

Bioremediation efficiencies of *Gracilaria verrucosa* cultivated in an enclosed sea area of Hangzhou Bay, China

Yuan Zi Huo · Shan Nan Xu · Yang Yang Wang ·
Jian Heng Zhang · Yin Jiang Zhang · Wei Ning Wu ·
Ya Qu Chen · Pei Min He

Received: 23 January 2010 / Revised and accepted: 20 August 2010 / Published online: 3 September 2010
© Springer Science+Business Media B.V. 2010

Abstract Due to the discharge of nutrients into the East China Sea, severe eutrophication has appeared in Hangzhou Bay. Therefore, we cultivated *Gracilaria verrucosa* on a large scale in the Jinshan enclosed sea with an area of 1.72 km² in the northern part of Hangzhou Bay to perform bioremediation. The Fengxian enclosed sea with an area of 2.3 km² and 50 km far from Jinshan was used as the control. The results showed that the Hangzhou Bay was severely eutrophicated before *G. verrucosa* cultivation. During the period of cultivation between August 2006 and July 2007, the annual growth rate of *G. verrucosa* was 9.42% day⁻¹, and the sea water quality was improved from worse than grade IV to grades II–III, with the concentration of dissolved inorganic nitrogen (DIN) and PO₄-P significantly lower than that in the Fengxian enclosed sea ($p < 0.01$). The concentration of NH₄-N, NO₃-N, NO₂-N, and PO₄-P after *G. verrucosa* cultivation was decreased by 54.12%, 75.54%, 49.81%, and 49.00%, respectively. The density of phytoplankton in the Jinshan enclosed sea with cultivation of *G. verrucosa* was 6.90–126.53 × 10⁴ cells m⁻³, which was significantly lower than that in the Fengxian enclosed sea. In addition, species diversity, richness, and evenness was significantly increased after

cultivation of *G. verrucosa* in the Jinshan enclosed sea compared with that in the Fengxian enclosed sea. The density of *Skeletonema costatum*, *Prorocentrum micans*, and *Prorocentrum donghaiense*, which were the usual species of red tides at the coastal sea of China, in the Jinshan enclosed sea with cultivation of *G. verrucosa* was significantly lower. Based on these results, if the water quality in the Jinshan enclosed sea were to be maintained at grade I (DIN ≤ 0.20 mg L⁻¹) or II (DIN ≤ 0.30 mg L⁻¹), 21.8 t or 18.0 t fresh weight of *G. verrucosa* need to be cultivated, respectively. These results indicated that large-scale cultivation of *G. verrucosa* could play a significant role in the bioremediation of Hangzhou Bay.

Keywords Eutrophication · Rodophyta · Seaweeds · Red tides · Nutrient reduction efficiency · East China Sea

Introduction

Eutrophication is generally considered as the principal cause of red tides and the deterioration of marine coastal environments (Schramm et al. 1996). The coastal area is closely associated with human economic activities and has become an important region for human society. Over the last few decades, eutrophication of coastal seas has been aggravated by human activities (Schramm 1999; Capriulo et al. 2002). In coastal areas of China, rapid population increases and human activities such as agricultural production, discharge of industrial wastewater, urban runoff, and large-scale finfish and shrimp aquaculture have caused large inputs of nutrients (He et al. 2008), many times greater than those generated by natural processes (Victor et al. 2002), and many areas exhibit typical symptoms of eutrophication (Xu and He 2006). The detrimental effects

This paper was presented at the 7th Asia Pacific Congress on Algal Biotechnology, New Delhi 2009.

Y. Z. Huo · S. N. Xu · Y. Y. Wang · J. H. Zhang · Y. J. Zhang ·
W. N. Wu · P. M. He (✉)
College of Fisheries and Life Sciences,
Shanghai Ocean University,
Shanghai 201306, China
e-mail: pmhe@shou.edu.cn

Y. Q. Chen
East China Sea Fishery Research Institute,
Chinese Academy of Fishery Sciences,
Shanghai 200091, China

of eutrophication include harmful phytoplankton and unwanted macroalgae blooms (Cuomo et al. 1993; Naylor et al. 2000), as well as development of hypoxia and anoxia (Bonsdorff et al. 2002; Sfriso et al. 1992), which ultimately result in the breakdown of continuous economic development (Fei 2004).

Among different measures to control eutrophication, seaweed cultivation is receiving more attention, because of the low cost of cultivation and possible pollutant removal by the seaweeds (Xu and He 2006). Until now, most bioremediation studies have focused on the green seaweed *Ulva*, the red seaweeds *Gracilaria* (Anderson et al. 1999; Mariachiarra and Pierluigi 2002; Suzuki et al. 2005; Hernández et al. 2006; Yang et al. 2006), *Porphyra* (Chopin and Yarish 1999; Chopin et al. 1999; Chung et al. 2002; McVey et al. 2002; Kraemer et al. 2004; Carmona et al. 2006; Pereira et al. 2006) and the kelp, *Laminaria* (Chopin et al. 2003; Fei 2004; Yang et al. 2004). Many studies have shown that large-scale cultivation of macroalgae may be a feasible approach to reduce eutrophication by reducing the concentration of nutrients in the sea water. However, to our knowledge, few studies are available on the utilization of macroalgae for the bioremediation of low salinity sea areas close to estuaries.

Hangzhou Bay is located in the northern part of East China Sea. Water from Yangtze River, Qiantang River, Yong River, and Caoe River containing large amount of inland pollutants converge in Hangzhou Bay (Chen and Chen 2003). The Bulletin of Marine Environmental Quality of China issued by the State Bureau of Oceanic Administration from 2004 to 2008 showed that the concentration of dissolved inorganic nitrogen (DIN) in all the Hangzhou Bay sea area and the PO₄-P concentration in some sea areas of the Hangzhou Bay are worse than the water quality of grade IV (S.O.A. 2004–2008). In addition, these impacts tend to be most severe in Hangzhou Bay with poor water exchange, leading to severe eutrophication and frequent red tides. The purpose of this study was to cultivate *Gracilaria verrucosa* in a low salinity enclosed sea area of Hangzhou Bay close to the entrance of Yangtze River and evaluate its role in the bioremediation of the eutrophicated area. At the same time, the matched pattern of bioremediation for the utilization of *G. verrucosa* in an enclosed sea area was evaluated. The results of the present paper could provide references for the effective control of eutrophication and the consequent red tides.

