatman), the filtered water sample was measured for the extracellular MCs concentration with the enzyme-linked immunosorbent assay (ELISA) test kit. (Tokiwa Chemical Industries Co. LTD.) and the toxin measurement in ELISA method did not allow separate quantification of MC-LR or MC-LF but gave a total estimation of MCs presented in cyanobacterial suspensions **(Liu**

et al., 2013).

The results of physiological rates measured in these two experiments are shown in **Table 6-1**.

Table 6-1. Physiological rates of *A (S). woodiana* in the first two experiments.

Experiment	Food type	Filtration rate (mL g^{-1}FW h^{-1})	Absorption efficiency (%)	Respiration rate (μg O_2 g^{-1}FW h^{-1})
Experiment (1) (**Chapter 4**)	*Microcystis aeruginosa*	8.64	89	9.87
	Scedenesmus obliquus	6.03	39	2.66
	Micxture	14.38	41	14.37
Experiement (2) (**Chapter 5**)	Colonial and unicellular *Microcystis* spp.	4.51	94	17.10

6.2.3 Long-Term Grazing Experiment

The long-term comparative grazing experiment was carried out to evaluate the effects of toxic cyanobacteria on *A. woodiana*'s feeding and survival. The culturing part of this experiment was conducted in two tanks (40 cm × 25.2 cm × 26.2 cm) both containing 10 L of filtered water from a pond in Oita, Japan, with 10 mussels (shell length of 7.3 ± 0.3 cm) at controlled conditions (25 ± 1℃, 12 h light:dark cycle, 3400 lux) with continuous aeration.

Two types of diets: highly concentrated naturally blooming *Microcystis* spp. (colonial and unicellular *M. aeuginosa* concentrations were 4.5×10^6 cells mL^{-1} and 2.8×10^5 cells mL^{-1}, respectively, accounting for over 90% of the total population) containing concentrations (25.5 μg L^{-1}) of MCs; non-toxin green algae *Chlorella vulgaris* at the same Chl.a concentration of 424.5 μg L^{-1}, were supplied to Blooming *Microcystis* Group and *Chlorella* Group, respectively. The OD (Optical Density) value was measured and the corresponding diet was added every two days to keep the diet suspension at the constant concentration.

On 0-day, 3-day, 6-day, and 12-day, the grazing rates and metabolic rates were measured. The filtration rate measurements were performed through the 4-h feeding experiments. 6 mussels in each tank were collected on random for each diet suspension, and then the selected 6 mussels were transferred to 3 replicated 2 L beakers (2 mussels per beaker) for the filtration rate measurement. Each of the beakers contained 1 L corresponding diet suspension. After that, respiration rate, absorption efficiency and

ammonia excretion rate measurements were carried out as described in the following part. Finally, the scope for growth (SFG) values were calculated.

6.3 METHODS

6.3.1 SFG Measurement

6.3.1-1 Filtration rates measurement

The filtration rate (*FR*, mL g^{-1}FW h^{-1}) of each individual mussel, defined as the volume of water cleared per unit time was determined by the time course of the decrease of algal density due to mussel filtration. The experiment began when the shell valve of the mussel opened. The Chl.a concentration in the feeding beaker was measured every one hour, for a total of 4 hours on 0-day, 3-day, 6-day and 12-day.

The algae solution was stirring gently to keep diets in suspension and homogeneous. Each time the suspended diet was sampled at certain intervals of time. The Chl.a concentration was measured by fluophotometer, and *FR* was determined by the following formula, which has been used in modified form by many other authors (**Coughlan, 1969**):

$$FR = (V/[wt]) \ln[C_0/C_t] \quad (6\text{-}1)$$

In which *V* is the volume of the diet suspension (2 L), *w* is the fresh weight of the mussels in each beaker (g), *t* is the duration of the experiment (in hour), C_0 is the Chl.a concentration (mg L^{-1}) at 0 or one time step before *t* and C_t is the particle concentration at time *t*. In the beakers with mussels the particle concentration was corrected for changes observed in the control beakers.

The amount of ingested or consumed energy (*C*) was calculated for each individual by multiplying *FR* by the amount of particle organic material (*POM*) per liter in input water samples and assuming an energy content of algal material of water samples and assuming an energy content of algal material of 23 J mg^{-1} (**Widdows and Johnson, 1988**).

6.3.1-2 Respiration rates measurement

The respiration rate (μg O_2 g^{-1}FW h^{-1}) of mussels was measured by respirometers in enclosed chambers (1,200 mL) full of corresponding diets water used in filtration rates measurement experiments on 0-day, 3-day, 6-day and 12-day. The mussels were isolated from the treatment tanks after the filtration rate measurement and transferred into corresponding enclosed chambers. A chamber without a mussel was used as a control.

Pond water in the chamber was mixed by a magnetic stirrer placed on the bottom. The oxygen concentration in the pond water was detected by an oxygen electrode. Measurement of oxygen concentration commenced when the animals opened their shell valves and lasted for 2 hours in darkness. Oxygen concentration was plotted against time of measurement and respiration rate was calculated from the slope of the decrease of oxygen concentration over time during two hours. The amount of energy used in respiration (*R*) was calculated assuming an energy content of 0.456 J $\mu mol^{-1}O_2$ (**Gnaiger, 1983**).

6.3.1-3 Absorption efficiencies measurement

The absorption efficiency of the organic matter by mussels was determined by the ratio method (**Conover, 1966**). This method assumed that the absorption of the inorganic component of food was insignificant during the digestive process. After the respiration rate measurement, the mussels were placed into three beakers with 2 L aerated dechlorinated tap water. Any faeces egested were collected with pipettes after 12 hours. The faeces were filtered onto a preweighted GF/F filter (Whatman). The filters were subsequently ashed in a muffle furnace at 450℃ for 1 h, and the loss of organic matter was calculated. The absorption efficiency (*AE*) was calculated by comparing the ratio of organic and inorganic materials between the food and faeces, by the following equation (**Conover, 1966**):

$$AE(\%) = (F' - E')/[(1 - E') \times F'] \times 100 \quad (6\text{-}2)$$

Where *F'* is the ash-free dry weight:dry weight ratio (AFDW/DW) of the food and *E'* is the ash-free dry weight: dry weight ratio of faeces.

6.3.1-4 Excretion rates measurement

Another component of metabolic loss is presented by the products of excretion. The excretion rate (*ER*; mg NH_4^+-N h^{-1}) measurement was performed simultaneously with the respiration rate measurement. After the measurement of the respiration rate, the filtered water samples were measured for ammonium ion concentrations in the indophenol blue photometric method. The ammonium ion concentration was measured at 0-hour and 2-hour and the excretion rate was calculated from the increase of ammonium ion concentrations in 2 hours. The amount of energy used in excretion (*U*) was calculated for each individual by multiplying *ER* by assuming an energy content ammonium ion of 19.4

J $mg^{-1}NH_4^+$ (**Widdows and Johnson, 1988**).

6.3.2 SFG Calculation

The SFG, which defined the energy available for growth and reproduction and was calculated by the following equation (**Widdows and Johnson, 1988**):

$$\mathrm{SFG} = C \times AE - R - U \tag{6-3}$$

Where *C* is the consumed energy, *AE* is absorption efficiency, *R* is respired energy, and *U* is excreted energy.

6.3.3 Statistical Analysis

The repeated measures ANOVA was used to decide the differences in all the mussel physiological parameters and SFG values between the Blooming *Microcystis* Group and *Chlorella* Group during 12 days.

All statistical analyses were carried out with SPSS 16.0 for Windows.

6.4 RESULTS

6.4.1 Physiological Rates

6.4.1-1 Filtration rates

The Chl.a concentration changes during four hours in beakers for filtration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on different days are shown in **Fig. 6-1 (a)-(d)**.

The filtration rates of *A. woodiana* on toxic *Microcystis* spp. ranged from 0.53 to 2.23 mL g^{-1}FW h^{-1} and on non-toxic *Chlorella vulgaris* ranged from 0.55 to 1.92 mL g^{-1}FW h^{-1}, (**Fig. 6-1 (e)**), with mean values of 1.48 and 1.40 mL g^{-1}FW h^{-1}, respectively. A slightly decrease of filtration rates was observed on 3-day for both diet groups. However, *A. woodiana* filtration rates throughout the 12 days exposure to toxic *Microcystis* spp. did not decrease obviously (P>0.05, ANOVA), indicating there were no detrimental effects of cyanobacteria toxicity on the potential growth of *A. woodiana*.

Moreover, the weight-specific filtration rates of mussels for *Chlorella* Group and Blooming *Microcystis* Group throughout the 12 days were not significantly different (repeated measures ANOVA, P>0.05). The effectively filtering activity on the colonial *M. aeruginosa* has been found in the 4-h feeding experiments on the 0-day.

The algae sizes for two groups were different obviously (colonial *M. aeruginosa*>200 μm; unicellular *M. aeruginosa* 3-4.5 μm; *C. vulgaris* 3-6 μm). The weight-specific filtration rates between two groups indicated that the gill in this species of mussel can filter diets with a variety of sizes, which can be the adaptation response to the environment.

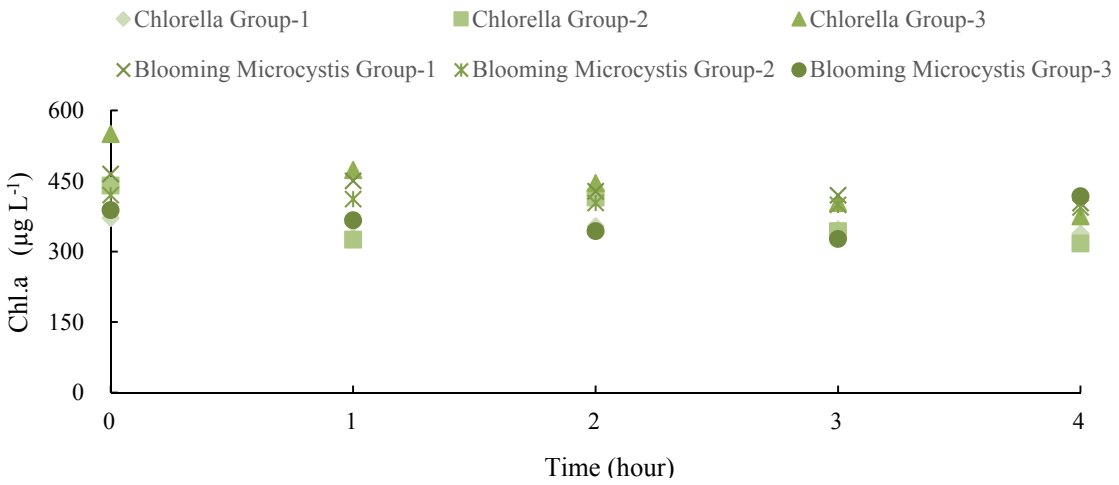

Fig. 6-1 (a). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on 0-day.

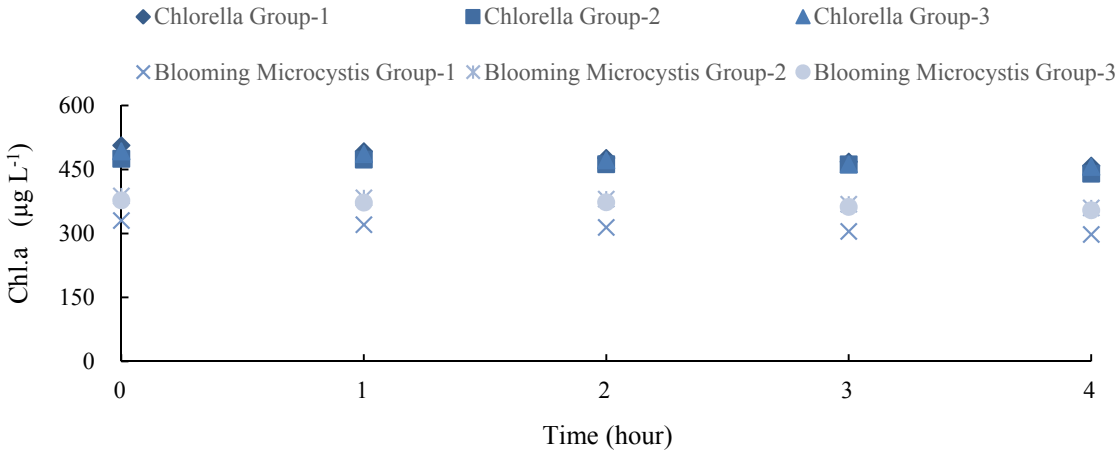

Fig. 6-1 (b). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on 3-day.

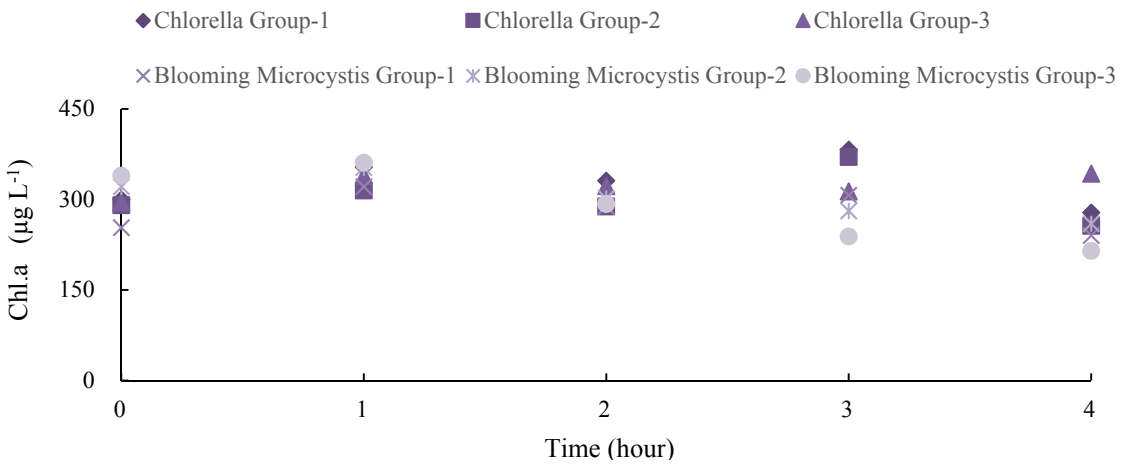

Fig. 6-1 (c). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on 6-day.

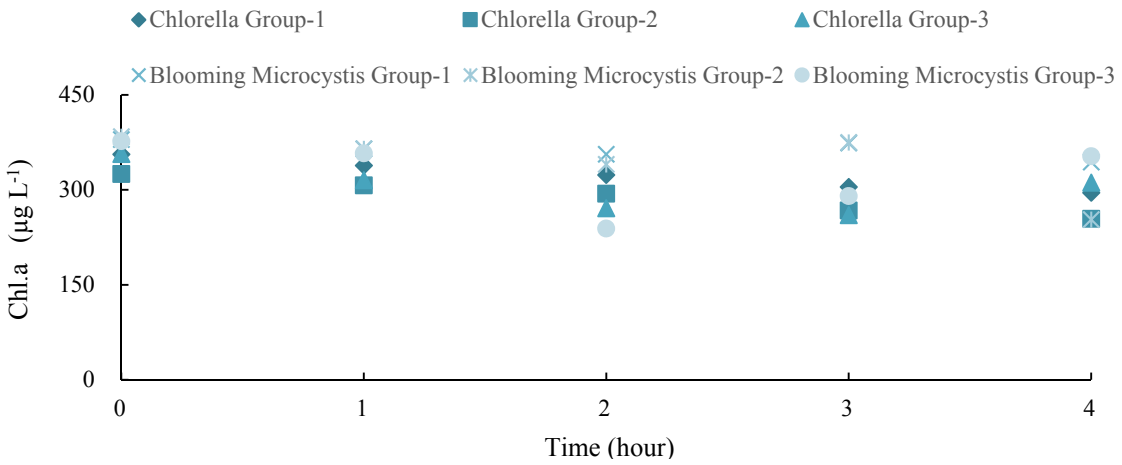

Fig. 6-1 (d). The Chl.a concentration changes during four hours in beakers for filtration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on 12-day.

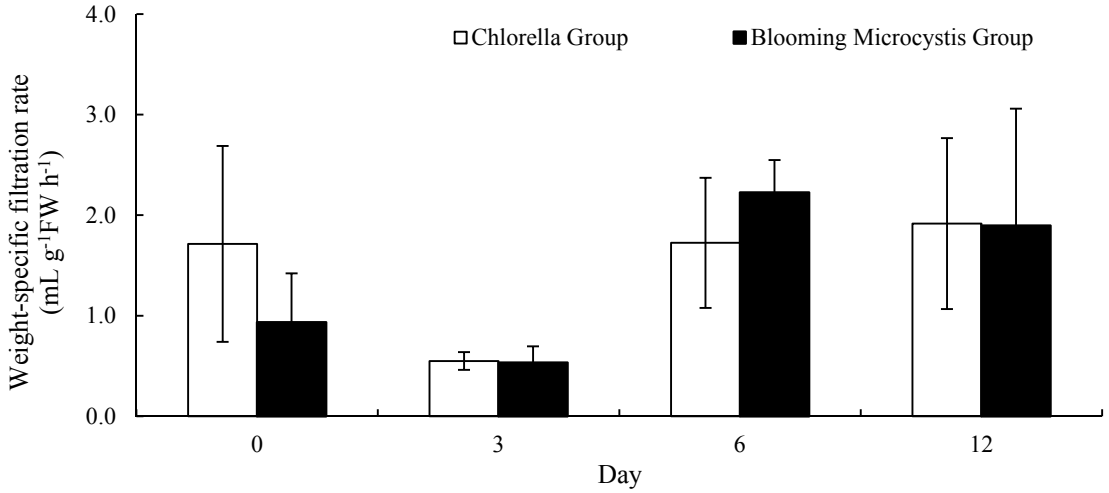

Fig. 6-1 (e). Weight-specific filtration rates (mL g^{-1}FW h^{-1}) of *Anodonta woodiana* on different days when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. Error bars are S.E. (N=6).

6.4.1-2 Absorption efficiencies

The AFDW/DW ratio of *Chlorella vulgaris* and blooming *Microcystis* spp. was measured and the averaged values are 0.90 and 0.97, respectively; in addition, the AFDW/DW ratio of faeces excreted by *Anodonta woodiana* on different days when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. are shown in **Table 6-2**.

