J Appl Phycol (2009) 21:649–656
DOI 10.1007/s10811-008-9398-1

Potential of the seaweed *Gracilaria lemaneiformis* for integrated multi-trophic aquaculture with scallop *Chlamys farreri* in North China

Yuze Mao · Hongsheng Yang · Yi Zhou · Naihao Ye · Jianguang Fang

Received: 17 August 2008 / Revised and accepted: 16 December 2008 / Published online: 28 January 2009

Abstract In this study the red alga, *Gracilaria lemaneiformis*, was cultivated with the scallop *Chlamys farreri* in an integrated multi-trophic aquaculture (IMTA) system for 3 weeks at the Marine Aquaculture Laboratory of the Institute of Oceanology, Chinese Academy of Sciences (IOCAS) in Qingdao, Shandong Province, North China. The nutrient uptake rate and nutrient reduction efficiency of ammonium and phosphorus from scallop excretion were determined. The experiment included four treatments each with three replicates, and three scallop monoculture systems served as the control. Scallop density (407.9±2.84 g m^{-3}) remained the same in all treatments while seaweed density differed. The seaweed density was set at four levels (treatments 1, 2, 3, 4) with thallus wet weight of 69.3±3.21, 139.1±3.80, 263.5±6.83, and 347.6±6.30 g m^{-3}, respectively. There were no significant differences in the initial nitrogen and phosphorus concentration between each treatment and the control group (ANOVA, $p>0.05$). The results showed that at the end of the experiment, the nitrogen concentration in the control group and treatment 1 was significantly higher than in the other treatments. There was also a significant difference in phosphorus concentration between the control group and the IMTA treatments (ANOVA, $p<0.05$). Growth rate, C and N content of the thallus, and mortality of scallop was different between the IMTA treatments. The nutrient uptake rate and nutrient reduction efficiency of ammonium and phosphorus changed with different cultivation density and time. The maximum reduction efficiency of ammonium and phosphorus was 83.7% and 70.4%, respectively. The maximum uptake rate of ammonium and phosphorus was 6.3 and 3.3 µmol g^{-1} DW h^{-1}. A bivalve/seaweed biomass ratio from 1:0.33 to 1:0.80 (treatments 2, 3, and 4) was preferable for efficient nutrient uptake and for maintaining lower nutrient levels. Results indicate that *G. lemaneiformis* can efficiently absorb the ammonium and phosphorus from scallop excretion and is a suitable candidate for IMTA.

Keywords *Gracilaria lemaneiformis* · Integrated Multi-trophic Aquaculture (IMTA) · Nutrient uptake rate · Nutrient reduction efficiency · Scallop

Y. Mao · H. Yang (✉) · Y. Zhou
Institute of Oceanology, Chinese Academy of Sciences,
Qingdao 266071, People's Republic of China
e-mail: hshyang@ms.qdio.ac.cn

Y. Mao
e-mail: maoyz@ysfri.ac.cn

Y. Mao · N. Ye · J. Fang (✉)
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Science,
Qingdao 266071, People's Republic of China
e-mail: fangjg@ysfri.ac.cn

Introduction

The cultivation of filter-feeding bivalves in China has developed quickly since the 1980s. In 2006, the production was close to 11.13×10^6 t, which is 77.03% of total mariculture production in China (China Fishery Statistical Yearbook, 2006). However, the rapid industrial growth of aquaculture has raised concerns about environmental problems and sustainable development regarding filter-feeding, digestion and absorption, respiration, excretion, and biodeposition (Naylor et al. 2000; Zhang 2003; Zhou et al. 2003; Mao et al. 2006a). Thus, ecological engineering in aquaculture is sought to satisfy a complex of environmental and social aims rather than only being concerned with maximizing short-term profitability (Ruddle and Zong 1988; Primavera 1991; Hishamunda and Ridler 2004).