Materials and methods

The Jinshan enclosed sea and Fengxian enclosed sea are located in the coastal region of northern Hangzhou Bay in the East China Sea (Fig. 1). This sea area has a subtropical

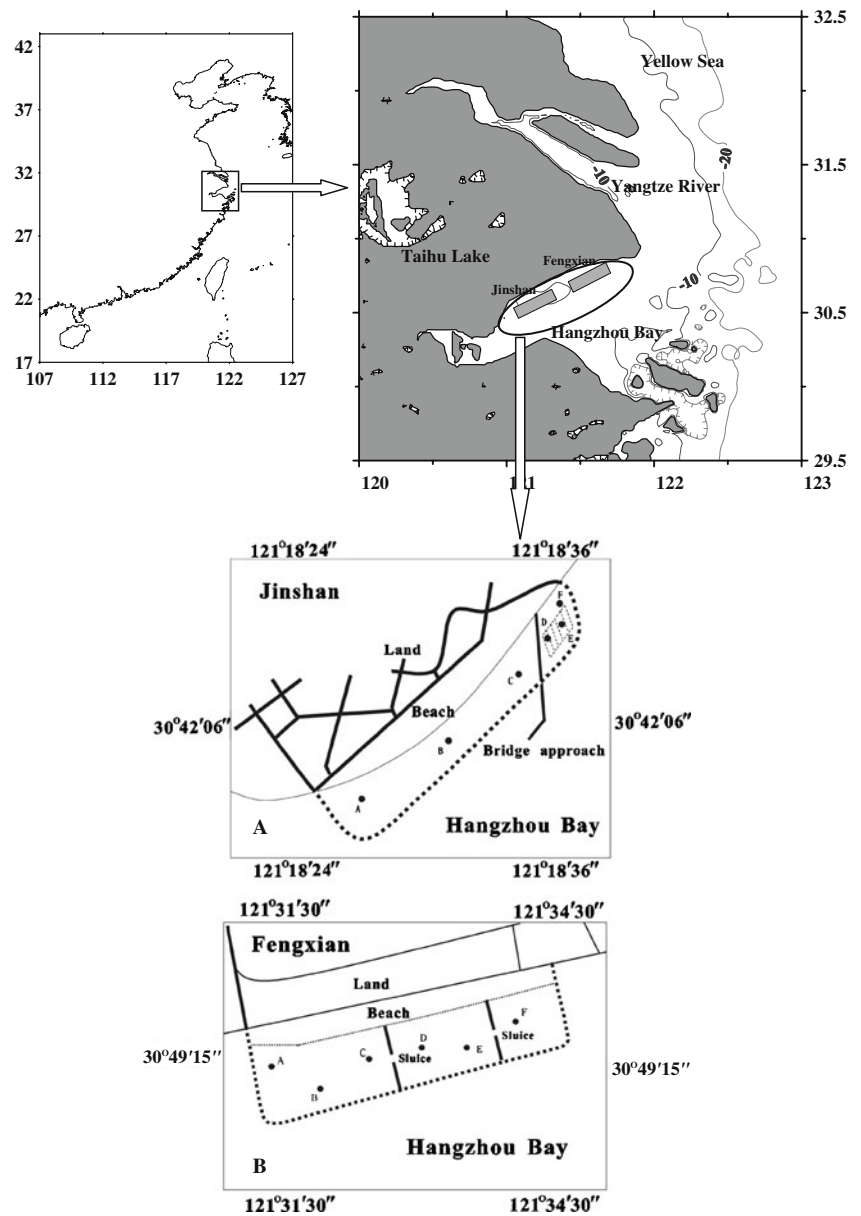
monsoon climate with an annual average temperature of 15.7–15.9°C and annual average precipitation of 988.1–1197.2 mm (Ji et al. 2004). The average salinity of Hangzhou Bay is only 11–15 psu due to the influence of fresh water from the Yangtze, Qiantang, Yong, and Caoe rivers. The length of the coastline and dam for the Jinshan enclosed sea is 3.16 km and 3.63 km, respectively. The Jinshan enclosed sea has an average water depth of 5 m and is divided into two parts by a ramp bridge (Fig. 1a). The east part of Jinshan enclosed sea was selected to cultivate *G. verrucosa* (dashed square in Fig. 1a) because the outside sea water containing high nutrients was introduced into this region. The length of the dam for the Fengxian enclosed sea was 3.83 km with a total area of 2.30 km² (Fig. 1b), which was used as control. A total of six monitoring sites (A, B, C, D, E and F) were established in the both of Jinshan and Fengxian enclosed sea. The monitoring sites D, E and F of the Jinshan enclosed sea were located in the area where *G. verrucosa* was cultivated.

Cultivation of G. verrucosa and the growth rate determination

The equipment for cultivating *G. verrucosa* was composed of 63 cables (each cable 250 m long). The distance between the cables was 3 m. The ends of each cable were fixed by bamboo stakes. The middle part of the cable was supported by five to six floats, forming floating ropes with a total area of 75,000 m². A 3.5-t fresh weight of *G. verrucosa* was transferred from a seaweed culture factory in the Xiangshan Harbor, Zhejiang province and placed in polyethylene bags with a mesh size of 0.5 cm, a length of 10 m, a width of 0.5 m and a height of 0.5 m. The polyethylene bags were hung on the cables with five to six bags per cable, giving a total of 350 bags initially containing an average 10 kg *G. verrucosa* per bag. The bags on adjacent cables were interlaced. By hanging heavy objects, bags were kept 1–2 m below the surface of water in order to avoid damage by strong light intensity. Cultivation of *G. verrucosa* started in August 2006 and finished in July 2007. Every 14 days, three to five bags of *G. verrucosa* were removed from the cables and spun for 3 min in a laundry-drier and weighed. The growth rate was calculated according to the following equation: $SGR = \frac{100 \times (\ln W_i - \ln W_{i-1})}{t}$ where SGR represents specific growth rate (% day⁻¹), W_i represents the fresh weight of the *G. verrucosa* collected at time i , W_{i-1} represents the fresh weight of the *G. verrucosa* collected at time $i-1$ and t represents days (d) between i and $i-1$. To prevent reduced growth rate of *G. verrucosa* in bags due to the crowding effect resulting from fast growth, the additional production was harvested periodically to keep 10–15 kg of *G. verrucosa* in one bag.