The absorption efficiencies of *A. woodiana* for two groups were shown in **Fig. 6-2**. Surprisingly *A. woodiana* absorption efficiencies of Blooming *Microcystis* Group were statistically higher than them of *Chlorella* Group (repeated measures ANOVA, $P<0.05$). *A. woodiana* absorption efficiency on toxic *Microcystis* spp. ranged from 38.4% to 86.4% and on non-toxic *Chlorella vulgaris* ranged from 11.5% to 39.0%, (**Fig. 6-2**), with mean

Table 6-2. The AFDW/DW ratio of faeces of *Anodonta woodiana* on different days when fed with *Chlorella vulgaris* and blooming *Microcystis* spp.

Group	0-day	3-day	6-day	12-day
Chlorella Group-1	0.81	0.89	0.89	0.89
Chlorella Group-2	0.86	0.87	0.87	0.80
Chlorella Group-3	0.87	0.89	0.90	0.87
Blooming *Microcystis* Group-1	0.87	0.78	0.93	0.76
Blooming *Microcystis* Group-2	0.91	0.84	0.95	0.79
Blooming *Microcystis* Group-3	0.86	0.85	0.95	0.82

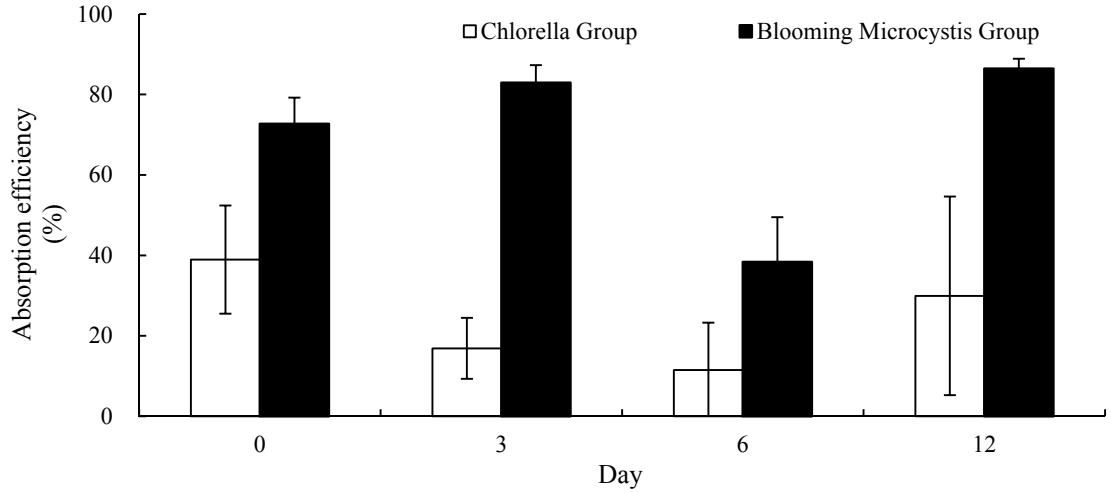

Fig. 6-2. Absorption efficiencies (%) of *Anodonta woodiana* on different days when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. Error bars are S.E. (N=6).

values of 70.1% and 24.3%, respectively.

6.4.1-3 Respiration rates

The DO concentration changes during two hours in beakers for respiration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on different days are shown in **Fig. 6-3 (a)-(d)**.

In addition, as shown in **Fig. 6-3 (e)**, respiration rates between two groups were not significantly different during this experiment (repeated measures ANOVA, $P>0.05$). *A. woodiana* respiration rates on toxic *Microcystis* spp. ranged from 20.0 to 51.0 μg O_2 g^{-1}FW and on non-toxic *Chlorella vulgaris* ranged from 22.0 to 73.0 μg O_2 g^{-1}FW, with mean values of 33.8 and 36.5 μg O_2 g^{-1}FW, respectively.

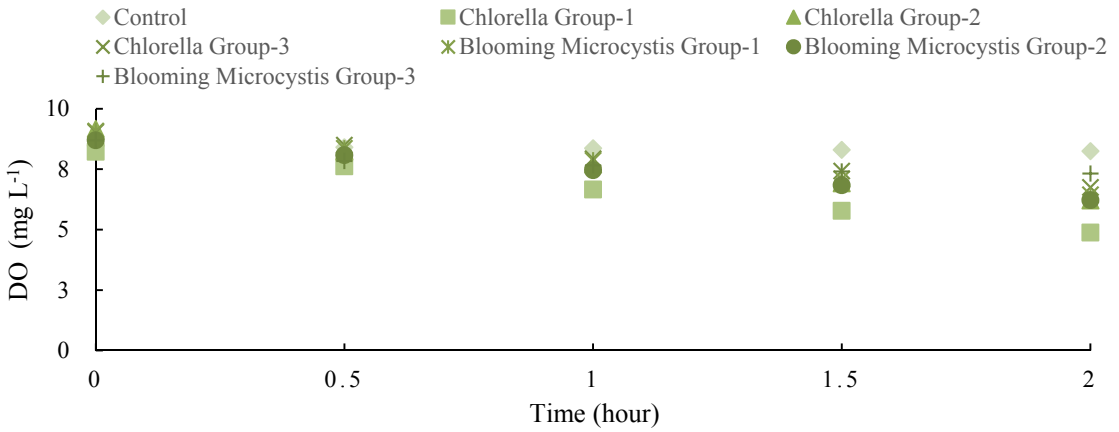

Fig. 6-3 (a). The DO concentration changes during two hours in beakers for respiration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on 0-day.

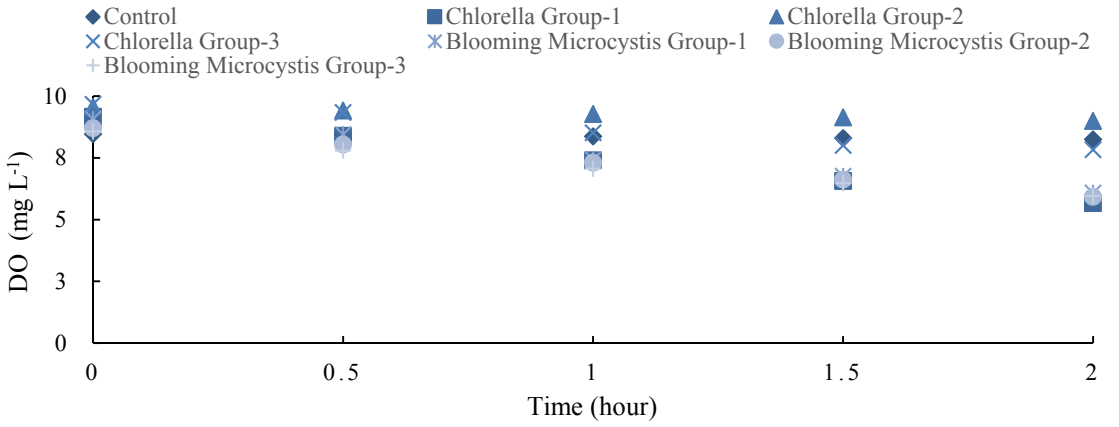

Fig. 6-3 (b). The DO concentration changes during two hours in beakers for respiration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on 3-day.

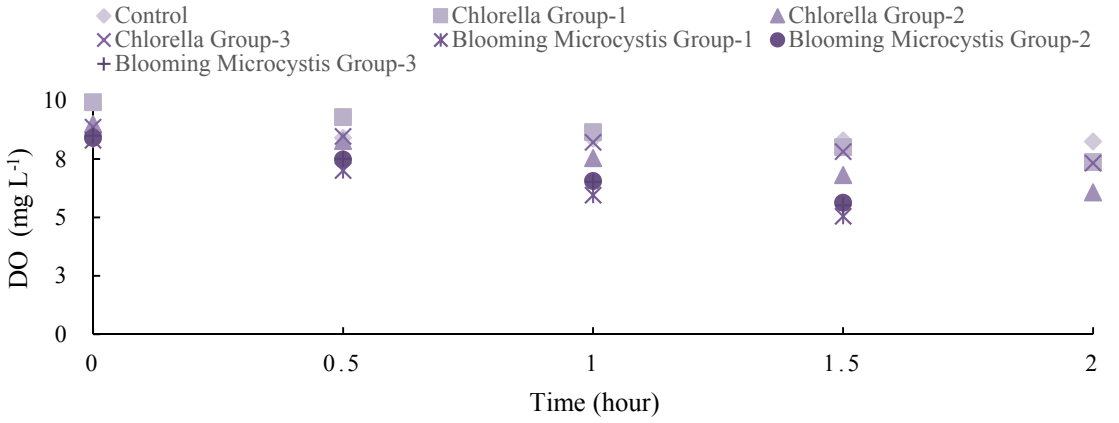

Fig. 6-3 (c). The DO concentration changes during two hours in beakers for respiration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on 6-day.

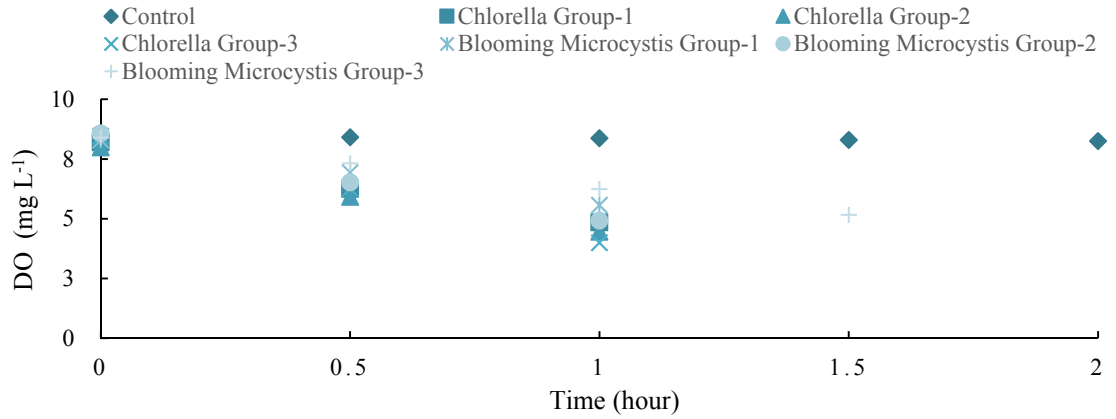

Fig. 6-3 (d). The DO concentration changes during two hours in beakers for respiration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on 12-day.

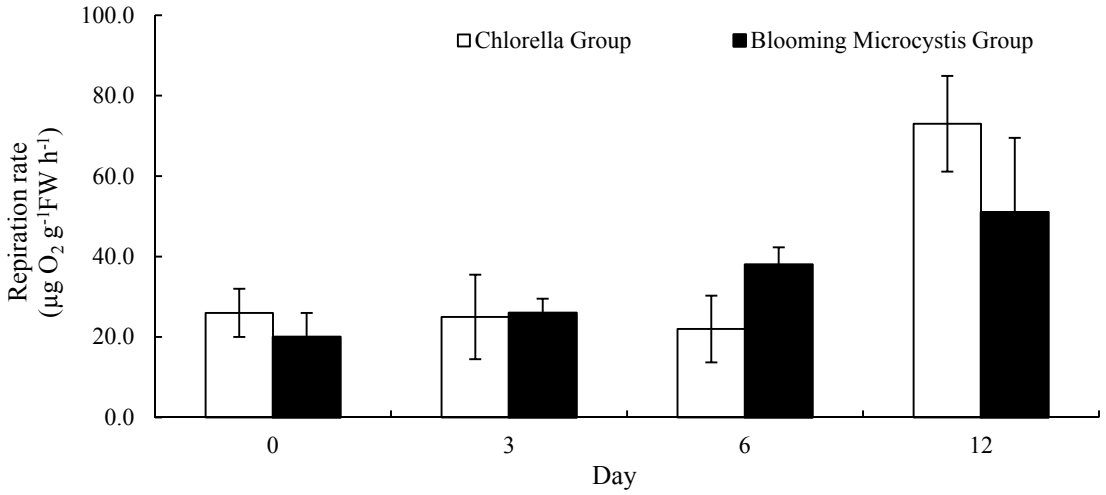

Fig. 6-3 (e). Respiration rates (μg O_2 g^{-1}FW h^{-1}) of *Anodonta woodiana* on different days when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. Error bars are S.E. (N=6).

6.4.1-4 Excretion rates

As shown in **Table 6-3**, the ammonium ion concentration changes during respiration rates measurement of mussels when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. on different days.

In addition, as shown in **Fig. 6-4**, *A. woodiana* excretion rates on toxic *Microcystis* spp. ranged from 0.85 to 19.25 μg NH_4^+ g^{-1}FW h^{-1} and on non-toxic *Chlorella vulgaris* ranged from 0.35 to 6.18 μg NH_4^+ g^{-1}FW h^{-1}, with mean values of 10.5 and 3.0, respectively.

The ammonia excretion rates of *A. woodiana* for two groups were shown in **Fig. 6-4**. Ammonia excretion rates of Blooming *Microcystis* Group were statistically higher than them of *Chlorella* Group (repeated measures ANOVA, $P<0.05$).

Table 6-3. Ammonium ion concentration changes for ammonia excretion rates measurement of *Anodonta woodiana* on different days when fed with *Chlorella vulgaris* and blooming *Microcystis* spp.

Group	Ammonium ion concentrations (mg L^{-1})							
	0-day		3-day		6-day		12-day	
	0-hour	2-hour	0-hour	2-hour	0-hour	2-hour	0-hour	2-hour
Chlorella Group-1	0	0.46	0.21	0.28	0.58	0.61	1.17	1.49
Chlorella Group-2	0	0.46	0	0.47	0.51	0.55	1.19	1.27
Chlorella Group-3	0	0.26	0.15	0.24	0.58	0.59	1.15	1.36
Blooming *Microcystis* Group-1	0.01	0.79	0.64	2.15	-	-	1.62	1.75
Blooming *Microcystis* Group-2	0.03	0.74	0.31	2.44	1.13	2.51	1.50	1.82
Blooming *Microcystis* Group-3	0.02	1.48	1.11	1.64	-	-	1.58	1.90

- means samples could not be measured.

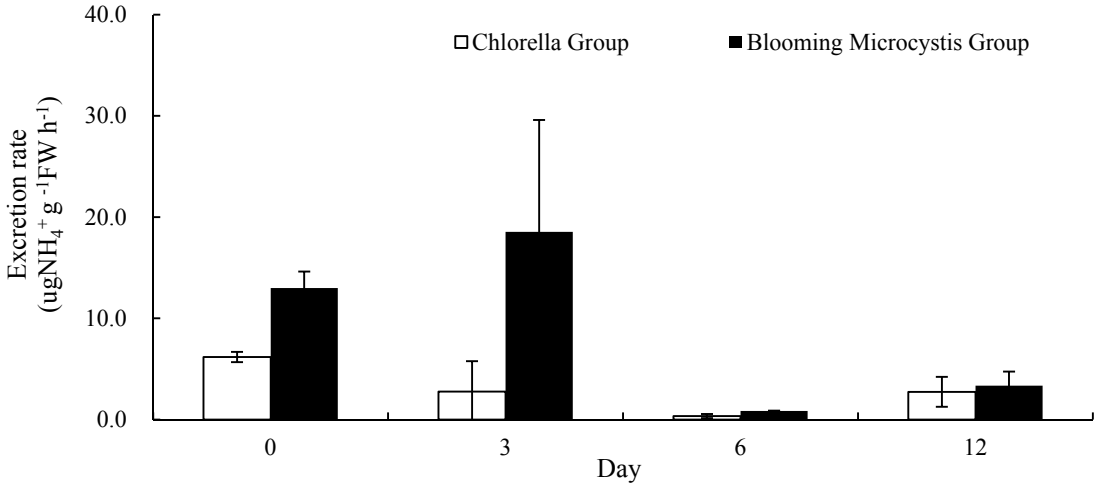

Fig. 6-4. Excretion rates (μg NH_4^+ $g^{-1}FW\ h^{-1}$) of *Anodonta woodiana* on different days when fed with *Chlorella vulgaris* and blooming *Microcystis* spp. Error bars are S.E. (N=6).

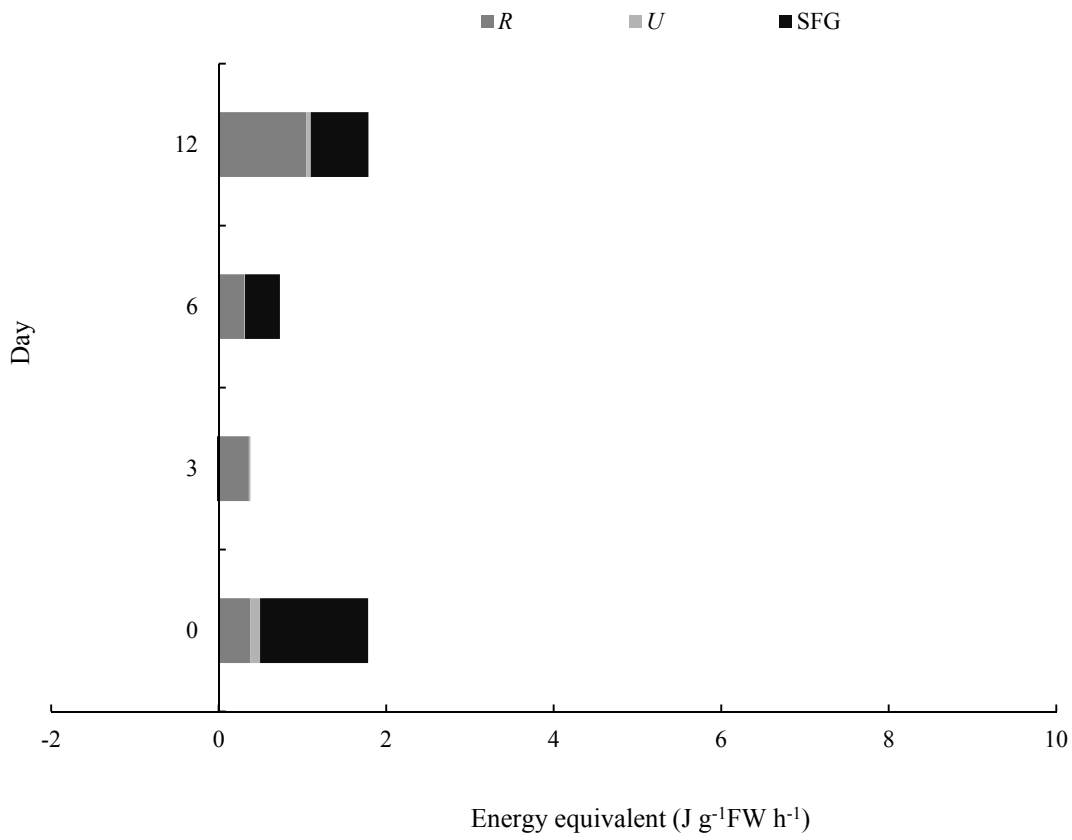

Fig. 6-5 (a). The energy equivalent of *Anodonta woodiana* on different days when fed with *Chlorella vulgaris* (*R*: energy consumed by respiration; *U*: energy consumed by ammonia excretion; SFG: energy available for growth).