Increased environmental concern about the rapid expansion of intensive mariculture systems has increased research into seaweed-based integrated techniques. Seaweeds can significantly absorb waste nutrients, control eutrophication and, consequently, improve the health and stability of marine ecosystems, and promote a sustainable development of aquaculture (Buschmann et al. 2001; Chopin et al. 2001; Troell et al. 2003; Fei 2004; Neori et al. 2004; Yang et al. 2005; Mao et al. 2005). The physiological responses and the biofiltering ecological functions of seaweeds has been studied in different culture systems, e.g., in filter-feeding bivalve culture systems (Fang et al. 1996a), in fish cage farms (Troell et al. 1997; Zhou et al. 2006; Hayashi et al. 2008), in shrimp culture ponds (Jones et al. 2001; Nelson et al. 2001; Xu et al. 2007), and in IMTA systems containing finfish, shellfish, and seaweed (Shpigel and Neori 1996; Neori et al. 2000; Chow et al. 2001; Shen et al. 2007).

IMTA has been proposed as a means of developing environmentally sound aquaculture practices and resource management through a balanced coastal ecosystem approach (Chopin et al. 2001; Troell et al. 2003; Neori et al. 2004). IMTA is potentially more sustainable than monoculture, because of the reutilization of waste products from one species by another (Petrell et al. 1993; Neori et al. 2007). Most studies have focused on seaweeds integrated with fish cultures (Petrell et al. 1993; Hirata and Kohirata 1993; Buschmann et al. 1994; Troell et al. 1997, 2003; Chopin et al. 2001; Neori et al. 2004; Zhou et al. 2006). Only few studies have investigated the possibilities of IMTA systems of bivalves and seaweeds (Qian et al. 1996; Evans and Langdon 2001; Langdon et al. 2004).

China has a long history of IMTA of filter-feeding bivalves and seaweeds (Zhang and Wang 1985; Wang et al. 1993; Fang et al. 1996b; Hawkins et al. 2002; Yang et al. 2000, 2005; Nunes et al. 2003; Mao et al. 2006b; Zhou et al. 2006). The temperate kelp species, *Laminaria japonica*, has been widely used for IMTA with scallops and oysters to mitigate the coastal eutrophication in Northern China (Fei 2004). But during summer and early autumn, the kelp is harvested, and no high-temperature-adapted seaweed has been cultured to replace the *L. japonica* during the warm seasons.

The high-temperature-adapted red alga *Gracilaria lemaneiformis* grows naturally on the coast of Shandong Peninsula, Northern China. After selective breeding at the IOCAS (Fei et al. 1998), this species has been successfully introduced in Fujian coast in the South China Sea (Tang et al. 2005), and cultivated widely due to its high growth rate, good adaptability, and higher concentration of agar.

Previous experiments showed that the seaweed also grows well in Sanggou Bay, in Shandong Province, North China. After 116 days of cultivation, the average wet weight of the algae increased 89 times from 0.1 kg rope^{-1} to 8.9 kg rope^{-1}, with an average specific growth rate (based on wet weight) of 3.9% per day (Yang et al. 2005). We have also reported the bioremediation potential of that species integrated with fish aquaculture (Zhou et al. 2006).

In this study, the nutrient (ammonium and phosphorus) uptake rates and nutrient reduction efficiency of *G. lemaneiformis* was determined in an IMTA system with the scallop *Chlamys farreri*. The objective of this study was to (1) determine if *G. lemaneiformis* can efficiently take up the dissolved ammonium and phosphorus from *C. farreri* excretion; and (2) evaluate the optimum ratio of seaweed to scallop for IMTA systems.

Materials and methods

To determine whether the seaweed can effectively utilize dissolved nutrients from scallop culture, a laboratory experiment was conducted in static systems at the Marine Aquaculture Laboratory of Institute of Oceanology, Chinese Academy of Science, located in Qingdao, Shandong Province, North China. The experiment was performed for 3 weeks in October 2002.