Water sample collection and the water quality Water samples were collected in November 2005 and in February,

Fig. 1 The location of the Jinshan and Fengxian enclosed sea in northern part of the Hangzhou Bay. **a** and **b** show the schematic diagram of the Jinshan and Fengxian enclosed sea, respectively. The *thick dashed line* around the enclosure was dam in **a** and **b**. The *dashed square* in the east part of **a** was selected to cultivate *G. verrucosa*. The *letters* indicate the location of monitoring sites



May, August 2006 prior to the cultivation of *G. verrucosa* and once every month during the *G. verrucosa* cultivation period. Temperature, pH, salinity, and dissolved oxygen (DO) were measured in the field by using a YSI multi-parameter water quality meter. Chemical oxygen demand (COD) and biological oxygen demand (BOD) were determined by using an AWA analyzer. Three water samples were collected at 0.5 m below the sea surface at every monitoring site for the determination of chlorophyll *a* (Chl *a*) and inorganic nutrients. The water sample (500 mL) used for Chl *a* determination was immediately filtered onto Whatman GF/F filters and then stored frozen. For the determination, the filter was extracted with 90% acetone and a Turner Designs fluorescent analyzer was used (Parsons et al. 1984). The water sample (250 mL) used

for dissolved inorganic nutrient determination was filtered through cellulose membranes (Millipore® HAWP 0.45 μm) and a small amount of HgCl₂ was added. The samples were transported to the laboratory in cold condition and nutrients were determined using SKALAR flow analyzer.

Collection and determination of phytoplankton Phytoplankton were sampled with a conical plankton net (0.37 m mouth diameter, 77 mm mesh size), which was towed vertically from near the bottom to the surface. Simultaneously, 1,000 mL sea water was also sampled at different depths of water at every site. The samples were immediately fixed with Lugol’s solution at a final concentration of 1%. After settling over 24 h and concentration, the phytoplankton species were determined and counted.

The dominance index (Y) of the phytoplankton species, species diversity index (H'), richness index (D) and evenness index (J) were calculated according the Wang et al. (2005), Shannon and Weaver (1963), Margalef (1968) and Pielou (1975). A species with a $Y > 0.02$ was considered as a dominant phytoplankton species.

Data analysis Average values obtained from the each monitoring site from the Jinshan and Fengxian enclosed seas were used for water quality and phytoplankton comparison. ANOVA was used to compare the phytoplankton index and water quality. Differences were considered significant when $p < 0.05$ and all the data are displayed as mean \pm standard deviation.

Results

Eutrophication of Hangzhou Bay

The concentration of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{PO}_4\text{-P}$ in the both Jinshan and Fengxian enclosed seas was similar during the four seasons prior to the cultivation of *G. verrucosa* (Table 1), with a range of 0.22–0.38 mgL^{-1} , 1.05–1.45 mgL^{-1} , 0.020–0.042 mgL^{-1} , and 0.018–0.037 mgL^{-1} , respectively. $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were the main forms of dissolved inorganic nitrogen, accounting for 14.55–23.26% and 74.29–84.12%, respectively. DO, COD, BOD, and Chl *a* were 5.92–6.96 mgL^{-1} , 4.17–5.15 mgL^{-1} , 4.21–5.13 mgL^{-1} and 9.25–16.14 μgL^{-1} , respectively, in the both enclosed seas. These results suggested that both Jinshan and Fengxian enclosed seas of Hangzhou Bay were severely eutrophicated.

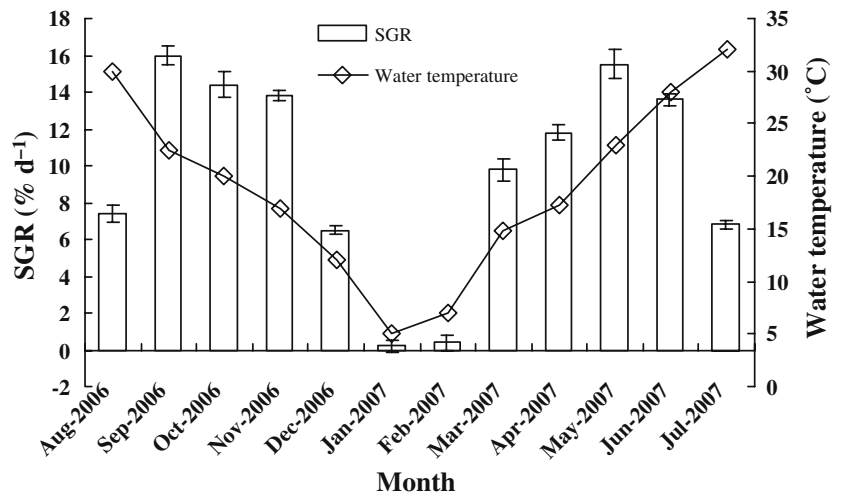
Growth rate of *G. verrucosa*

The water temperature varied between 28.93 and 31.80°C in summer, and 5.20 to 11.80°C in winter (Fig. 2). The growth rate of *G. verrucosa* in the Jinshan enclosed sea from September to November of 2006 and from April to June of 2007 was significantly higher than in other months ($p < 0.05$). The highest *SGR* of 15.88%·day⁻¹ and 15.16%·day⁻¹ occurred in September and May. At the end of December 2006, most of the *G. verrucosa* was harvested and nearly 1 t fresh weight of *G. verrucosa* was left in order to observe the growth during the winter. In January, the sea water temperature could drop to 5°C, and the growth of *G. verrucosa* stopped or was negative. However, when the water temperature increased in February, *G. verrucosa* growth increased and the biomass was doubled at the end of February. The additional production of *G. verrucosa* was used as seedlings for extensive cultivation. And at the end

Table 1 Concentrations of DIN, $\text{PO}_4\text{-P}$, DO, BOD, COD and Chl *a* in both Jinshan and Fengxian enclosed seas before the cultivation of *G. verrucosa*