6.4.2 SFG of Mussels

As shown in **Fig. 6-5 (a)** and **(b)**, on the energy level, the toxic *M. aeruginosa* supplied significantly more potential growth energy for the mussel *A. woodiana* than *Chlorella vulgaris* (repeated measures ANOVA, $P<0.05$).

SFG of *A. woodiana* on Blooming *Microcystis* Group ranged from 1.7 to 8.0 J g^{-1}FW h^{-1} and on *Chlorella* Group ranged from -0.1 to 1.3 J g^{-1}FW h^{-1}, with mean values of 4.2 and 0.6 J g^{-1}FW h^{-1}, respectively. No mussel mortality was registered for both Blooming *Microcystis* Group and *Chlorella* Group.

A slightly decrease of SFG was observed on 3-day for both diet groups. However, *A. woodiana* SFG throughout the 12 days exposure to toxic *Microcystis* spp. did not decrease significantly ($P>0.05$, ANOVA), indicating there were no negative effects of cyanobacteria toxicity on *A. woodiana* growth, probably due to the presence of the

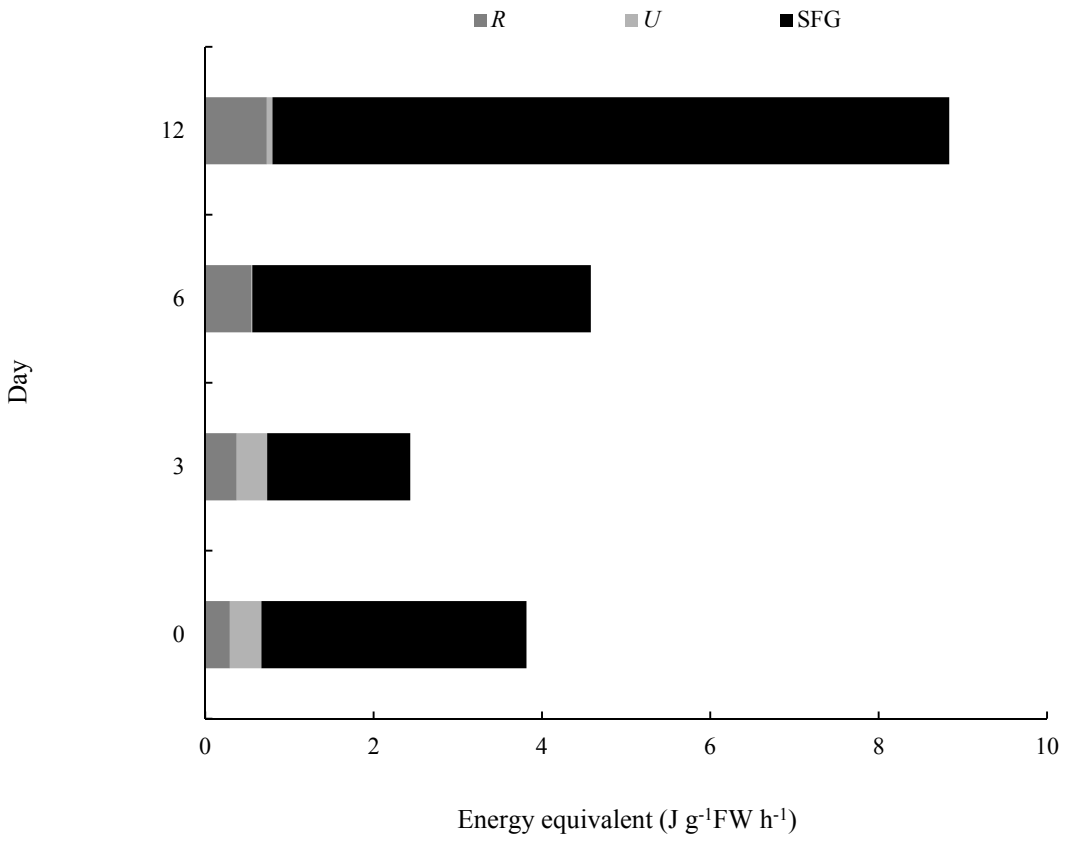

Fig. 6-5 (b). The energy equivalent of *Anodonta woodiana* on different days when fed with blooming *Microcystis* spp. (*R*: energy consumed by respiration; *U*: energy consumed by ammonia excretion; SFG: energy available for growth).

detoxification ability.

The extended long-term comparative grazing experiment showed that there was not a decrease in *A. woodiana* SFG under exposure to toxic *Microcystis* spp. It indicated that *A. woodiana* assimilated cyanobacteria cells during the long-term comparative grazing experiment and no toxic effect could be observed.

It was also noticed that compared with the non-toxin green algae *Chlorella vulgaris*, the toxic naturally blooming water supplied much higher potential growth energy for the mussels.

In addition, no mussel mortality was registered. The assimilation of highly concentrated toxic *Microcystis* spp. by *A. woodiana* suggested that this mussel presents survival mechanisms in face of toxins. Therefore, cyanobacteria toxicity has no negative effect on *A. woodiana* survival and growth.

6.5 DISCUSSION

6.5.1 Feeding Behavior and Selective Mechanism

6.5.1-1 Filtration selectivity factors

There were no significant differences in weight-specific filtration rates between the *Chlorella* Group and Blooming *Microcystis* Group throughout the 12 days. **Table 6-4** indicates the results after the correlation analysis between the diet characters and physiological rates, which were calculated based on all the measurement results of *A (S). woodiana* used in these three experiments. With regard to the affecting factors of filtration rates, as shown in **Table 6-4,** there was an obvious negative correlation between the initial diet concentration and the filtration rates ($P<0.001$). It has already reported by many authors that with increasing diet concentrations the filtration rate frequently decreases for a large number of mussel species examined: e.g. *Dreissena polymorpha1* (**Sprung and Rose, 1988**) and *Mytilus edulis* (**Widdows et al., 1979**).

In addition, as mentioned in **Chapter 3**, when the algae solution concentration was lower than the critical cell density, the filtration rates increased as the increase of algae concentrations till the critical cell density was reached, at which the pseudofaeces were expelled; above the critical cell density, the filtration rates decreased sharply as the algae concentration increased. Probably, most of the initial diet concentrations used in the experiments in **Table 6-4** were above the critical cell density, which induced the obvious negative correlation between the initial diet concentration and the filtration rates.

However, as indicated in **Table 6-4**, the inhibiting effect of MCs on the filtration rates of *A. woodiana* was not obvious ($P>0.01$).

It was pointed out by Jørgensen that higher concentrations of suspended particles in the ambient water elicit secretion of mucus. When mucus is produced, the chance of particles being caught, and thus ejected as pseudofaeces, depends upon size, shape and other physical characteristics of the particles, and not quality, i.e. diet value (**Jørgensen, 1996**). As we know that the MCs concentration belongs to the diet value, thus it did not induce excessive pseudofaeces excretion, which can reduce the filtration rate significantly, consequently, obvious inhibitory effect of MCs on the filtration rates of *A. woodiana* was not observed in the long-term grazing experiment.

Filtration rates in filter-feeding mussels are basically automatized process, and they are not subject to physiological regulation at the organismic level, e.g. according to

Table 6-4. Correlation matrix (*r*) among diet characters and physiological rates.

r	Initial Chl.a concentration ($\mu g\ L^{-1}$)	Microcystin ($\mu g\ L^{-1}$)	Filtration rate ($mL\ g^{-1}FW\ h^{-1}$)	Absorption efficiency (%)	Respiration rate ($\mu g\ O_2\ g^{-1}FW\ h^{-1}$)	Excretion rate ($\mu g\ NH_4^+\ g^{-1}FW\ h^{-1}$)
Initial Chl.a concentration	1	0.40	-0.93***	-0.36	0.61***	0.36
Microcystin		1	-0.38	0.59***	0.18	0.59***
Filtration rate			1	0.34	-0.47	-0.47
Absorption efficiency				1	-0.16	0.39
Respiration rate					1	-0.06
Excretion rate						1

***$P<0.001$.

Table 6-5. Characteristics of the experimental cyanobacteria diets fed to *Anodonta woodiana*.

Cyanobacteria	Shape	Origin	Intracellunar Microcystin ($\mu g\ L^{-1}$)	Extracellunar Microcystin ($\mu g\ L^{-1}$)	Total Microcystin ($\mu g\ L^{-1}$)	Chl.a ($\mu g\ L^{-1}$)
Microcystis aeruginosa NIES-90 (cultured)	Unicell	Chapter 4	-	-	1.55	38.7
Microcystis spp. (naturally blooming)	Colony and Unicell	Chapter 5	-	0.36	-	120.0
Microcystis spp. (naturally blooming)	Colony and Unicell	A pond, Oita, Japan	25.14	0.36	25.50	424.5

nutritional needs, but they are determined by the capacity of the pump and concentration of diet in the ambient water (**Jørgensen, 1996**).

6.5.1-2 Absorption selectivity factors

The distinctly higher *A. woodiana* absorption efficiencies of Blooming *Microcystis* Group than them of *Chlorella* Group found in the long-term grazing experiment are consistent with them reported in the laboratory experiment - *A. woodiana*'s acute physiological responses to different microalgae diets described in **Chapter 4**. In that experiment, it is found that the absorption efficiencies of *A (S). woodiana* on *M. aeruginosa* were obviously higher than them on *S. obliquus* (**Liu et al., 2014**).

Moreover, Peirson observed a low absorption efficiency value in *Argopecten* spp. of 17% for *Chlorella autotrophica* (**Peirson, 1983**), and in the oyster *Crassostrea virginica*, *Tetraselmis suecica* was digested and absorbed with an efficiency of only 6% (**Romberger and Epifanio, 1981**). These low absorption efficiency values have been attributed to the thick indigestible cell walls of these Chlorophyte species (**Gosling, 2003**).

With regard to the effect of MCs on the absorption efficiency, as shown in **Table 6-3**, the absorption efficiency of *A. woodiana* had an obvious positive correlation with MCs concentration. It shows that the absorption efficiency increased with the elevation of the toxic algae *Microcystis* concentration, indicating that *A (S). woodiana* prefers to digest toxic *Microcystis* spp.

As discussed in **Chapter 4**, the possible reason was that Chlorophyta cells have thick cell walls, which make them indigestible in the digested tracks; another possibility was that it is the different digestive enzyme activity in *A (S). woodiana*'s digestive tract that induced the prefer ingestion of *M. aeruginosa* to the green algae. Amylase is the digestive enzyme that can break starch in food down into smaller carbohydrate molecules; while protease is a general term of enzyme that can break down protein into its building blocks, amino acids (**Joanne Marie and Demand Media, 2011**). The ratio of amylase to protease enzyme activities in the stomach of *A (S). woodiana* reported by Fei et al. (**Fei et al., 2006**) was quite high, and it indicated that *A (S). woodiana* has stronger digestive ability on cyanobacteria that mainly consist of starch than green algae that mainly consist of protein (**Liu et al., 2014**).

6.5.2 Impacts of Microcystins on Mussels

With regard to the microcystins (MCs) effect on *A. woodiana's* physiological behavior, the characteristics of the experimental cyanobacteria diet used in our three different experiments were shown in **Table 6-5**. All the toxin measurement in the enzyme-linked immunosorbent assay (ELISA) method did not allow separate quantification of MC-LR or MC-LF but gave a total estimation of MCs presented in cyanobacterial suspensions.

In the experiment with cultured *Microcystis aeruginosa* NIES-90 as mentioned in **Chapter 4**, which contained the total MCs in a small quantity (1.55 μg L^{-1}) in the short-term grazing effect experiment, it was found that the toxic *M. aeruginosa* did not restrain the grazing behavior of *A(S). woodiana*, instead supplied the highest SFG value for the mussel's growth. Furthermore, the possible reason was supplied that in some degree, the mussel *A(S). woodiana* could accumulate and depurate MCs, although, we could not distinguish whether it was MC-LR or the MC-LF variant (**Liu et al., 2013**).

In the 6-day feeding experiment on naturally blooming water in this study, the extracellular MCs concentration was measured and the value was 0.36 μg L^{-1} in this study. As reported by Dittmann and Borner that the extracellular MCs are usually <10% of total MCs (**Dittmann and Borner, 2005**), thus the total MCs of the diet suspension used in this study may be estimated higher than 3.6 μg L^{-1}.

After 6-day exposure to the toxic *Microcystis* blooming water (MCs>3.6 μg L^{-1}), significantly increased SFG for *A. woodiana* was observed, mainly due to the increasing filtration rate. It can be inferred that MCs in this study did not restrain the filtration rate and the potential growth of *A. woodiana*.

The extended long-term comparative grazing experiment showed that there was not a decrease in *A. woodiana* SFG under exposure to toxic *Microcystis* spp. It indicated that *A. woodiana* assimilated cyanobacteria cells during the 12-day experiment and no toxic effect could be observed. It was also noticed that compared with the non-toxin green algae *Chlorella vulgaris*, the toxic naturally blooming water supplied much higher potential growth energy for the mussels.

In addition, no mussel mortality was registered. The assimilation of highly concentrated toxic *Microcystis* spp. by *A. woodiana* suggested that this mussel presents survival mechanisms in face of toxins. Therefore, cyanobacteria toxicity has no negative effect on *A. woodiana* survival and growth.

A similar experiment with zebra mussel indicated higher filtration rates on toxic

Microcystis aeruginosa than on non-toxic diet (*Nannochlopsis limnetica*) with no mussels mortality in a 3-week assimilation period (MC-LR concentration = 11.7 μg L^{-1} (**Dionisio Pires et al., 2004**), which endorse our results.

In previous studies, the ability of mussels to accumulate and store toxins has been demonstrated (**Yokoyama and Park, 2003**). One possible explanation is that microcystins can be detoxified through the conjugation of the toxin with glutathione (GSH), an intracellular tripeptide, via the enzyme soluble GST (glutathione-S-transferases), which was shown in several aquatic organisms, including zebra mussel (**Pflugmacher et al., 1998**).

6.5.3 Ammonia Excretion Rates with Possible Detoxification Process

Ammonia excretion rates of Blooming *Microcystis* Group were obviously higher than them of *Chlorella* Group, in addition, the toxic *M. aeruginosa* supplied more potential growth energy for the mussel throughout the long-term grazing experiment**,** indicating there were no negative effects of cyanobacteria toxicity on *A. woodiana* growth, probably due to the presence of the detoxification ability.

Regarding to the affecting factors on ammonia excretion rates, as shown in **Table 6-4**, there was an obvious positive correlationship between the ammonia excretion rate and the MCs concentration ($P<0.001$). This is possibly due to the process that the accumulated MCs can be degraded and finally excreted as ammonium.

MCs are monocyclic heptapeptides. The detoxification of MC-LR in the liver is known to occur via conjugation to GSH by GST activity (**Kondo et al., 1996**) and is transported to the kidneys and intestine for excretion (**Ito et al., 2002**).

The hepatic metabolism of MCs, produced by cyanobacteria, was studied by injection in mice and rats by Kondo et al. In his study, two metabolites, GSH and cysteine (Cys) conjugates of MCs from mouse and rat livers were found, and the GSH conjugate detected at 3-h was transformed to the Cys conjugate at 24-h. These results indicated that the conjugation of MCs with GSH are important in the metabolic pathway for MCs detoxification through the Cys conjugates formation (as illustrated in the No.1 process of **Fig. 6-6**). (**Kondo et al., 1996**)

In the experiment performed with mice by Ito et al., the conjugation of MCs with Cys, MCLR-Cys was detected the most actively excreted from the kidney. Furthermore, it was pointed out by Ito et al. that the appearance of toxicity by MCs depends on the balance between accumulation and metabolism in the liver (**Ito et al., 2002**). These results suggested that through MCLR-Cys formation, the detoxification process can be done by

liver and then, the conjugation MCLR-Cys can be transferred to kidney and be excreted in the form of ammonium (as shown in **Fig. 6-6**). However, all these studies were performed with mice rather than mussels.

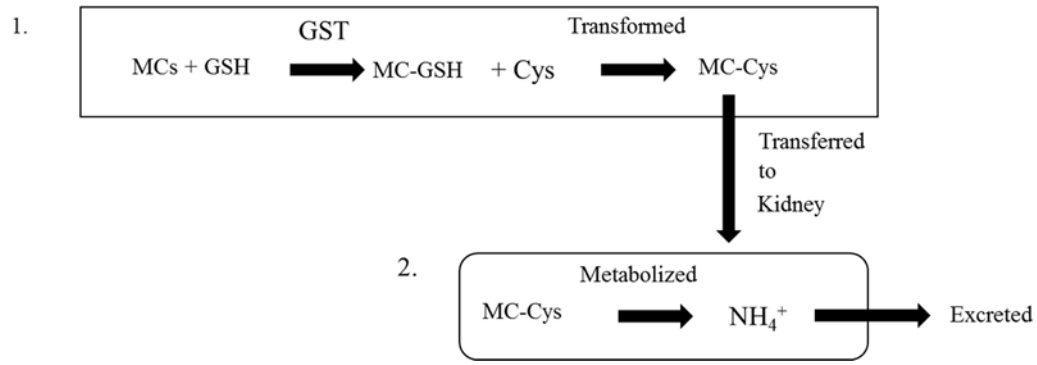

Fig. 6-6. The detoxification process occurred in the liver (the No.1 process) and in the kidney (the No.2 process). Adapted from **Kondo et al.**, **1996** and **Ito et al.**, **2002**.

Most research related to MCs toxicity has been focused on acute effects on mammals and fish. Nevertheless, the study on the MCs metabolization process in mussels was seldom, only in the recent two years, some research results were reported.