Both the seaweed *G. lemaneiformis* and scallop *C. farreri* were collected form Sanggou Bay, Northern China. They were carried to the laboratory in temperature preservation cases with ice. After being disinfected and cleaned of any visible fouling organisms by washing with filtered seawater, they were acclimatized in separate tanks for one week. During acclimatization, seawater was changed once per day, and the scallops were fed with 5 g dry algal powder (*Spirulina* sp.) twice a day (at 9:00 and 16:00).

G. lemaneiformis and *C. farreri* were cultivated in 15 cylindrical Plexiglas tanks (3 m^3, 1.8 m diameter, 1.2 m height, 1.1 m water depth), as shown in Fig. 1. The scallops were cultured in a lantern polyethylene net cage, which was tied to a bamboo bar over the bank. The cage was separated

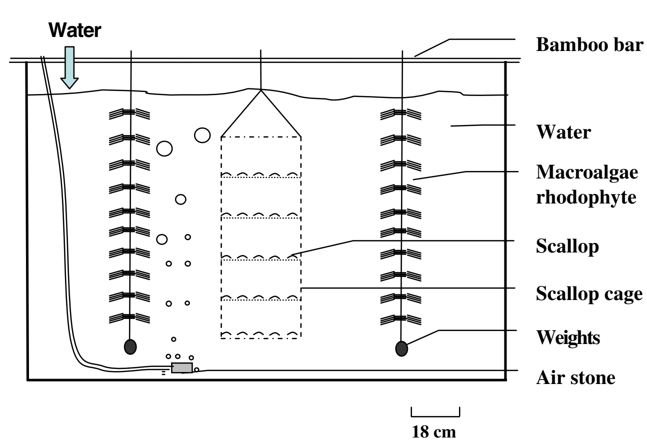

Fig. 1 IMTA system for seaweed and scallop

into five chambers by plastic discs 30 cm in diameter, 18 cm apart, with some round holes on them. Each chamber contained ten scallops. The seaweed thalli (15 cm length) were nipped at 8–10 cm intervals in 1.5-m long ropes, which were tied to the bamboo bar and positioned vertically around the scallop cage in the tanks. Two air stones were placed in the bottom of each tank. The experiment was conducted in outdoor conditions with plastic shade cloth (light transmittance 80%). All tanks were aerated, and no water was exchanged except for the supplement of evaporated water with fresh seawater.

The experiment included four treatments each with three replicates. Three scallop tanks served as control. Scallops (24.6 g wet weight, 57.0 cm shell height, 2 years of age) were of the same density in all treatments (ANOVA, $p<0.05$) while seaweed density differed: 2, 5, 8, and 10 ropes tank^{-1} or 69, 139, 264, and 348 g m^{-3}, respectively (Table 1).

Seawater used in this experiment was filtered seawater. Water temperature, salinity, irradiance, pH, and dissolved oxygen concentration (DO) were measured at 10:00 A.M. and 15:00 P.M. every day by surface thermometer, HD-3 model salinometer, Li-250 model illuminometer, 520 Aplus model pH meter, and the Winkler method, respectively. During the experiment, the temperature ranged from 20 to 13°C with an average value 16.5°C; pH ranged from pH 7.8 to 8.2; salinity ranged from 31.8 to 32.2 ppt; irradiance ranged from 70 to 201 μmol photons m^{-2} s^{-1}; and DO ranged from 9.2 to 10.2 mg L^{-1}. Scallop survival in every tank was carefully checked every day, dead individuals were removed and replaced by scallops of the same size, and mortality was calculated for each treatment.