Districts (Concentrations)	Jinshan enclosed sea				Fengxian enclosed sea			
	Nov-05	Feb-06	May-06	Aug-06	Nov-05	Feb-06	May-06	Aug-06
$\text{NH}_4\text{-N}$ (mgL^{-1})	0.28±0.02	0.22±0.04	0.27±0.03	0.38±0.02	0.23±0.02	0.35±0.01	0.22±0.04	0.36±0.03
$\text{NO}_3\text{-N}$ (mgL^{-1})	1.27±0.19	1.18±0.09	1.05±0.28	1.30±0.23	1.33±0.17	1.45±0.25	1.22±0.33	1.15±0.31
$\text{NO}_2\text{-N}$ (mgL^{-1})	0.02±0.007	0.032±0.008	0.039±0.005	0.041±0.010	0.021±0.011	0.042±0.008	0.038±0.009	0.038±0.007
$\text{PO}_4\text{-P}$ (mgL^{-1})	0.023±0.005	0.032±0.008	0.028±0.007	0.037±0.012	0.018±0.012	0.027±0.006	0.025±0.007	0.023±0.009
DO (mgL^{-1})	6.96±1.58	6.93±1.33	6.61±2.14	6.40±2.14	5.92±2.03	6.45±1.55	6.77±1.98	6.19±1.73
COD (mgL^{-1})	4.35±0.78	4.17±1.03	4.64±1.86	4.93±1.21	4.57±1.67	4.28±1.54	4.46±2.04	5.15±1.09
BOD (mgL^{-1})	4.33±1.34	5.02±0.77	4.69±1.76	5.11±1.05	4.93±0.77	4.21±1.33	5.13±0.55	4.97±0.96
Chl <i>a</i> (μgL^{-1})	14.53±2.33	10.25±3.78	13.67±3.39	16.14±3.84	13.67±2.67	9.25±3.28	14.34±2.19	15.83±4.56

Fig. 2 Variations of the specific growth rate of *G. verrucosa* in the Jinshan enclosed sea



of the experiment, a cumulative 15 t dry weight of *G. verrucosa* was harvested from the Jinshan enclosed sea. Our results suggested that *G. verrucosa* could survive in both winter and summer in Hangzhou Bay.

Concentrations of dissolved inorganic nutrients

The monthly changes of NH₄-N, NO₃-N, NO₂-N and PO₄-P concentrations in the water column of the Jinshan and

Fengxian enclosed seas are shown in Fig. 3a–d. Before *G. verrucosa* cultivation (prior to August 2006), there was no significant difference in the concentration of these nutrients between the two seas, with the exception of PO₄-P in the Jinshan enclosed sea which was significantly higher than in the Fengxian enclosed sea ($p < 0.01$). During the period of *G. verrucosa* cultivation from September 2006 to July 2007, the concentrations of dissolved inorganic nutrients in the Jinshan enclosed sea were significantly lower than those

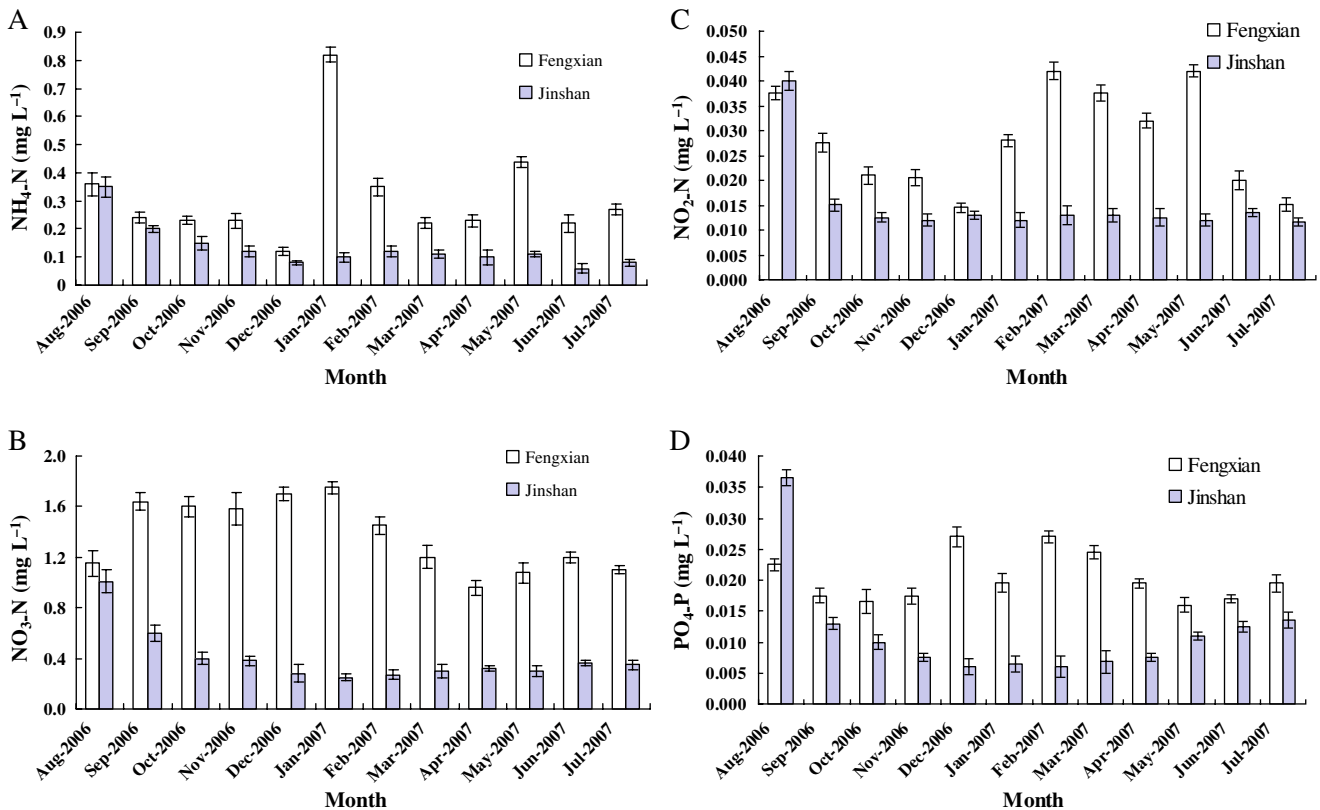


Fig. 3 Concentration of NH₄-N (a), NO₃-N (b), NO₂-N (c), and PO₄-P (d) of the sea water in the Jinshan and Fengxian enclosed sea in difference months

in the Fengxian enclosed sea ($p < 0.05$) with the concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{PO}_4\text{-P}$ decreased by 54.12%, 75.54%, 49.81%, and 49.00%, respectively.

Biomass and species diversity of phytoplankton

The highest phytoplankton density in the Fengxian enclosed sea occurred from June to August with an average density of $8584.91 \times 10^4 \text{ ind. m}^{-3}$, while the lowest densities occurred from January to February with an average density of $9.52 \times 10^4 \text{ ind. m}^{-3}$ (Fig. 4). During the period of *G. verrucosa* cultivation in the Jinshan enclosed sea, the average density of phytoplankton was $126.53 \times 10^4 \text{ ind. m}^{-3}$ in summer/fall and $6.90 \times 10^4 \text{ ind. m}^{-3}$ in winter/spring, which was significantly lower than that in the Fengxian enclosed sea ($p < 0.05$).