Biotransformation via GST detoxifies MCs conjugation to GSH enhances the water solubility of the toxin for better excretion. In the experiment conducted by Burmester et al*.,* the freshwater mussels *Dreissena polymorpha* and *Unio tumidus* demonstrated biotransformation (*via* the enzyme GST) and excretion capacity for cyanobacterial toxin MCs. *D. polymorpha* is capable of detoxification of MCs up to 50 μg L^{-1} via the GST system for at least 7-d exposure duration as could be observed during a cyanobacterial bloom. (**Burmester et al., 2012**)

In the experiment performed by Sabatini et al. with the freshwater mussel *Diplodon chilensis patagonicus*, the results indicated that this species would be able to consume toxic cyanobacteria for about 3 weeks, which is a considerable time for a cyanobacterial bloom, without much accumulation of MC-LR, without suffering visible biochemical damage for at least 4 weeks. Thus, it was suggested in their study that this species can tolerate the exposure to seasonal toxic cyanobacterial blooms. (**Sabatini et al., 2011**)

Therefore, possibly, like in other organisms, in *A. woodiana* the MCs were conjugated to GSH by the GST system to form the Cys conjugates in the liver, through which MCs were detoxified, and then they were transported to the kidneys and intestine, where they were metalized into ammonia and excreted. Moreover, the MCs metabolism process is more active than the accumulation process in the liver, thus the toxicity by MCs

was not appeared in *A. woodiana*. However, in order to verify the detoxification process in the mussel *A. woodiana* inferred in this study, further research deserves to be performed in future.

6.6 CONLUSIONS

The results from the long-term grazing experiment, combined with the experiments mentioned in **Chapter 4** and **5**, indicated that for the mussel *A. woodiana*:

(1) Filtration selectivity factors - MCs did not restrain the feeding behavior of *A. woodiana*; instead the exorbitant initial diet concentration could inhibit the filtration rates.

(2) Absorption selectivity factors - thick cell walls of Chlorophyta cells made them indigestible in the digested tracks; probably it is the different digestive enzyme activity in *A. woodiana*'s digestive tract that induced the prefer ingestion of *M. aeruginosa* to the Chlorophyta cells, indicated by about 3 times higher absorption efficiency of *M. aeruginosa* than that of the Chlorophyta cells.

(3) The possible detoxification process - in the mussel liver, through the MC-Cys formation, MCs could be detoxified and then they were transferred to kidney and were excreted in the form of ammonium. In addition, more MCs were metabolized efficiently rather than accumulated in the liver, thus the MCs toxicity effect in *A. woodiana* did not reveal.

Therefore, due to all these physiological adaptation mechanisms, *A. woodiana* can survive in *Microcystis*-blooming water bodies up to specific densities, including resistance to toxic chemicals.

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CHAPTER 7

Interactions Between Mussels and Submerged Macrophytes

CHAPTER 7

Interactions Between Mussels and Submerged Macrophytes

Abstract

As indicated by previous chapters, the mussel *Anodonta woodiana* can be proposed to inhibit the over growth of cyanobacteria. Nevertheless, excessive stocking density of mussels may induce DO deficiency in the sediment, and excessive NH_4^+-N regeneration in the water column can deteriorate water quality. On the other hand, as mentioned in the first chapter, water clarity is always the key factor for successful submerged macrophytes restoration. Therefore, in this chapter, the experiments with the mussel *A. woodiana* combined with the submerged macrophyte *Vallisneria asiatica* were performed to figure out the interactions between them. The results indicated that (1) mussel culture sites should have aerobic sediments, thus mussels stocking density should be in a range named "ecological carrying capacity"; (2) the presence of submerged macrophytes can supply the required aerobic condition for mussels to play the effective role of biomanipulation tool in the eutrophic freshwaters; (3) for the restoration of submerged macrophytes in the littoral zone, the sediment characteristics, as a vital factor need to be taken in to account; (4) turbidity caused by the wind-induced sediment disturbance can be improved significantly with the existence of mussels with sufficient biomass, which finally increases the possibility of successful restoration. Consequently, there is high feasibility of the combined application of *A. woodiana* and *V. asiatica* in the littoral zone in practice as an optimal ecological restoration method for Lake Taihu.

7.1 INTRODUCTION

In the previous chapters, through a series of laboratory experiments, it has been found that not only the pure cultured *Microcystis aeruginosa* but also the naturally blooming toxic *Microcystis* spp. of unicell and colony can be grazed efficiently by *Anodonta woodiana*; moreover, it was inferred that there are possible detoxification mechanisms in the mussel *A. woodiana*, which assisted the mussel in obtaining the energy for its potential growth in exposure to high concentrations of microcystins. Thus, *A. woodiana* as a local species of mussels in Lake Taihu, the increase of biomass can be encouraged in order to inhibit the over growth of cyanobacteria through the Top-Down effect.

However, there are several potential problems for mussels when they are applied in practice that should draw our attention: the biomass of mussels should be controlled in an appropriate range, or else, excessive amounts of mussels may excrete excessive NH_4^+, which can threaten the growth of aquatic organisms and further deteriorate water quality in the culture site; furthermore, excessive amounts of mussels can cause the anoxia environment in the benthic zone; in addition, the excreted algae included in the pseudofaeces still have activity, which can regrow and be blooming in suitable circumstances.

On the other hand, as mentioned in the first chapter, water clarity is always the key factor that can decide the success of submerged macrophytes restoration. In the littoral zone in Lake Taihu, water clarity is always quite low with a rather poor light field, caused by the wind disturbance-induced sediment resuspension, which limits the growth of submerged macrophytes, and hence results in the failure of submerged macrophytes restoration.

Therefore, in this chapter, in order to find an optimal ecological method for the formation of aquatic macrophytes-dominated stable state in Lake Taihu, which can solve the potential problems induced by excessive excrements of mussels used for biomanipulation, meanwhile, can improve the water transparency condition for the growth of submerged macrophytes, the experiments with the mussel *A. woodiana* combined with the submerged macrophyte *Vallisneria asiatica* were performed. Thus, in the first experiment in this chapter, the objectives are: (1) to clarify the relationship between mussels stocking density and potential accumulation of NH_4^+; (2) to testify whether the introduction of submerged macrophytes can benefit the removal of excessive NH_4^+ excreted by mussels. Thereafter, in the following out-door experiment, the

relationship between submerged macrophytes, turbidity and mussels will be verified: (1) whether the growth of submerged macrophytes can be affected by turbidity or not; (2) whether mussels can improve the turbidity condition and furthermore benefit the growth of submerged macrophytes.

In addition, the results can give insight to the interactions between mussels and submerged macrophytes, which can further supply the necessary knowledge for the feasibility of the combined application of *A. woodiana* and the submerged macrophyte *V. asiatica* in the littoral zone in practice.

7.2 MATERIALS AND METHODS

The mussels of *A. woodiana* were supplied by Gunma Prefecture in Japan. The mussels were kept at 4℃ before transporting them to the laboratory. Immediately upon arrival, the mussels were transferred to 35 L aquaria with 20 L aerated dechlorinated tap water at controlled temperature 25℃, under a light:dark regime of 12h:12h for at least one week, before being used for experiments. The water was completely refreshed three times per week. Mussels were chosen with similar sizes to remove the effects caused by physiological sizes. Before introduction to the aquaria, mussels were gently cleaned with a brush under running de-ionized water to remove phytoplankton adhered to the shell and not fed for 2 days in clean aerated dechlorinated tap water to clear the guts.

The submerged macrophytes, *V. asiatica*, were collected in River Onga, Fukuoka, Japan, and then cultured in dechlorinated tap water for 5 days to wash attachments on plants. Only the plants with healthy growth and even size were chosen for the experiment.

7.2.1 Experiment 1 (Mussels-Nutrients-Submerged Macrophytes)

Dechlorinated tap water which contained 4.34 mg L^{-1} DTN and 0.12 mg L^{-1} DTP was used for the experiment. Different concentrations of N and P were chose to determine the physiological effect of water column nutrients on *V. asiatica*. N and P concentrations were determined based upon concentrations found in Lake Taihu. Then the NH_4Cl and KNO_3 were used as N source, and KH_2PO_4 was used as P source to adjust the concentrations of N and P so as to achieve the set values.

There were 6 tanks indicating 6 different conditions, depending on the different biomass of the mussel *A. woodiana* and the submerged macrophyte *V. asiatica*, all of which were filled of 30 L aerated tap water with initial nutrients as described in **Table 7-1**.

The objectives in the Experiment 1 were:

(1) To clarify the relationship between mussels and nutrients-potential problems induced by mussels: the accumulation of excreted NH_4^+ and the regrowth of the excreted algae in the pseudofaeces;

(2) To testify the introduction of submerged macrophytes can benefit the removal of excessive NH_4^+ excreted by mussels.

Table 7-1. Materials and experimental conditions for the Experiment 1.

Materials		Tanks					
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Aerated tap water	Volume (L)	30					
	Initial DO (mg L^{-1})	9.2					
	Initial pH	7.8					
Initional nutrients (mg L^{-1})	NH_4^+-N	0.42					
	NO_3^--N	3.92					
	DTN	4.34					
	DTP	0.12					
Vallisneria asiatica	Freshweight (g)	0	200	0	200	200	200
Anodonta woodiana	Freshweight (g)	0	0	107	107	215	414
	Individual numbers	0	0	2	2	4	8
Artificial food (mainly constituted of algae and several proteins)	Freshweight (g)	0.25	0.25	0.25	0.25	0.5	1
	Input frequency	Every 3 days					
	N contents (mg N g^{-1})	32.8					
	P contents (mg N g^{-1})	7.4					
Conditions	Light intensity (Lux)	3400					
	Light:Dark	12 h:12 h					
	Temperature (℃)	25					
	Period (day)	12					

The Experiment 1 was performed as shown in the following pictures in **Fig. 7-1**.

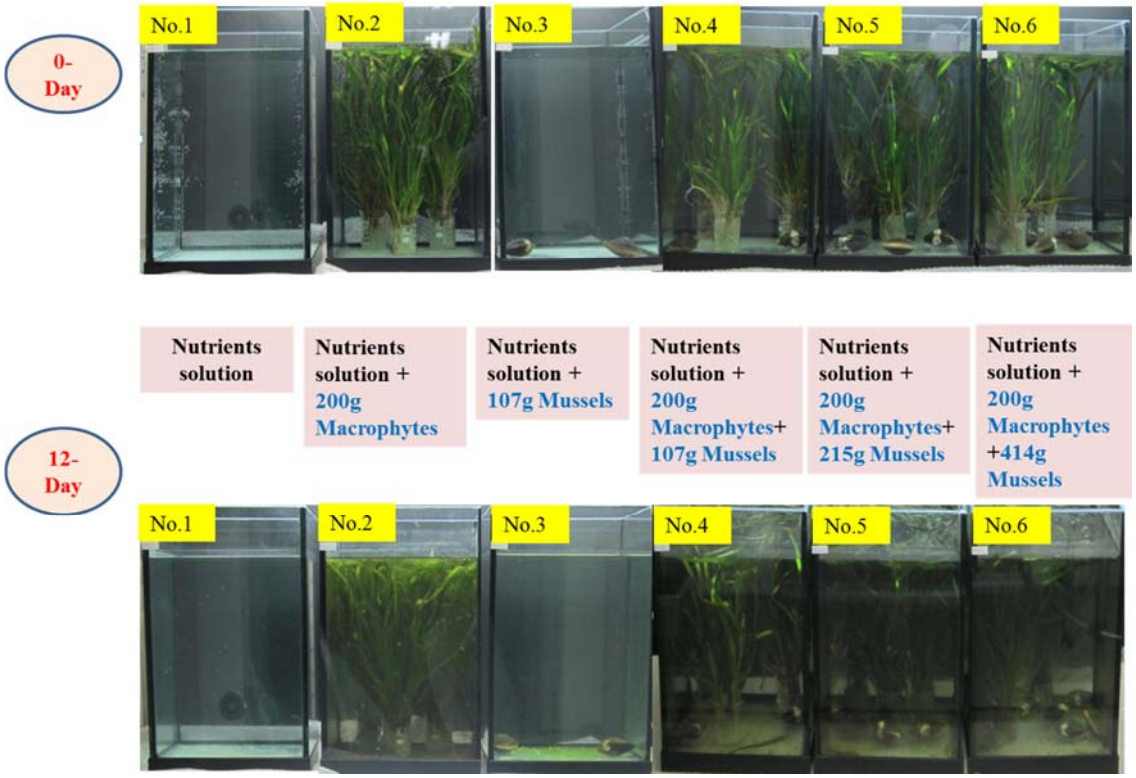

Fig. 7-1. The experimental set up for each tank and the situations on 12-day for each tank.

7.2.2 Experiment 2 (Submerged Macrophytes-Turbidity-Mussels)

The objectives in Experiment 2 were to testify, when submerged macrophytes are to be restored especially in the turbid water body, the mussels can significantly improve water clarity, which will potentially stimulate the growth of submerged plants.

5 buckets were used in this out-door experiment, each of which was filled with 100 L solution. The solution for No.1 bucket was from Ongagawa River, where the submerged macrophytes were collected; while the solution for the other 4 buckets were all from Morota eutrophic pond, where *Microcystis* spp. bloom severely in every summer. The information of *A. woodonta*, *V. asiatica* and sediment was listed in **Table 7-2**.

The experiment was performed out-door with natural illumination for 50 days, at the temperature from 10.8 to 26.5℃, with the light intensity from 1830 to 5940 lux. The set up for each bucket was shown in **Fig. 7-2**.

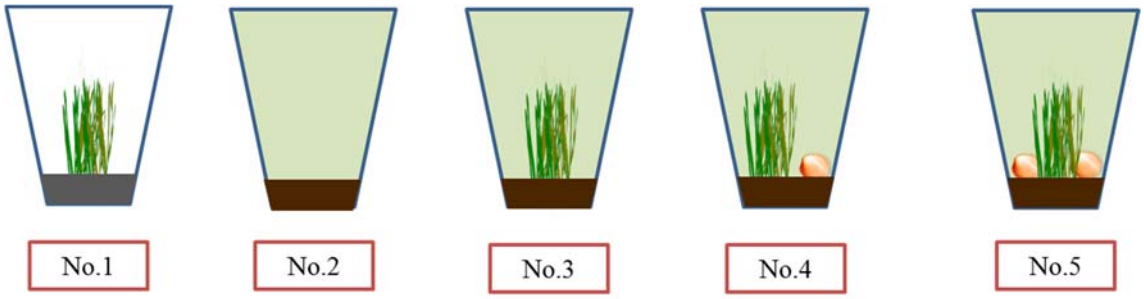

Fig. 7-2. The Experiment 2 set up for each bucket.

Table 7-2. Materials and experimental conditions for the Experiment 2.

Materials		Tanks				
		No. 1	No. 2	No. 3	No. 4	No. 5
Solution	Volume (L)	100				
	Initial DO ($mg\ L^{-1}$)	8.3				
	Initial pH	8.0	7.3			
	Turbidity (NTU)	1.0	11.0	9.3	11.4	8.8
	Source	Ongagawa River	Morota eutrophic pond			
Vallisneria asiatica	Freshweight (g)	250	0	250	250	250
Anodonta woodiana	Freshweight (g)	0	0	0	260.9	524.2
	Individual numbers	0	0	0	6	12
Sediment	Source	Ongagawa River	Morota eutrophic pond			
Out-door conditions	Light intensity (Lux)	1830~5940				
	Light:Dark	12 h:12 h				
	Temperature (℃)	10.8~26.5				
	Period (day)	50				

NTU: Nephelometric Turbidity Unit.

7.2.3 Sampling Analysis Methods

The Experiment 1 lasted for 12 days and the Experiment 2 lasted for 50 days. pH, DO, and turbidity were tested by pH meter (D-52, Horiba, Japan), Portable LDO Probe and Meters (Model HQ10, Hach, USA) and Portable Turbidimeter (2100P, Hach, USA), respectively. NH_4^+-N, NO_3^--N and DTP were determined by salicylate method, chromotropic acid method, and acid persulfate digestion method respectively (DR/2400 portable spectrophotometer, Hach, USA) for the water quality analysis.

7.3 RESULTS

7.3.1 Water Quality (Mussels-DO-Submerged Macrophytes-Pseudofaeces)

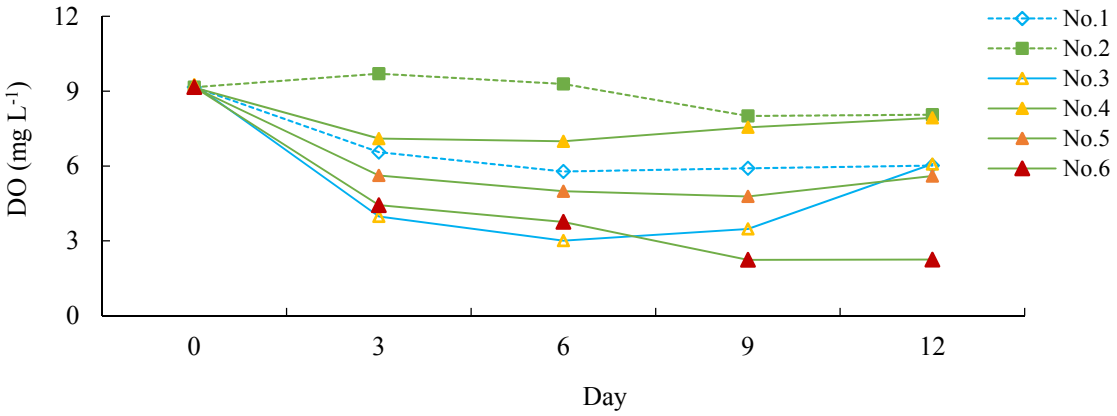

Fig. 7-3. DO changes in 6 tanks during the experiment. (No.1-with only nutrients solution; No.2-with nutrients solution + 200 g plants; No.3-with nutrients solution + 2 mussels; No.4-with nutrients solution + 200 g plants + 2 mussels; No.5-with nutrients solution + 200 g plants + 4 mussels; No.6-with nutrients solution + 200 g plants + 8 mussels.)

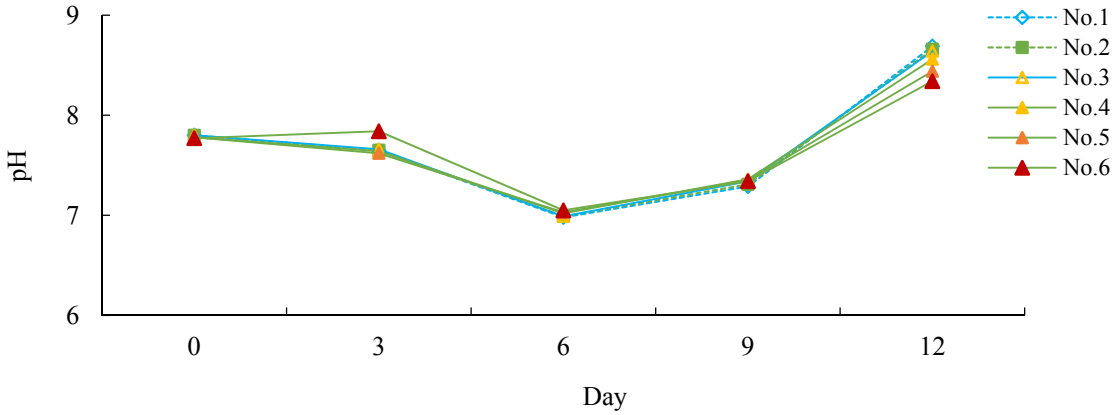

Fig. 7-4. pH changes in 6 tanks during the experiment. (No.1-with only nutrients solution; No.2-with nutrients solution + 200 g plants; No.3-with nutrients solution + 2 mussels; No.4-with nutrients solution + 200 g plants + 2 mussels; No.5-with nutrients solution + 200 g plants + 4 mussels; No.6-with nutrients solution + 200 g plants + 8 mussels.)