Water samples were collected once every week for determination of NH_4-N and PO_4-P. NH_4-N was measured by indophenols blue method and PO_4-P by phosphorus–molybdenum blue method (Grasshoff et al. 1983). The nutrients were analyzed with a continuous flow analyzer (Skalar San Plus System; Netherlands). At the start and end of the experiment, the seaweed thalli were sampled for determination of carbon and nitrogen with a Perkin Elmer Model 240c CHN analyzer standardized with acetanilide. The nutrient uptake rate and nutrient reduction efficiency of ammonium and phosphorus were calculated as follows:

$$\mathrm{NUR} = (C_t - M_t)V/\mathrm{DW}/t$$

$$\mathrm{NRE} = (C_t - M_t)/C_t \times 100$$

where NUR (μ mol g^{-1}DW h^{-1}) is the nutrient (ammonium or phosphorus) uptake rate, NRE (%) is the nutrient (ammonium or phosphorus) reduction efficiency, C_t and M_t (μM) is the nutrient concentration of control (no seaweed) and treatment tanks at time t since the beginning, respectively; V (l) is the volume of the tanks; DW (g) is the dry weight of the seaweeds; and t (day) is the experimental time.

The growth rates were estimated as follows:

$$\mathrm{SGR} = 100(\ln W_t - \ln W_0)/t$$

where SGR is the specific growth rate (% day^{-1}); W_0 is the initial wet weight; and W_t is the wet weight at time t since start of experiment.

Differences between treatments were tested for significance using one-way analysis of variance (ANOVA), and then the data were sorted and compiled using Excel and analyzed using the SPSS version 11.5 statistical package.

Results

Variation of ammonium and phosphorus concentration in different IMTA treatments

There were no significant differences in ammonium and phosphorus concentrations between the control and any of the treatments at the beginning of the experiment (ANOVA, $p<0.05$). The ammonium concentration of the control group increased from 2.9 μM at the start of experiment to 12.1 μM at the end of the experiment (Fig. 2a). The ammonium concentration in treatment 1 increased from 2.7 to 10.1 μM. In the first week, the ammonium concentration increased slowly, whereas in the second week, it increased rapidly. The ammonium concentration of the other three treatments

Table 1 Density of *C. farreri* and *G. lemaneiformis* in the different treatments (wet weight, g m^{-3})

Group	Control		Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Scallop	Seaweed	Scallop	Seaweed	Scallop	Seaweed	Scallop	Seaweed	Scallop	Seaweed
A	406.5	0.0	428.6	67.3	441.6	144.4	429.2	272.1	443.6	352.4
B	411.9	0.0	444.5	66.9	417.5	137.4	432.9	255.4	437.3	338.7
C	405.4	0.0	453.4	73.9	417.7	135.6	423.1	262.9	413.8	351.7
Mean	407.9	0.0	442.2	69.3	425.6	139.1	428.4	263.5	431.6	347.6
Ratio			1 : 0.16		1 : 0.33		1 : 0.61		1 : 0.80	