The dominant species in the Jinshan enclosed sea were *Cerataulina bergonii*, *Chaetoceros subtilis*, *Chaetoceros subsecundus*, *Melosira moniformis*, *Coscinodiscus oculus-iridis*, and *Pleurosigma formosum*. The most abundant species was *C. bergonii* with an average density of $2850.35 \times 10^4 \text{ ind. m}^{-3}$. While the dominant species in the Fengxian enclosed sea was *Skeletonema costatum*, *C. subsecundus*, *M. moniformis*, *Prorocentrum micans* and *Prorocentrum donghaiense*. In spring, the average density in the Fengxian Sea was $3050.82 \times 10^4 \text{ ind. m}^{-3}$. In addition, the abundance of dinoflagellates was much higher than that of diatoms, and *P. micans* accounted for 72.74% of the total phytoplankton. In summer, the average abundance of phytoplankton was $8584.91 \times 10^4 \text{ ind. m}^{-3}$ and the dominant species was *S. costatum*, accounting for 70.30% (Table 2). The annual diversity index (H') and richness index (D) in the Jinshan enclosed sea were significantly higher than in the Fengxian enclosed sea ($p < 0.05$; Table 3). However, there

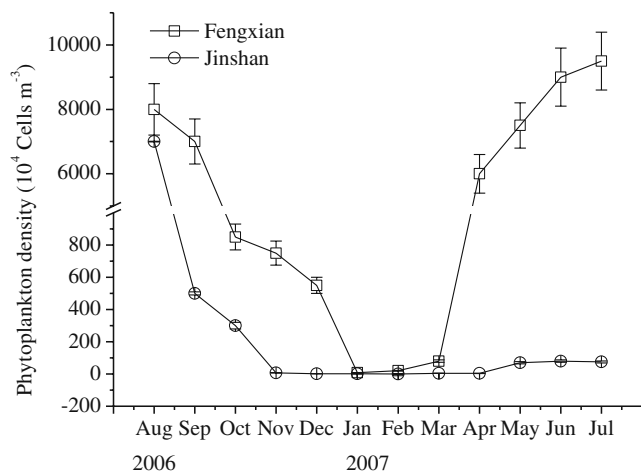


Fig. 4 Monthly changes of phytoplankton density in the Jinshan and Fengxian enclosed sea

was no significant difference in the evenness index (J ; $p > 0.05$; Table 3).

Discussion

Eutrophication of Hangzhou Bay

From November 2005 to August 2006, the sea water of Hangzhou Bay was considered as severely eutrophicated. The COD had reached to the level of grade IV or worse than grade IV level. Although DIN ($29.56\text{--}45.36 \mu\text{mol L}^{-1}$) was lower than in other coastal areas in China ($57.1\text{--}108.0 \mu\text{mol L}^{-1}$), e.g., coastal areas of Lvsu harbor in Jiangsu province, Xiangshan Harbor in Zhejiang province and Yaling Bay in Guangdong province (Ye et al. 2002; Jia et al. 2005; He et al. 2008), it also reached the level of grade IV or worse than grade IV level. The high concentration of DIN in Hangzhou Bay was mainly caused by inland activities and nutrient discharge or runoff to the coastal area. According to the Bulletin of Marine Environmental Quality of China issued by State Bureau of Oceanic Administration (S.O.A. 2006–2007), in 2006 and 2007, COD introduced into the sea water through Yangtze River was 5.0×10^6 and $4.9 \times 10^6 \text{ t year}^{-1}$ and nutrients were 1.2×10^6 and $1.4 \times 10^6 \text{ t year}^{-1}$, respectively. In addition, nutrients introduced by mariculture are also an important factor causing high concentrations of inorganic nutrients in Hangzhou Bay. Until the end of 2004, the total yield of aquatic products in Zhejiang province where the Hangzhou Bay is located reached $4.9 \times 10^6 \text{ t}$, among which shellfish was $0.75 \times 10^6 \text{ t}$, accounting for 80.75% of the total yield (Xu et al. 2007). Cultivation of shellfish produces large amount of biological deposition, which easily results in eutrophication. In addition, industrial and domestic sewage also plays a role in the introduction of nutrients. Although the nutrients introduced through wet precipitation accounted for a small percentage of the total nutrients in the entrance of Yangtze River, sudden precipitation can cause eutrophication on the surface of the seawater (Zhang et al. 2003). Our results showed that the concentration of $\text{PO}_4\text{-P}$ was relatively low ($0.19\text{--}0.39 \mu\text{mol L}^{-1}$). The overall N:P indicated that P was the main limiting nutrient in the coastal waters of Hangzhou Bay. Even the lowest N:P ratio (44.75:1) surpassed the threshold at which phosphorus becomes limiting (about 25:1; Guildford and Hecky 2000). These results indicate that any efforts to reduce eutrophication in the coastal waters of Hangzhou Bay must focus on nitrogen removal.

Growth of *G. verrucosa* in Hangzhou Bay

The growth of *G. verrucosa* was significantly affected by the water temperature. When the water temperature ranged

Table 2 Dominant species as well as values of dominance index (*Y*) of phytoplankton in both Jinshan and Fengxian enclosed seas in different seasons

Phytoplankton species	Jinshan enclosed sea				Fengxian enclosed sea			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Diatom								
<i>Cerataulina bergonii</i>	0.06	0.32	0.27		0.1			
<i>Skeletonema costatum</i>		0.03			0.08	0.69		
<i>Chaetoceros subtilis</i>	0.07	0.35	0.03			0.05		
<i>Chaetoceros subsecundus</i>	0.03		0.3				0.46	
<i>Chaetoceros affinis</i>		0.22			0.07			
<i>Melosira moniiformis</i>	0.14			0.36				0.56
<i>Melosira granulata</i>				0.09				
<i>Coscinodiscus oculusiridis</i>		0.04	0.05					
<i>Coscinodiscus granii</i>			0.06					
<i>Coscinodiscus asteromphalus</i>		0.03					0.04	
<i>Biddulphia sinensis</i>	0.04							
<i>Nitzschia paradoxa</i>	0.06							0.23
<i>Pleurosigma formosum</i>	0.08			0.06				
<i>Pleurosigma pelagicum</i>	0.06							
Dinoflagellate								
<i>Prorocentrum micans</i>					0.71	0.17		
<i>Prorocentrum donghaiense</i>							0.03	