During the 12 days, DO in the tank No.2 cultured with only submerged macrophytes was the highest compared with that in the other tanks. Furthermore, DO in the tank No.4 (mussels and submerged macrophytes) was obvious higher than that of the tank No.3 during the 12 days. Along with the increase of mussels culture density as shown in tanks No.5 and No.6, the corresponding DO value decreased during the experiment period. Especially the DO value as shown in No.6 fell down all the time, and from 9-day, the DO value was as low as about 2 mg L^{-1}, which can inhibit the growth of mussels.

The DO values in the tanks No.3, No.4 and No.5 demonstrated the similar trend with time, decreased at first and then increased.

The pH values in all the tanks demonstrated the similar trend with time, decreased during the first 6 days and then increased, all of which were above 7.

7.3.2 Nutrients (Mussels-N-Submerged Macrophytes)

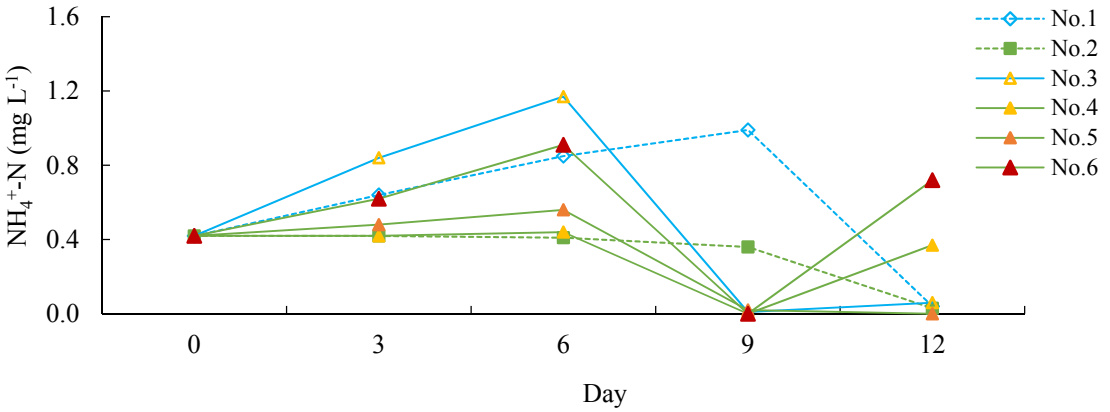

Fig. 7-5. NH_4^+-N (mg L^{-1}) concentration changes in 6 tanks during the experiment. (No.1-with only nutrients solution; No.2-with nutrients solution + 200 g plants; No.3-with nutrients solution + 2 mussels; No.4-with nutrients solution + 200 g plants + 2 mussels; No.5-with nutrients solution + 200 g plants + 4 mussels; No.6-with nutrients solution + 200 g plants + 8 mussels.)

In the tank No.2, the NH_4^+-N concentration declined gradually during the experiment period.

As illustrated in **Fig. 7-5**, the trend of NH_4^+-N concentrations with time in the tanks No.3, No.4, No.5 and No.6 was the same, all of which climbed up from 0-day to 6-day, thereafter dropped to the bottom on the 9-day, followed by an increase between 9-day and 12-day.

The comparison of NH_4^+-N concentrations during the experiment period between the tanks No.4, No.5 and No.6 revealed that, the values of NH_4^+-N concentrations increased with the mussels stocking density. Especially as shown in the values of the tank No.6, although NH_4^+-N can be declined from 6-day to 9-day, whereas, thereafter it increased sharply.

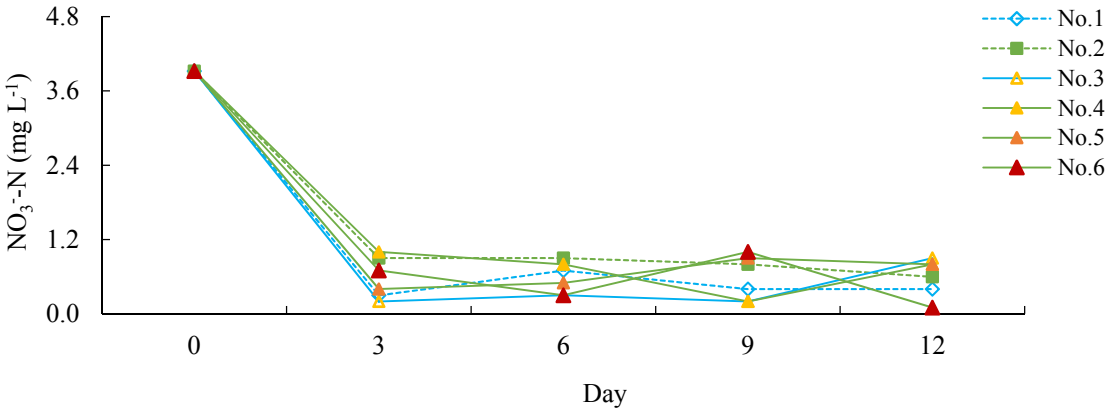

Fig. 7-6. NO_3^--N (mg L^{-1}) concentration changes in 6 tanks during the experiment. (No.1-with only nutrients solution; No.2-with nutrients solution + 200 g plants; No.3-with nutrients solution + 2 mussels; No.4-with nutrients solution + 200 g plants + 2 mussels; No.5-with nutrients solution + 200 g plants + 4 mussels; No.6-with nutrients solution + 200 g plants + 8 mussels.)

As shown in **Fig. 7-6**, the NO_3^--N concentration declined sharply in the tank No.3.

As illustrated in **Fig. 7-5** and **Fig. 7-6**, in the first three days in the tank No.2, compared with NH_4^+-N, NO_3^--N can be removed more significantly by the submerged macrophytes *V. asiatica*.

As shown in **Fig. 7-7**, no obvious trend was found in PO_4^{3-}-P concentration changes in 6 tanks during the experiment.

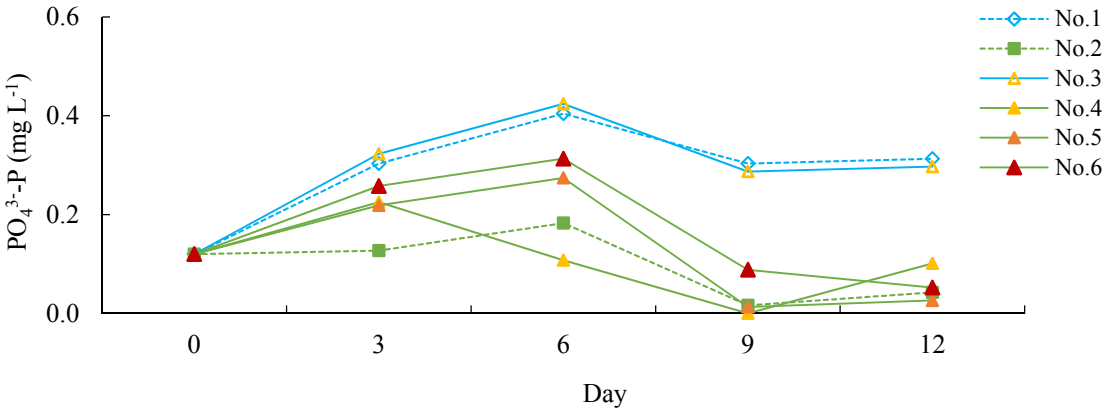

Fig. 7-7. PO_4^{3-}-P (mg L^{-1}) concentration changes in 6 tanks during the experiment. (No.1-with only nutrients solution; No.2-with nutrients solution + 200 g plants; No.3-with nutrients solution + 2 mussels; No.4-with nutrients solution + 200 g plants + 2 mussels; No.5-with nutrients solution + 200 g plants + 4 mussels; No.6-with nutrients solution + 200 g plants + 8 mussels.)

7.3.3 Turbidity (Macrophytes-Turbidity-Mussels)

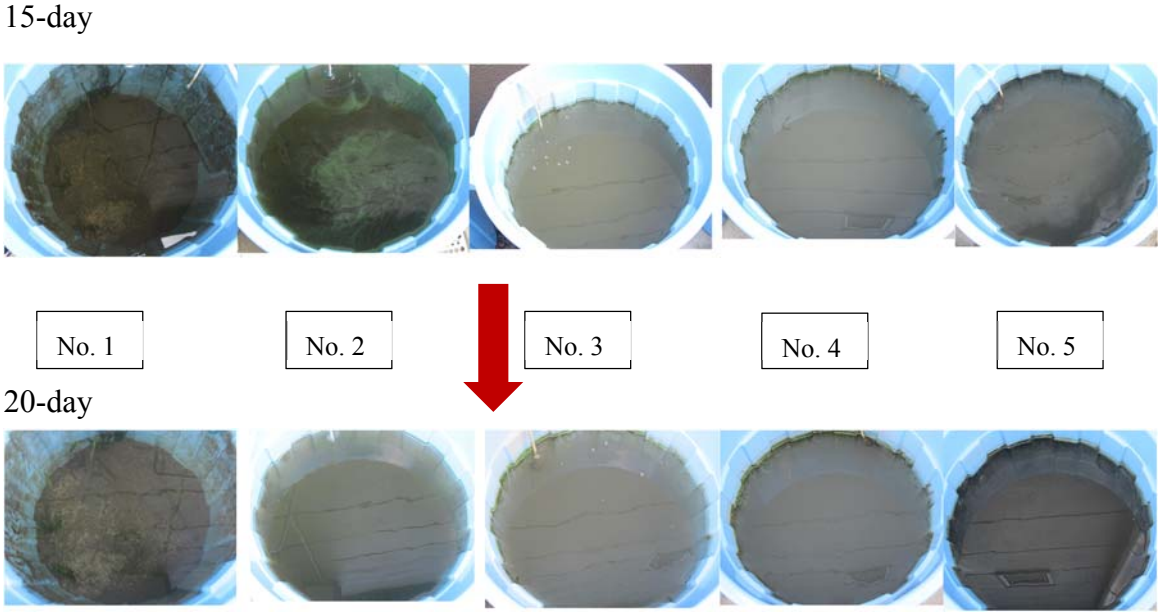

Fig. 7-8. The water clarity in each experimental bucket on 15-day and 20-day, respectively.

As shown in **Fig. 8**, the water clarity in the bucket No.5 was clearer than that either in No. 3 or No.4 on 15-day and 20-day, indicating the impact of mussels on turbidity.

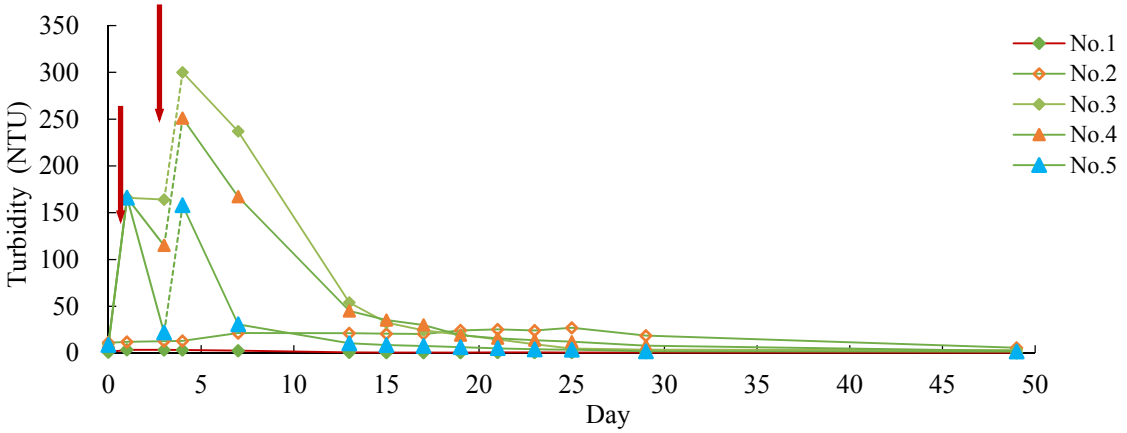

Fig. 7-9. Turbidity (NTU) changes in 5 buckets during the experiment. (No.1-with plants in Ongakawa River environment; No.2-blooming water in Morota Pond environment; No.3-plants + blooming water in Morota Pond environment; No.4- plants + blooming water in Morota Pond environment + 6 mussels; No.5-plants + blooming water in Morota Pond environment + 12 mussels; furthermore, the corresponding sediments were disturbed on 0-day and 3-day while sampling, which represents the wind-induced disturbance in the littoral zone in Lake Taihu. (The red arrows mean that after the water sampling, on 0-day and 3-day, the submerged macrophytes *V. asiatica* were sampled in the tank No.1, No.3, No.4 and No.5, respectively.)

The comparison of the turbidity changes induced by the sediment disturbances of the submerged macrophytes sampling (the disturbance forces during the sampling process performed by the same person were approximately closer) in the buckets No.1 and No.3 illustrates that there was only a slight change in the bucket No.1, whereas there was a rapid increase in the bucket No.3.

After the sediment disturbance on 3-day, turbidity in the bucket No.5 cultured with mussels only behaved a slightly increase until 7-day, whereas in the bucket No.3 it climbed up significantly. In addition, there were no significant differences of the turbidity between the buckets No.3 and No.4.

In addition, as shown in **Fig. 7-9**, after 30 days, the turbidity of the bucket No.2 declined gradually.

7.4 DISCUSSION

7.4.1 The Effect of Mussels and Submerged Macrophytes on DO

7.4.1-1 Submerged macrophytes and DO

As mentioned in the previous part, the only difference of constitution between the tanks No.3 and No.4 was the existence of submerged macrophytes. It indicated that the existence of submerged macrophytes can supply the oxygen circumstance for the mussels' culture sites. Submerged macrophytes through the photosynthetic process, oxygenate the water more effectively than floating-leaved macrophytes (**Pokorny and Rejmankova, 1983**). Also, they can oxidize their rhizospheres, photosynthetically-produced oxygen diffuses to the roots through the aerenchyma, and subsequently diffuses across the epidermis into the sediment (**Oremland and Taylor, 1977**).

7.4.1-2 Mussels stocking density, DO and sediment

Carrying capacity, originally, it was a concept in ecology that was applied to the population density achieved at the asymptote in the logistic population growth equation (**Dame and Prins, 1998**). "Ecological carrying capacity" was proposed for mussels culture defined as "the standing stock of suspension-feeding mussels where the consumption of phytoplankton, enhancement of nutrient removal, and other ecosystem services are maximized without negatively affecting water quality, sediment biogeochemistry, and overall ecosystem function." (**Newell I.E. R., 2007**)

The DO comparisons between the tanks No.4, No.5 and No.6, indicate that the mussels stocking density should be in a range named "ecological carrying capacity", concerning the possible problem induced by mussels-DO deficiency.

At very high density of mussels, the mussels' respiration and the microbial respiration resulted from the intense biodeposition may be so intense that reduces the oxygen content of the surrounding water column and sediments, moreover, this can cause the sediment anoxia, thereby altering benthic community composition. Excess biodeposition, especially in low water flow environments, has the potential to stimulate bacterial respiration to such an extent that sediments become anaerobic, thereby inhibiting coupled nitrification-denitrification (**Fig. 7-10**) and causing sediment-bound P to be mobilized.

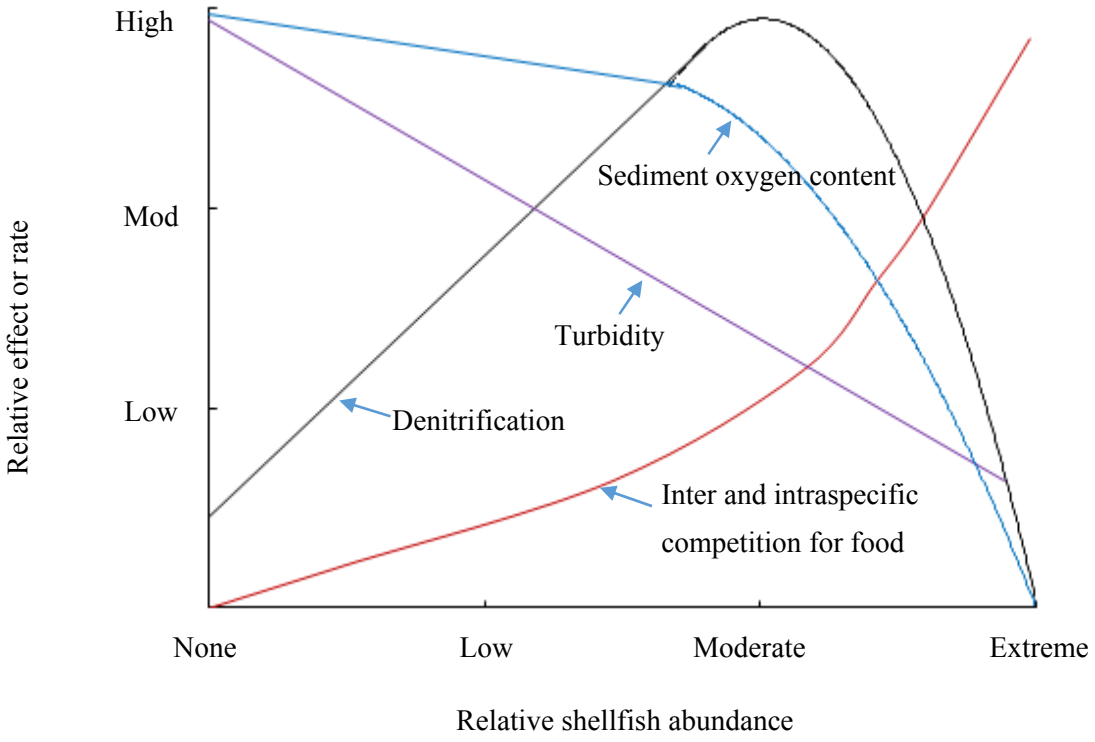

Fig. 7-10. Conceptual figure illustrate how increasing abundance of mussels feeding on phytoplankton and inorganic particles reduce water column turbidity in eutrophic coastal waters. At high mussel abundances, this may lead to intra- and interspecific competition for phytoplankton with other suspension-feeding herbivores. Biodeposits, containing undigested POM, are deposited into the sediment surface where they are microbially metabolized, thereby affecting sediment oxygen content. In locations where there is either extremely heavy biodeposition or little water flow and mixing, sediment oxygen content may decline, thereby reducing coupled nitrification-denitrification. (**Newell and Koch, 2004**)

The adverse effects of sediment over enrichment by mussel biodeposits have often been observed in sediment underlying mussels in suspended raft culture. For example, **Ito and Imai** (**1955**) reported that intensive oyster aquaculture resulted in underlying sediments becoming anaerobic, and these effects appeared cumulative because the longer oysters were cultivated in a location, the more frequently sediment anoxia occurred. Such reduction in sediment oxygen content will reduce rates of bacterially mediated nitrification and increase the proportion of N released as NH_4^+. When sediments become completely anaerobic, the increasingly formation of H_2S can kill the aerobic nitrifying bacterial community. Consequently, even if aerobic conditions in the surface sediments are restored, nitrification will not restart without the regeneration of the nitrifying bacteria

community (**Henriksen and Kemp, 1988**).