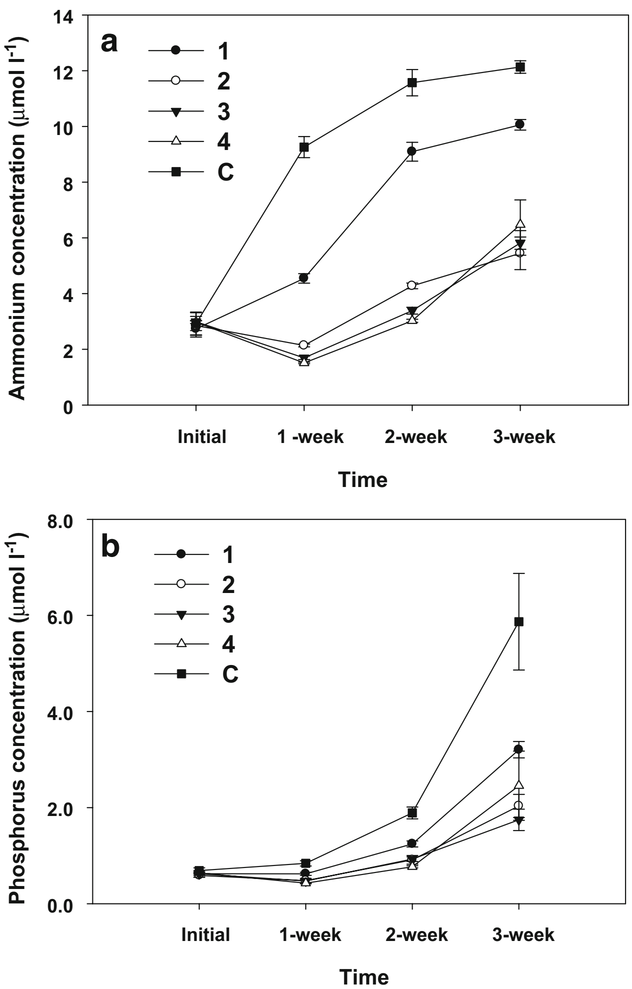

Fig. 2 Variation of ammonium (**a**) and phosphorus (**b**) in IMTA systems for the seaweed and scallop

showed a similar trend: decreasing in the first week, increasing slowly in the second week, and then increasing rapidly in the third week from 4.3 to 5.4 μM, 3.4 to 5.8 μM and 3.0 to 6.5 μM, for treatments 2, 3, and 4, respectively.

After a 1-week cultivation, the phosphorus concentration of the control group had increased slightly from 0.69 to 0.84 μM, while it decreased slightly in all treatments. In the second week, the phosphorus concentration of the control group increased more rapidly, from 0.84 to 1.89 μM, compared to the treatment groups which increased from 0.62 to 1.24 μM, 0.48 to 0.91 μM, 0.47 to 0.93 μM, and 0.43 to 0.47 μM in treatments 1, 2, 3, and 4, respectively. In the third week, the concentration in all groups increased, but the control group increased sharply from 1.89 to 5.87 μM.

Uptake rates of ammonium and phosphorus

In the first week, the ammonium uptake rates decreased with the increasing algal density (Fig. 3a). The uptake rates were 6.3, 3.8, 2.5, and 2.3 μmol g^{-1} dry weight h^{-1} for treatments 1, 2, 3, and 4, respectively. Analysis of variance (ANOVA) indicated that there were significant differences between the treatments, except between treatments 3 and 4. In the second and third week, the uptake rates increased with the increase of algae density, and reached a maximum in treatment 2, then decreased with the increasing of algae density. There were no significant differences among any treatments in the second week (ANOVA, $p>0.05$). In the third week, Student–Newman–Keuls (S-N-K) multiple comparison test indicated that there was a significant difference between treatment 1 and 4 ($p<0.05$).

The trend of the phosphorus uptake rate differed from that of the ammonium uptake rate (Fig. 3b). In the first and second week, the uptake rate decreased with the increasing algal density and reached the minimum in treatment 3, then increased again. S-N-K test indicated that there was a significant difference between treatment 1 and treatments 3 and 4 in the first week; there was a