from 16 to 29°C, SGR could reach 15.88% day⁻¹. In winter, the low water temperature inhibited the growth of *G. verrucosa*. Therefore high temperatures in summer and low temperature in winter are not appropriate for the growth and survival of *G. verrucosa*. We adopted a series of measures including utilization of polyethylene bags and cultivation of *G. verrucosa* in a lower depth of water. The results showed that *G. verrucosa* could survive the low temperatures in winter and once the temperature increased in spring, the seedlings of *G. verrucosa* can be used for extensive cultivation. The annual average growth rate of *G. verrucosa* in Hangzhou Bay was 9.42% day⁻¹, which was lower than that in Xiangshan Harbor cage culture sea area (11.52% day⁻¹) and lower than the growth rate of *G. lemaneiformis* (11~13% day⁻¹) (Zhou et al. 2006; Yang et al. 2006). However, the growth rate of *G. verrucosa* was

higher than that of *G. chilensis* (5.6–6.7% day⁻¹; Troell et al. 1997) and *G. longissima* (6% day⁻¹) (Hernández et al. 2006) in the cage culture sea areas. Therefore, *G. verrucosa* can be successfully cultivated and used for bioremediation of eutrophication in Hangzhou Bay.

Bioremediation by *G. verrucosa*

The high value of *Gracilaria* in human food, abalone feed, and agar production has led to the development of farming systems in the sea and in ponds in Brazil, Chile, China, Israel, Mexico, South Africa, Taiwan, and the United States (Mclachlan and Bird 1986; Neto 1987; Fei et al. 1999; Buschmann et al. 1995, 2001; Wakibia et al. 2001). In China, *Gracilaria* has been cultivated on a large scale in Guangdong, Shandong, Hainan, Guangxi, Fujian, Zhejiang,

Table 3 Seasonal changes in *H'*, *D* and *J* of phytoplankton in both Jinshan Jinshan and Fengxian enclosed seas

Index	Jinshan enclosed sea					Fengxian enclosed sea				
	Spring	Summer	Autumn	Winter	Annual average	Spring	Summer	Autumn	Winter	Annual average
<i>H'</i>	2.6	1.55	2.18	1.85	2.04	0.57	0.46	0.67	0.78	0.62
<i>D</i>	0.8	0.65	0.71	0.57	0.68	0.36	0.27	0.16	0.22	0.25
<i>J</i>	0.62	0.36	0.73	0.48	0.55	0.53	0.38	0.46	0.61	0.50

Jiangsu, and Liaoning provinces, etc. (Fei et al. 1998; Tseng 2001; Yang and Fei 2003; Yang et al. 2004, 2006). In addition to the production of agar, many studies have shown that *Gracilaria* can effectively remove nutrients (e.g., N and P) in the polyculture system of fish, or scallop, or shrimp with algae (Buschmann et al. 1996; Troell et al. 1997; Neori et al. 1998; Jones et al. 2001; Yang et al. 2005, 2006; Hernández et al. 2006; Zhou et al. 2006; Xu et al. 2008a, b; Mao et al. 2009; Jiang et al. 2010). In the cage culture sea area of Dongshan Island in Zhejiang Province, *G. lemaneiformis* could remove more than 80% of DIN and PO₄-P (Tang et al. 2005). Yang et al. (2006) found that after 40 days of *G. lemaneiformis* cultivation, the removal rate of NH₄-N and PO₄-P reached 69.45% and 26.74%, respectively. Xu et al. (2007) reported that the concentrations of inorganic nitrogen and inorganic phosphate in the polyculture ponds of *Gracilaria lichenoides* with *Litopenaeus vannamei*, and with *Epinephelus awoara* were lower and more stable than those in the ponds without *Gracilaria*. Mao et al. (2009) reported that the maximum reduction efficiency of ammonium and phosphorus was 83.7% and 70.4%, respectively, in one polyculture system of *G. lemaneiformis* and scallop *Chlamys farreri*.

The present study also demonstrates that cultivation of *G. verrucosa* played a significant role in the bioremediation of eutrophicated water in the Jinshan enclosed sea of Hangzhou Bay. Bioremediation effects of *G. verrucosa* cultivated in eutrophicated seas were achieved through absorption and utilization of excessive nutrients (Xu and He 2006). Over the period of *G. verrucosa* cultivation water quality in the Jinshan enclosed sea was transformed from grade IV (from August to October in 2006) to grades II–III (from November 2006 to July 2007). From August to December in 2006, the dissolved inorganic nutrients decreased to the lowest level because of absorption by *G. verrucosa* during the period of fast growth. During winter from December 2006 to February 2007, even though the most of *G. verrucosa* biomass was harvested, the dissolved inorganic nutrients still remained at the low level due to less rainfall and no water exchange with the external sea water. From March to July 2007, although the external sea water containing relatively high concentrations of nutrients the water quality still remained good because of the fast growth of *G. verrucosa*. The nutrient removal efficiency achieved by *G. verrucosa* cultivation in the Jinshan enclosed sea was higher than that achieved by *G. lemaneiformis* cultivation (Tang et al. 2005) or that achieved by *G. verrucosa* cultivated in the cage culture sea area of Dongshan Island (Tang et al. 2005).

The dam of the Jinshan enclosed sea prevented the effect of Hangzhou Bay tides on internal seawater of enclosure. The current of the water in the enclosed sea was slow and mainly initiated by the wind. Exchange of water was artificially controlled and the enclosed sea was regularly

replenished with external sea water to maintain stable water level. These provided relatively stable conditions to investigate the bioremediation efficiencies of *G. verrucosa* cultivated in one eutrophicated sea. In addition, these stable conditions increased the efficiency of nutrient uptake and growth rate of *G. verrucosa*. The results of the present paper also show that *G. verrucosa* could inhibit the growth of phytoplankton but increased phytoplankton species diversity. Cultivation of *G. verrucosa* could not only control the eutrophication, but also prevent and reduce the occurrence of red tides.