These findings suggest that extremely dense mussel communities can adversely affect sediment microbial processes by shifting them from aerobic to anaerobic metabolism as a result of increased POM (particular organic matter) loading. (**Fig. 7-10**)

Overall, the stocking density of mussels in the tank No. 6 was over the "ecological carrying capacity" of the ecosystem composed of 414 g mussels and 200 g submerged macrophytes in 30 L liquid.

7.4.1-3 Regrowth of excreted algae in pseudofaeces and submerged macrophytes

For the tanks No.4 and No.5, the similar DO trend with time suggested that the mussels density were in the range of ecological carrying capacity, due to the appropriate arrangement fresh weight ratio of mussels to submerged macrophytes, 107 g : 200 g for the tank No.4 and 215 g : 200 g for the tank No.5, respectively, thus DO could rose again. In the case of the tank No.3, the increase of DO since 6-day can be explained by the regrowth of the excreted algae included in the pseudofaeces which were observed with obvious green color at the bottom of the tank.

It was reported in some studies that food particles have remained alive after being expelled in pseudofaeces by mussels. It has been observed that colonial cyanobacteria *Gloeotrichia echinulate* were viable after rejection by zebra mussel and could return unharmed to the water column and migrate vertically due to their possession of gas vacuoles (**Naddafi et al., 2007**). In addition, pseudofaeces from zebra mussel containing diatoms have been reported to resuspend under turbulent mixing during seasonal circulation and autumn turnover (**Baker et al., 1998**). It was also observed that algae from zebra mussel pseudofaeces when cultivated in a laboratory continued to grow.

Therefore, the selective feeding of mussels could promote the return of non-selected food to sediments and favor species rejected that possibly remain alive in the bottom of systems, which under appropriate conditions can regrow.

However, in the other tanks (No.4, No.5 and No.6) cultured with not only mussels but also submerged macrophytes, no benthic algae were observed at the end of the experiments. This phenomenon suggested that the introduction of submerged macrophytes in the mussels' culture site can inhibit the regrowth of the excreted algae included in the pseudofaeces of mussels, probably due to the competition for nutrients and shading effect. Moreover, in the 6-day feeding responses experiment mentioned in **Chapter 5**, after the 6-day, when the solution inside the treatment tank with mussels was emptied, on the surface of the sediment and the inside walls of the tank, attached algae were observed, there were possibilities that some of these attached algae were from the

excreted algae included in the pseudofaeces of mussels.

7.4.2 The Effect of Mussels and Submerged Macrophytes on Nitrogen Transformation

7.4.2-1 Submerged macrophytes and N

The existence of *V. asiatica* can benefit the removal of NH_4^+-N in the water body, probably due to the direct absorption and the activated metabolization of microorganisms attached around the roof zone of *V. asiatica*.

As reported in the absorption experiments with submerged macrophyte *V. asiatica*, among all the nutrients components, NH_4^+-N was absorbed by the macrophyte *V. asiatica* preferentially (**Liu et al., 2011**). In that experiment, after washing carefully under running tap water for five times, 10.5±0.5 g of *V. asiatica* was selected and submerged in culture solution of 1 L without sediment. Three different culture solutions with three different quantities of the mixture KNO_3, NH_4Cl, KH_2PO_4 dissolved in the tap water were supplied. When the initial concentration of NH_4^+-N is 2.51 mg L^{-1} in the culture solutions, the NH_4^+-N was found to be completely removed by *V. asiatica* in the first 7 days.

7.4.2-2 Mussels and N

For the tank No.3 cultured only with mussels, the rise of NH_4^+-N concentrations in the first 6 days can be explained by the metabolization process of mussels and ammonification process of microorganisms: mussels fed on the artificial food mainly composed of particle nitrogen and phosphorus, which was added into the tank every three days, and after the metabolization, the particle nitrogen was excreted mainly in the form of NH_4^+ and into the water body; meanwhile, the microorganism can actively perform the ammonification process, which accelerated the transformation of particle organic nitrogen in the added artificial food to the NH_4^+-N.

Therefore, in addition to the direct “Top-Down” control that mussels can exert on phytoplankton stocks as mentioned in the foregoing chapters, they may also exert “Bottom-Up” control by changing rates and process of nutrient regeneration (**Fig. 7-11**) mussels, by virtue of their high clearance rates, filter phytoplankton from large volumes of water. This has the effect of focusing nutrients that are then regenerated in the sediments around the population, hence increasing nutrient concentrations within the zone. Nonetheless, the total amount of nutrients regenerated directly by mussel excretion and the microbial degradation of their biodeposits cannot be any greater than if the

phytoplankton was being degraded solely by planktonic organisms.

In the study of the eastern oysters fed on natural seston ranging in concentration from 5 to 20 mg L^{-1}, it is reported that 50% of the PON (particular organic nitrogen) cleared from the water column was assimilated, and the remainder was accumulated in biodeposits (**Newell and Jordan**, **1983**).

Of the N absorbed by mussels from the ingested food, the majority is used for tissue growth and some is excreted as urine (70% of which is NH_4^+, 0% to 13% urea, and 5% to 20% amino-N; **Bayne et al., 1976**). Measured rates of NH_4^+-N flux from natural mussel communities (direct excretion plus regeneration from biodeposits in sediments) can be substantial, ranging from 1 to 5 mmol N m^{-2} h^{-1} (**Dame et al., 1992**). The nitrogen released comes not only from ingested phytoplankton but also non-phytoplankton material, such as N-rich bacterial and flagellates (**Asmus and Asmus, 1991**), which are readily captured by mussels (**Kreeger and Newell, 2001**). This release of nitrogen increases the water column dissolved nitrogen pool and hence can support new phytoplankton and microphytobenthos production (**Kaspar et al., 1985**).

Thereafter, the regrowth of the benthic algae from the 6-day as mentioned in the foregoing discussion can absorb the dissolved nitrogen in NH_4^+, which induced the sharp decrease of NH_4^+-N concentrations.

7.4.2-3 NH_4^+-N excreted by mussels stocking density and ecological carrying capacity

As indicated by the comparison results of NH_4^+-N concentrations during the experiment period between the tanks No.4, No.5 and No.6, the values of NH_4^+-N concentrations increased with the mussels stocking density. Especially as shown in the values of the tank No.6, although NH_4^+-N can be declined from 6-day to 9-day, whereas, thereafter it increased sharply and exceeded the initial value, implying that the stocking density of mussels in the tank No.6 has be over the NH_4^+-N process ability of the aquatic ecosystem even with the presence of submerged-macrophytes.

This means the stocking intensity of mussels was over the “ecological carrying capacity” of the ecosystem composed of 414 g mussels and 200 g submerged macrophytes in 30 L liquid. Thereby, high levels of NH_4^+-N accumulation probably occurred in the tank No.6, which can enhance rates of primary production and phytoplankton biomass, in addition NH_4^+ can be a problem in an aquatic environment, because it is difficult to remove in short term and a high level of NH_4^+ can be toxic to fish and other biota (**Wee et al., 2007**). Therefore, more attention should be paid on the potential risks of the excessive NH_4^+ excreted by over biomass of mussels.

7.4.2-4 Mussels stocking density and nitrogen microbiological process in sediments

The case of the tank No.6 can be imagined in field, mussels of too intense stocking density in the aquatic ecosystem with the presence of submerged macrophytes, can bring about the potential problems: DO deficiency in the sediment, excessive NH_4^+-N regeneration in the water column. If the mussels were cultured of the density within the absorption ability of submerged macrophytes, the DO deficiency in the sediment can be avoided due to the photosynthetically-produced oxygen, which diffuses to the roots through the aerenchyma, and subsequently diffuses across the epidermis into the sediment (**Oremland and Taylor, 1977**).

At the different conditions of oxygen content in the sediment induced by different stocking densities of mussels, from aerobic to anaerobic, the nutrient metabolization process can be totally different: as shown in the **Fig. 7-11**.

When DO is deficient in sediment induced by the extensive stocking density, the nitrogen would be recycled as illustrated in **Fig. 7-11 (a)**. In eutrophic conditions, mussels filter large amounts of phytoplankton, a large part of which is rejected as pseudofaeces, faeces and pseudofaeces are then transferred to the sediment surface (**Newell, 2004**). Under anaerobic conditions, any remaining PON is subject to microbial degradation that can lead to some NH_4^+-N being generated to the water column which enters the water-column dissolved inorganic nitrogen (DIN) pool (**Newell, 2004**). The part of nitrogen excreted by mussels entered into the DIN pool in water column and can be used for the growth of phytoplankton, if it was accumulated to high concentrations, can induce threat to the aquatic system again.

When the mussels were cultured of the density within the absorption ability of submerged macrophytes, the surficial sediment can be fully oxygenated due to the photosynthetically-produced oxygen, which can supply the aerobic conditions for microbially mediated nitrification, and this step is crucial precursor to denitrification in the underlying anaerobic sediments (**Newell, 2004**).

Thus, as illustrated in **Fig. 7-11 (b)** when mussel biodeposits settle to the sediment surface, any remaining PON is subject to microbial degradation that can lead to some NH_4^+-N being generated to the water column. Some N that is not microbially metabolized can become buried in the accumulating sediments. (**Newell, 2004**). In this range of stocking density of mussels, the surficial sediments contain sufficient oxygen, then aerobic nitrifying bacteria can oxidize nitrogen compounds within the biodeposits to NO_2^- and NO_3^- (**Newell, 2004**). As shown in **Fig. 7-11 (b)**, some of this NO_2^- and NO_3^- diffuses out of the sediment and enters the water-column DIN pool and some diffuses down into the underlying anaerobic sediments (**Newell, 2004**).

Meanwhile, within the underlying anaerobic sediments, denitrifying bacteria reduce the NO_2^- and NO_3^- to N_2 gas (**Henriksen and Kemp, 1988**). Absent N fixation, this gaseous N_2 is in a form unavailable to plankton and so it passes to the atmosphere without stimulation further primary production (**Newell, 2004**). Furthermore, it was reported that under aerobic conditions, coupled nitrification-denitrification was promoted, resulting in denitrification of ~20% of the total nitrogen from food resource for mussels (**Newell et al., 2002**).

Due to the fact that denitrification can only occur where there is a close juxtaposition between oxygenated conditions that support nitrifying bacteria and anaerobic conditions that support denitrifying bacteria (**Kaspar et al., 1985**), thus the ecological benefits associated with aquaculture of mussel suspension-feeders, such as the nitrogen removal through promoting coupled nitrification-denitrification and Top-Down control on phytoplankton, are largely depended on having aerobic sediments. As a consequence, the degree of sediment oxygenation around the culture site must be regularly monitored. In some cases, remedial actions should be taken to maintain the aerobic conditions needed to sustain both nitrifying bacteria and the benthic bioturbator community. For example, when it is necessary, the use of a paddle-wheel system can increase the rate of mixing of surface oxygenated water to the bottom.

In future further study, the biomass of *A. woodiana* can be accurately calculated based on the model with the important parameters, including NH_4^+ excreted and the oxygen consumed by mussels, the filtration ability of mussels.

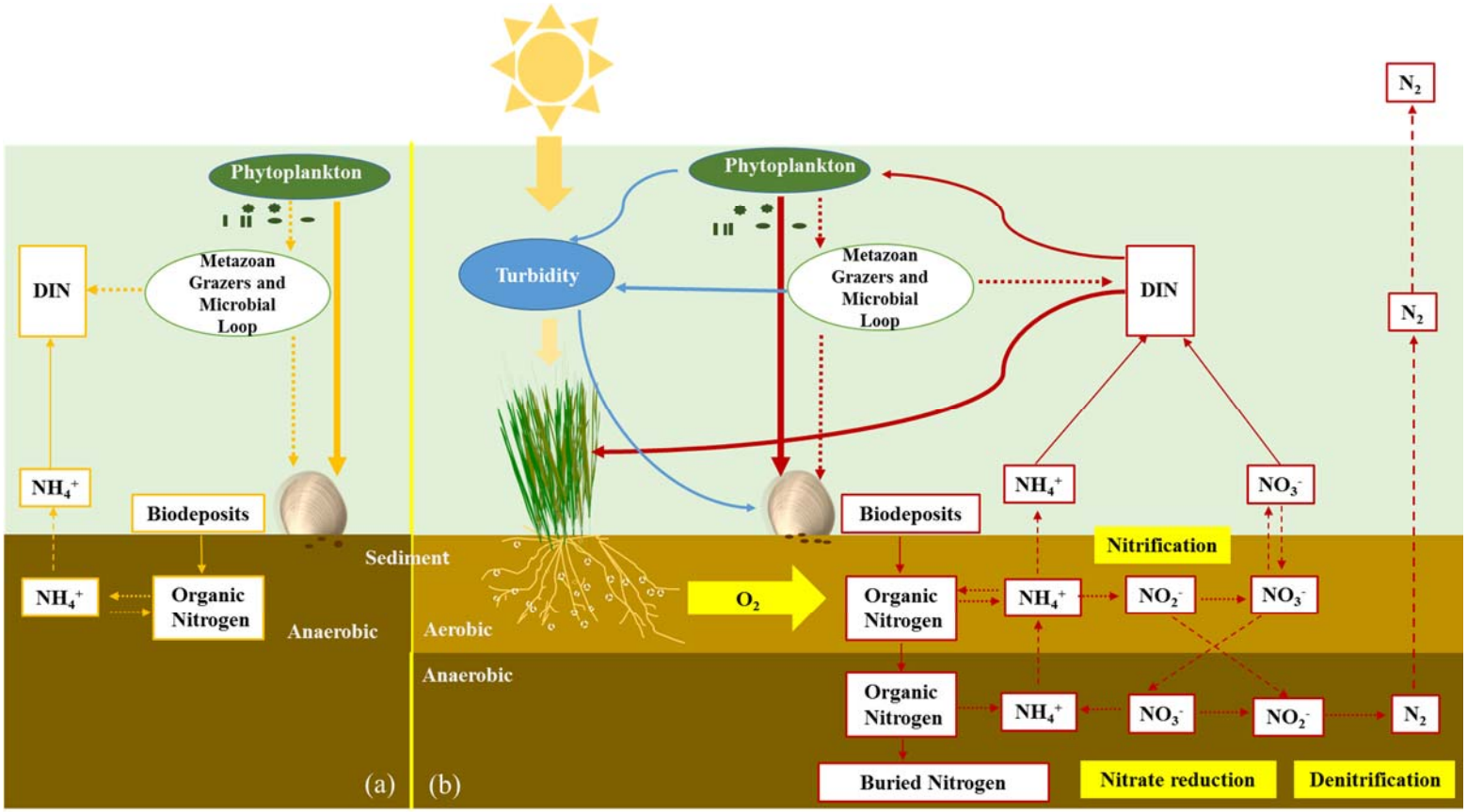

Fig. 7-11. Conceptual diagram of how mussels serve to transfer undigested POM to the sediment surface and reduce turbidities in the water column with or without the presence of submerged macrophytes illustrated in (a) and (b) part, respectively. Within the aerobic layers, the microbially mediated process of nitrification occurs, which, when linked to denitrification within the underlying anaerobic sediment, leads to the release of N_2 gas. The presence of submerged macrophytes can stimulate the nitrification process in the aerobic layers. Solid lines indicate movement of materials; dashed lines indicate diffusion of materials; dotted lines indicate microbially mediated reactions. (Modified from **Newell et al., 2002**)

7.4.2-5 Submerged macrophytes and N microbiological process

As shown in **Fig. 7-6**, the NO_3^--N concentration declined sharply in the tank No.3 and this is consistent with the DO trend in this tank without plants shown in **Fig. 7-3**. The sharp decline of the NO_3^--N concentrate can be caused by the active denitrification process of microorganisms. Furthermore, In the absence of submerged macrophytes, the DO concentration in the tank No.3 decreased significantly, especially in the first six days, and thus the sharply decreased DO accelerated the denitrification process, and a large amount of NO_3^- was transformed by denitrifying bacteria into N_2 and diffused from the tank water into the atmosphere.

As illustrated in **Fig. 7-5** and **Fig. 7-6**, in the first three days in the tank No.2, compared with NH_4^+-N, NO_3^--N can be removed more significantly by the submerged macrophytes *V. asiatica.* Possibly can be explained by the obviously higher initial concentration of NO_3^--N than that of NH_4^+-N in the tank. The absorption efficiency of the submerged macrophytes *V. asiatica* on different forms of nitrogen can be effected by the corresponding concentrations of nitrogen.

Therefore, the effect of submerged macrophytes on the mussels can be summarized as shown in **Fig. 7-12**.

7.4.3 The Effect of Mussels on Submerged Macrophytes by Virtue of Turbidity

7.4.3-1 Sediments characteristics and resuspension

The comparison results indicate that the characteristics of sediments can affect the degree of sediment resuspention, and thus affect the turbidity changes in the buckets. For instance, in the bucket No.1, the sediment was from Ongakawa River, which was constituted mainly of gravels, whereas in the bucket No.3, the sediment was from Morota eutrophcation pond and contained large quantities of fine clay, which was easily disturbed by outside forces.

Therefore, for the restoration of submerged macrophytes in the littoral zone, the sediment characteristics, such as composition and the particle size, as vital factors need to be taken in to account.