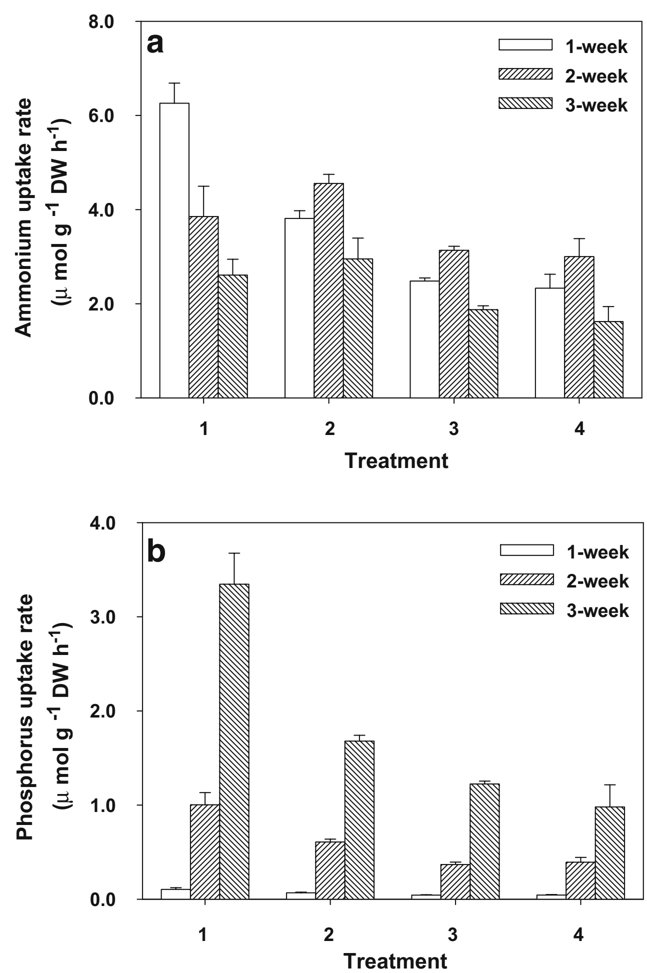

Fig. 3 Ammonium (**a**) and phosphorus (**b**) uptake rates of *G. lemaneiformis* in different IMTA treatments

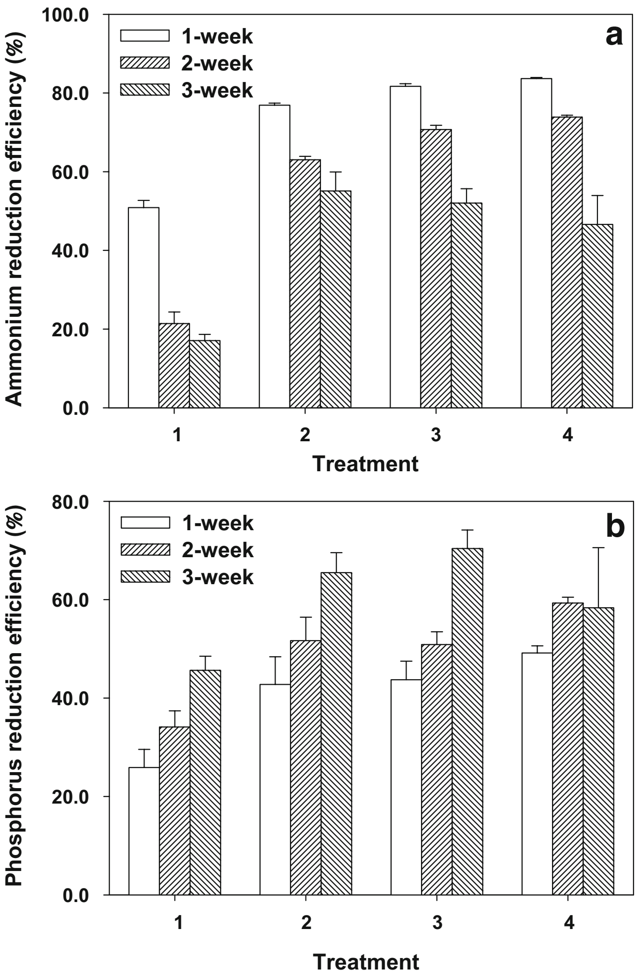

Fig. 4 Ammonium (**a**) and phosphorus (**b**) reduction efficiency of *G. lemaneiformis* in different IMTA treatments

significant difference between treatment 1, treatment 2, and treatments 3 and 4 in the second week ($p<0.05$). The uptake rate decreased with increasing algal density in the third week. Within each treatment, the uptake rate increased with the cultivation period, and reached a maximum of 3.3 μmol g^{-1} dry weight h^{-1} in the third week of treatment 1. There was a significant difference between treatment 1 and the other 3 treatments ($p<0.05$).