To maintain the low level of DIN in the Jinshan enclosed sea, the matched pattern of *G. verrucosa* cultivated could be evaluated based on the results of present study. The volume of the seawater in the Jinshan enclosed sea is adjusted by periodically artificial sluicing, so according to the method of calculating sea water exchange rate (Makoto 1984), the horizontal distribution of the nutrients inside and outside of the enclosed sea, and the assuming that the concentration of nutrients in the enclosed sea did not change once the nutrients and water exchange are balanced, 0.78 mg·L⁻¹ average DIN could be calculated in the Jinshan enclosed sea. The annual average growth rate of *G. verrucosa* was 9.42% day⁻¹, the average nitrogen content in the tissue was 4.80%, and dry/wet ratio was 0.169 (Xu et al. 2008a, b). In addition, on the basis of the enclosure area of 1.72 km² and average water depth of 5 m, it was evaluated that 21.8 t of *G. verrucosa* need to be cultivated to achieve a grade I water quality (DIN ≤0.20 mg·L⁻¹) and 18.0 t of *G. verrucosa* need to be cultivated to achieve a grade II water quality (DIN ≤0.30 mg·L⁻¹). However, this calculation is only based on average growth rate, average DIN concentration and average nitrogen content of *G. verrucosa*. The precise requirement of *G. verrucosa* cultivation in different months in the Jinshan enclosed sea needs to be further studied.

Acknowledgment This study was supported by National Natural Science Foundation (30371101), the Project for Excellence Disciplines Leader (08XD14037), International Cooperation Project (08540702600), Pujiang Project (05PJ14086), in Shanghai Science Commission, and Superiority Subject Sponsored Projects in Shanghai Municipal Education Commission (S30701). Thanks are due also to two anonymous reviewers for their valuable comments and suggestions on the manuscript.

References

- Anderson RJ, Smit AJ, Levitt GJ (1999) Upwelling and fishfactory waste as nitrogen sources for suspended cultivation of *Gracilaria gracilis* in Saldanha Bay, South Africa. *Hydrobiologia* 398/399:455–462
- Bonsdorff E, Römberg C, Aarnio K (2002) Some ecological properties in relation to eutrophication in the Baltic Sea. *Hydrobiologia* 475/476:371–377

- Buschmann AH, Westermeier R, Retamales CA (1995) Cultivation of *Gracilaria* on the sea-bottom in southern Chile: a review. *J Appl Phycol* 7:291–301
- Buschmann AH, Troell M, Kautsky N, Kautsky L (1996) Integrated cultivation of salmonids and *Gracilaria chilensis* (Gracilariiales, Rhodophyta). *Hydrobiologia* 326/327:75–82
- Buschmann AH, Correa JA, Westermeier R, Hernandez-Gonzalez MC, Norambuena R (2001) Red algal farming in Chile: a review. *Aquaculture* 194:203–220
- Capriulo GM, Smith G, Troy R, Wikfors G, Pellet J, Yarish C (2002) The planktonic food web structure of a temperate zone estuary, and its alteration due to eutrophication. *Hydrobiologia* 475/476:263–333
- Carmona R, Kraemer GP, Yarish C (2006) Exploring northeast American and Asian species of *Porphyra* for use in an integrated finfish—algal aquaculture system. *Aquaculture* 252:54–65
- Chen JY, Chen SL (2003) Environmental changes of Yangtze river and opinions on estuary improvement. *Water Resour Hydropower Eng* 34:19–25, in Chinese with English abstract
- Chopin T, Yarish C (1999) Aquaculture does not only mean fin-fish monoculture....seaweeds must be a significant component for an integrated ecosystem approach. *Bull Aquacult Assoc Can* 99:35–37
- Chopin T, Yarish C, Wilkes R, Belyea E, Lu S, Mathieson A (1999) Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. *J Appl Phycol* 11:463–472
- Chopin T, Bastarache S, Belyea E, Haya K, Sephton D, Martin JL, Eddy S, Stewart I (2003) Development of the cultivation of *Laminaria saccharina* as the extractive inorganic component of an integrated aquaculture system and monitoring of therapeutants and phycotoxins. *J Phycol* 39(S1):10
- Chung I, Kang YH, Yarish C, Kraemer G, Lee J (2002) Application of seaweed cultivation to the bioremediation of nutrient-rich effluent. *Algae* 17:187–194
- Cuomo V, Merrill J, Palomba I, Perretti A (1993) Systematic collection of *Ulva* and mariculture of *Porphyra*: biotechnology against eutrophication in the Venice Lagoon. *Int J Environ Stud* 43:141–149
- Fei XG (2004) Solving the coastal eutrophication problem by large scale seaweed cultivation. *Hydrobiologia* 512:145–151
- Fei XG, Lu S, Bao Y, Wilkes R, Yarish C (1998) Seaweed cultivation in China. *World Aquac* 29:22–24
- Fei XG, Bao Y, Lu S (1999) Seaweed cultivation: traditional way and its reformation. *Chin J Oceanol Limnol* 7:193–199
- Guildford SJ, Hecky RE (2000) Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: is there a common relationship? *Limnol Oceanogr* 45:1213–1223
- He PM, Xu SN, Zhang HY, Wen SS, Dai YJ, Lin SJ, Yarish C (2008) Bioremediation efficiency in the removal of dissolved inorganic nutrients by the red seaweed, *Porphyra yezoensis*, cultivated in the open sea. *Water Res* 42:1281–1289
- Hernández I, Pérez-Pastor A, Vergara JJ, Martínez-Aragón JF, Fernández-Engo MÁ, Pérez-Lloréns JL (2006) Studies on the biofiltration capacity of *Gracilariopsis longissima*: from microscale to macroscale. *Aquaculture* 252:43–53
- Ji HH, Ye SF, Wang JH, Huang XQ (2004) Variation and assessment of nutrients in the sea area of Jinshan three-islands marine ecological natural reserve. *Mar Environ Sci* 23:38–39, in Chinese with English abstract
- Jia HL, Wen YM, Xie J (2005) Self-pollution status of cage culture in Yaling Bay. *Mar Environ Sci* 24(2):5–8, in Chinese with English abstract
- Jiang ZJ, Fang JG, Mao YZ, Wang W (2010) Eutrophication assessment and bioremediation strategy in a marine fish cage culture area in Nansha Bay, China. *J Appl Phycol* 22:421–426
- Jones AB, Dennison WC, Preston NP (2001) Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: a laboratory scale study. *Aquaculture* 193:155–178
- Kraemer GP, Carmona R, Chopin T, Neefus C, Tang XR, Yarish C (2004) Evaluation of the bioremediatory potential of several species of the red alga *Porphyra* using short-term measurements of nitrogen uptake as a rapid bioassay. *J Appl Phycol* 16:489–497
- Makoto K (1984) The concept of tidal exchange and the tidal exchange ratio. *J Oceanol Soc Jpn* 40:135–147
- Mao YZ, Yang HS, Zhou Y, Ye NH, Fang JG (2009) Potential of the seaweed *Gracilaria lemaneiformis* for integrated multi-trophic aquaculture with scallop *Chlamys farreri* in North China. *J Appl Phycol* 21:649–656
- Margalef DR (1968) Perspectives in ecological theory. University of Chicago Press, Chicago, pp 1–111
- Mariachiara N, Pierluigi V (2002) Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy). *J Exp Mar Biol Ecol* 269:65–83
- McLachlan J, Bird CJ (1986) *Gracilaria* (Gigartinales, Rhodophyta) and productivity. *Aquat Bot* 26:27–49
- McVey JP, Stickney R, Yarish C, Chopin T (2002) Aquatic polyculture and balanced ecosystem management: new paradigms for seafood production. In: Stickney RR, McVey JP (eds) Responsible aquaculture. CAB International, Oxon, pp 91–104
- Naylor RL, Goldberg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. *Nature* 405:1017–1024
- Neori A, Ragg NLC, Shpigel M (1998) The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based system: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system. *Aquac Eng* 17:215–239
- Neto CC (1987) Seaweed culture in Rio Grande do Norte, Brazil. *Hydrobiologia* 151/152:363–367
- Parsons TR, Maita Y, Lalli GM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon, pp 101–122
- Pereira R, Yarish C, Sousa-Pinto I (2006) The influence of stocking density, light and temperature on the growth, production and nutrient removal capacity of *Porphyra dioica* (Bangiales, Rhodophyta). *Aquaculture* 252:66–78
- Pielou EC (1975) Ecological diversity. Wiley, New York, pp 16–51
- S.O.A. (2004–2008) Marine environment quality bulletin of China. State Oceanic Administration, China. http://www.soa.gov.cn/hyjww/hygb/hyhjzlg/A020707/index_1.htm. Accessed 12 Feb 2009
- Schramm W (1999) Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. *J Appl Phycol* 11:69–78
- Schramm W, Lotze H, Schories D (1996) Eutrophication and macroalgal blooms in inshore waters of the German Baltic coasts: the Schlei Fjord, a case study. In: Rijstenbil JW, Kamermans P, Nienhuis PH (eds) EUMAC Synthesis Report and Proceedings of the second EUMAC Workshop. Sete, France, pp 18–73
- Sfriso A, Pavoni B, Marcomini A (1992) Macroalgae, nutrient cycles and pollutants in the Lagoon of Venice. *Estuaries* 15:517–528
- Shannon CE, Weaver W (1963) The mathematical theory of communication. University of Illinois Press, Urbana, pp 1–117
- Suzuki Y, Kametani T, Maruyama T (2005) Removal of heavy metals from aqueous solution by nonliving *Ulva* seaweed as biosorbent. *Water Res* 39:1803–1808
- Tang KX, You XP, Lin YS, Chen ME, Shen DL, Lin SB (2005) A study on bioremediation of eutrophication of mariculture waters by *Gracilaria lemaneiformis*. *Acta Ecol Sin* 25(11):3044–3051, in Chinese with English abstract