7.4.3-2 Filtering behavior of mussels and turbidity

The different responses of turbidity observed in the buckets No.3 and No.5 until 7-day indicated that with the existence of mussels, the aquatic ecosystem behaved more resistant to the disturbance, furthermore, mussels of enough biomass can be in favor of

the stable state in the aquatic ecosystem.

The unobvious difference between the buckets No.3 and No.4 can be resulted from the insufficient biomass of mussels in relative to the 100 L suspended solution.

Mussels due to their strong filtration feeding behavior as examined in the foregoing chapters, can accelerate the bio sedimentation process, resulting from the bond between the suspended particles in the water body and the mucus secreted by mussels during the filtering process. Thus, turbidity caused by the sediment disturbance can be improved significantly with the existence of mussels in enough biomass, which can result in the obvious transparency improvement, which finally benefits the growth of submerged macrophytes and increase the possibility of successful restoration.

7.4.3-3 The growth of submerged macrophytes and turbidity

Depth, latitude, temperature, and water levels are the important factors, among which light is of paramount importance, because it exerts a major control on phytosynthesis and declines with water depth due to attenuation, scattering, and absorption (**Hudon et al., 2000**). It was also pointed out that light is a main limiting factor for submerged macrophyte growth in turbid lakes (**Scheffer, 2001**). Therefore, any increase in light penetration is likely to affect the plant community (**Lammens et al., 2004**).

Dreissenid mussels are commonly reported to increased water clarity in rivers and lakes (**Baldwin et al., 2002**). The mussels increase water clarity by filtering particles from the water and consuming them or binding them in pseudofaeces. Greater water clarity leads to deeper light penetration and thus would be expected to influence the distribution and community composition of submerged macrophytes (**Wetzel, 1983**). It was demonstrated that the increase of water clarity in Oneida Lake from 1972 to 2002 was primarily associated with zebra mussels, following which are greater maximum depth of colonization and more suitable growing area for submerged macrophytes (**Zhu et al., 2006**). Thereby, submerged macrophytes increased in diversity and frequency of occurrence and shifted composition from shade-tolerant species to species that can live at a wider variety of light levels.

In addition to increasing light penetration, there are numerous ways that dreissenid mussels may affect submerged macrophytes (**Fig. 7-12**) The growth of submerged macrophytes depends mostly on nutrients such as P and N in the sediments (**Carignan and Kalff, 1990**). The mussels can relocate P and N in the particles from the water column to the sediments through the production of pseudofaeces and faeces (**Hecky et al., 1993**). Thus, a potentially important net effect of dreissenid mussels activity is the conversion of

particulate forms of nutrients to dissolved chemical elemental forms in sediments, and this process may benefit submerged macrophytes (**Reusch et al., 1994**)

In summary, the presence of mussels in the submerged macrophytes restoration sites can stimulate the growth of submerged macrophytes, due to light penetration increase and the nutrients conversion for dissolved chemical elements forms in sediment.

In this experiment, the turbidity of the bucket No.2 cultured with only eutrophic blooming water without any disturbance was far lower than that of the bucket No.3, which indicates that the sediment disturbance played more vital role on the turbidity value than the Chlorophyll-a concentration.

On the final day, it was observed that in the bucket No.1, there were no obvious differences in the appearance of *V. asiatica* from them on the initial day; nevertheless, there was so serious decay for the ones growing in the buckets No.3 and No.4, that no macrophytes samples can be collected there; in addition, there were still a small amount of them in the bucket No.5. The results further indicated that the turbidity condition can affect the growth of submerged macrophytes greatly and the existence of mussels with enough biomass can stimulate the growth of submerged macrophytes in some degree through turbidity improvement.

7.5 CONCLUSIONS

In this chapter, the experiments with the mussel *A. woodiana* combined with the submerged macrophyte *V. asiatica* were performed to figure out the interactions between them. The results indicated that:

(1) Mussels stocking density should be in a range named "ecological carrying capacity", concerning possible problems induced by mussels-DO deficiency, NH_4^+ excretion and the pesudofaeces algae blooming.

(2) As mentioned in the discussion part, the beneficial effect associated with the mussel culture on the aquatic ecosystem depends on having aerobic sediments, thus the degree of sediment oxygenation around the culture site must be regularly monitored. In some cases, remedial actions should be taken to maintain the aerobic conditions, which are needed to sustain both nitrifying bacteria and the benthic bioturbator community.

(3) The presence of submerged macrophytes can supply the required aerobic condition for mussels to play the effective role of biomanipulation tool in the eutrophic freshwaters. Submerged macrophytes, through their photosynthesis, can moderate the decline of DO as a result of mussels respiration and remove the extra NH_4^+ through direct absorption or the metabolization activities of the attached microcosms around the root zone; through competition with the algae included in pseudofaeces, they can also inhibit the regrowth of the algae excreted by mussels as pseudofaeces. Furthermore, the oxygen diffused into the sediment can assist the activities of nitrifying bacteria, thus help the nitrogen-denitrification process, and can finally help the removal of about 20% of the nitrogen in filtered particles by mussels through formation of N_2 from the aquatic ecosystem diffused into the air.

(4) For the restoration of submerged macrophytes in the littoral zone, the sediment characteristic, such as composition and the particle size, as vital factors need to be taken in to account.

(5) Turbidity caused by the wind-induced sediment disturbance can be improved significantly with the existence of mussels of sufficient biomass, which can assist in the obvious improvement of water clarity, thus the growth of submerged macrophytes can be improved and the possibility of successful restoration can be increased finally.

Overall, the interactions between mussels and submerged macrophytes examined in

this chapter can be described in **Fig. 7-12.**

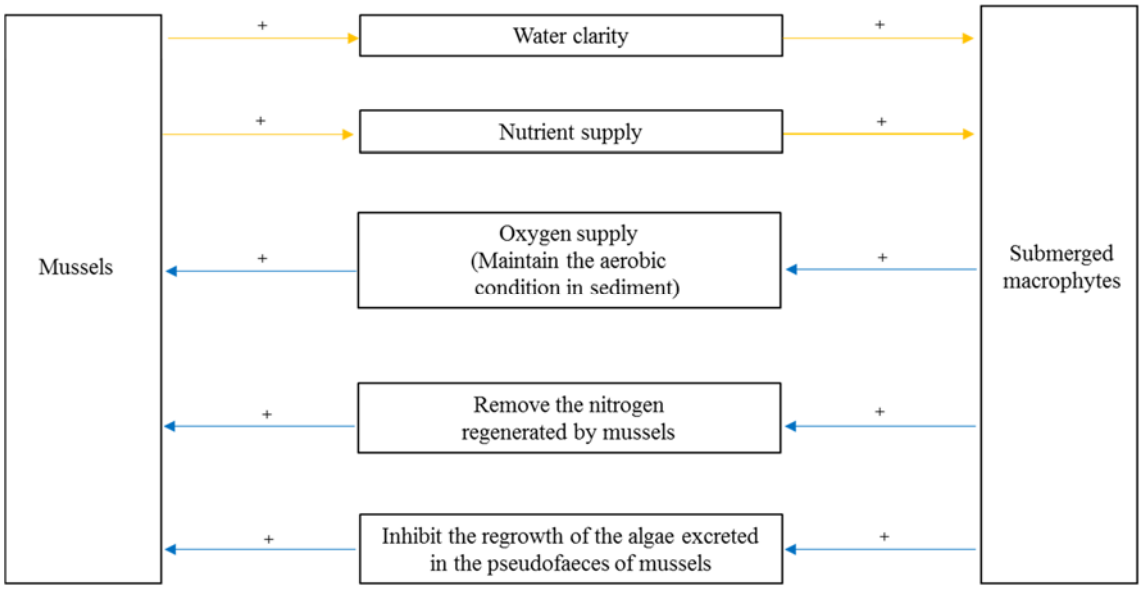

Fig. 7-12. Possible interactions between the mussel *Anodonta woodiana* and the submerged macrophytes *Vallisneria asiatica* in eutrophic freshwater ecosystems based the experiments conducted in this study. Beneficial effects are indicated with a + symbol.

Consequently, it is promoted that in Lake Taihu, especially in the littoral areas with wind-induced disturbance, the biomass increase of *A. woodiana* can be encouraged to be combined with the restoration of submerged macrophytes in practice. This combination method could assist in the formation and maintenance of submerged macrophytes-dominated stable state in Lake Taihu.

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CHAPTER 8
Conclusions

CHAPTER 8

Conclusions

8.1 SUMMARY OF DISSERTATION

The research goals of the dissertation are directed toward the applicability evaluation in the physiological energetics method of the mussel *Anodonta woodiana* as a biomanipulation tool in Chinese Lake Taihu.

Firstly, in **Chapter 1**, through literature review and the field investigation, the information of the eutrophication situation in Lake Taihu was obtained. Then the alleviating eutrophication methods applied in practice and deficiency were summarized, furthermore, the research of biomanipulation methods and the application prospect of freshwater mussels were mentioned. Accordingly, the research objectives in this dissertation were proposed as: to clarify the feeding behavior of the mussel *A. woodiana* in freshwater ecosystems, especially on toxic *Microcystis* spp., which are the dominant algal species in Lake Taihu when algae blooming occurs; meanwhile, to evaluate the effect of toxic *Microcystis* spp. on *A. woodiana* with the physiological energetics method-scope for growth (SFG); furthermore, to clarify the interactions of *A. woodiana* and submerged macrophytes, and all these results can contribute to our understanding of the integrated ecological role of the mussel *A. woodiana* in the eutrophic lake, in addition the applicability of the mussel *A. woodiana* as a biomanipulation tool in Lake Taihu restoration, pushing the lake from algae-dominated turbid state to macrophytes-dominated clear state.

Thereafter, in **Chapter 2**, the physiological evaluation method used in this research, SFG was explained, in which the significance, calculation methods, affecting factors were summarized.

As an important part of SFG, feeding behavior and the affecting factors of mussels were examined and further discussed in **Chapter 3**. The results in this chapter indicated that the filtration rates increased as the increase of algae concentrations till the critical cell density was reached, at which the pseudofaeces were expelled; above the critical cell density, the filtration rates decreased sharply as the algae concentration increased. The mussel *A. woodiana* reached the critical cell density at 4.3×10^7 cells mL^{-1} and 3.4×10^6 cells mL^{-1}, when the temperature was 20℃ and 25℃, respectively in this experiment.

Based on the first three chapters, the application feasibility of the mussel *A. woodiana* was clarified through the following experiments described from **Chapter 4** to **7**, and the relationship between these experiments is illustrated in **Fig. 8-1**.

In **Chapter 4**, in order to examine whether *A. woodiana* can exert grazing pressure

on *Microcystis* spp. and to evaluate the different effects of each algae diet on mussels' potential growth, a comparative study was carried out on the acute physiological responses to variable microalgae diets including toxic microcystin-producing cyanobacteria *M. aeruginosa* and non-toxic green algae *Scenedesmus obliquus*. The results showed that the mussel *A. woodiana* has a higher grazing ability on the toxic *M. aeruginosa* compared with the green algae *S. obliquus*; furthermore, the effects of different algae diets on SFG of *A. woodiana* demonstrated that the toxic *M. aeruginosa* may supply 4.5 times more energy for *A. woodiana*'s potential growth than that *S. obliquus* could do.

Thereafter, in **Chapter 5**, the interactions between *A. woodiana* and algae blooming water were examined, in which the grazing of mussels on phytoplankton in naturally blooming pond water dominated by toxic colonial *Microcystis* spp. was studied. The 6-day feeding responses experiment was carried out with naturally blooming pond water and the mussels in the laboratory. These results indicated that toxic *Microcystis* spp. of colony and unicell in natural eutrophic water can be removed greatly by *A. woodiana*; moreover, the toxic *Microcystis* spp. were found to supply about 1.5 times more energy for *A. woodiana*'s potential growth after 6-day exposure, thus the mussels have the strong adaptation ability when they were exposed to toxic natural eutrophic water. If toxic cyanobacteria are ingested by *A. woodiana*, it is vital to know whether the cyanobacterial toxins will induce a threat to their survival, or whether there are some possible survival mechanisms of *A. woodiana* in exposure to microcystins.

Subsequently, in the long-term grazing experiment in **Chapter 6**, the mussels demonstrated a strong survival ability during exposure to natural eutrophic water containing high concentrations of MCs for 12 days. In order to clarify the survival mechanisms (feeding selective and detoxification mechanisms), all the conducted experiments from **Chapter 4** to **6** were summarized and an correlation analysis between the diet characters and mussels' physiological rates was performed and the results suggested that (1) filtration selectivity factors - MCs did not restrain the feeding behavior of *A. woodiana*; instead the exorbitant initial diet concentration could inhibit the filtration rates; (2) absorption selectivity factors - thick cell walls of Chlorophyta cells made them indigestible in the digested tracks, probably it is the different digestive enzyme activity in *A. woodiana*'s digestive tract that induced the prefer ingestion of *M. aeruginosa* to the green algae; (3) the possible detoxification process - in the liver of mussels, through the MC-Cys (cysteine conjugates of MCs) formation, MCs could be detoxified and then they were transferred to kidney and were excreted in the form of ammonium. In addition, more MCs were metabolized efficiently rather than accumulated in the liver, thus the MCs

toxicity effect in *A. woodiana* did not reveal. Therefore, due to all these physiological adaptation mechanisms, *A. woodiana* can survive in *Microcystis*-blooming water bodies up to specific densities, including resistance to toxic chemicals. From these laboratory experiments, all the results indicate that the mussel *A. woodiana* can graze the toxic *Microcystis* spp. both of unicell and colony in blooming water, thereafter can still obtain the energy for its potential growth.

Accordingly, in **Chapter 7**, the interactions between *A. woodiana* and submerged macrophytes were clarified. In this chapter, the experiments were performed with the mussel *A. woodiana* combined with the submerged macrophyte *Vallisneria asiatica* to figure out the interactions between them. The results indicated that (1) the beneficial effect associated with mussels culture on the aquatic ecosystem depends on having aerobic sediments and thus mussels stocking density should be in a range named "ecological carrying capacity"; (2) the presence of submerged macrophytes can supply the required aerobic condition for mussels to play the effective role as a biomanipulation tool in the eutrophic freshwaters. Through their photosynthesis, they can moderate the decline of dissolved oxygen as a result of mussels respiration, remove the extra ammonium and also inhibit the regrowth of the algae excreted by mussels as pseudofaeces; furthermore, the oxygen diffused into the sediment can assist the activities of nitrifying bacteria in the nitrogen-denitrification process, and finally can help about 20% of the nitrogen in filtered particles by mussels through the formation of N_2 from the aquatic ecosystem diffused into the air; (3) for the restoration of submerged macrophytes in the littoral zone, the sediment characteristics, such as composition and grain sizes, as vital factors need to be taken in to account; (4) turbidity caused by the wind-induced sediment disturbance can be improved significantly with the existence of mussels in sufficient biomass, which can assist in the obvious improvement of water clarity, benefit the growth of submerged macrophytes and finally increase the possibility of successful restoration. Consequently, there is high feasibility of the combined application of *A. woodiana* and the submerged macrophyte *V. asiatica* in the littoral zone in practice as an optimal ecological method for the formation of submerged macrophytes-dominated clear state in Lake Taihu.

Consequently, in **Chapter 8**, based on the integrated ecological effects of *A. woodiana* indicated in the forgoing chapters, the integrated ecological restoration method is proposed to be applied in Lake Taihu, especially in the littoral areas. This integrated method includes the Ecological Dam (ED) and the Adjustable-Submerging Bed (ASB), through which the algae-dominated turbid state can be pushed into submerged macrophytes-dominated clear state in Lake Taihu.

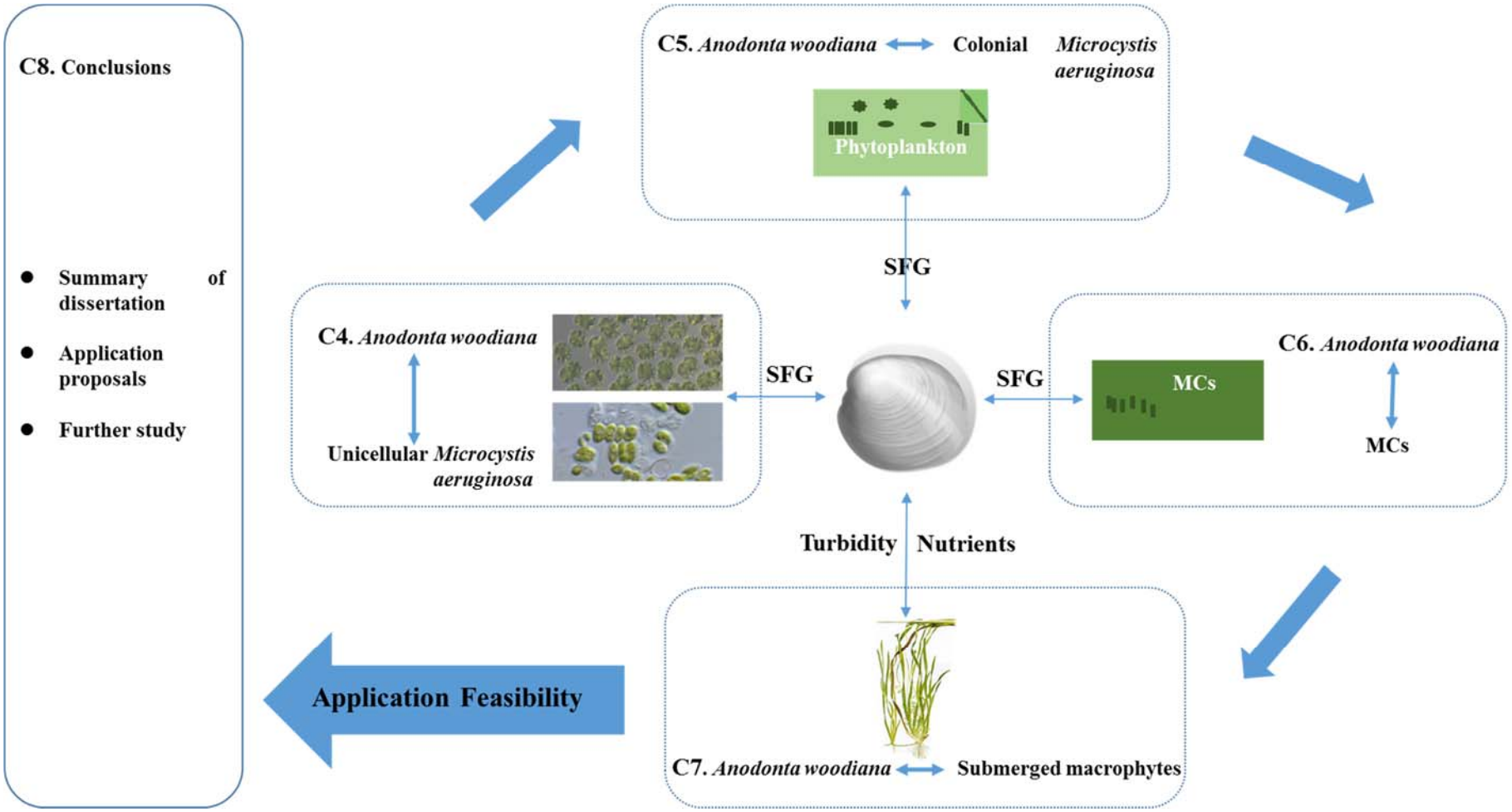

Fig. 8-1. The relationship between experiments mentioned in **Chapter 4-7** and conclusions.