Reduction efficiency of ammonium and phosphorus

Nutrient reduction efficiencies by the thalli in IMTA treatments were calculated based on the difference in nutrient concentration between the control and IMTA treatments (Fig. 4). In the first week, ammonium reduction efficiency in treatment 1 was 50.8%; while in treatments 2, 3, and 4, the reduction efficiency was around 80%. In the second week, the reduction efficiencies in all treatments were less than in the first week with 21.4, 63.0, 70.7 and 73.9%, respectively, but the trend was the same as that of the first week. In the third week, the ammonium reduction efficiency increased with increased algal density and reached the maximum with 55.1% in treatment 2, then decreased with increased algal density. The phosphorus reduction efficiency increased generally with the cultivation density and period of cultivation except for treatment 4 (Fig. 4b). The phosphorus reduction efficiencies in treatments 1, 2, 3, and 4 were 40.0, 65.5, 70.4 and 58.4% after 3 weeks, respectively.

Growth and mortality

The mortality of scallop in the IMTA systems was lower than in monoculture (Table 2). There were significant differences between the control group and treatments 2, 3, and 4; however, there were no significant differences between the IMTA treatments (ANOVA, $p<0.05$).

The average thallus SGRs in treatments 1, 2, 3, and 4 were 0.73±0.13, 2.52±0.31, 1.25±0.09 and 0.55±0.22% day^{-1}, respectively. The N contents in dry thalli in the IMTA systems ranged from 3.61 to 4.07% (mean 3.87%), and were markedly higher than those at the beginning of the experiment (mean 2.35%; ANOVA, $p>0.05$). The C contents in dry thalli in the IMTA systems ranged from 29.6 to 32.1% (mean 30.9%), were higher than those at the beginning of the experiment (mean 28.1%), but there were

Table 2 Mortality rates of *C. farreri* and specific growth rate, carbon and nitrogen content of *G. lemaneiformis* (in dry matter) in the different IMTA treatments (n=3)

Treatment	Mortality (%)	SGR (% d^{-1})	C %	N %
Initial/control	12.67±2.49 ^{a}		28.1±1.54 ^{a}	2.35±0.11 ^{a}
Treatment 1	8.67±2.49 $^{a, b}$	0.73±0.13 ^{a}	32.1±1.68 ^{a}	4.07±0.32 ^{b}
Treatment 2	3.33±1.70 ^{b}	2.52±0.31 ^{b}	31.7±1.38 ^{a}	3.94±0.22 ^{b}
Treatment 3	2.67±1.70 ^{b}	1.25±0.09 ^{c}	30.2±1.26 ^{a}	3.84±0.24 ^{b}
Treatment 4	4.67±1.89 ^{b}	0.55±0.22 ^{a}	29.6±0.19 ^{a}	3.61±0.17 ^{b}

Same superscript letters in column indicate that there are no significantly differences between the treatments not significant difference among the treatments (ANOVA, $p>0.05$).

Discussion

Most previous studies on nutrient excretion by clams and mussels have selectively focused on ammonium and phosphorus, and the excretion of nitrate+nitrite has commonly been neglected or found to be not significant. So in this study, only the ammonium and phosphorus excretion by scallop in the system were determined, and the nutrient uptake rate and nutrient reduction efficiency of ammonium and phosphorus by the seaweed were calculated. Our experiments have shown that scallop aquaculture could greatly increase nutrient (ammonium and phosphorus) levels in the water column. In the scallop control tanks, the ammonium and phosphorus concentrations in water increased, and after 3 weeks, were about four and nine times higher than the initial concentrations, respectively.

The nutrient reduction efficiency is defined as the average reduction (%) in nutrient concentration in water. Nutrient uptake rate, on the other hand, is defined as the amount of nutrients removed per unit area of, e.g., seaweed per unit time. Both these concepts are important; they will vary depending on culture conditions such as depth, light, stocking density, and water turnover rates (Buschmann et al. 2001). Our experiment showed that both nutrient reduction efficiency and uptake rate varied with seaweed density and cultivation period. In the IMTA system, the seaweed *G. lemaneiformis* was an efficient nutrient scrubber capable of removing much of the nutrients from the system. Ammonium reduction efficiency increased with the algal density within the first 2 weeks of cultivation. In the third week, the ammonium reduction efficiency reached its maximum in treatment 2. Phosphorus reduction efficiency increased generally with the cultivation time and the algal density in all treatments.