- Troell M, Halling C, Nilsson A, Buschmann AH, Kautsky N, Kautsky L (1997) Integrated marine cultivation of *Gracilaria chilensis* (Gracilariales, Rhodophyta) and salmon cages for reduced environmental impact and increased economic output. *Aquaculture* 156:45–61
- Tseng CK (2001) Algal biotechnology industry and research activities in China. *J Appl Phycol* 13:375–380
- Victor ND, Elliott M, Orive E (2002) Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. *Hydrobiologia* 475/476:1–19
- Wakibia JG, Anderson RJ, Keats DW (2001) Growth rates and agar properties of three gracilarioids in suspended open-water cultivation in St. Helena Bay, South Africa. *J Appl Phycol* 13:195–207
- Wang YL, Yuan J, Shen XQ (2005) Ecological character of phytoplankton in spring in the Yangtze River estuary and adjacent water. *J Fish Sci China* 12:300–306, in Chinese with English abstract
- Xu SN, He PM (2006) Analysis of phenomena for frequent occurrence of red tides and bioremediation by seaweed cultivation. *J Fish China* 30(4):554–561, in Chinese with English abstract
- Xu SN, Chen ZZ, He PM (2007) Influencing analysis of human activities on frequency of red tides (HABs) in Zhejiang Sea Area. *Mar Environ Sci* 26:232–236, in Chinese with English abstract
- Xu SN, Wen SS, Wu WX, He PM (2008a) Bioremediation of caged fish aquaculture by the red alga *Gracilaria verrucosa* in an integrated multi-trophic aquaculture system. *Acta Ecol Sinica* 28:1467–1475, in Chinese with English abstract
- Xu YJ, Fang JG, Wei W (2008b) Application of *Gracilaria lichenoides* (Rhodophyta) for alleviating excess nutrients in aquaculture. *J Appl Phycol* 20:199–203
- Yang YF, Fei XG (2003) Prospects for bioremediation of cultivation of large-sized seaweed in eutrophic mariculture areas. *J Ocean U Qingdao* 33:53–57, in Chinese with English abstract
- Yang YF, Li CH, Nie XP, Tang DL, Chung IK (2004) Development of mariculture and its impacts in Chinese coastal waters. *Rev Fish Biol Fish* 14:1–10
- Yang HS, Zhou Y, Mao YZ, Li XX, Liu Y, Zhang FS (2005) Growth characters and photosynthetic capacity of *Gracilaria lemaneiformis* as a biofilter in a shellfish farming area in Sanggou Bay, China. *J Appl Phycol* 17:199–206
- Yang YF, Fei XG, Song JM, Hu HY, Wang GC, Chung IK (2006) Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. *Aquaculture* 254:248–255
- Ye Y, Xu JL, Ying QL, Wei DY, Chen QZ, Ning XR (2002) Changes of nutrient in net aquaculture area of Xiangshan Harbor. *Mar Environ Sci* 21(1):39–41, in Chinese with English abstract
- Zhang GS, Chen HT, Zhang J, Liu SM (2003) Nutrient elements in the atmospheric wet deposition in Changjiang River estuary. *Chin J Appl Ecol* 14:1107–1111
- Zhou Y, Yang HS, Hu HY, Liu Y, Mao YZ, Zhou H, Xu XL, Zhang FS (2006) Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. *Aquaculture* 252:264–276