8.2 APPLICATION PROPOSALS

It was reported that when the ratio of water depth to Secchi depth (SD) is larger than 5.26, most of aquatic macrophytes cannot grow (**Chen et al., 2000**), and transparency at this time becomes the crucial factor to decide whether the aquatic macrophytes can grow or not. In order to choose the targeted cites in Lake Taihu for the application of the combined ecological method proposed in this thesis, Lake Taihu was divided into nine zones. In addition, the water depth, SD and the ratio of water depth to SD was calculated based on data from the references as shown in **Table 8-1**. Furthermore, the nine different zones of Lake Taihu were marked with three different colors, based on the ratio shown in **Table 8-1**. Thus, zones with the ratio that is less than 5.26 were colored with light blue, such as Gonghu Bay, and East Taihu; zones with the ratio that is in the range of 5.26-11 were marked with blue, such as Meiliang Bay, Wuli Bay, Eastern littoral zone and other zone; zones with the ratio that is in the range of 11-15 were marked with dark blue, such as the southern parts of Central zone and Southern littoral zone as shown in **Fig. 8-2**.

Therefore, in order to reach the macrophytes-dominated clear state, transparency should be improved urgently in Lake Taihu, apart from the zones East Taihu Lake and Gonghu Bay.

Consequently, Wuli Bay, a shallow hypereutrophic bay of Lake Taihu is chosen as one of our targeted area for submerged macrophytes restoration. It is located in Wuxi City in the Jiangsu Province in China. The mean depth is 2.1 m, and after sediment dredging for the whole lake performed in 2003, the maximum depth is 3.4 m and a surface area is 5.15 km^2. (**Chen et al., 2009**) In the 1950s, the coverage of submerged macrophytes was up to 98% in this lake, and the water was clear (SD is approximately 2 m) (**Wu, 1962**). In the 1960s, the area decreased by reclamation for fish ponds, and submerged macrophytes disappeared with the enhanced discharge of sewage into this lake, mainly from fish ponds around the lake and Wuxi City. The algae biomass was high, a mean value of 81.0 μg L^{-1} Chlorophyll a in 2001, and SD was only 0.25-0.48 m (**Chen et al., 2009)**, which inhibits the growth of submerged macrophytes.

Based on the results obtained in my PhD study, the integrated ecological restoration method is proposed to be applied in Lake Wuli firstly, especially in the littoral areas. This integrated method includes the Ecological Dam (ED) and the Adjustable-Submerging Bed (ASB) (**Fig. 8-3**). ED is constituted of *A. woodiana* and floating plants (*Ipomoea aquatica*) as the filtering zone, through which the blooming water inflow can be filtered

Table 8-1. The water depth, SD and ratio (Min. and Max.) for the different zones of Lake Taihu.

Lake zone	Zhushan Bay	Meiliang Bay	Gonghu Bay	Central zone	Eastern Littoral zone	East Taihu Lake	Southern littorarl zone	Western littoral zone	Wuli Bay
Water depth (m)	1.86-2.1	1.65-2.90	1.42-2.34	2.35-3.02	1.64-2.1	1.42-1.64	1.86-2.1	2.1-2.52	2.40-3.10
SD (m)	0.25-0.40	0.30-0.55	0.52-0.66	0.20-2.50	0.2-0.4	1.07-1.18	0.17-0.25	0.2-0.4	0.25-0.48
Ratio (Min.)	4.65	3.00	2.15	0.93	4.10	1.20	7.44	5.25	5.00
Ratio (Max)	8.40	9.70	4.50	15.10	10.50	1.53	12.35	12.60	12.40

SD: Secchi depth; Ratio: the ratio of water depth to SD. (Modified from **Qin et al., 2014**)

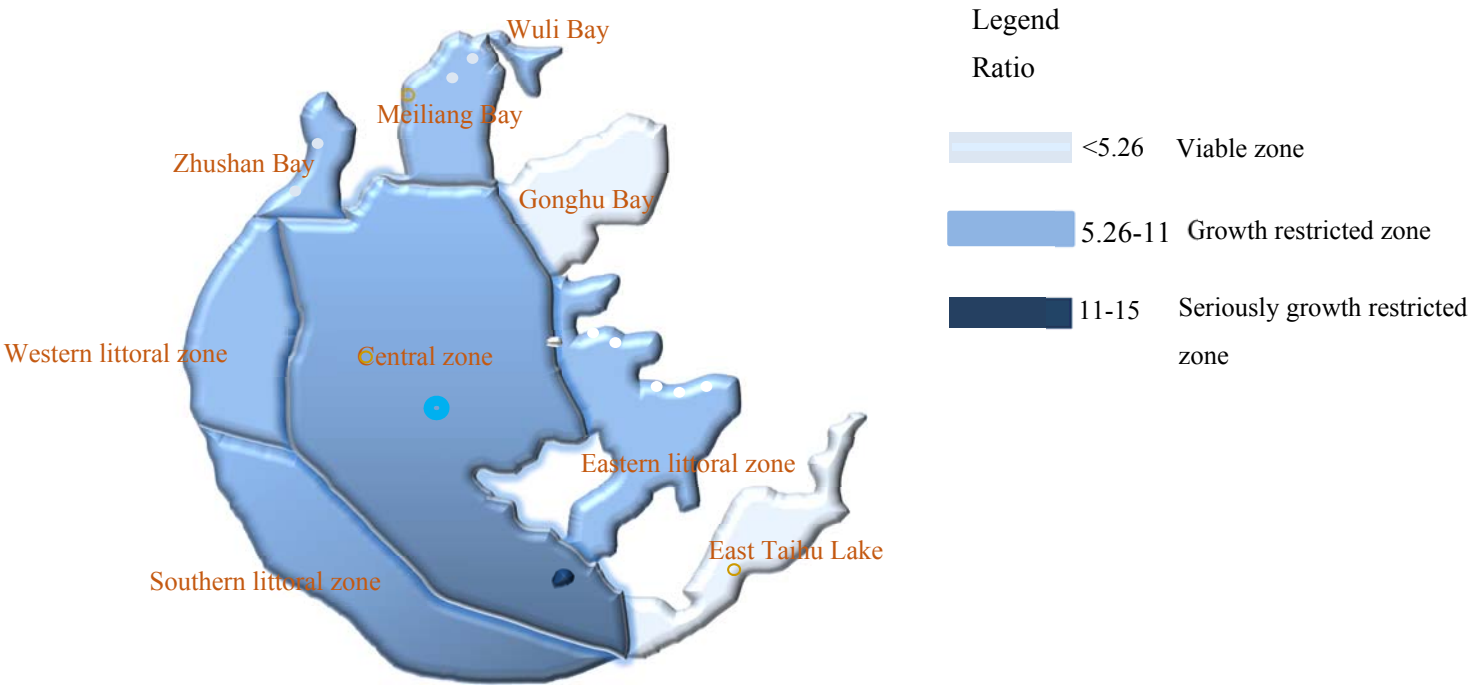

Fig. 8-2. The ratio of water depth to Secchi depth in the different zones of Lake Taihu.

effectively; thereafter, the filtered blooming water is flowing into the ASB, where the submerged macrophytes *Vallisneria asiatica* are planted. The submerging depth can be adjusted according to the transparency changes, through which the submerged macrophytes *V. asiatica* can be maintained under light depth of compensation.

Therefore, the restoration method can be carried out following these steps:

(1) Substrate of the sediment will be covered with gravel and sand with mud, and gentle slope will be constructed in shore area in March before the appearance of *Microcystis* spp., thus the sediment condition can be improved for the growth of *V. asiatica*.

(2) Stocking of ED as the arc-shaped filtering curtains separating the restoration enclosure from open area of the Lake Wuli water in March, before the occurrence of *Microcystis* spp. blooms; in addition, the DO condition of the sediment in the cultured site should be monitored, and in some cases, remedial actions should be taken to maintain the aerobic conditions, which are needed to sustain both nitrifying bacteria and the benthic bioturbator community.

(3) Cultivation of *V. asiatica* in ASB inside the whole enclosure near the shore line in April when the temperature is over 10℃ and suitable for the growth of *V. asiatica*; as the transparency improved by ED, the submerging depth of ASB can be gradually adjusted to the sediment. In addition, the cultivation area of *V. asiatica* can be extended close to ED zone gradually, and this is one of the important features of the integrated method proposed in this study, which allows the culture site of *V. asiatica* movable.

(4) *I. aquatica* can be harvested in the later growth season for vegetable, and mussels can be harvested as food for crab farms.

This integrated ecological restoration method can result not only in the improvement of water quality and the restoration of submerged macrophytes, but also in ecological benefits. Higher grazing pressure of mussels on phytoplankton may improve the water transparency that facilitate the growth of submerged macrophytes in the littoral area in Lake Wuli, and in return water quality can be improved after the reestablishment of aquatic macrophytes and finally the algae-dominated turbid state can be pushed into submerged macrophytes-dominated clear state in Lake Wuli. Thereafter, this method can be applied in other zones of Lake Taihu, such as Meiliang Bay and Eastern littoral zone.

For other western zones of Lake Taihu, such as Zhushan Bay, Western littoral zone and Southern littoral zone, inflowing rivers to Lake Taihu are mainly located in these zones, which contains a large amount of nutrients, thus more attention should be paid to the nutrients control in inflowing rivers in these zone. The details for the proposals for

these zones are listed in **Table 8-2**.

Table 8-2. The proposals and details for the main lake zones in Lake Taihu.

Lake zone	Characteristics	Proposals	Details
Zhushan Bay (Pollution control region)	1. This zone receives a large pollutant discharge; 2. Non-point pollutant sources have caused serious pollution of the aera; 3. Inflowing river brings pollutants into the lake	1. Industrial wastewater treatment; 2. Enginering for domestic wastewater treatment; 3. Treatment of agricultural pollution source; 4. Engineering for less-polluted farmland its management technology	1. Industrial wastewater discharge is only permitted after meeting discharge standards; 2. To decrease pollution from farmland by developing farming style, fertilization and irrigation techniques
Meiliang-Wuli Bay (Heavily polluted control region)	1. Most seriously polluted waters; 2. Heavily pollutant loads and intensified human activities; 3. Aquatic macrophytes have been destroyed in recent decades; 4. Pen-fish started in the 1980s, causing in-lake pollution; 5. It is the main drinking water source;	1. Pollution control; 2. Engineering for aquatic macrophytes restoration; 3. Forbid pen-fish; 4. Engineering for protection of drinking water	1. Domestic wastewater treatment industrial sewage discharge after meeting the discharge criteria; 2. To restore mainly submerged macrophytes with ED and ASB proposed in this study; 3. To reduce the in-lake pollutant load with ED and ASB; 4. To protect the drinking water sources with ED and ASB;
Gonghu Bay	1. It is the drinking water source; 2. Point and non-point source pollution;	1. Industrial wastewater treatment; 2. Enginering for domestic wastewater treatment; 3. Treatment of agricultural pollution source;	1. To treat 26 industrial sources to attain discharge standard; 2. To treat domestic waste water; 3. Developing eco-agriculture to control pollution from livestocks;
Eastern littoral zone	1. Comparatively good water quality 2. Low transparency for submerged-macrophytes	Engineering for aquatic macrophytes restoration;	1. To restore mainly submerged macrophytes with ED and ASB proposed in this study; 2. To reduce the in-lake pollutant load with ED and ASB;
East Taihu Lake	1. It is an important drinking water source, with comparatively good water quality; 2. There is increasingly more pen-culture for crabs and fish	1. Enginering for domestic wastewater treatment; 2. Control the aera of pen culture	1. To treat domestic waste water 2. To reduce inlake pollution
Western and Southern littoral zones (Pollution control regions)	1. This zone receives a large pollutant discharge; 2. Non-point pollutant sources have caused serious pollution of the aera; 3. Inflowing river brings pollutants into the lake	1. Industrial wastewater treatment; 2. Enginering for domestic wastewater treatment; 3. Treatment of agricultural pollution source; 4. Engineering for less-polluted farmland its management technology	1. Industrial wastewater discharge is only permitted after meeting discharge standards; 2. To decrease pollution from farmland by developing farming style, fertilization and irrigation techniques

(Modified from **Jin et al., 2003**)

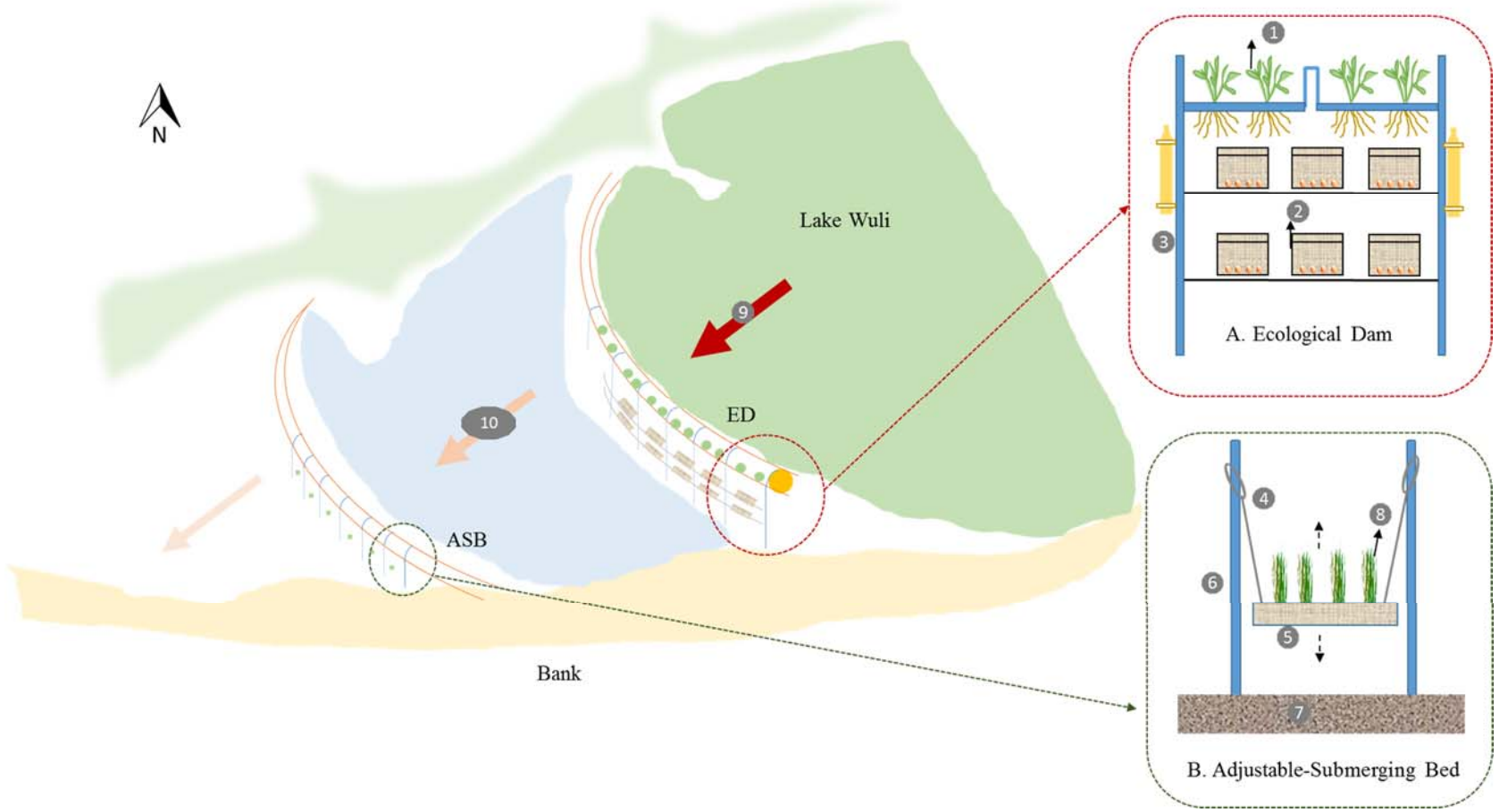

Fig. 8-3. Schematic diagram of Ecological Dam (ED) and Adjustable-Submerged Bed (ASB) applied in Lake Wuli. (1-*Ipomoea aquatic*; 2-*Anodonta woodiana*; 3-Buoyant bottles; 4-Depth adjusting ring; 5-Submerging bed; 6-Spud pile; 7-Substrate consisted of coarse gravel, sand and mud; 8-*Vallisneria asiatica*; 9-Blooming water inflow; 10-Blooming water filtered by Ecological Dam.

8.3 FURTHER STUDY

In **Chapter 6**, the possible detoxification process was inferred - in the mussel liver, through the MC-Cys formation, MCs could be detoxified and then they were transferred to kidney and were excreted in the form of ammonium. In addition, more MCs were metabolized efficiently rather than accumulated in the liver, thus the MCs toxicity effect in *A. woodiana* did not reveal.

However, this inference was based on the obvious positive correlation between ammonia excretion rates and microcystin concentrations and the survival phenomenon in that long-term feeding experiment on toxic *Microcystis* spp. in **Chapter 6**, in order to verify the detoxification process in the mussel *A. woodiana* inferred in this study, further research deserves to be performed in future.

In addition, in **Chapter 7**, the "ecological carrying capacity" for mussel aquaculture was mentioned. In future study, a well-parameterized model should be developed, which will allow a comprehensive assessment of the major interactions between mussels and the ecosystem. In this model, the main five ecosystem processes need to be included: (1) Top-Down control on phytoplankton and microzooplankton; (2) sediment hypoxia; (3) inorganic nutrient cycling; (4) reduction in turbidity; (5) provision of food resources and habitat for other organisms. A well-developed model can help us to predict the moderate the stocking density of mussels, at which the positive ecological services supplied by mussels can be maximized with the minimized adverse effect on the ambient ecosystem.

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