Many studies have shown that seaweeds can assimilate ammonium generated by fish culture (Cohen and Neori 1991; Neori et al. 1996; Buschmann et al. 1996; Zhou et al. 2006). For instance, the reduction efficiency of *Ulva rigida* treated with waste water was 76% (Jiménez del Río et al. 1994). Kelp (*Laminaria saccharina*) removed approximately 26–40% of the incoming dissolved inorganic nitrogen (DIN) from salmon farm effluent (Subandar et al. 1993). Ammonium reduction efficiency of *Ulva lactuca* at all three stages treated with effluent was 85–90% in an integrated shellfish–fish–seaweed culture system (Neori et al. 2003). *G. lemaneiformis* removed more than 90% (in spring–summer experiment) and 80% (in autumn experiment) of the DIN generated by fish in an IMTA system of fish–seaweed (Zhou et al. 2006). In our study, the maximal reduction efficiency of ammonium and phosphorus were 83.7 and 70.4%, respectively.

In this study, the SGR of the thallus in the IMTA treatments first increased with the increase in the thallus density, reaching a maximum of 2.52% in treatment 2, and then decreased with the thallus density increasing. The growth rate was lower than that in field-cultured trials in Sanggou Bay (average 3.9% d^{-1}), and also lower than that in an integrated system with fish in a laboratory experiment (3.73–4.49% d^{-1}; Zhou et al. 2006). A possible explanation may be that the fish–seaweed systems had higher TIN concentration (64.85 μM in fish monoculture system). Static water, lower temperature, and light limitation may also have affected the growth of the seaweed.

High nutrient uptake rates are achieved by supplying the seaweed culture with high areal (per unit area) loads of nutrients, conditions that also maximize seaweed areal yield and seaweed protein content. Under these conditions, however, reduction efficiency is low, and therefore a large fraction of the dissolved nutrients remains in the water. To achieve high nutrient reduction efficiency, a seaweed culture should be "starved"—supplied with a low areal load of nutrients, a situation that supports low seaweed areal yields with low protein content (Buschmann et al. 1994). In an integrated fish/bivalve farm, it is therefore necessary to optimize the aerial nutrient load to the seaweeds, to reach acceptable levels of both nutrient uptake rate and reduction efficiency. In this study, the bivalve and seaweed wet weight ratio of treatments 1, 2, 3, and 4 was 1:0.16, 1:0.33, 1:0.61 and 1:0.80, respectively. With a ratio from 1:0.33 to 1:0.80, the nutrient concentrations in the tanks were considerably lower than in the control tank, indicating high nutrient reduction efficiency. Further, the SGR was highest in treatment 2 showing it was optimal for keeping both a healthy water environment and a high algal growth.

In summary, this study demonstrated that the red alga *G. lemaneiformis* has high nutrient bioremediation efficiency and assimilative capacity, and its IMTA with the bivalve *C. farreri* could be an effective and environmentally friendly method to reduce nutrient loading from bivalve culture. We conclude that environmental advantages can be achieved by integrating the seaweed cultivation with bivalve farming in the coastal waters of North China.

Acknowledgments This work was supported by the Special Funds for the Major State Basic Research Projects No. 2006CB400608, the HI-TECH research and development Program of China under contract No. 2006AA10Z414, No. 2006AA100304, the National Science & Technology Pillar Program No. 2006BAD09A02, and the National Marine Public Welfare Research Project No. 200805069. The manuscript was greatly improved due to the constructive comments by several reviewers. We thank Jon Funderud from Norwegian University of Science and Technology for checking the English.

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